Declaration

This thesis was composed by myself and is based on work carried out at the University of Edinburgh between September 1990 and December 1993.

Signed

Date 5/4/94
To mum and dad; thanks.
We are galactic dust,
and to galactic dust we will return.

P. W. Atkins
Abstract

The work described in this thesis is concerned with the development and application of laser desorption laser photoionisation time-of-flight mass spectrometry (L²TOFMS). This technique has been used to enable photoionisation mass spectra of a very wide variety of involatile and thermally labile molecules to be recorded. The instrument used for this work is described along with an overview of the fundamental principles behind this methodology.

A number of specific classes of molecules have been studied using L²TOFMS. These include polyaromatic hydrocarbons, porphyrins, dyestuffs and a variety of analytically important staining agents. The advantages of this approach for analysing complex mixtures, which yield relatively simple mass spectra, have been demonstrated for both environmental systems and commercially important mixtures. It has also been shown that L²TOFMS can be used for the direct interrogation of target systems adsorbed onto organic substrates.

L²TOFMS has been used to probe the photophysics of both porphyrin molecules and a series of azo dyes. Ionising wavelength dependent fragmentation was observed for a number of metallotetraphenylporphyrins and metallo-octaethylporphyrins. Using 193 nm laser photoionisation, molecular dissociation, involving loss of the macrocycle side groups, was shown to be similar to that obtained by electron impact ionisation. Whereas, at 266 nm, fragmentation via a neutral intermediate state, resulting in the loss of the metal from the macrocycle, competes with further photon absorption. Characteristic azo-bond photoreductive cleavage has been observed for azo molecules when using 266 nm laser photoionisation. This behaviour is linked to the cis-trans photoisomerisation of the azo bond.

Finally, the value of L²TOFMS as a general analytical tool is considered. Its utility in comparison with competing techniques is discussed, along with suggestions as to the development of this technique and directions for further work.
This thesis would not have been produced without generous contributions from a number of different sources. Firstly, I wish to thank my supervisor, Pat Langridge-Smith, for introducing me to the project and for his guidance and friendship through the course of this work. I also wish to give particular thanks to Anita Jones for many valuable discussions and her enduring interest in this work.

Thanks must also go to Unilever Plc. and a number of personnel there. Unilever sponsored my SERC CASE award and provided much needed financial support for the project. Kevin Costello not only designed and built the instrument used in this work but provided hands-on experience and many opportunities for robust discussion. Phil Cummins and Ken Lee have also provided both moral support and confidence by their faith in this research project.

A special thanks must be given to my coworkers. As a novice, George Keenan introduced me to practical L²TOFMS, taught me how to operate the instrumentation and revealed its potential significance. Trevor Ridley has provided ready assistance with the operation and alignment of the excimer pumped dye laser. My fellow research group members deserve a special mention here. Jon Miller has provided unique inspiration. Jim MacDonald, Craig Redpath and Scott Wright have also inspired and amused, always being ready to lend advice and a helping hand. Also, thanks to Ali, Graeme, Cameron and all the folk in room 6.

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# Table of Contents

1. Introduction 17

2. L²TOFMS - Background Theory 25
   2.1 Introduction .................................. 25
   2.2 Laser Desorption ................................ 26
      2.2.1 Background and Mechanisms .............. 26
      2.2.2 Laser Desorption of Neutral Molecules .... 36
   2.3 Laser Multiphoton Ionisation .................... 39
      2.3.1 Background ................................ 39
      2.3.2 Theoretical Description ................... 44
      2.3.3 Analytical Advantages of MPI ............. 52
   2.4 Time-of-Flight Mass Spectrometry ................ 59
      2.4.1 Introduction .................. 59
      2.4.2 Resolution Limiting Factors .......... 64
      2.4.3 The Reflectron TOFMS .................. 70
   2.5 Concluding Remarks ............................ 75

3. L²TOF Mass Spectrometry: Instrumentation 85
   3.1 Introduction ............................... 85
Table of Contents

3.2 Reflectron Mass Spectrometer Vacuum System ..................... 86
3.3 Laser Desorption Source .................................. 90
3.4 Time-of-Flight Ion Optics .................................. 93
3.5 Ion Signal Detection .................................. 95
3.6 Laser Systems .................................. 96
   3.6.1 Alltec 854MS CO₂ Laser .......................... 96
   3.6.2 JK HyperYAG HY750 Nd³⁺:YAG Laser .................. 97
   3.6.3 Lumonics TE-861T-4 Excimer Laser .................. 98
3.7 Experimental Control and Data Acquisition .................. 99
   3.7.1 Control Hardware ................................ 99
   3.7.2 Transient Digitiser ................................ 101
   3.7.3 Control Software ................................ 102
3.8 Data Acquisition Modes ................................ 103

4. A Survey into the Analytical Applications of L²TOFMS .......... 111
   4.1 Introduction ................................ 111
   4.2 Small Organic and Biological Analytes .................. 114
   4.3 Peptide Analysis ................................ 117
   4.4 Nucleosides and Nucleotides .......................... 122
   4.5 Polymers and Additives .............................. 123
   4.6 Quantitation and Sensitivity Measurements ............ 125
   4.7 Microscopic Organic Analysis ......................... 126
   4.8 Concluding Remarks .............................. 127
5. L²TOFMS of Polycyclic Aromatic Hydrocarbons (PAHs) .................................................. 133
   5.1 Introduction .................................................................................................................. 133
   5.2 L²TOF Mass Spectra of Pure PAH Analytes ............................................................... 136
   5.3 Limits of Detection (LOD) for PAH Analytes ............................................................. 151
   5.4 Direct Determination of PAHs in Environmental Matrices .......................................... 158
       5.4.1 Mass Spectra of Contaminated Soils ................................................................. 158
       5.4.2 Mass Spectra of Contaminated Engine Oils ....................................................... 167
       5.4.3 Mass Spectra of Coal Soot ................................................................................. 172
       5.4.4 Mass Spectra of Creosote .................................................................................. 173
   5.5 Concluding Remarks ...................................................................................................... 176

6. L²TOFMS of Porphyrins ..................................................................................................... 182
   6.1 Introduction .................................................................................................................... 182
   6.2 L²TOFMS of Porphyrins Using 193 nm Laser Photoionisation ..................................... 185
       6.2.1 L²TOFMS of Octaethylporphyrins (OEPs) ......................................................... 186
       6.2.2 L²TOFMS of Biological-Type Porphyrins ......................................................... 196
       6.2.3 L²TOFMS of Tetraphenylporphyrins (TPPs) ....................................................... 216
   6.3 Laser Photodissociation of Metalloporphyrins ............................................................. 226
       6.3.1 L²TOFMS of Metallotetraphenylporphyrins using 266 nm Laser Photoionisation .................................................. 227
       6.3.2 L²TOFMS of Metallo-Octaethylporphyrins using 248 nm Laser Photoionisation .................................................. 236
   6.4 Concluding Remarks ...................................................................................................... 240

7. L²TOFMS of Dyes ............................................................................................................. 248
   7.1 Introduction .................................................................................................................... 248
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2 L²TOFMS of Azo Dyestuffs</td>
<td>250</td>
</tr>
<tr>
<td>7.2.1 High-Purity Azo Dyes</td>
<td>250</td>
</tr>
<tr>
<td>7.2.2 Low Purity Azo Dyes</td>
<td>262</td>
</tr>
<tr>
<td>7.3 L²TOFMS of Phthalocyanine Dyes</td>
<td>273</td>
</tr>
<tr>
<td>7.4 L²TOFMS of Anthraquinone and Coumarin Dyes</td>
<td>281</td>
</tr>
<tr>
<td>7.5 Concluding Remarks</td>
<td>286</td>
</tr>
<tr>
<td>8. Application of L²TOFMS to Complex Systems</td>
<td>293</td>
</tr>
<tr>
<td>8.1 Introduction</td>
<td>293</td>
</tr>
<tr>
<td>8.2 Investigation of Complex Stain Systems</td>
<td>295</td>
</tr>
<tr>
<td>8.2.1 Adsorbed Dyestuffs</td>
<td>296</td>
</tr>
<tr>
<td>8.2.2 Chlorophyll a</td>
<td>300</td>
</tr>
<tr>
<td>8.2.3 Curcumin, Turmeric and Curry</td>
<td>304</td>
</tr>
<tr>
<td>8.2.4 Tea and Coffee</td>
<td>314</td>
</tr>
<tr>
<td>8.2.5 Malvin and Red Wine</td>
<td>320</td>
</tr>
<tr>
<td>8.2.6 The Fate of the Substrate</td>
<td>326</td>
</tr>
<tr>
<td>8.3 Concluding Remarks</td>
<td>327</td>
</tr>
<tr>
<td>9. L²TOFMS - Concluding Remarks</td>
<td>332</td>
</tr>
<tr>
<td>9.1 Thesis Summary</td>
<td>332</td>
</tr>
<tr>
<td>9.2 Advantages of L²TOFMS</td>
<td>334</td>
</tr>
<tr>
<td>9.3 Current Limitations of L²TOFMS</td>
<td>337</td>
</tr>
<tr>
<td>9.4 L²TOFMS - Analytical Future</td>
<td>339</td>
</tr>
<tr>
<td>A. Courses and Conferences Attended</td>
<td>344</td>
</tr>
<tr>
<td>B. Publications</td>
<td>346</td>
</tr>
</tbody>
</table>
List of Figures

1-1 Schematic representation of the instrument developed for L\(^2\)TOFMS studies. A - desorption chamber; B - ionisation chamber; C - reflectron time-of-flight mass spectrometer. ............................................... 20

2-1 Schematic representation of the most commonly used photoionisation processes. a) direct photoionisation, b) non-resonant photoionisation, c) and d) resonant two-photon ionisation (R2PI), e) [2+1] resonant enhanced multiphoton ionisation (REMPI). ........ 41

2-2 Simplified schematic diagram summarising the possible competing photophysical and photochemical processes at the intermediate excited state. ................................................................. 45

2-3 L\(^2\)TOFMS spectra of lead acetate [Pb(CH\(_3\)CO\(_2\)] using a) 193 nm laser, b) 450.3 nm laser photoionisation and c) 193 nm laser photoionisation after desorption in an enclosing faceplate to encourage cluster formation. ......................... 48

2-4 Ladder switching model of multiphoton ionisation. ................. 51

2-5 L\(^2\)TOFMS spectra of a mixture of caffeine, paracetomol and aspirin produced using a) 266 nm and b) 193 nm laser photoionisation. ... 55

2-6 L\(^2\)TOFMS mass spectra of paracetomol obtained under a) soft and b) hard ionisation conditions using 193 nm laser photoionisation. . 58

2-7 Schematic diagram of the a) single-field, and b) two-field Wiley-McLaren type, TOF mass spectrometers. .............................. 61
List of Figures

2-8 $L^2$TOFMS spectra of a polystyrene polymer distribution (av. mol. wt. $M_n = 687$ amu) obtained using a linear TOF mass spectrometer and 266 nm laser photoionisation. The spectra were recorded using successively higher deflection plate potentials; a) 100 V, b) 200 V, c) 400 V and d) 500 V. ............................................. 65

2-9 $L^2$TOFMS mass spectrum of a polystyrene polymer distribution (av. mol. wt. $M_n = 687$ amu) obtained using a collinear reflectron TOF mass spectrometer and 266 nm laser photoionisation. .......... 66

2-10 Schematic diagram of a reflectron TOF mass spectrometer based on the geometric design of Boesl, after that of Mamyrin. ....... 72

3-1 Plan schematic of the $L^2$TOF mass spectrometer. .............. 88

3-2 Elevation schematic of the $L^2$TOF mass spectrometer. .......... 89

3-3 Schematic of the desorption/entrainment assembly. .............. 91

3-4 Schematic of reflectron TOFMS ion extraction optics. All dimensions are in mm. ........................................... 93

3-5 Schematic of reflectron TOFMS ion mirror. All dimensions in mm. 94

3-6 Schematic of CAMAC based experimental control system. ......... 100

3-7 Typical trigger pulse timing set-up for $L^2$TOFMS experiments. All times in microseconds. ......................... 104

3-8 Desorption profile of carbazole obtained using timescan mode. .... 106

3-9 Temporal profile of the aniline seeded He molecular beam obtained using timescan mode. ................................. 106

3-10 $L^2$TOF [2+1] resonant photoionisation spectrum of the atomic lead $6p^7p(^3P_0) \rightarrow 6p^2(^3P_0)$ transition. ................ 107

3-11 Fixed frequency scan of perylene demonstrating postionisation signal instability when sample is deposited onto the square faced sample probe slot from solution. Accumulation of sample material at both ends of the slot is clearly apparent. ............ 108
3-12 Fixed frequency scan of carbazole, demonstrating postionisation signal stability on consecutive passes of the sample. The sample was prepared as a tacky paste in glycerol and pressed firmly into the square face slotted sample probe. ........................................... 109

5-1 L²TOF mass spectra of selected PAHs obtained using 266 nm laser photoionisation: a) coronene, b) perylene, c) pyrene, d) tetracene, e) pentacene. ................................................................. 137

5-2 L²TOF mass spectra of selected PAHs obtained using 193 nm laser photoionisation. a) coronene, b) perylene, c) pyrene, d) tetracene, e) pentacene. ................................................................. 140

5-3 L²TOF mass spectrum of perylene obtained using 193 nm laser photoionisation. Resolution (m/δm) = 1350. ................................................................. 141

5-4 L²TOF mass spectra of perylene using 266 nm laser photoionisation with incident laser power densities of a) 0.5 MW cm⁻² and b) 5 MW cm⁻². ................................................................. 146

5-5 L²TOF mass spectra of pyrene using 193 nm laser photoionisation with incident power densities of a) ≤ 0.8 MW cm⁻², b) 1.7 MW cm⁻² and c) 12 MW cm⁻². ................................................................. 148

5-6 L²TOFMS spectra of fluoranthene using 193 nm laser photoionisation with incident fluences of a) ≤ 0.8 MW cm⁻², b) 1.7 MW cm⁻² and c) 12 MW cm⁻². ................................................................. 149

5-7 L²TOF mass spectra of carbazole using 193 nm laser photoionisation with incident power densities of a) ≤ 0.8 MW cm⁻², b) 1.7 MW cm⁻² and c) 12 MW cm⁻². ................................................................. 152

5-8 1000 shot L²TOF mass spectra obtained for a) coronene and b) perylene, using successively reduced sample probe loadings. ...................... 156

5-9 L²TOF mass spectra of ca. 1 mg of contaminated soil obtained using a) 266 nm and b) 193 nm laser photoionisation. .................................... 160
5-10 PAH species previously determined present in contaminated soil by GC-MS following CH₂Cl₂ extraction. ....................... 162

5-11 Expansion of L²TOF mass spectrum of contaminated soil between 170 and 320 amu to show more clearly the resolved mass spectral peaks. Confirmed mass assignments from GC-MS are 1, 178, phenanthrene/anthracene; 2, 202, pyrene/fluoranthene; 3, 228, chrysene/benzo[a]anthracene; 4, 252, benzo[a]fluoranthene/benzo[a]pyrene; 5, 276, indeno[1,2,3-cd]pyrene/benzo[ghi]perylene. ............... 163

5-12 Plot of 178 amu : 202 amu signal intensity ratio versus spiked concentration of phenanthrene for a contaminated soil sample. Extrapolation to zero spiking concentration yields an approximate concentration of phenanthrene/anthracene contamination. ............... 166

5-13 L²TOF mass spectra of clean, unused engine oil obtained using 193 nm laser photoionisation; spectrum a) shows the entire mass range up to 380 amu, and spectrum b) an expansion of the region between 240 amu and 520 amu. ....................... 168

5-14 L²TOF mass spectra of contaminated, used engine oil obtained using 193 nm photoionisation. In spectrum a) a series of contaminants in the mass “window” in the spectrum shown in Figure 5.13a between 170 amu and 300 amu can be clearly identified. These are shown more clearly in the expanded spectrum b). The assignment of these peaks to a series of alkylated PAHs is indicated (see text). 170

5-15 L²TOF mass spectrum of coal soot obtained using 193 nm laser photoionisation. ....................... 173

5-16 L²TOF mass spectrum of coal-tar creosote obtained using 193 nm photoionisation. ....................... 174

6-1 L²TOF mass spectra of a) OEP, b) CoOEP and c) NiOEP obtained under soft ionisation conditions using 193 nm laser photoionisation. 189
6-2 $L^2$TOF mass spectra of a) OEP, b) CoOEP and c) NiOEP obtained under hard ionisation conditions using 193 nm laser photoionisation. 191

6-3 $L^2$TOF mass spectra of ZnOEP obtained using 193 nm laser ionisation with increasing laser pulse energies; a) ca. 0.5 mJ/shot, b) ca. 3 mJ/shot and c) ca. 6 mJ/shot. 194

6-4 $L^2$TOF mass spectra of CuOEP obtained under a) soft and b) hard ionisation conditions using 193 nm laser photoionisation. 195

6-5 $L^2$TOF mass spectra of etioporphyrin I dihydrobromide obtained under a) soft and b) hard ionisation conditions using 193 nm laser photoionisation. 199

6-6 $L^2$TOF mass spectrum of etioporphyrin I dihydrobromide obtained under partially hard ionisation conditions using 193 nm laser photoionisation. The species labelled with an A correspond to aniline and its clusters. 201

6-7 $L^2$TOF mass spectra of hematoporphyrin IX obtained under a) soft and b) hard ionisation conditions using 193 nm laser photoionisation. The species labelled with an A corresponds to the aniline molecular ion. 202

6-8 Scheme summarising the principal fragmentation products obtained from the hematoporphyrin IX molecular ion using 193 nm laser photoionisation. All masses shown are in amu. 204

6-9 $L^2$TOF mass spectra of hemin obtained under a) soft and b) hard ionisation conditions using 193 nm laser photoionisation. 206

6-10 Scheme summarising the principal fragmentation products obtained from the hemin molecular ion using 193 nm laser photoionisation. 207

6-11 $L^2$TOF mass spectra of chlorophyll a obtained under a) soft and b) partially hard ionisation conditions using 193 nm laser photoionisation. 210
6-12 Scheme summarising the principal fragmentation products obtained from the chlorophyll a molecular ion using 193 nm laser photoionisation. ............................................. 212

6-13 L²TOF mass spectra of the trisodium salt of coppered chlorophyllin obtained under partially hard ionisation conditions. ................... 213

6-14 Scheme summarising the principal fragmentation products obtained from the trisodium salt of coppered chlorophyllin using 193 nm laser photoionisation. ............................................. 215

6-15 L²TOF mass spectra of a) TPP, b) CoTPP and c) NiTPP obtained under soft ionisation conditions using 193 nm laser photoionisation. 219

6-16 L²TOF mass spectra of a) TPP, b) CoTPP and c) NiTPP obtained under hard ionisation conditions using 193 nm laser photoionisation. 220

6-17 L²TOF mass spectrum of ZnTPP obtained under hard ionisation conditions using 193 nm laser photoionisation. ..................... 224

6-18 L²TOF mass spectra of 5,10,15,20 tetrakis (4-methoxyphenyl) 21H,23H porphine iron (III) chloride under a) soft and b) partially hard ionisation conditions using 193 nm laser photoionisation. ................. 225

6-19 L²TOF mass spectra of copper TPP under a) soft and b) hard ionisation conditions obtained using 266 nm laser photoionisation. 229

6-20 L²TOF mass spectra of a) CoTPP, b) NiTPP and c) VOTPP obtained using 266 nm laser photoionisation ................................. 230

6-21 L²TOF mass spectra of VOTPP obtained using 266 nm laser photoionisation of power density a) 0.2 MW cm⁻², b) 0.6 MW cm⁻² and c) 3.6 MW cm⁻². ............................................. 232
6-22 Schematic representation of the photophysical processes involved in:
   a) Class A photoionisation of ZnTPP at 266 nm. Absorption of two 266 nm photons results in formation of the molecular ion \([\text{ZnTPP}]^+\).
   b) Class B photoionisation of NiTPP at 266 nm. Photoexcitation is followed by rapid intramolecular conversion of electronic to vibrational energy to produce a vibrationally hot ground state species, \([\text{NiTPP}]^+\); dissociation followed by photoionisation yields the metal-free fragment ion \([\text{TPP}]^+\).

6-23 L^2TOF mass spectrum of ZnOEP obtained using 248 nm laser photoionisation. ............................................. 238

6-24 L^2TOF mass spectrum of CuOEP obtained using 248 nm laser photoionisation. ............................................. 239

6-25 L^2TOF mass spectra of a) NiOEP and b) CoOEP obtained using 248 nm laser photoionisation ...................... 241

7-1 L^2TOF mass spectra of Disperse Red 1 using a) 266 nm and b) 193 nm laser photoionisation. ...................... 254

7-2 Schematic representation of the principal fragmentation pathways resulting from either 266 nm or 193 nm laser photoionisation of Disperse Red 1. ............................................. 255

7-3 L^2TOF mass spectra of a) azobenzene, b) methyl red, c) 4-phenylazoaniline and d) 4-phenylazophenol, obtained using 266 nm laser photoionisation. ............................................. 257

7-4 Schematic representations of the principal fragmentation pathways observed for azobenzene, methyl red, 4-phenylazoaniline and 4-phenylazophenol following 266 nm laser photoionisation. ...................... 258

7-5 Postulated cyclic transition state required to enable loss of \(\text{N}_2\) from cis-azobenzene. ............................................. 259

7-6 L^2TOF mass spectrum of Disperse Orange 1 produced by 266 nm laser photoionisation. ............................................. 264
7-7 Schematic representations of the principal fragmentation pathways resulting from 266 nm laser photoionisation of Disperse Orange 1 and Disperse Orange 3 ........................................ 265

7-8 L²TOF mass spectrum of Disperse Yellow 3 produced by 266 nm photoionisation........................................... 266

7-9 Schematic representation of the principal fragmentation pathways resulting from 266 nm laser photoionisation of Disperse Yellow 3. ............................. 267

7-10 L²TOF mass spectra of a) Disperse Orange 1 and b) Disperse Orange 3 using 193 nm laser photoionisation. ...................... 270

7-11 L²TOF mass spectrum of Disperse Yellow 3 produced by 193 nm photoionisation........................................... 271

7-12 Schematic representation of the principal fragmentation reactions resulting from 193 nm laser photoionisation of Disperse Yellow 3. ............................. 271

7-13 L²TOF mass spectra of azobenzene derivatives mixed with Disperse Orange 3 (95% dye content), a) Disperse Red 1, b) methyl red, c) 4-phenylazoaniline and d) 4-phenylazophenol, obtained using 193 nm laser photoionisation. ............................. 274

7-14 L²TOF mass spectra of magnesium phthalocyanine obtained using a) 193 nm and b) 266 nm laser photoionisation...................... 276

7-15 L²TOF mass spectrum of magnesium phthalocyanine obtained, under hard ionisation conditions, using 193 nm laser photoionisation. ............................. 279

7-16 L²TOF mass spectrum of cobalt phthalocyanine obtained, under hard ionisation conditions, using 193 nm laser photoionisation. ............................. 280

7-17 L²TOF mass spectrum of Alcian Blue 8GX obtained using 193 nm laser photoionisation........................................... 281

7-18 L²TOF mass spectra of a) Disperse Blue 1, b) Disperse Orange 11, and c) Basic Blue 47 using 193 nm laser photoionisation............................. 284
7-19 $L^2$TOFMS spectra of Disperse Blue 1 at 266 nm under soft and hard ionising conditions. Ionising laser power densities are a) $0.25 \times 10^6$ Wcm$^{-2}$ and b) $1 \times 10^6$ Wcm$^{-2}$. .................................................. 285

7-20 $L^2$TOF mass spectra of a) Coumarin 47, b) Coumarin 102 and c) Coumarin 152a, using 266 nm laser photoionisation. ............... 287

8-1 $L^2$TOF mass spectrum of methyl red obtained using 266 nm laser photoionisation after IR laser desorption from a cotton substrate. . 297

8-2 $L^2$TOF mass spectra of magnesium phthalocyanine obtained using 266 nm laser photoionisation after IR laser desorption from a) polyester, b) nylon and c) cotton substrates. ......................... 298

8-3 $L^2$TOF mass spectra of chlorophyll a obtained using 193 nm laser photoionisation after a) IR desorption from a stainless steel substrate and b) IR desorption from a cotton substrate. ............... 302

8-4 Structure and principal fragmentation products observed in the $L^2$TOF mass spectra of a 1,3-diphenyl-1,3-propanedione derivative when using 193 nm laser photoionisation. ......................... 303

8-5 $L^2$TOF mass spectra of chlorophyll a obtained using 193 nm laser photoionisation after IR laser desorption from a cotton substrates. The amount of material required in the sample cloth to produce each spectrum was a) 11 nanomoles, b) 3 nanomoles and c) 600 picomoles. .................................................. 305

8-6 $L^2$TOF mass spectra of curcumin obtained using a) 193 nm and b) 266 nm laser photoionisation. .................................................. 307

8-7 $L^2$TOF mass spectrum of curcumin obtained using 266 nm laser photoionisation after IR laser desorption from a cotton substrate. . 309

8-8 $L^2$TOF mass spectra of turmeric obtained using a) 193 nm and b) 266 nm laser photoionisation. .................................................. 310
List of Figures

8-9 L²TOF mass spectrum obtained using IR desorption of a curry mixture followed by 266 nm laser photoionisation. .................................. 311

8-10 L²TOF mass spectrum of a curry stain obtained using 266 nm laser photoionisation after IR laser desorption from a cotton substrate. 312

8-11 L²TOF mass spectrum of curry residue, extracted from a cotton substrate with acetone, obtained using 266 nm laser photoionisation. 313

8-12 L²TOF mass spectrum of black tea residue obtained using 266 nm laser photoionisation. .......................................................... 314

8-13 Example structures of a variety of polyphenolic species thought to be present in tea/coffee residues. ............................................. 317

8-14 L²TOF mass spectrum of coffee residue obtained using 266 nm laser photoionisation. .......................................................... 319

8-15 L²TOF mass spectra of a tea/coffee mixture obtained using 266 nm laser photoionisation after desorption from a) the stainless steel probe and b) knitted polyester. ............................................. 321

8-16 L²TOF mass spectrum of a tea/coffee mixture obtained using 266 nm laser photoionisation after IR laser desorption from a cotton substrate. .......................................................... 322

8-17 Molecular structure of malvin. .................................................. 322

8-18 L²TOF mass spectra of malvin obtained using a) 193 nm and b) 266 nm laser photoionisation. .................................................. 324

8-19 L²TOF mass spectrum of malvin obtained using 266 nm laser photoionisation after IR laser desorption from a cotton substrate. ........ 325

8-20 L²TOF mass spectrum of concentrated red wine obtained using 266 nm laser photoionisation. .................................................. 326
### List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-1</td>
<td>Power supplies used for ion extraction and reflection electrodes.</td>
<td>95</td>
</tr>
<tr>
<td>5-1</td>
<td>Molecular weights, IP’s, and skeletal structures of selected PAHs. (IP data: a - [29], b - [30])</td>
<td>138</td>
</tr>
<tr>
<td>5-2</td>
<td>A comparison between relative mass spectral peak intensities measured from the mass spectra in Figure 5.2 and those calculated from the natural isotopic abundance of $^{13}$C.</td>
<td>145</td>
</tr>
<tr>
<td>5-3</td>
<td>L$^3$TOFMS mass spectral peaks of pyrene and fluoranthene using 193 nm laser photoionisation (relative intensities in parentheses).</td>
<td>150</td>
</tr>
<tr>
<td>5-4</td>
<td>L$^2$TOFMS mass peaks of carbazole obtained using 193 nm laser photoionisation (relative intensities in parentheses).</td>
<td>153</td>
</tr>
<tr>
<td>5-5</td>
<td>PAH assignment for the L$^3$TOF mass spectrum of contaminated soils obtained using 193 nm laser photoionisation.</td>
<td>161</td>
</tr>
<tr>
<td>5-6</td>
<td>PAH concentrations determined for soil sample using GC-MS [35]. The combined concentration of phenanthrene and anthracene (309.8 ppm) can be compared with that obtained using L$^2$TOFMS and the method of standard additions (see text). NF - determinand not found.</td>
<td>165</td>
</tr>
<tr>
<td>5-7</td>
<td>Peak assignments for L$^2$TOF mass spectrum of contaminated engine oils obtained using 193 nm laser photoionisation.</td>
<td>171</td>
</tr>
<tr>
<td>5-8</td>
<td>Predominant polycyclic aromatic hydrocarbons in coal tar creosote.</td>
<td>175</td>
</tr>
</tbody>
</table>
6-1 The structures and masses of the octaethylporphyrins investigated using L^2TOFMS. ........................................... 187

6-2 The structures and masses of biological-type porphyrins investigated using L^2TOFMS. For details concerning the entries marked with an asterisk refer to the complete chlorophyll structure shown in Figure 6.11a. .................................................. 197

6-3 Composition of principal fragment ions produced by L^2TOFMS using 193 nm laser photoionisation for a variety biological porphyrins. 217

6-4 The structures and masses of the tetraphenylporphyrins (and a tetramethoxyphenyl derivative) investigated using L^2TOFMS, ... 218

7-1 Structural classification of dyestuffs. ........................................... 251

7-2 Comparison of PB EI (Particle Beam Electron Impact) mass spectral peaks and photoionisation mass spectral peaks for Disperse Red 1 (Relative intensities in parentheses) ......................................... 261

7-3 L^2TOFMS mass spectral peaks of class I dyes using 266 nm laser photoionisation. ........................................... 263

7-4 L^2TOFMS mass spectral peaks of class I dyes obtained using 193 nm laser photoionisation (Relative intensities in parentheses). ... 268

7-5 Principal L^2TOFMS fragment peaks of phthalocyanine dyes obtained, under hard ionisation conditions, using 193 nm laser photoionisation. ........................................... 277

8-1 Black tea beverage composition. ........................................... 315

8-2 The percentage composition of roasted Arabica coffee. .......... 318
Chapter 1

Introduction

One of the major challenges for the analytical chemist is the identification and structural characterisation of involatile, thermolabile, polar, and/or high-molecular-weight organic compounds. Frequently, these are only available in small quantities and often accompanied by impurities or contained in a complex matrix. Mass spectrometry is an important weapon in the armoury of analytical techniques used to tackle such problems. Any mass spectrometric technique, however, may have to satisfy a wide variety of stringent criteria in order to provide solutions to a particular analytical problem. Ideally, a mass spectrometric technique should be able to deliver information on the molecular weight of the target molecule as well as the functionalities and basic structural features. A soft ionisation technique alone is, therefore, often not sufficient to provide a satisfactory solution. However, on the other hand as the generation of fragments implies a reduction in parent ion intensity and an increase in spectral complexity, some control over the degree of fragmentation is desirable. The chosen mass spectrometric technique should also allow measurements in the high mass range with good resolution and reasonable mass measurement accuracy. It must be sensitive and selective, enabling the detection of minor components of mixtures and perhaps the eliminate purification steps. A wide variety of mass spectrometric techniques are now available that can fulfil at least some of these demands. The scope of their analytical application is further increased by coupling to different separation techniques to yield an immense variety of hyphenated methodologies. Even so, no universal mass spec-
trometric methodology is presently available, and new techniques are required to address the mass spectrometry of the types of system described above.

The most severe limitation in the mass spectral analysis of thermally labile or highly polar compounds is that mass spectrometric characterisation must be preceded by thermal evaporation of the sample. Frequently, the energy required to enable evaporation exceeds that which promotes thermal degradation. This is the fundamental reason why, until recently, many important molecular species have proved impossible to analyse successfully by mass spectrometric means. One approach to overcome this problem has been to apply mass spectrometry to the analysis of molecular overlayers, using particle impact desorption methods to desorb/sputter ions from a matrix by surface bombardment. These developments include secondary ion mass spectrometry (SIMS) [1,2], fast atom bombardment (FAB) [3], $^{252}$Cf-plasma desorption ($^{252}$Cf-PD) [4], laser desorption (LD) [5,6,7] and more recently the burgeoning field of matrix assisted laser desorption (MALD) [8]. In addition, direct chemical ionisation (DCI) [9], field desorption (FD) [10], thermal desorption [11], thermospray [12] and electrospray [13] have all been increasingly employed for the investigation of large molecules.

Although these techniques differ widely in their technical details they have one principal feature in common: sample desorption and ionisation are both accomplished in a single step. Their overall efficiency is therefore limited by the combined efficiency of both these processes. As the energy available for ionisation in desorption techniques is directly linked to that required for desorption, it is difficult to control the ionisation process, and thus control the degree of fragmentation. Furthermore, the molecular ion is often not observed: instead several adduct ions are found which stem from the addition of protons and/or alkali cations to the molecule. There is also the possibility of ionising any matrix species present, producing spectra of increasing complexity, thereby preventing ready interpretation of the resulting data. However, perhaps the most significant disadvantage is the fact that these methods are not always efficient at generating ions, often producing greater concentrations of gas-phase neutral molecules [14,15]. An alternative approach, therefore, is to ignore the nascent ions generated from such processes and
Chapter 1. Introduction

to exploit the higher density of gas-phase neutrals present by postionising them. This allows the desorption and ionisation steps to be independently optimised. The work presented in this thesis concerns the development of a two-laser mass spectrometric technique which combines laser desorption with postionisation by laser multiphoton ionisation followed by time-of-flight mass spectrometric analysis of the photoions. The key advantage of this methodology is the spatial and temporal separation of the desorption and ionisation steps which allows for independent optimisation of each process with respect to both laser wavelength and fluence.

Laser desorption with a pulsed infrared laser, in common with FAB and SIMS, produces many more neutral molecules than ions, as mentioned earlier: typically, the ratio has been reported to be about $10^4:1$ [16,17]. The rate of surface heating by an infrared laser pulse is known to be about seven orders of magnitude faster than conventional methods (ca. $10^7$ Ks$^{-1}$). This favours the desorption of intact molecules over decomposition [18]. To make use of the large ratio of neutral molecules to ions, a tandem arrangement of laser desorption of intact neutral molecules into a molecular beam and subsequent multiphoton ionisation is used. A schematic representation of the instrumentation used is shown in Figure 1. This combination of techniques will be given the acronym L$^2$TOFMS, where appropriate, in the remainder of this thesis.

Laser photoionisation is an ideal tool for the postionisation of laser desorbed neutrals. For many gas-phase molecules a minimum energy of the order of 7 to 10 eV is required to exceed the ionisation limit [19]. This can only be accomplished by the absorption of an energetic vacuum ultraviolet (VUV) photon in a single photon ionisation scheme. Radiation in this region of the spectrum is relatively difficult to work with, requiring evacuated light paths and expensive specialist optics. Alternatively, a multiphoton ionisation (MPI) scheme can be employed, whereby, a molecule absorbs two or more, less energetic, visible or ultraviolet (UV) photons exciting it from, say, the ground state to above the ionisation limit. Radiation at these wavelengths is readily available from a wide variety of lasers and is much more convenient to work with than VUV radiation. Similarly, resonance enhanced multiphoton ionisation (REMPI), an optical ionisation technique in which
Figure 1-1: Schematic representation of the instrument developed for L^2TOFMS studies. A - desorption chamber; B - ionisation chamber; C - reflectron time-of-flight mass spectrometer.
Chapter 1. Introduction

the electronic absorption features of a molecule are exploited for resonant excitation, can be used to provide unique identification and discrimination of molecules in a mixture by wavelength selective ion production. In addition, laser MPI is a versatile ionisation source which can be used for either soft or hard ionisation by simply controlling the laser beam intensity. Particular attention has been paid to the wavelength dependent fragmentation channels observed in the experimental work presented later.

Laser desorption combined with laser postionisation was primarily developed as a mass spectrometric technique by three separate research groups. Two of these groups, Schlag's [20] and Lubman's [21], have adopted instrumentation with a geometry similar to that shown in Figure 1. In this case, the desorbed material is entrained by a pulsed molecular beam of an inert carrier gas and transported to the ionisation region. Zare's group, on the other hand, photoionise the desorbed molecules directly above the surface of the sample [22]. The detection sensitivity is considerably enhanced in this latter configuration. However, an attractive option is to utilise the optical selectivity inherent in resonance-enhanced multiphoton ionisation (REMPI). To be effective this requires that the internal degrees of freedom of the target molecules are cooled. This is best achieved using the jet-cooling obtained on entraining the desorbed molecules in a molecular beam. The combination of laser desorption with jet-cooling has been previously demonstrated for biomolecules and complex organic species in these two laboratories [23, 24, 25]. Thus, wavelength, a further dimension, can be added to the mass axis of a conventional mass spectrum.

The principal objective of the work described in this thesis was to develop the analytical applications of the laser desorption laser photoionisation time-of-flight mass spectrometric technique (L2TOFMS). The following Chapter contains a more detailed account of the three fundamental components of the methodology, namely: laser desorption, laser photoionisation and time-of-flight mass spectrometry. Each of these subjects will be considered in turn, restricting the discussion to those details most concerned with the experimental results presented later. Chapter 3 contains a description of the experimental methods and equipment employed
in these studies, whilst Chapter 4 completes the technical background with a survey of work performed previously using this technique. The rest of the thesis is dedicated to the presentation and analysis of the results obtained from the application of L\textsuperscript{2}TOFMS to a variety of target systems. Chapter 5 describes the L\textsuperscript{2}TOF mass spectrometry of a range of polynuclear aromatic hydrocarbons as pure compounds along with an assessment of the technique for the assay of these species in complex environmental matrices. In Chapter 6 the photoionisation mass spectra of a variety of analytically interesting porphyrin and metalloporphyrin compounds are presented. The influence of ionisation laser wavelength on the nature of the resulting mass spectra is examined, resulting in the observation of a variety of photophysical phenomena. Chapter 7 documents the use of L\textsuperscript{2}TOFMS for measuring the mass spectra of a range of commercial dyestuffs, including a discussion concerning the photofragmentation behaviour observed for azo molecules. The penultimate chapter, Chapter 8, describes initial studies using the L\textsuperscript{2}TOFMS to investigate a number of interesting molecular systems which present interesting, real world analytical problems. In particular, the application of this novel technique directly to sample systems which conventionally require a combination of techniques or are not readily amenable to analysis without complex sample extractions and separations: specifically these involve the analysis of staining agents as complex mixtures along with preliminary investigations concerning the application of L\textsuperscript{2}TOFMS to the in situ analysis of organic materials adsorbed onto organic substrates. In conclusion, Chapter 9 provides a summary of the advances made in the analytical applications of L\textsuperscript{2}TOFMS, an assessment of the place of this technique in the analytical laboratory and suggestions for the future directions of this work.
Bibliography


Chapter 2

$L^2$TOFMS – Background Theory

2.1 Introduction

The technique of laser desorption laser photoionisation mass spectrometry relies upon the vaporisation of intact neutral species which can be postionised and subsequently mass analysed. The first stage, namely laser desorption, underpins the effectiveness of the entire $L^2$TOFMS methodology. Laser multiphoton ionisation has been exploited in its own right elsewhere. Similarly, time-of-flight mass spectrometers have been established for many years. It is the coupling of these techniques with a flexible and efficient mode of volatilisation that has broadened the field of mass spectrometric enquiry to include a vast array of large, involatile or fragile molecules as potential target systems. This chapter covers the theoretical background of the three main components of the technique and provides a preamble to the following chapters which describe the results obtained. The following section contains a brief discussion concerning the history of laser desorption mass spectrometry, along with a description of some of the existing ideas and models that seek to explain the mechanisms governing the laser desorption of organic molecules. The section concludes with some comments concerning the importance of desorbing predominantly intact neutral species, enabling laser desorption to be ideally mated to laser postionisation. The nature of laser multiphoton ionisation is then discussed in the following sections, including both a theoretical description and a discussion relating to the advantages of this technique over other ionisation methods. The penultimate section contains a brief outline of the principles behind time-of-flight mass analysis along with an assessment of the advantages
of this technique in combination with laser multiphoton ionisation. Finally, the main features of the technique will be summarised prior to a description of the instrumentation used to perform the experiments described in the latter half of this thesis.

2.2 Laser Desorption

2.2.1 Background and Mechanisms

Conceptually, laser desorption (LD) mass spectrometry of organic compounds dates from 1970, when Vastola et al. [1] reported the detection of cationised molecular sodium hexylsulfonates without fragmentation or decomposition. However, the 1978 paper by Posthumus et al. [2] was perhaps the most influential in generating interest in the use of LD for the analysis of polar, thermolabile and relatively high molecular weight organic compounds. Numerous laser desorption experiments have been performed since that time in an attempt to understand the laser desorption process. The exact role of many of the experimental parameters - such as laser power and wavelength, pulse duration, substrate, matrix, sample thickness and orientation of the incident laser beam - in the mechanism of laser desorption remains controversial. A wide variety of experimental parameters have been employed in different laboratories during the investigation of LD. As a result, the concepts invoked to rationalise the results from the various experiments range from resonant desorption [3], thermal desorption [4] to shock wave or nonthermal desorption models [5]. The first of these mechanisms occurs where the analyte itself undergoes an electronic transition under laser irradiation. The thermal model of desorption appears to proceed as a function of substrate temperature whilst the third mechanism refers to the nonthermal explosive ablation of target species. It is now generally accepted that the mass spectral information generated from LD-MS experiments is the result of one or more of these desorption mechanisms.

Any adequate description of the laser desorption mechanism must account for a number of experimental observations:
higher kinetic energy particles (tens of electron volts) are emitted immediately after the desorption laser pulse. These particles are often atomic or smaller molecular fragments,

- many desorbed species are protonated or cationised and have relatively low kinetic energies,

- many more neutral particles than ions are emitted and the release of neutral molecules occurs for longer times.

Theoretical attempts to describe desorption mechanisms have mainly focussed on energy transfer or deposition and subsequent disintegration mechanisms. The latter step is critical in the volatilisation of large molecules. If disintegration occurs too slowly or too late energy equilibration can lead to high vibrational excitation and decomposition of the larger molecular species. Most recently, Vertes and Gijbels [6] have surveyed efforts to understand the desorption mechanism, emphasising the role of restricted energy transfer pathways as an explanation for the volatilisation of nondegraded large molecules. Less attention has been dedicated to describing the actual ionisation mechanisms which produce ions directly from the laser desorption process. As only a small fraction of the desorbed species are actually ionic, and the principal concern of the $L^3$TOFMS technique is the desorption of neutrals, ion formation mechanisms will only be considered where appropriate. Even so, the majority of studies into laser desorption have been performed using mass spectrometry and have, therefore, directly utilised the laser desorbed ions for analysis.

**Resonant Desorption**

It is generally accepted that the actual wavelength used to perform the laser desorption is of some importance. For example, Southon et al. [7] demonstrated that at 532 nm the laser irradiation of organic materials produced pyrolysis products, whilst structurally relevant information could be obtained at 266 nm. It has also been determined that under resonant desorption conditions the laser fluence
threshold for the detection of aliphatic and aromatic amino acids is lowered relative to nonresonant conditions [8]. The observation of predominantly molecular radical cations generated from PAHs, when desorbed at 266 nm, also provides support for a resonant desorption mechanism initiated by electronic excitation of the sample [9]. Furthermore, work on the LD of amino acids, has shown that using a desorption wavelength which coincides with the presence of a molecular absorption band of the analyte can dramatically improve the molecular ion to fragment ion ratio [8,10,11,12].

Observations of this kind, particularly those which pointed towards an enhanced ion yield for nonadsorbing molecules if presented in an absorbing mixture, rapidly increased the degree of interest in the LD technique. The concept of resonant laser desorption has been exploited to greatest effect in the realm of matrix-assisted laser desorption (MALD). If the target sample is chemically homogeneous only direct absorption of radiation is possible. Direct excitation of this kind offers limited possibilities for the desorption of large molecules as the energy is deposited in the target species itself. This is especially true in the case of nonresonant desorption, where the high laser fluences necessary come close to promoting the onset of plasma generation. This can lead to the dissociation of large molecules and effectively limits the size of molecules that can be successfully desorbed as intact species. A more successful strategy is to promote indirect energy deposition. This is the mechanism used in MALD.

The use of absorbing matrices has proved to be one of the most significant recent developments in laser mass spectrometry. The technique generates quasi-molecular ions of non-absorbing materials by dissolving them prior to analysis in absorbing matrices. The first reported use of this methodology was presented in 1987 by Tanake et al. [13], and showed the mass spectra of proteins with high molecular masses. Subsequently, several groups confirmed the matrix assisted laser desorption effect [14,15,16,17]. This particular area of research has exploded, with a stream of publications reporting the discovery of new matrix materials and the observation of successively higher mass molecules [3,18]. The development of commercial instrumentation has also added to the wealth of MALD data being
generated and reports now regularly include the mass spectra of molecules with masses in excess of 100,000 amu. It has proved particularly successful for the mass analysis of large biomolecules, such as enzymes and proteins, along with the analysis of high molecular weight polymers.

Efficient and controllable energy transfer to a sample material requires the incident laser radiation to be resonantly absorbed by the analyte or matrix molecules. Consequently, lasers emitting in the UV, which can couple to electronic states, or in the far-IR, which can excite rovibrational states, have been shown to give the best results. However, Overberg et al. [19,20] have convincingly demonstrated that resonance conditions can be met at almost any wavelength in the UV to IR region (200 nm to 10 μm) if there is sufficient coupling of energy to the sample. Above the threshold desorption laser fluence, under nonresonant conditions, energy transfer between the incident laser radiation and the analyte occurs via nonlinear absorption [11]. Excitation with a resonant absorption wavelength, on the other hand, permits direct transfer of energy into the target molecules. However, experimental results indicate that this linear absorption is not the only process that can occur. For example, when UV radiation is used for desorption, a wide range of physical processes can occur. The absorption of such high energy photons results in electronic excitation of the molecules, which in turn, can lead to a number of processes such as the formation of excitons, electrons and holes in the conduction band of the organic crystals. Also, direct photofragmentation may occur as a consequence of electronic singlet state excitation of the target molecules. As a result of fast (picosecond) non-radiative relaxation processes, such as internal conversion, the electronic energy of the molecule can be transformed into vibrational molecular energy. The phonon modes of the sample lattice are excited in this way. An external force field can then destabilise the system leading to explosive desorption [21]. A similar effect is thought to be involved in the matrix assisted laser desorption of nonabsorbing molecules. However, in this case, where the host matrix molecules are the absorbers, the guest molecules are found to be less susceptible to thermal decomposition. This phenomena can be explained by the homogeneous bottleneck model (HMB) [21]. This argues that a frequency mismatch between the guest-
host interaction frequency and the internal vibrational frequencies of the guest molecules causes an energy transfer bottleneck. Vertes et al. [22] showed, using a simple kinetic model, that with an appropriately high rate of matrix sublimation the guest molecules can be liberated internally cold and intact. An energy transfer bottleneck is also thought to be involved in the laser induced thermal desorption mechanism. This is discussed in more detail below. The concept of restricted energy flow appears to be an important consideration in describing the processes which influence the outcome of laser desorption. Essentially, such energy transfer bottlenecks reflect an extremely low value for a particular transfer coefficient, e.g. that for transfer of surface phonon vibrational energy into adsorbate vibrations, or transfer of internal molecular vibrations into lattice vibrations.

A frequent feature of LD mass spectra is the presence of molecular ions produced by cationisation or protonation rather than electron abstraction. In the negative ion mode, deprotonation rather than electron attachment is usually observed. Radical ions are not often detected. Any neutral molecules which leave the surface can interact further with the UV radiation resulting in their photoexcitation, fragmentation or ionisation. At sufficiently high intensities of laser radiation, the situation can become still more complex due to the possibility of nonlinear processes in both the adsorbed organic crystals and the desorbed species. For example, at $10^{10}$ Wcm$^{-2}$, photoionisation in the vapour phase above the sample can give rise to a plasma leading to involved ion molecule reactions and the destruction of molecular identity. LAMMA (LAser Microprobe Mass Analysis) and LIMA (Laser Ionisation Mass Analysis) are two commercially available instruments which use high UV laser power densities. These mass spectrometers operate in both conventional reflection mode and transmission mode. In the latter configuration, the samples are deposited on a thin foil and the UV laser beam is tightly focussed onto the opposite side of the film from the sample. The desorption of ions is prompt yielding both negative and positive ions. A variety of molecular classes have been studied using the LAMMA technique, including polycyclic aromatic hydrocarbons [23], organic acids [24], peptides [25], glycosides [49] and polymers [27]. Although the mechanisms of ion formation are not well understood,
Van Vaek et al. [28] have attempted to formulate a framework to rationalise the analytical results obtained from microprobe applications. Firstly, it is considered that an energy gradient is created along the sample surface in a similar manner to that suggested earlier by Hercules et al. [29]. This starts a sequence of processes; disintegration in the central area resulting in the formation of non-specific elemental cluster ions, neighbouring softer conditions result in desorption of non-ionic molecules as neutrals which are subsequently ionised in the selvedge region or intermediate region between the solid state and gas phase. Ionisation is thought to be effected by electron interaction yielding odd-electron molecular ions, $M^+$ or $M^-$, or adduct formation by combination with co-desorbed alkali ions. The so-called pressure effects are related to the density of neutrals present in the selvedge region and are dependent on the volatility of the sample.

An alternative mechanism of disintegration, following the absorption of UV photons, has been suggested by Vertes [21]. Following absorption, a molecule is excited electronically and the resulting state can be an antibonding state, a higher lying excited state or an ionised state. As a result of the Franck-Condon principle, these excited states are not in their equilibrium geometry. The adsorbates in these configurations are frequently in the repulsive range of the interaction potential. It is the peculiarity of this completely nonthermal mechanism that in the case of coherent electronic excitation the energy conversion leads to coherent translational motion of the desorbed particles. The removed particles exhibit velocity distributions oriented strongly toward the surface normal [30] resulting in the formation of well defined pits.

**Thermal Desorption**

As a model for desorption ionisation, resonance mechanisms have proved very useful in suggesting technical innovations such as MALD. However, it appears that a thermal mechanism is also an important contributor to the efficiency of the desorption process. This mechanism is thought to dominate under low fluence conditions, ca. $10^8 \text{ Wcm}^{-2}$, where desorption proceeds as a function of substrate temperature. It should be noted that the thermolability of organic molecules is a relative
concept, and is dependent on the heating rate. Thus, the liberation of intact thermally labile molecules from the solid state is not necessarily incompatible with the high temperatures attained by ultrafast heating during laser irradiation. Daves et al. [31] and Beuhler et al. [4] exploited this in the flash desorption technique, developed as an alternative to conventional resistive heating. The phenomenon can be rationalised assuming that an Arrhenius-like equation applies to the desorption of intact neutrals and to the generation of the thermal degradation products.

\[ k = A e^{-\frac{E_a}{RT}} \]  

(2.1)

The parameter \( k \) is the rate of desorption or thermal degradation, \( E_a \) is the activation energy for the process and \( T \) is the temperature. A nonvolatile compound can therefore be defined as a species for which the activation energy for desorption exceeds that for decomposition. By plotting the logarithmic rate of desorption against \( 1/T \) and the logarithmic rate of decomposition against \( 1/T \), it can be seen that there is a point of intersection between the two curves, indicating that at higher temperatures, the rate of desorption becomes favoured [32]. Essentially, the fast-heating approach means that the time spent in the low temperature range, where decomposition prevails, is minimised and the relative contribution of the intact molecules in the gas phase is increased.

The absorption of infrared laser radiation by a solid leads directly to its heating. The rate of heating induced in a MACOR ceramic or glass surface by the IR output of a CO\(_2\) laser has been calculated by Zare et al. [33] as being approximately \( 10^8 \) Ks\(^{-1}\). The same group have measured the temperature induced in a dielectric surface by irradiation with a CO\(_2\) laser, using a time-resolved resistance thermometer, to show heating rates in excess of \( 10^9 \) Ks\(^{-1}\) for quartz and glass surfaces [34]. This compares with traditional resistive heating rates of \( 10 \) Ks\(^{-1}\) or less. It is, therefore, logical to assume that the desorption ionisation mechanism should be linked in some way to the heating of the sample. A wide variety of equipment has been used in the investigation of IR-LD. Kistemaker and coworkers [2] have used both pulsed CO\(_2\) and Nd:glass lasers with a home-built magnetic sector mass.
spectrometer. In a later study [35], they also employed a cw CO₂ laser. Cotter initially employed a pulsed CO₂ laser with a double-focussing mass spectrometer before switching to a time-of-flight mass analyser, whilst Stoll and Rollgen [36] used a quadrupole mass spectrometer to study ion formation using cw laser desorption. A generally accepted model for the IR laser desorption of organic species coated on a surface is that first proposed by Kistemaker [37]. In this model, it is assumed that for a substrate having high thermal absorptivity, the presence of the organic film does not influence the substrate temperature distribution, i.e. the organic adsorbate film is considered to be optically transparent. It was observed that less laser power was required to form ions from nonabsorbing samples on metal surfaces, which have high thermal conductivities, than from nonabsorbing samples on substrates with poor heat transfer properties [37]. This suggested that the laser energy is mainly absorbed by the substrate and then transferred to the organic layer. Other experiments provided evidence that the rapid heating of a surface enables the desorption of internally cool molecules [38,39,40]. Zare and Levine [41] have proposed a mechanism which attempts to explain the thermal desorption of internally cool molecules, as described below.

A molecule physisorbed to a surface is bound by a weak, van der Waals type bond. This bond, therefore, has a low vibrational frequency, similar to those of the surface phonons of the substrate. On the other hand, the chemical bonds in the physisorbed species will have much higher frequencies. If the substrate absorbs the incident laser radiation, the physisorption bond is expected to be readily pumped. A bottleneck in the energy flow from the surface-adsorbate van der Waals bond to the chemical bonds in the physisorbed molecule may occur at this stage. This is similar in principle to the bottleneck invoked in the homogeneous bottleneck model for MALD. The energy flow out of the physisorption bond and into the molecule will therefore be slow and relatively cold molecules will be desorbed. Thus it is possible to break the surface-adsorbate bond preferentially, even if this is not the weakest bond in the free molecule. A term \( \tau \) was defined as the time required for the hot surface to transfer energy to the physisorption bond in excess of its dissociation energy. As energy will also flow from the physisorption bond to
the internal molecular modes, the molecule may not actually dissociate, or desorb, after time \( \tau \). However, the conclusion reached by Zare and Levine was that the molecules will desorb if the heating rate is sufficiently rapid. The criterion then derived for the desorption of cool molecules from a surface is,

\[
\tau \nu \exp(-\varepsilon) < 1
\]  

(2.2)

where \( \nu \) is the frequency of the physisorption bond and \( \varepsilon \) is the adiabatic parameter, which represents the ability of the adsorbate molecule to resist changes in vibrational excitation. The parameter \( \varepsilon \) is related to the ratio of the vibrational frequency of a chemical bond to that of a van der Waals bond. It represents the ability of the adsorbate molecule to resist changes in vibrational excitation. Even if the condition in Equation 1.2 is not satisfied, the molecule may still desorb but will carry in its internal degrees of freedom any energy that has been transferred in excess of the physisorption bond energy, \( D \). Experimental evidence from the unimolecular decomposition of van der Waals adducts \([42,43,44]\) suggests that values of \( \varepsilon > 11 \) are not uncommon. With \( \varepsilon > 10 \), heating rates of \( 10^{11}-10^{12} \) Ks\(^{-1}\) can be used to desorb even reasonably strongly bound molecules with reasonably low internal energies. Heating rates of \( 10^{15} \) Ks\(^{-1}\) have been reported with femtosecond laser pulses \([45]\) suggesting that molecules may be thermally desorbed from even tighter-binding surfaces.

There is also evidence that a thermal model is appropriate for the consideration of ion formation. Ions can be observed using both pulsed and cw radiation. If a thin layer of sample molecules is used the efficiency of ion formation appears to be almost independent of the IR wavelength used. Inspection of laser desorption mass spectra suggests that the most favoured channel of molecular ion formation is cationisation. The formation of quasi-molecular ions in IR-LD is always accompanied by intense emission of alkali ions. The frequently observed \( \text{Na}^+ \) and \( \text{K}^+ \) ion peaks usually result from the heating of the sample or substrate surface to a temperature in excess of 700 °C, at which point impurities in the probe surface are ionised, or ions in the sample are mobilised \([37]\). It is proposed that \((\text{M}+\text{c})^+\) ions,
where c is Na⁺ or K⁺, are most probably formed as a result of attachment of an alkali ion to the intact neutral molecule [2,36,46]. Protonated molecular ions are also usually abundant in positive ion spectra. It seems likely that protonated ions are formed on the surface or in the solid phase by proton hopping and alkali cation additions are mostly the products of gas phase ion-molecule reactions [47]. Experiments in which the organic molecules are physically separated from the alkali sources have demonstrated that gas-phase cationisation is indeed possible, though other ionisation processes have not been ruled out [48].

Shock-Wave and Mechanical Desorption

Seydel and Lindner [49] have proposed an alternative mechanism to the thermal and resonant models, called the volcano model or shock-wave-driven desorption. Using an instrument with transmission geometry they measured mixtures of saccharides and alkali salts at a variety of power densities and sample thicknesses. Extensive fragmentation was observed when a fluence of 10⁸ Wcm⁻² was incident on a 1 μm thick sample, whereas cationised signals predominated on irradiation of 20 μm layers at a fluence of 10¹¹ Wcm⁻². Desorption in the former case appears to conform to a thermal model whilst under the latter conditions it is attributed to a shock-wave mechanism. The heating rate in these experiments, of 10¹¹ Ks⁻¹, exceeds the limit of 10⁹ Ks⁻¹ above which an explosive vaporisation (phase explosion) can occur. The result of this thermal ablation is a shock wave which traverses the solid and leads to the desorption of intact molecules and of alkali ions from the opposite surface via vibrational disturbance of the binding potentials. As the molecules are not coupled to the lattice long enough to absorb sufficient internal energy, thermally labile molecules can be ejected intact. A similar phenomenon is that of disintegration via mechanical stress. Here inhomogeneous heating of the sample produces thermal expansion resulting in mechanical stress [50]. Even under moderate laser irradiances this thermally induced stress may exceed a critical value where cracks can form and mechanical fragmentation is observed.
2.2.2 Laser Desorption of Neutral Molecules

In the previous section a summary of some of the models used to describe and explain both the disintegration and ion formation mechanisms involved in laser desorption was presented. The mechanism deemed most appropriate to explain a particular experiment can be seen to rely on a variety of experimental parameters, such as laser wavelength and power density, sample thickness and the use, or not, of selected sample matrices. The majority of the experiments described in this thesis were performed using IR radiation, from a CO₂ laser, to desorb relatively thin layers of organic samples from a stainless steel sample probe. If any mechanism predominates here it would seem likely to be that corresponding to thermal desorption. However, not all the laser desorption experiments discussed in this thesis used this parameter set. In some cases, samples were presented either in their natural matrices (see Chapter 5) or in specially selected matrices, such as glycerol and/or alumina. Here the samples could be of thicknesses up to ca. 0.5 mm. The mechanism of desorption, in these cases, is not so easily assigned to a particular model and is most likely to be a combination of the models discussed above.

Although the desorption of ions has proved very important for one-stage LD-MS analysis, and has enabled the desorption processes to be studied, it is the desorption of neutral molecules which is of greatest importance for the technique of L²TOFMS. Several investigators using LD-MS noted that neutral molecules are desorbed from a surface by laser irradiation in tandem with ion production [1,51,52,53,54]. The original LD experiment performed by Vastola and Pirone [1] revealed that neutral molecules were desorbed and that these were the major product of the desorption process, neutral emission occurring over a longer period than that of ions. This is predicted by the thermal model of desorption. The rate of desorption of ions (or neutrals) depends only on the provision of sufficient energy to remove them from the surface:

\[ \frac{dn(\pm)}{dt} \propto [c] \exp\left(-\frac{E_d}{kT}\right) \]  \hspace{1cm} (2.3)
where \( n^{(+)} \) is the number of ions or neutrals in the gas phase, \([c]\) is the surface concentration of species, \(E_d\) is the activation energy for desorption of an ion or neutral molecule, and \(T\) is the surface temperature. The activation energy for the desorption of neutral molecules will generally be lower than that for ions, so neutrals will be emitted at lower surface temperatures and for longer times than ions [52]. The ratio of ions to neutrals produced in a laser desorption experiment is determined by the rapid jump in surface temperature. This ratio can be calculated to a first approximation by the Langmuir-Saha equation,

\[
\frac{n^+}{N^0} = \exp\left[\frac{(W - I)}{kT}\right]
\]

(2.4)

where \(W\) is the work function of the substrate, \(I\) is the ionisation potential of the various sample molecules and \(T\) is the temperature. This equation, which describes a gas-phase thermal equilibrium process between ions and neutrals, has been applied with some success. As the surface temperature is dependent on the laser power density, the ratio of ions to neutrals also varies with the desorption laser power density [55]. For example, at power densities below \(10^8\) Wcm\(^{-2}\), the ratio of ions to neutrals is approximately \(10^{-5}\), whilst with power densities of \(10^9\)-\(10^{10}\) Wcm\(^{-2}\), this ratio has been reported to be as high as 0.01-0.1. Cotter has investigated this phenomenon [51,52]. He reported that with a 40 ns, 700 mJ CO\(_2\) laser pulse and power densities in the region of \(10^8\) Wcm\(^{-2}\), ions were emitted from the surface for about 1 \(\mu\)s, whilst neutrals were desorbed over a period of 100 \(\mu\)s. The ratio of ions to neutrals was heavily biased in favour of the neutrals. Using EI to detect neutrals it was found that in fact the neutral molecules form the vast majority of the desorption product.

As mentioned in Chapter 1, it would appear, in the light of these facts, that to generate analytically useful and sensitive data it is more efficient to utilise the desorbed neutrals by postionising them than simply analysing the nascent ion yield. Several groups have developed the technique of using laser photoionisation to postionise laser desorbed molecules. Chapter 4 contains a review of the various systems to which the technique has been applied. The remainder of this section
is therefore confined to a brief description of the various experimental parameter combinations that have been used for the desorption of neutrals.

Since 1985, six other groups have become involved in the development of the technique of laser photoionisation of laser desorbed molecules, combined with TOF mass spectrometry [56,57,58,59,60,61]. Lubman [56] has used two different laser systems for the desorption of neutral molecules, namely a pulsed CO$_2$ laser (\(\lambda = 10.6 \ \mu\text{m}\)), and the second harmonic from a Nd:YAG laser (\(\lambda = 532 \ \text{nm}\)). Grote-meyer et al. [58] also used predominantly CO$_2$ laser desorption. More recently, however, this group investigated the resonant vs nonresonant laser desorption of organic molecules using the four harmonic wavelengths of a Nd:YAG laser (266, 355, 532 and 1064 nm) [62]. They confirmed that the wavelength used for desorption was an important parameter. It was observed that, in a manner reminiscent of the LD of ions, resonant desorption of neutrals could be achieved at lower laser fluence thresholds with stronger post-ion yields than could be obtained using nonresonant desorption. Initially, both these groups employed rather thick sample films of material deposited from a suitable solvent by evaporation. Lubman’s group reverted to the use of a glycerol matrix to provide even sample coverage and to enhance shot-to-shot stability. Meanwhile, Zare’s group [57] have used a CO$_2$ laser to desorb very thin layers of sample material from absorbing matrices, such as glass or MACOR ceramics. Levy’s group [61] have tried a variety of sample preparations, including pellets doped with laser dye (the latter added to strongly absorb the 532 nm laser radiation used for desorption), before settling on thin films deposited from methanol. These films were ca. 10 \(\mu\text{m}\) thick corresponding to several thousand monolayers. Kistemaker’s group [59] use a CO$_2$ laser to desorb their samples from a fixed stainless steel probe.

Recent developments in L$^2$TOFMS have focussed on laser mass microscopy innovations, in which spatially resolved desorption enables microparticles to be examined individually. De Vries et al. [60] have used a UV waveguide laser for desorption (248 nm) with reflective cassegrainian optics to achieve a spatial resolution of 1 \(\mu\text{m}\). This group, along with Zare’s, have dispensed with the use of supersonic molecular beam entrainment for transportation of the desorbed mate-
rial from the desorption to the ionisation regions. Instead they have chosen to photoionise the desorbed material directly above the sample surface.

In the experiments described in this thesis, a supersonic molecular beam is used for the transportation of desorbed material. This has three advantages. The entrainment of the lukewarm, desorbed molecules into an expanding supersonic molecular beam helps to internally and translationally cool them. This reduces the possibility of delayed fragmentation. If sufficient cooling can be obtained it also allows for the possibility of selective jet-cooled gas-phase spectroscopy as discussed in Section 2.3.3. Translational cooling also reduces the initial kinetic energy distribution of the ions produced by MPI, leading to improved mass spectral resolution. A disadvantage, however, is a consequence of the inefficiency of the molecular beam as a molecular "pick-up" source. Therefore, the utilisation of this mechanism severely compromises the intrinsic detection sensitivity of such an instrument.

2.3 Laser Multiphoton Ionisation

2.3.1 Background

Laser photoionisation can proceed via a variety of different mechanisms. Figure 2.1 summarises some of the more common photoionisation schemes. Direct, single-photon ionisation (SPI) has been used as an ionisation scheme many times in the past (Figure 2.1a) [63,64]. Here, ionisation results if the energy of the absorbed photon is greater than the ionisation potential (IP) of the molecule. This absorption process can be strongly allowed since the ejected photoelectron can remove both energy and angular momentum. Typically, high energy photons from vacuum ultraviolet (VUV) sources (ca. 10 eV, 100 nm) are required for SPI. VUV lamps have only low output intensity. Therefore, the resultant ion yields are too low for sensitive mass spectrometry. The recent development of VUV laser sources has dramatically increased the potential photoion yield. Kung et al. [65], have recently produced 10.5 eV (118 nm) radiation by frequency tripling the third harmonic of
an Nd:YAG laser (355 nm) in a phase-matched mixture of xenon and argon. This technique has subsequently been used as an ionisation scheme for time-of-flight mass spectrometric studies [66,67]. However, the use of VUV, even VUV laser sources, remains a non-trivial matter. This emerging field of technology needs further development to enable more general application. A more widespread and routine photoionisation methodology is multiphoton ionisation (MPI).

The multiphoton ionisation technique depends upon the absorption of several photons by a molecule on irradiation with an intense visible or ultraviolet light (UV) source. A molecule will only ionise if the sum of the energies of the absorbed photons exceeds the ionisation potential. There are a number of important practical advantages concomitant with the use of MPI processes. In MPI schemes the photon wavelengths required are ordinarily in the visible or the UV regions of the electromagnetic spectrum. This means that the light sources can be operated in air, and at a convenient distance away from the experiment. VUV sources, on the other hand, require an evacuated or purged beam path and must therefore be located as close to the ion source as possible.

A general expression for the rate of an n-photon absorption process, \( W_n \), can be expressed as follows,

\[
W_n = \sigma_n I^n
\]  

(2.5)

where \( \sigma_n \) is the cross-section for n-photon absorption (cm\(^2\)s\(^{-1}\)) and \( I \) is the instantaneous photon flux (photons cm\(^{-2}\)s\(^{-1}\)). When this equation is transformed into logarithmic form it can readily be seen that a power dependence plot, using a fixed ionisation frequency, gives a crude estimate of a molecule's ionisation potential (IP). A plot of \( \ln W \) (or, in practical terms, the logarithm of the photoion yield) vs ln \( I \) should give, for low photon fluxes and well-characterised systems, a straight line of slope \( n \). Clearly, the IP is then to be found in the region \((n-1)h\omega < \text{IP} < nh\omega\). Deviations from linearity at higher photon flux result from resonant saturation effects or volume saturation effects in the region of a tightly focussed laser beam.
Figure 2-1: Schematic representation of the most commonly used photoionisation processes. a) direct photoionisation, b) non-resonant photoionisation, c) and d) resonant two-photon ionisation (R2PI), e) [2+1] resonant enhanced multiphoton ionisation (REMPI).
The simplest multiphoton process, non-resonant multiphoton ionisation, involves the simultaneous absorption of two photons. The transition rate, in this case, reflects the requirement for a coherent two-photon interaction with the molecule,

$$W_2 = \sigma_2 I^2$$  \hspace{1cm} (2.6)

where $\sigma_2$ is the two-photon absorption cross-section (cm$^4$s) and $I$ is the radiation flux (photons cm$^{-2}$s$^{-1}$). Cross sections for strongly-allowed single-photon transitions are of the order of $10^{-17}$ to $10^{-18}$ cm$^{-2}$. However, those for allowed two-photon absorptions are much smaller, typically only $10^{-50}$ cm$^4$s. For simultaneous two-photon absorption one commonly refers to the presence of a so-called "virtual" state at the one photon level. This "virtual" state is not a real eigenstate of the molecule. The interaction time required for this coherent two-photon process is on the order of the flight time of the photon past the molecule, ca. 1 femtosecond. This process can only occur if the photon flux is sufficiently high. UV arc-lamps, which can typically provide $10^{15}$ photons cm$^{-2}$ s$^{-1}$, are not sufficiently powerful to drive such non-resonant processes. The alternative choice, a narrow bandwidth pulsed laser, can provide fluxes in excess of $10^{28}$ photons cm$^{-2}$s$^{-1}$ (ca. 1 GW cm$^{-2}$), when tightly focussed. This is sufficient to drive a simultaneous two-photon absorption process. Unfortunately, at such high photon fluxes, background gas in the mass spectrometer may be ionised efficiently and extreme fragmentation is likely to occur, both of which are analytically undesirable.

If the energy of an incident photon is equivalent to the energy difference between the molecular ground state and a real excited state of a molecule, the situation is radically different. This regime is called resonance enhanced multiphoton ionisation (REMPI). A more general definition states that an MPI process is resonant when the energy of an integral number, $n$, of photons approaches closely the energy of an $n$-photon allowed transition. As real excited states have much longer lifetimes than "virtual ones", typically between $10^{-9}$ and $10^{-6}$ seconds, the probability of absorbing successive photons is increased by six orders of magnitude.
It is important to note that the efficiency of a resonant process depends upon the precise character of the intermediate state and its absorption spectra. Choosing a strongly resonant transition over a weakly resonant transition can improve the ionisation efficiency by several orders of magnitude. As the probability of further photon absorption is increased relative to non-resonant absorption, REMPI requires a much lower photon density than non-resonant ionisation, often around 1 MW cm$^{-2}$. This has the experimental consequence that at relatively low laser pulse energies the resonance enhancement of the ion current is important. At very high laser powers the non-resonant contribution becomes dominant, and any selective spectroscopic information present in the data is lost. It remains the case, however, that any mass spectral fragmentation derived from non-resonant processes can still reveal information on the molecular structure of the target species. The major concern is to prevent confusion between the data resulting from the interaction with the target species of interest, and that which is related to impurities and background species.

The MPI method that has found most extensive application, and the one that is predominantly employed in the work described here, is resonant two-photon ionisation (R2PI). This is shown schematically in Figures 2.1c and d. One photon excites a molecule to an excited state, that is $S_1 \leftrightarrow S_0$, and a second photon ionises the molecule. The sum of the two photon energies in this case must be greater than the IP of the molecule. Figure 2.1c shows the case where both absorbed photons have the same wavelength. However, the same process can be driven using photons of different wavelengths as shown in Figure 2.1d. Experimentally, this can prove a more arduous task, requiring both the temporal and spatial overlap of the two laser beams. This is commonly referred to as a two-colour ionisation experiment. As most organic molecules of interest have ionisation potentials which lie between 7 and 13 eV, R2PI requires photons whose wavelengths lie in the range between the far and near UV. The spectra shown in this thesis were generally obtained in one-colour R2PI experiments. The UV wavelength sources used, although not selected purposely to coincide with specific molecular resonances, can be considered resonant to some degree as a consequence of the high density of vibronic
states present in the relatively large aromatic systems studied. Little information is presently available on the exact nature of gas-phase vibronic structure for most involatile organic molecules as, prior to development of desorption techniques it proved difficult to obtain intact gas-phase neutrals for spectroscopic studies.

Finally, other possible MPI processes include those in which two photons are required to reach the first resonant state of the molecule (Figure 2.1e). Such a process has a much lower efficiency than R2PI, as at least one photon is non-resonant and must interact with a "virtual" intermediate state. However, an n-photon method like this can be useful for probing electronic states that are forbidden in a single-photon absorption because of symmetry restrictions but are allowed via a two-photon transition [70,71].

2.3.2 Theoretical Description

Multiphoton ionisation can be considered to comprise three fundamental photophysical processes, namely excitation, ionisation and fragmentation. These processes each compete with other decay processes for the various excited state populations. In all cases, ionisation and/or photochemistry is the result of a single molecule absorbing an integral number of photons. Therefore, the yield of ions will have a nonlinear dependence on the light intensity. The significance of nonlinear photochemistry rests in the fact that an MPI process requiring 20 eV (e.g. 5×4 eV photons), for example, will not necessarily have the same photochemical consequence as produced by absorption of a single 20 eV photon.

Generally, the primary excitation proceeds via the first excited singlet state of the molecule (S\textsubscript{1}) which usually has the best characterised and simplest spectroscopic features. Whilst the lifetime of a virtual level is too short to allow photochemical transformation, in a real excited state various photophysical and photochemical processes can occur in competition with further photon absorption by the target species (up-pumping). These different processes are summarised schematically in Figure 2.2. The excited neutral molecule could possibly undergo, i) molecular decomposition to form neutral fragment species, ii) intramolecular re-
Figure 2-2: Simplified schematic diagram summarising the possible competing photophysical and photochemical processes at the intermediate excited state.

arrangement, iii) intersystem crossing (ISC) to the triplet electronic state, which cannot be ionised by a photon of the same energy or, iv) excited state decay via fluorescence on a timescale which can compete effectively with ionisation. The net result of these competing processes is an overall reduction in the ionisation yield.

Gedanken [72] classified two extremes of behaviour at the resonant excited state, i.e. photochemical decomposition versus ionisation. The behaviour observed is dependent on the relative rates of up-pumping, leading to ionisation, and dissociation. "Class A" systems are designated as those which exhibit ionisation followed by fragmentation, and "Class B" molecules are those in which dissociation to
yield neutral fragments precedes ionisation. The majority of molecules examined in this work, organic aromatic species, exhibit Class A behaviour. However, some compounds, such as inorganic complexes, e.g. \( \text{Fe}(	ext{CO})_5 \) and some organometallic compounds, exhibit Class B behaviour and dissociate after the absorption of a single photon [73]. In these latter cases no molecular ion is observed in the mass spectra.

In \( L^2 \) TOFMS experiments it can be difficult to determine whether a molecule exhibits Class B behaviour during laser photoionisation, or whether fragmentation is the result of thermal or photodissociation occurring [74,75] during the laser desorption process. For example, a number of organo-lead molecules were investigated using the \( L^2 \) TOFMS methodology. These appeared, initially, to exhibit Class B behaviour, yielding mass spectra containing characteristic fragments and no molecular ion. As an example of this type of behaviour, Fig2.3a shows the \( L^2 \) TOFMS spectrum obtained for lead acetate. In view of the absence of any molecular ion it is postulated that the gas-phase organometallic species had undergone Class B, neutral dissociation after the absorption of a single photon and the resulting fragments had been subsequently photoionised. The presence of atomic lead in the ionisation region of the mass spectrum was then demonstrated by selectively ionising this species via one of its atomic resonances [76]. This is shown in Figure 2.3b for lead acetate. A single-colour photoionisation scheme was used, consisting of excitation by means of a two-photon transition, followed by absorption of one more photon to promote ionisation. A visible wavelength of 450.3 nm, generated using a \( \text{XeCl} \) excimer pumped dye laser, was used. Although it would require the absorption of at least four photons in the ionisation stage to induce both dissociation and subsequent ionisation, such a process cannot be discounted. Lichten et al. [77] have reported that the successive absorption of up to ten photons in a single laser pulse is not uncommon. At this point, however, it remains unclear exactly when the lead acetate is disintegrating. It appears possible that the lead atoms observed may be a result of dissociation during the desorption stage and are then transported, along with the other fragments to the ionisation region. In order to test this hypothesis the desorption probe was enclosed in a faceplate.
The desorption was thus performed in the throat of a supersonic expansion. It is well known that these conditions promote clustering of laser ablated atomic species [78]. As can be seen in Figure 2.3c, lead clusters were generated. This result suggests that neutral lead atoms are, in fact, produced during the desorption process. At this point further work is required to ascertain unequivocally whether the lead acetate dissociates during the ionisation or the desorption process, or during both processes.

In order to confirm that fragmentation occurs in the desorption stage two experiments must be performed. These consist of varying the ionisation laser intensity and varying the laser wavelength. On increasing the desorption laser intensity the relative fragment to parent ion yield may be expected to increase whilst changing the desorption wavelength may change the nature of the fragmentation observed. If the fragments were a result of parent ion photodissociation, a plot of log(relative abundance) vs log(i onising fluence) should give slopes of integral number. This number will correspond to the number of additional photons required to promote fragmentation. The situation is more complex if dissociation is occurring in both stages of the experiment. It is important to note that for the majority of aromatic species studied in this work, this problem does not arise. As discussed in the Section 2.2.2, infrared pulsed laser desorption of these molecules produces predominantly intact, neutral gas phase species. Any fragmentation observed in the mass spectra can, therefore, be directly attributed to multiphoton absorption in the ionisation region, i.e. Class A photochemistry.

Class A behaviour is exhibited by benzene and many of its derivatives. Benzene has proven to be an archetypal molecule for studying MPI mechanisms and fragmentation. Boesl et al. [79], used a UV laser to produce benzene molecular ions via the $S_1$ state. These ions were then irradiated 17 ns later by a second laser, yielding fragment ions. The total ion current was found to be the same, both with and without the influence of the second laser. They therefore concluded that the molecular ion must have been the precursor for all the observed fragment ions. This is typical Class A behaviour and is expected to predominate for organic aromatic molecules.
Figure 2-3: L^2 TOFMS spectra of lead acetate [Pb(CH\textsubscript{3}CO\textsubscript{2})\textsubscript{2}] using a) 193 nm laser, b) 450.3 nm laser photoionisation and c) 193 nm laser photoionisation after desorption in an enclosing faceplate to encourage cluster formation.
Molecules with groups that induce radiationless transitions, such as chlorinated and brominated groups on aromatic rings, generally exhibit less efficient ionisation than their unsubstituted counterparts. Intersystem crossing, for example, generally results in the population of highly excited rovibrational states of the first excited triplet electronic state of the molecule. From there, either the Franck-Condon factors for the ionisation step from these are so low that efficient R2PI does not occur, or vibrational relaxation down the triplet manifold means that the second photon is insufficiently energetic to promote the molecule into the ionisation continuum. Photochemical and photophysical behaviour is not limited to the extremes described above, and neither are these types of behaviour mutually exclusive. In the latter chapters of this thesis, the influence of the intermediate eigenstate on the nature of the mass spectra will be dealt with further for specific classes of target species. Typically, however, vibronic states of the \( S_1 \) system of organic aromatic molecules do not dissociate, and display relatively slow non-radiative relaxation processes. Thus, the predominant process, following single photon excitation, is further photon absorption by the resonant intermediate state, exciting a molecule into the ionisation continuum.

Two processes must be considered for the ionisation step, namely direct ionisation and autoionisation. In direct ionisation a molecule is excited directly to the ionisation continuum, with the result that an electron is ejected leaving the molecular ion either in its ground state or an electronically excited state. In the case of autoionisation, although the total electronic energy is greater than the IP, no single electron has sufficient energy to be ejected. A super-excited neutral state, known as the autoionising state, is populated above the molecular ionisation potential. The electronic configuration of this state can change spontaneously, so that one electron acquires sufficient energy to enable ionisation to occur. This is known as electronic autoionisation. Molecular ions are formed in well defined vibronic levels, usually the vibrationless ground electronic state [83]. Any excess energy supplied in the ionisation step is removed as kinetic energy by the ejected electron, leaving the molecular ions with a narrow energy spread.

Further photon absorption can occur after a molecule has already absorbed
energies above the IP. An important consideration here is whether or not autoionisation proceeds at a fast enough rate to precede further absorption, i.e. is any further photon absorption by the neutral molecule or the molecular ion. A simple calculation, assuming an autoionisation rate of $10^{13} \text{s}^{-1}$ [84] and a cross section for absorption by the super-excited state of $10^{-16} \text{cm}^2$, suggests that autoionisation will dominate under typical laser ionisation conditions ($I < 10^{29} \text{photons cm}^{-2} \text{s}^{-1}$). This conclusion is supported by the work of Boesl et al. [79]. The MPI mass spectrum of benzene was found to be independent of the delay (0–17 ns) between the ionising and fragmenting laser pulses. In 17 ns, any autoionising state would have autoionised, so that any further photon absorption must be by the molecular ion. As the same mass spectrum was observed with no delay between the two lasers the same mechanism can be assumed to operate.

The dissociation of most polyatomic molecular ions seems to follow statistical models. These allow for a generalised description of the dissociation with only a few parameters changing for different molecular ions. Several mechanisms have been proposed [85,86,87,88,89] to explain the fragmentation of the molecular ion. The generally accepted model is the "ladder-switching" model of Dietz et al. [89], which is shown schematically in Figure 2.4.

In this model, the neutral molecule absorbs two or more photons on its absorption ladder resulting in photoionisation. At high incident photon densities the molecular ion can then absorb one or more additional photons. In common with the quasi-equilibrium theory of conventional mass spectrometry [90], fragmentation occurs once certain energy thresholds are exceeded. The "ladder switching" model takes into account the energy take-up of fragment ions by photon absorption. Unimolecular dissociation according to statistical decay is assumed to occur on a short timescale ($10^{-11} \text{s}$), and competes effectively with photon absorption over the duration of a laser pulse (5-10 ns). This places an effective maximum energy on the molecular ion absorption ladder, and causes the system to switch to a new ladder of products. These products can then absorb further photons in turn, until their maximum energy is reached when they themselves undergo ladder switching. This successive ladder switching strongly influences the possible
Figure 2-4: Ladder switching model of multiphoton ionisation.
fragmentation pathways. Boesi and coworkers [91] have produced experimental evidence to support this model. They selectively irradiated primary fragment ions from benzene with a second laser and observed mass spectra similar to those generated in a single laser photoionisation experiment. They concluded that the fragmentation pathway involved absorption by these fragment ions, directly supporting the ladder switching model. Deitz et al. [89] and Rebentrost and Ben-Shaul [88] have shown that absorption of one photon by the molecular ion of benzene, up to ca. 5 eV, results in up-pumping, whilst absorption of two photons results in dissociation becoming dominant. This energy is much lower than the appearance potential of small ionic fragments such as C\textsuperscript{+}, so the ladder switching model must be invoked to explain their appearance in MPI mass spectra of benzene in intense laser fields. In more recent experiments, Schlag's group have followed this fragmentation ladder step by step using a new tandem time-of-flight technique [92,93]. In parallel with the fragmentation rules deduced for EI [80], Pandolfi et al. [82] have determined that the fragments generated by organic aromatic species will occur so as to leave the positive charge on the larger fragment. The same group have also demonstrated that for nanosecond laser pulses, as used all in the work described in this thesis, absorption and ionisation of the neutral fragments do not play an important role as a channel for further fragmentation.

2.3.3 Analytical Advantages of MPI

The primary advantage of resonance-enhanced MPI is that it is a very efficient means of producing molecular ions of target species. As discussed previously, photon absorption by molecular species is a process of low probability unless the incident photon energy is in resonance with one of the real electronic states of the molecule. R2PI can typically provide ionisation efficiencies of several percent or higher within the laser beam volume during the period of the laser pulse. The efficiency is limited by the duty cycle of the laser source (ca. 10 ns pulses at a repetition rate of 10 Hz). Ultimate limitations to efficiency depend upon the absorption and ionisation cross sections at a particular ionising wavelength. Boesl et al. [94] have measured the ionisation cross sections for a number of organic
molecules in an effusive molecular beam. For broad band excitation (1 cm\(^{-1}\)) at 10\(^7\) W cm\(^{-2}\), approximately 25% of the ground-state population resonant with the laser bandwidth could be ionised. In contrast, ionisation efficiencies in electron impact (EI) mass spectrometry are much lower, generally in the order of 10\(^{-4}\) [95]. Furthermore, soft ionisation in EI can only be achieved at low electron beam energies, resulting in a loss of ionisation efficiency.

In addition to conferring a high ionisation efficiency, the use of R2PI means that a molecule can be identified not only by its mass spectrum, but also by its resonant ionisation wavelength. This is a truly unique property of MPI. Essentially, the ionisation cross-section over a range of wavelengths reflects the absorption-excitation spectrum over the same wavelength range. The significance of this is that, in a mixture of molecules, any component with an absorption band is resonance with the incident wavelength will be preferentially ionised. Lowering the power density of the ionisation laser to a suitable level enables complete discrimination against the non-resonant ionisation of other components. At fluences of ca. 1 MW cm\(^{-2}\), soft ionisation mass spectra, containing only the molecular ion of the target species, can be produced allowing the direct determination of trace species in mixtures. R2PI, with both mass and wavelength discrimination possible, is a truly two-dimensional technique.

The two-dimensional nature of the technique allows intermediate state spectra for a selected mass to be measured. The gas-phase R2PI spectra of polyatomic molecules at room temperature show broad features due to the thermal population of rovibronic states of the molecule. In order to maximise the optical selectivity, the spectral congestion must be minimised. The standard means of achieving this is to make use of the adiabatic cooling available in supersonic molecular beams [99, 97, 100]. Briefly, molecules in a high pressure reservoir are allowed to expand into a vacuum through a very small orifice, undergoing many two-body collisions. The result is that enthalpy is converted into directed mass flow velocity. This has the effect of collapsing the room temperature Maxwell-Boltzmann velocity distribution to a narrow range centered on the directed mass flow velocity. The translational temperature which describes the width of the “jet-cooled” distribution can be
as low as 0.1K. Rotational and vibrational temperatures come into equilibrium with the translational temperature, leading to temperatures of ca. 10K and 100K respectively. Molecules of interest are generally seeded, at low concentrations, into expansions of monatomic (e.g. He), diatomic (e.g. N\textsubscript{2}) or polyatomic gases (e.g. CO\textsubscript{2}). At these low temperatures the number of rotational and vibrational states populated are reduced, simplifying the optical absorption spectra.

Mass-selective intermediate state spectra of the $^6\Pi_0$ cold band in the $S_1(^1B_{2u}) \leftarrow S_0(^1A_{1g})$ transition of benzene isotopes of low concentration have been measured by Boesl et al. [96]. Similarly, the R2PI jet-cooled spectra for the origin band of the $S_1 \leftarrow S_0$ spectrum of two aniline isotopes (mass 93/mass 94 = 100/13.87%) have been recorded by Costello et al. [97]. Here, it was demonstrated that by carefully tuning the laser to a wavelength corresponding to the absorption maximum of one of the isotopes, it was possible to discriminate between each of the isobaric components of a natural isotopic mixture.

In our experiments, target species are swept into a pulsed supersonic flow downstream of the pulsed nozzle, after being laser desorbed from a freely-hanging sample probe. In this way the sample is transported into the ionisation chamber. Using this method of entrainment it is uncertain how much cooling is conferred to the desorbed species. However, even with only limited cooling in our source, wavelength selective ionisation is still possible. This has been demonstrated already in Figure 2.3b, for atomic lead. Atomic systems, of course, require no jet-cooling to enable selective photoionisation. In this case a single-colour process consisting of excitation by means of a two-photon transition to the resonant excited state $^3P_0$, followed by photoionisation with a further 450.3 nm photon was used [81]. This [2+1] scheme was utilised to selectively ionise atomic lead, discriminating against the other organic or cluster fragment species seen when using 193 nm excitation, see Figure 2.3a. Another example, demonstrating the discrimination between molecular components in a mixture, is shown in Figures 2.5 a and b. These spectra show the laser desorption MPI mass spectra of a mixture containing aspirin, paracetomol and caffeine at ionising wavelengths 266 nm and 193 nm respectively.
Figure 2-5: L²TOFMS spectra of a mixture of caffeine, paracetomol and aspirin produced using a) 266 nm and b) 193 nm laser photoionisation.
The mass spectra are clearly different from one another. At 266 nm, we observe strong molecular ion signals for both the caffeine and paracetamol species. A lower intensity peak at 109 amu corresponds to the partial loss of the amide side chain from the paracetamol molecule. When using 193 nm laser photoionisation, fragment signals directly attributable to the aspirin component dominate the mass spectrum. No signals of significant intensity are observed for either paracetamol or caffeine. In fact, the peaks observed correspond to the characteristic mass spectrum of the aspirin molecule at this ionisation wavelength. It is clear from these spectra that the different molecular components are being selectively ionised at each of the two wavelengths. It is also obvious that for a complete analysis of such a mixture, the mass spectra at both wavelengths are required. As it is unlikely that much cooling is conferred to the molecules under the conditions employed here, the absorption characteristics of the components must be significantly different. It is possible to obtain substantial internal cooling of such molecules by desorbing in an enclosing faceplate. This maximises the number of two-body collisions occurring in the throat of the supersonic expansion. This has been demonstrated previously for both tryptophan and perylene using the apparatus described in Chapter 3 [98].

Alongside the potential for selectivity, R2PI can be used to control the fragmentation pattern obtained. It is now widely known that by judicious control over the ionising laser fluence soft ionisation mass spectra can be obtained. This fact has been exploited to provide soft ionisation mass spectra of a wide variety of important organic aromatic analytes in tandem with laser desorption. These investigations are described in more detail in Chapter 4. However, soft ionisation mass spectra which contain only a single peak corresponding to the molecular ion, or minimal fragmentation, facilitate simple mass spectral interpretation. Also, the interpretation of the mass spectra of multi-component mixtures is much simplified. It is important to remember that soft ionisation spectra of this nature will not be obtained for Gedanken’s Class B compounds, where fragmentation is the result of the dissociation of the excited neutral intermediate prior to ionisation.

An increase in the ionising laser power density promotes further absorption of photons and induces fragmentation of the molecular ion. This process is now
well understood through the ladder switching mechanism. At high laser powers extensive fragmentation can occur, even down to individual carbon atoms. As the laser power varies so does the ratio of the different fragments in the mass spectrum. Figures 2.6a and b show the soft and hard (laser fluences of ca. 1 MWcm$^{-2}$ and ca. 10 MWcm$^{-2}$ respectively) ionisation mass spectra of paracetomol using 266 nm laser photoionisation. It is clear from these spectra that the extent of fragmentation that can be induced, by varying the laser fluence, is wide ranging. It is possible, in certain cases, to distinguish isomers by their fragmentation patterns. This was demonstrated by Kuhlwind et al. [101] for three butyl iodide isomers. Additionally, the nature of the fragmentation observed in the mass spectrum of a specific target molecule may be radically different for differing ionising laser wavelengths (see Chapter 7).

The fragmentation patterns produced by laser MPI are a result of the absorption of UV photons. This means that the transitions will be Franck-Condon controlled, i.e. vertical transitions without any change in nuclear coordinate. Therefore, only transitions to certain states will be allowed, limiting the nature of the fragmentation observed. Electron impact studies, on the other hand, employ typically 70 eV electrons, corresponding to radiation of 0.15 nm, so strong perturbation of the molecule will occur. Not surprisingly, many more states are accessible with the potential for more interesting fragmentation channels [102]. However, in EI, the 70 eV of energy is deposited in the molecule instantaneously, leaving the different dissociation pathways to compete with each other. This results in an effective loss of control over the degree of fragmentation observed in the mass spectrum.

It can readily be seen that the combination of pulsed laser desorption and laser MPI offers exciting analytical possibilities, and opens up the field of mass spectrometry to a wide range of molecular types. One of the limitations of MPI is that the target molecule requires a UV chromophore in order to be able to absorb the UV laser radiation. Fortunately, the majority of the molecules targetted by this technique have strongly UV absorbing chromophores. The following section will focus on the use of time-of-flight mass spectrometry for the analysis of multiphoton ionised molecules.
Figure 2-6: L$_2$TOFMS mass spectra of paracetomol obtained under a) soft and b) hard ionisation conditions using 193 nm laser photoionisation.
2.4 Time-of-Flight Mass Spectrometry

2.4.1 Introduction

In the following section, the principles behind the operation of a time-of-flight (TOF) mass spectrometer are introduced along with a description of the advantages of this technique in combination with pulsed ionisation sources. Two TOF mass spectrometers, utilising the same source chamber, were used in the work described here: a linear TOFMS and a reflecting geometry TOFMS (reflenton). These are shown diagramatically in Figures 3.1 and 3.2. As a result of the improved performance of the reflectron instrument this was generally used in preference to the linear instrument. A description of the actual TOF mass spectrometer used to perform the experiments described in Chapters 5-8 is given in the following chapter. The remainder of the present section is confined to a discussion concerning the factors which limit the resolution of a TOF mass spectrometer and concludes with a theoretical description of the reflecting geometry TOF mass spectrometer which circumvents some of these limitations. A more complete theoretical description of the TOF instruments, along with a consideration of their operational limitations can be found elsewhere [97].

In the preceding sections, the background and mechanisms involved in laser desorption and laser photoionisation have been considered. The lasers employed for both these processes have pulsed outputs. It is therefore logical to utilise a mass spectrometric technique which most efficiently processes ions created in a pulsed fashion. The mass spectrometric method that really takes advantage of the pulsed nature of ion production by MPI is time-of-flight mass spectrometry. As opposed to all other mass spectrometers, a TOF mass spectrometer can enable a complete mass spectrum to be recorded after every laser pulse. Although scanning mass spectrometers have been used in conjunction with MPI, there are a number of drawbacks associated with these. Sector and quadrupole instruments are usually coupled to continuous ion sources: the separation of ions of different masses is
accomplished by scanning electric or magnetic fields so that different masses impinge on the detector. This is clearly inappropriate for the low duty cycle pulsed laser photoionisation experiments employed in the work described here.

TOFMS is one of the simplest, yet most versatile, mass spectrometric techniques. The small size, low cost and ease of construction of TOF instruments have made them the primary choice of researchers involved in laser ionisation experiments. However, it is not a new technique. In 1955, Wiley and McLaren [103] published their first design for a TOF mass spectrometer. This has proved to be the prototype instrument for over 30 years. The design has high ion transmission and a theoretically unlimited mass range, along with the advantages noted above. The principal disadvantage, compared with other MS instruments, is the limited mass resolution achievable in a TOF mass spectrometer. The reason for this limitation, together with a discussion of the measures available to improve the resolution, are described in the following section.

The fundamental components of a TOF mass spectrometer are the ion source, the drift region (or flight tube) and the detector. In the simplest case, ions are formed in the ion source and are immediately extracted by a fixed potential. This electric field accelerates the ions into a longer, field-free drift region. In the ideal case, all ions with the same charge enter the drift region with the same kinetic energy (KE). This is given by:

\[ KE = zeE_s s \]  

(2.7)

where \( e \) is the charge on an electron, \( z \) is the number of such charges, \( E_s \) is the electric field and \( s \) is the distance between the repeller and ground plates in the source region of the spectrometer. A simple single-field linear TOFMS of this kind is shown schematically in Figure 2.7a. Also shown, in Figure 2.7b, is the schematic diagram of a Wiley-McLaren type TOF mass spectrometer. In this latter case, the kinetic energy is furnished using a two-step acceleration field [103], which provides first-order focussing of the ion packets. This means that ions created at different positions in the source region arrive at the detector simultaneously,
a) Single Field
Linear TOFMS

b) Double Field
Linear TOFMS

Figure 2-7: Schematic diagram of the a) single-field, and b) two-field Wiley-McLaren type, TOF mass spectrometers.

$V_b =$ molecular beam velocity
thereby increasing the mass resolution of the mass spectrometer with respect to that of a single field device. This is discussed further in the following section.

The ions entering the drift region with the same kinetic energies will have velocities that depend on their mass, \( m \):

\[
v = \left( \frac{2ZeEs}{m} \right)^{\frac{1}{2}}
\]  

(2.8)

Therefore, the time required to traverse the drift region will also depend on the mass of the ion:

\[
t = \left( \frac{m}{2ZeEs} \right)^{\frac{1}{2}} D
\]  

(2.9)

A mass spectrum recorded over time can therefore be converted directly to a mass spectrum:

\[
\frac{m}{z} = 2eEs \left( \frac{t}{D} \right)^2
\]  

(2.10)

The lighter, faster moving ions reach the detector before the slower, heavier ones. By recording intensity as a function of time, a time-of-flight mass spectrum is generated.

A complete mass spectrum is thus generated for each laser desorption laser photoionisation experimental cycle. This allows for facile optimisation of the desorption/ionisation conditions without excessive sample depletion. A further advantage afforded by TOFMS is improved sensitivity compared to sector and quadrupole instruments. These latter instruments use spatial, rather than temporal separation of ions of differing mass. Thus, all ions not focussed onto the detector at a particular (RF or magnetic) field setting are rejected. The transmission in a TOF mass spectrometer is potentially much higher, since a mass spectrum is recorded with static fields and all ions of a particular mass are extracted along the same trajectory, to a first approximation. The sensitivity is therefore enhanced, being limited only by the physical transmission efficiency of
the grids employed in the ion extraction optics. In a real TOF mass spectrometer, there are several other mechanisms which can lead to reduced ion transmission. For example, the ions may collide with background gas molecules and be deflected, or their velocity components perpendicular to the direction of extraction, due to the velocity distributions of their neutral precursors, may be sufficient for them to miss the detector.

These problems are alleviated, in the reflectron instrument described in Chapter 3, by the adoption of a collinear molecular beam/reflectron geometry. This allows a greater number of ions to be transmitted into the drift tube than would be the case if ion extraction was orthogonal to the molecular beam. Previous investigations have shown that the maximum signal intensities recorded in the reflectron were twice those recorded under similar conditions using the linear TOF available on the same instrument (see Figures 3.1 and 3.2) [97]. A further advantage of the collinear geometry is that it has a (theoretically) mass independent transmission function. This is not the case for TOF mass spectrometers in which ion extraction is orthogonal to the direction of the molecular beam. L²TOFMS experiments were performed using both the linear and reflectron geometry on a polymeric distribution of polystyrene oligomers with average molecular weight 687 amu. Figure 2.8 shows the resulting mass spectra obtained from the linear TOFMS using a series of increasing deflection voltages. As can be clearly observed there is an effective mass "window", above and below which ions are not detected. Increasing the deflection voltages has the effect of increasing the highest observable mass. However, at the same time the lower mass signals are diminished in intensity or disappear entirely. This behaviour is a consequence of the momentum possessed by the neutral precursor molecules in the molecular beam. On ionisation, the linear TOFMS must extract the ions orthogonal to the direction in which the neutral species were travelling. Heavy species are not turned sufficiently in the extraction field and miss the detector, being lost to the walls of the flight tube. The deflection plates located above the extraction optics are able to compensate for this momentum. Increasing the deflection plate voltage it is possible to compensate for the momenta of increasingly heavier species. Unfortunately, by doing so, the
lighter species are overcompensated for and are lost to the opposite walls of the flight tube. The advantages of the collinear geometry are immediately apparent in Figure 2.9. In this case the whole polymer distribution can be observed simultaneously. The majority of mass spectra presented in later chapters were obtained using the reflectron TOF mass spectrometer. Therefore, the “windowing” effect observed in the linear TOF mass spectrometer is not important.

Mass calibration of the time-of-flight mass spectra is carried out by fitting the time of arrival for ions of known mass using Newton’s equations of motion. The time of arrival of a species can be fitted to the correct mass using the function,

\[ t = a + bm^2 \] (2.11)

where \( a \) and \( b \) are constants [104]. The time-of-arrival of a particular ion, measured in terms of transient digitiser channel number, is fitted to its equivalent mass, using a least-mean squares program, to obtain the coefficients \( a \) and \( b \). Calibration in L\textsuperscript{2}TOFMS experiments is usually performed using an internal standard or a calibrant gas such as aniline to give well-characterised mass peaks. Typically, dependent on the mass range of interest, one species at the low mass end and one species at the high mass end are utilised to provide quick mass spectral calibration for unknown samples.

### 2.4.2 Resolution Limiting Factors

As already noted, the principal disadvantage of TOFMS instrumentation has always been its relatively low resolving power. In a TOF mass spectrometer the resolution is reflected in the temporal width of the signal produced on detection of the ion packets. The mass resolving power (resolution) of a TOF mass spectrometer is defined as,

\[ R = \frac{m}{\Delta m} = \frac{t}{2\Delta t} \] (2.12)
Figure 2-8: L²TOFMS spectra of a polystyrene polymer distribution (av. mol. wt. $M_v = 687$ amu) obtained using a linear TOF mass spectrometer and 266 nm laser photoionisation. The spectra were recorded using successively higher deflection plate potentials; a) 100 V, b) 200 V, c) 400 V and d) 500 V.
Figure 2-9: L²TOFMS mass spectrum of a polystyrene polymer distribution (av. mol. wt. $M_v = 687$ amu) obtained using a collinear reflectron TOF mass spectrometer and 266 nm laser photoionisation.

where $m$ is the ion mass, $\Delta m$ is the FWHM spread in the ion packet mass, $t$ is the ion flight time and $\Delta t$ is the temporal width (FWHM) of the ion packet. Schlag and coworkers [105] introduced an alternative definition of mass resolution,

$$R = \frac{m(t_2 - t_1)}{\Delta t}$$

where $(t_2 - t_1)$ is the difference in time-of-flight for two neighbouring mass peaks, of mass $m$ and $m + 1$, and $\Delta t$ is the temporal width of the ion packets. Clearly, the resolving power of a TOF mass spectrometer also depends on the difference in arrival times of two adjacent ion packets. As seen from Equation 2.9, flight times are proportional to $m^{1/2}$. Therefore, the difference in flight times between adjacent mass peaks decreases as mass increases. At high masses, the resolving power of the instrument is degraded until eventually the FWHM of the individual ion packets is equal to the temporal difference between the neighbouring peaks, and it is no longer possible to resolve ions separated by 1 amu. The maximum resolvable mass is increased by a narrow ion packet width and long flight time. Design parameters
will dictate the flight-time, whilst the temporal width of an individual ion packet is limited by the initial temporal, spatial and kinetic energy distributions of the ion and properties of the recording system. These latter broadening mechanisms are discussed further below.

Spatial Resolution

In an ideal TOF mass spectrometer, all the ions formed in a single ionising laser shot will extract the same kinetic energy from the electric field in the source region of the mass spectrometer. This requires that all the ions are formed in exactly the same plane, perpendicular to the electric field gradient. As it is impossible to focus a laser beam to an infinitely thin plane or a single point, the ions are actually created over a finite spatial volume in the ion source. If two ions of the same mass-to-charge ratio and initial kinetic energy are created at the same time, but at different positions along the spectrometer axis, they will be extracted from the source with different kinetic energies and thus enter the drift region with different velocities. The ion formed towards the rear of the ionisation source falls through a larger electrical potential and is accelerated to a higher kinetic energy than one formed near the front. The same would be true of two ions created in the same plane but which experience different potentials due to field inhomogeneities. This limiting effect is known as the spatial resolution of the mass spectrometer.

This type of energy spread is the main limiting factor on the achievable mass resolution in simple linear TOF instruments. Clearly, the use of a focussed or spatially apertured laser beam to create ions limits the range of potentials over which the ions are created, and therefore reduces the spatial broadening contribution. However, this can lead to other broadening mechanisms such as space-charge effects. A method of improving the spatial resolution was described by Wiley and McLaren in 1955 [103]. They introduced the spatially-focussing double field ion source to compensate for these flight-time distributions.

In a simple, single accelerating field instrument there is a point in the field-free drift region at which the decrease in flight-time due to the larger velocity of
ions formed at higher potentials is compensated for by the increased distance over which they have been accelerated. Usually, such a flight time correction is only first order. With an ion detector positioned at the space-focus, one can observe an energy-compensated mass spectrum. However, for this simple two-electrode source the distance to the space focus is much too short for practical use. A double field source, in contrast, allows the position of the space focus to be shifted by varying the electric fields in the two-stage acceleration region. This results in much longer ion-flight times and allows the mass spectrometer to be refocussed by simply adjusting the fields in the ionisation region. An important feature, therefore, of the double field extraction region is that the spatial resolution of the mass spectrometer is increased with respect to that of the single-field device.

**Temporal Resolution**

In a simple TOF mass spectrometer it is not possible to produce ion packets which have temporal widths less than that characteristic of the ionisation technique employed. If two ions were formed at the same point in the source region, one at the leading edge and one at the trailing edge of an ionising pulse; they would have the same velocities but arrive at the detector separated in time by an amount $\Delta t$. This remains a constant separation through the drift region, even if all other broadening mechanisms are ignored. Before the advent of pulsed lasers, poor temporal resolution was the principal reason for the low mass resolution of TOF instruments. Pulsed lasers, however, are routinely available with pulse widths in the 10-20 ns range making laser ionisation an ideal ion source for TOFMS.

The effect of timing resolution can be minimised by simply increasing the overall flight time. This can be accomplished by reducing the accelerating voltage or increasing the drift tube length. As high accelerating voltages improve ion transmission and energy focussing, it has been common to use longer flight lengths. A more effective way of reducing the ion packet temporal width would be to use still shorter ionisation pulses, either picosecond or femtosecond pulse lengths. This is better than extending the drift tube length, since for large flight distances the
Kinetic Energy Resolution

The resolution of a TOF device is also affected by the initial velocity distribution of the ions parallel to the spectrometer axis. Consider the case of two ions with the same mass-to-charge ratio which are formed at the same position in the source but with initial velocities (derived from the velocities of the neutral precursors) which are directly opposed along the axis of the instrument. For example, one ion with initial velocity, +v, moving towards the detector and one ion with initial velocity, -v, moving away from the detector. The ion moving away from the source exit will travel against the field, stop, turn around, and be accelerated to the same energy as the ion moving initially towards the source exit. These two ions will have the same drift velocity and will, therefore, maintain a constant difference in arrival times. This is known as the “turn-around” time, the magnitude of which depends on the ion energy and the electric field strength in the ion source. Similarly, if two ions of the same mass-to-charge ratio are formed in the same location but with different initial kinetic energies, the difference in their flight-time, and therefore arrival time at the detector, increases with flight tube length. Essentially, a distribution of kinetic energies for the initial neutral molecules results in ion packets with a finite width. The limited resolution due to these effects is known as energy resolution.

In a simple, single-field TOF device, the effects of the initial energy distribution on resolution can be minimised by using high extraction fields to increase the ratio of the ion energy to the initial kinetic energy spread. This would require a long drift tube to ensure sufficient time separation between adjacent masses. The narrow velocity distributions afforded by a supersonic molecular beam propogating either parallel or perpendicular (the latter being most effective) to the flight-tube axis of a TOF mass spectrometer helps to substantially improve the energy resolution.

Wiley and McLaren [103] described a method of energy focussing, using their double-field source, known as “time-lag-focussing”. Here, the ions are allowed
to spread out in the field-free ion source before the extraction field is switched on. By choosing a delay time between ion formation and ion extraction, different velocities give rise to different spatial positions in the final extraction field and thus to different ion kinetic energies. The resulting variations in the flight time can then partially compensate for the effect of the initial velocities. However, the gain in energy resolution in this case was offset by a loss in spatial resolution since the device had to be operated away from space focusing conditions. An alternative technique for overcoming the effects of the initial velocity distribution is "impulse-field-focussing" [106]. A fast, short, high voltage pulse accelerates the ions in a very short time to velocities much higher than their initial velocities, before the normal acceleration field is switched on. Further novel methods of achieving improved energy resolution are summarised in a recent review by Boesl et al. [107].

All these different techniques have their inherent problems. A much better solution is to use the first-order space focus of an ion source as a pseudo-ion origin with minimised spatial distribution. The ions can therefore be considered to originate from the focus with a flying start and with only kinetic energy distribution uncompensated for. The flight-time broadening in the drift region behind the space focus, due to the kinetic energy spread, can now be corrected by a further energy compensating device. One such device is an ion reflector or reflectron mass spectrometer. Long flight paths and therefore good resolution can now be realised independently of the ion source; it is now possible to optimise the real ion source for maximum spatial resolution, without the need for a long flight path from the point of ion formation to the space focus.

2.4.3 The Reflectron TOFMS

Reflectron Energy Focussing

In 1973, Mamyrin and coworkers [108] introduced the reflectron device as a means of correcting the effects of initial kinetic energy distributions. A schematic diagram showing the characteristic features of a reflectron mass spectrometer is shown in
Chapter 2. I$^2$TOFMS – Background Theory

source overlaps the space focus of the reflector, being separated in space only by the tilt of the ion trajectories relative to the reflector axis.

A reflectron can only compensate for the flight-time spread due to kinetic energy differences. The temporal distributions cannot be corrected for in this way. In other words, the ion reflector images the flight-time distribution at the space focus of the ion source onto the surface of the detector. Therefore, the peak widths are kept narrow, whilst the time interval between adjacent masses is significantly increased over linear mass spectrometers, due to the extended flight time. Therefore, high mass resolution can be obtained with the extraction fields in the ion source region being unchanged.

The temporal distribution at the pseudo-ion source, or the time interval over which ions of a single mass pass the pseudo-ion source, is due to:

- an incompletely corrected energy distribution at this space focus, i.e. only first-order space focussing, or
- the time spread within the ion source due to the ion formation time and the "turn-around time".

Turn-around times can be effectively reduced by using high extraction fields. The non-optimal energy compensation in the ion source space focus is now negligible because of the small flight time to the space focus compared to those through the rest of the mass spectrometer. Thus, the minimisation of the "turn-around times" can be achieved by the ion source and the kinetic energy distribution can be primarily compensated for by the reflector.

Limitations of a Reflectron TOFMS

The conclusion that can be drawn from the previous section is that the final ion peak width in a reflectron TOF mass analyser results from the minimised "turn-around time", higher order energy terms not compensated for at the first-order space focus and intrinsic time spreads due to factors such as the ion formation
time. Several other factors which can also limit the mass resolution are discussed below.

Generally, a real ion beam will have a divergence due to inhomogeneities in the extraction fields and space charge effects. The maximum divergence leading to degradation of the mass resolution is given by the ratio of the effective drift length and the diameter of the ion detector surface. This can lead to a large difference in the drift length for two extreme ion trajectories if the ion detector is perpendicular to the ion beam. This effect can be compensated for by aligning the detector parallel to the grids of the ion reflector. This, however, introduces a new uncertainty. A real beam has finite width and therefore parallel shifted trajectories. Thus, aligning the detector parallel to the ion mirror grids will introduce different path lengths across the width of the ion beam and, consequently, varying flight times. This is a much smaller effect, however, than the influence of divergence. The instrument described in this thesis has a detector placed parallel to the ion beam. The resolution could thus be improved by either changing the orientation of the detector or reducing the divergence using an einzel lens. This latter technique may introduce uncertainties in the ion trajectories. In most cases it is sufficient to minimise the angle of reflection and thereby minimise the effects of a divergent beam.

Another factor which influences the resolution of the mass spectrometer is space-charge effects. For laser MPI the space-charge effect due to the high ion density at the point of ion formation can be a major problem. It causes an acceleration of ions away from the centre of the ion cloud; this occurs in the first millimeter of the ion extraction region until the ion cloud expands so far that the coulombic forces are negligible. The result is an additional component to the ion velocity distribution. Velocities perpendicular to the mass spectrometer axis increase beam divergence. Velocities parallel to the axis cause more severe time-dependent “turn-around time” effects. The best way to minimise these effects is to choose a high extraction field in the ion source, as suggested previously for minimising the “turn-around time” effect resulting from the initial kinetic energy distributions of the ions. Alternatively, lower ion densities can be produced by
using a larger laser focus. The concomittant increase in ion kinetic energy distribution due to spatial spread can be readily compensated by a reflectron TOFMS.

Finally, any ion detector has a finite response time following the impact of ions on its active area. In the case of microchannel plates, which are used in the instrument described in the following chapter, the rise-time is usually in the sub-nanosecond regime [110]. Therefore, this is not generally a factor which limits the resolution of the TOF mass spectrometer.

2.5 Concluding Remarks

The technique of L²TOFMS consists of three fundamental components: laser desorption, laser photoionisation and time-of-flight mass spectrometric analysis.

Laser desorption facilitates the volatilisation of fragile, involatile molecules as intact neutral species which can be postionised. The use of IR laser radiation at power densities of ca. $10^8$ Wcm$^{-2}$ has proved an effective method for sample vaporisation. The subsequent entrainment into a supersonic molecular beam helps minimise fragmentation and results in a relatively cold beam of intact neutral molecules, which can be ionised using laser multiphoton ionisation techniques.

Resonant laser molecular multiphoton ionisation has a number of unique analytical properties:

- selectivity in ionisation, especially when coupled to a jet-cooling facility.
- high sensitivity.
- controllable fragmentation.

A time-of-flight mass spectrometer ideally complements pulsed laser ionisation. The advantages of such a mass analyser are the almost unlimited mass range, the high transmission and high sensitivity, and the ability to collect a complete mass spectrum with each experimental cycle. Furthermore, the development of
reflectron TOF instrumentation has enabled reasonably high mass resolution to be achieved.

In conjunction with one another, these techniques provide a powerful mass spectrometric tool, L²TOFMS, capable of handling a wide variety of previously problematic sample systems. The following chapter contains a description of the instrument designed and built in Edinburgh for the purposes of exploiting this technique.
Bibliography


[75] M. J. Dale, S. J. Wright, A. C. Jones C. R. Redpath, P. R. R. Langridge-Smith, unpublished results


Chapter 3

L²TOF Mass Spectrometry:
Instrumentation

3.1 Introduction

The experiments described in Chapters 5 to 8 were performed on a purpose built molecular beam research instrument [1]. This modular, differentially-pumped time-of-flight mass spectrometer (TOFMS) consists of three vacuum chambers: the desorption chamber, the laser ionisation chamber and a third chamber containing a reflecting-geometry time-of-flight (RETOF) mass analyser. The mass spectrometer, shown schematically in Figures 3.1 and 3.2, was designed to have interchangeable linear and reflecting-geometry (reflectron) time-of-flight configurations. In both cases the experimental details were similar. Neutral molecules were desorbed from a sample probe in the source chamber (SC) and transported in a molecular beam to the ionisation chamber (IC), where ions were created by laser photoionisation and subsequently mass analysed.

The majority of the results presented in this thesis were obtained using the molecular beam apparatus with reflectron time-of-flight mass analysis. However, some experiments have been performed using an alternative molecular beam apparatus from which the instrument described here is derived. This instrument has only a linear time-of-flight mass analyser and has been described in detail elsewhere [2]. The design and function is similar to the instrument described in the following sections. The use of this alternative instrument will be indicated where necessary in the following chapters.

85
The following sections in this chapter contain a description of the vacuum hardware, lasers, data acquisition and experimental control modules used in the operation of the reflectron time-of-flight mass spectrometer. Lastly, a brief description of the three possible experimental modes of operation will be given.

3.2 Reflectron Mass Spectrometer Vacuum System

The vacuum system, shown schematically in Figures 3.1 and 3.2, consists of two cuboid chambers and a reflectron chamber (RC). All three chambers were constructed from 304 stainless steel, and equipped with demountable flanges. These flanges were also constructed of 304 stainless steel, apart from those on the source chamber. These larger flanges were fabricated from aluminium to minimise weight and thus facilitate their easy removal and handling.

The main source chamber, which has a volume of 47.3 l, also housed the laser desorption source, molecular beam valve and molecular beam skimmer. This chamber was pumped by a half-chevron baffled 10" oil diffusion pump (CVC PMC-10, Convoil 20 pump fluid) with a pumping speed for air of 2600 l s\(^{-1}\). To ensure background pressure was maintained as low as possible, and to speed up the roughing of the SC, the diffusion pump was backed by a mechanical booster/rotary pump combination (Edwards EH250/E2M40) capable of pumping 8.5 mbar l s\(^{-1}\) at a typical foreline pressure of ca. 0.1 mbar. The chamber pressure was monitored by an Edwards CP25EK Penning gauge head attached to an Edwards 505 gauge readout. The base pressure in the chamber was typically 5×10\(^{-6}\) mbar which rose to ca. 1×10\(^{-3}\) mbar on operation of the pulsed valve with 4.5 atmospheres stagnation pressure of helium at a repetition rate of 10 Hz. The SC could be isolated from the diffusion pump by a manually operated gate valve (Vacuum Research Company LP series), and the pump could be entirely isolated by means of a pneumatically-operated gate valve (Airco Temescal 5230). This pump isolation procedure allowed the SC to be evacuated by the rotary pump via an independent
roughing line, without requiring the diffusion pump fluid to cool down. This was essential to ensure rapid sample turnaround.

The ionisation chamber housed the ion extraction optics for the reflectron time-of-flight (RETOF) mass spectrometer. This 17 l cube was pumped by an Edwards Diffstak 160/700 diffusion pump, with a pumping speed of 700 l s⁻¹ for air, using Edwards L9 pump fluid. This pump was backed by an Edwards E2M18 rotary pump which was also used to back the reflectron chamber via a common foreline.

The drift tube of the linear TOFMS, 113 cm long, was mounted on the top flange of this chamber with the MCP port blanked off. This was not used for the duration of this project. However, the lower 65 cm of the drift tube was surrounded by a double-skinned liquid nitrogen dewar, the bottom of which penetrated into the IC chamber. This cryopumping effectively reduced the pressure in the IC by an order of magnitude. The ion optics for either the linear or the reflectron TOF configurations were mounted on bed bars running along the bottom of the IC and were protected on three sides by a copper cryoshield mounted on the base of the liquid nitrogen dewar. Pressures were measured in this chamber by an Edwards CP25K Penning gauge and a PRL 10 Pirani gauge. The background pressure in this chamber was typically less than 10⁻⁷ mbar rising to ca. 1×10⁻⁶ mbar under experimental conditions.

The reflectron chamber (63.5 cm long and 19 cm I.D.) was connected to the IC by a stand-off tube (31.2 cm long and 6.3 cm O.D.) which mated to the manually operated gate valve employed to mutually isolate these chambers. The ion mirror optics were mounted on the rear flange of the RC, tilted at an angle of 4° to the common molecular beam-ion extraction axis, to direct ions down the second arm of the flight tube. The axis of this second drift region was angled at 8° with respect to the first. A second stand-off section connected the RC to the MCP detector. This stand-off comprised two smaller tubes, the first of length 5.1 cm and 6.3 cm O.D., the second of length 8.9 cm and 5.1 cm O.D. The RC was pumped by an Edwards Diffstak CR 160/700 diffusion pump (fluid Edwards L9), which was equipped with both a liquid nitrogen-cooled cryotrap and a quarter swing butterfly isolation valve. A double skinned liquid nitrogen dewar was located on the top of
FIGURE 2.1: Plan schematic of the T2 TOP mass spectrometer.

- **DP** = Diffusion pump
- **EO** = Extraction optics
- **RO** = Reflection optics

Legend:
- **CO₂** Laser
- **UV** Laser
- **SP** = Sample probe
- **L** = Lens
- **L1** = Lens
- **XYZ** = Source Chamber (SC)
- **DP** = Diffusion pump
- **EO** = Extraction optics
- **RO** = Reflection optics
- **Gate Valve**
- **Valve**
- **Pulsed Valve**
- **Trans.**
Figure 3-2: Block diagram of the LT TOP mass spectrometer.
Chapter 3. $L^2$TOF Mass Spectrometry: Instrumentation

the RC for the purposes of cryopumping. The base chamber pressure, as measured by an Edwards CP25K Penning gauge, was below $10^{-7}$ mbar, whilst under typical operating conditions it rose to ca. $5 \times 10^{-7}$ mbar.

3.3 Laser Desorption Source

The desorption source situated in the SC, shown schematically in Figure 3.3, consisted of a molecular beam valve and a moveable sample probe. Desorption was effected by the use of infrared laser radiation propagating orthogonal to the direction of the molecular beam. In order to allow the infrared laser beam into the SC, a 50 mm diameter NaCl window was mounted on the side flange of the chamber. This window was held in place by PTFE clamps and sealed to the flanges using a viton O-ring. The material desorbed from the sample probe was entrained in a synchronised molecular beam pulse and transported through a skimmer to the IC.

The molecular beam valve was mounted on an XYZ translator. This was essential to enable alignment of the molecular beam with respect to the skimmer aperture, which defines the axis of the machine. A commercial pulsed molecular beam valve was employed, General Valve Corporation series 9. This valve consisted of an iron actuator, with a Teflon plunger attached, which was seated against a 1 mm orifice. Sealing was achieved through the action of a spring. The valve was opened by application of a current pulse to a single solonoid which pulled back the actuator. The pulse duration was determined by the length of time the current was applied to the actuator. Under normal experimental conditions the valve was operated with a pulse duration setting of between 450 and 650 µs and a stagnation pressure of between 3.5 and 4.5 bar.

Solid, low vapour pressure samples were introduced into the machine via stainless steel sample probes. Two different sample probes were routinely used in these experiments. The first was a cylindrical, solid stainless steel rod ca. 20 cm long and 6 mm in diameter. In this case, samples in solution were dropped onto
Rod rotated and translated by stepper motor.

Rod translated vertically by linear actuator.

Pulsed molecular beam to entrain desorbed material.

Cylindrical Rod
(Stainless steel)

Slotted, Square Rod
(Stainless steel)

Figure 3-3: Schematic of the desorption/entrainment assembly.

the horizontally rotating probe rod, the solvent being then allowed to evaporate leaving a solid residue on the surface of the probe. The probe was subsequently mounted beneath a stepper motor driven screw mechanism which was designed to permit continuous translation and rotation of the rod for the duration of the experiment. This ensured that a fresh area of sample was presented to each pulse of the desorption laser, extending the sample lifetime and thereby reducing the need for frequent sample replacement. A sample of this type could be expected to last for up to 15 minutes of constant use. The stepper motor was controlled using a Hytec 1604 stepper motor controller (SMC). This SMC supplied pulses to a chopped bipolar driver card (Mclennan TM162C) mounted in the rack unit to drive the sample probe stepper motor.

The second type of sample probe used was a square-faced stainless steel bar (6 mm x 6 mm square). This had a shallow slot ca. 1.5×40 mm² in the target face which contained the sample material. This probe proved to be more flexible, since samples could be deposited in solution as before, but the slot could also be
used to contain samples in various matrices. After sample deposition this probe was suspended below a stepping linear actuator which was controlled from a free standing variable speed driver unit, consisting of an RS unipolar 2A driver board and a type 320-24A RS power supply. This was generally operated on the slowest speed and with only half-steps in order to increase the lifetime of the samples. A sample prepared in this way could last up to 10 minutes.

The lower sections of both sample rods were contained by brass guiding channels attached to the molecular beam valve faceplate. This prevented the target probes moving out of the desorption laser focus during an experiment. The position of the rods relative to both the molecular beam axis and the nozzle orifice is an important experimental parameter. At short distances from the nozzle orifice there is a strong likelihood of setting up shock waves in the beam as the desorbed species try to enter the high density region of the molecular beam. This was found to reduce the signal intensity. A similar phenomenon has been observed by Li and Lubman [4]. Also, the distance from the probe to the molecular beam axis determines the ability to obtain good entrainment without disrupting the molecular beam. Typically, the distance between the orifice and point of desorption was ca. 4.5 mm and the distance between the molecular beam axis and point of desorption was ca. 5 mm.

In general, shot-to-shot stability was difficult to achieve for thin sample layers on stainless steel surfaces. Using thick samples, pressed in the slotted rod, an improved stability was obtained. However, large amounts of sample are required to perform experiments in this way. The mass spectral data reported in Chapters 5 to 8 most usually represents the accumulated waveform generated by summing data from between 200 and 500 laser shots.

The desorbed and entrained species pass through a skimmer before entering the ionisation chamber. This molecular beam skimmer (Beam Dynamics, 5 mm orifice diameter, 50 degrees included angle [5]) was mounted on the inner wall of the SC opposite the nozzle, at a distance of about 2.8 cm from the nozzle. The skimmer allowed a pressure differential of about 500 between the SC and the IC to be maintained under typical operating conditions. The skimmer apex was situated
approximately 9.5 cm upstream of the chamber wall closest to the IC in order to reduce any disruption of the molecular beam caused by skimmed gas reflected back from the wall [6].

3.4 Time-of-Flight Ion Optics

A schematic of the ion extraction optics used for the reflecting field geometry TOF instrument is shown in Figure 3.4. These optics followed the design originally described in 1955 by Wiley and McLaren [7] for a double-field spatially focusing TOFMS. These fields are defined by the repeller, draw-out and flight grid-plates. Also, a pair of deflection plates were included as a means of adjusting the ion trajectories. In the experiments described later only the horizontal deflection plates were used routinely.

The ion optics were home-built, and were fabricated from gold-plated aluminium. All the extraction optic plates were 1.8 mm thick and consisted of 8 cm
Figure 3-5: Schematic of reflectron TOFMS ion mirror. All dimensions in mm.

squares. The repeller plate had a 7.7 mm diameter hole cut in it to admit the molecular beam to the ion source region. This was not covered by mesh, which would have reduced transmission considerably. The draw-out and flight grids had 22.4 mm diameter apertures, covered in 90 percent transmitting mesh. The four deflectors were 30 mm square and 5 mm thick. The optics were supported with aluminium rods and precision machined Delrin spacers, with the deflection plates mounted on PTFE spacers.

A schematic of the reflecting field ion optics is shown in Figure 3.5. The ion mirror consisted of six stainless steel rings, 12 cm outer diameter and 6 cm internal diameter. The backplate was a solid stainless steel disk of the same outer diameter. The reflecting field was split into two distinct regions defined by the distances $d_t$ and $d_k$. These two fields, the retarding field and the reflecting field respectively, were supplied by two independent power supplies. The apertures of the first two rings were covered by grids (Buckbee Mears, 90% transmission), whilst the remainder acted as guard rings to improve the field homogeneity between the backplate and the mirror entrance. The first grounded ring was designed with a
Electrode | Power Supply
--- | ---
Repeller | Stanford PS 325
Draw out | Stanford PS 350
Horizontal deflectors | Stanford PS 350
Retarding field at ion mirror | Stanford PS 350
Reflecting field at ion mirror | Stanford PS 350

Table 3-1: Power supplies used for ion extraction and reflection electrodes.

The voltages were supplied to the various elements for the RETOF configuration described above using the power supplies listed in Table 3.1.

Typical voltages used when running the experiment are noted in Figures 3.4 and 3.5.

3.5 Ion Signal Detection

The TOFMS ion signal was detected by a dual microchannel plate [8] (MCP) and amplified before being fed into a transient digitiser. This MCP (R. M. Jordan) was of a dual chevron design, with two Galileo MCP-18B plates back to back, each having an active area of 2.5 cm². A divider chain was constructed to drop a maximum of 1000V across each plate, which had a gain of $10^3$ at 1kV and a gain of $10^2$ at a typical operating voltage of 700 V.

The detector was operated in grounded anode mode, thereby allowing easy coupling to the digitiser. This method requires that the front channel plate be maintained at a large negative voltage, but has the added advantage of increased gain for positive ions, which are accelerated into this plate. The disadvantage of
this is that negative ions cannot be detected, and an earth grid is required in front of the first plate in order to define the field-free drift region. In the design of the Jordan MCP, this was a grid with a transmission of 82%.

The ion signal at the MCP anode was amplified ten times (Ortec Model 134) before being fed into the the low level input of a transient digitiser.

3.6 Laser Systems

\(^2\text{L}\)\(^2\text{TOFMS}\) experiments require a minimum of two laser systems: one to desorb the sample molecules and a further laser to effect their photoionisation. Previous investigations have shown that infrared radiation at 10.6\(\mu\)m is very suitable for the desorption process, whilst UV wavelengths are required to produce multiphotonionisation. In this work, photoionisation relied principally on the production of three fixed UV wavelengths: 248 nm, 193 nm and 266 nm from the Krf and ArF lines of an excimer laser, and the fourth harmonic of a Nd:YAG laser, respectively. The lasers used to perform these experiments are described in more detail in the following sections.

3.6.1 Alltec 854MS CO\(_2\) Laser

The Alltec 854MS was a transverse excitation atmospheric (TEA) CO\(_2\) laser capable of generating ca. 100 ns pulses of 10.6 \(\mu\)m radiation at a maximum repetition rate of 50 Hz. A gas mixture of 12% CO\(_2\), 4% CO and a balance of He was automatically fed to the laser as required. On this fill, the laser was capable of producing a maximum output of 400 mJ. However, more typical pulse energies used were ca. 100 mJ or less at a repetition rate of 10 Hz. These energies were measured using a Coherent 210 power meter. Operational control of the laser was performed using a remote control module on an umbilical. External control over the operation of this laser required the provision of a 5V pulse of 50 \(\mu\)s duration.
The output from this laser was introduced into the SC via two gold plated mirrors and focussed using a 30 cm focal length NaCl lens mounted outside the chamber. Attenuation of the infrared beam was effected using a combination of nickel mesh grids and plastic attenuators. Taking into account of losses at the gold mirrors, the lens and window, a typical desorption laser power density is ca. $25 \times 10^6 \text{ Wcm}^{-2}$.

### 3.6.2 JK HyperYAG HY750 Nd$^{3+}$:YAG Laser

The HY750 was a pulsed Nd$^{3+}$:YAG laser used principally to generate 266 nm for ionisation, but also in some cases to generate 532 nm radiation for desorption purposes. The Q-switched fundamental output (1064 nm) of this oscillator-amplifier type laser was specified as 800 mJ per pulse at a repetition rate of 10 Hz. Second and third harmonics could be generated by the use of thermally-stabilised CDA and KDP crystals; typical pulse energies at these wavelengths were 320 and 170 mJ pulse$^{-1}$, respectively. Separation of the desired 532 nm wavelength radiation from the fundamental was achieved using two Brewster-angled gull wing prisms placed after the harmonic generating crystals.

The production of the fourth harmonic, at 266 nm, was achieved using an oven-housed KDP crystal to double the second harmonic 532 nm radiation. The fundamental and second harmonic were separated from the 266 nm output by a Pellin Broca prism assembly. Each prism in the assembly is arranged to turn the 266 nm radiation through 90 degrees but the unwanted beams are turned through smaller angles because of the wavelength dispersion of the prism. The optimised output for the fourth harmonic was 70 mJ pulse$^{-1}$.

The laser required two 15 V trigger pulses, to trigger both the flashlamps and the Q-switch. The Q-switched pulse energy could be varied by altering the time delay between the two pulses. However, usually the fine tuning of the pulse energy was achieved by reducing the amplifier charging voltage.

The laser output was introduced to the ionisation chamber via a series of broad band quartz prisms, each turning the beam through approximately 90 degrees.
Typically, an iris was used to reduce the beam diameter to ca. 2 mm. The pulse energy used in these experiments, measured with a Scientech 672 power meter, was ca. 2-3 mJ. A higher power was delivered by increasing the amplifier voltage and focussing the laser beam with a 30 cm quartz lens. This enabled power densities in the range $10^4$-$10^8$ W cm$^{-2}$ to be used. The laser spot size, when focussed was estimated to be ca. 300 μm [1].

3.6.3 Lumonics TE-861T-4 Excimer Laser

The TE-861T-4 excimer laser was used as an alternative photoionisation laser to the Nd$^{3+}$:YAG. This thyatron-switched laser was capable of operating on a variety of halogen-noble gas mixtures. For the experiments described here it was operated on either the ArF line (193 nm) or the KrF line (248 nm). Initially, experiments were carried out using MgF$_2$ stable resonator optics. The measured output at 193 nm was ca. 70 mJ pulse$^{-1}$ in a 8-10 ns pulse, whilst at 248 nm the output was typically 90 mJ pulse$^{-1}$ in a 12-16 ns pulse. For all experiments the laser was operated at a repetition rate of 10 Hz and the excimer power level was measured using a Coherent 210 average power meter.

The majority of experiments, however, were carried out using unstable resonator optics in the laser. These optics are used to decrease the output beam divergence. This is accompanied by a decrease in the total pulse energy. The reduction in beam divergence using these optics meant that tight focussing could be obtained for efficient multiphoton ionisation. In this configuration the output took the form of a well collimated beam of cross-section 3×15 mm$^2$ at 193 nm and 6×15 mm$^2$ at 248 nm. The maximum pulse energies measured under these conditions were ca. 16 mJ pulse$^{-1}$ at 193 nm and ca. 45 mJ pulse$^{-1}$ at 248 nm. The laser beam was further collimated using razor edged slits, of varying widths, before it entered the IC. The precise details of beam dimensions used in different experiments will be recorded where important in Chapters 5 to 8.

To externally operate the laser two 15 V trigger pulses were required. The first pulse triggered a "charge on demand" which initiated the capacitor charging
cycle, whilst the second, delayed by ca. 12 ms, triggered the thyratron. Varying the charging period or the peak charging voltage allowed the output pulse energies to be varied.

3.7 Experimental Control and Data Acquisition

The pulsed laser desorption laser photoionisation experiments required precise control over many experimental events and generated large amounts of data in a short period of time. A sophisticated computer-based system was developed to enable both optimisation of experimental parameters and acquisition and storage of data. A CAMAC based system was used in these experiments [9]. This involved using a range of hardware located in a crate which was controlled by software from a microcomputer. The computer also enabled data to be displayed as the experiment proceeded and subsequently digitally stored. The development of this integrated data acquisition and control software was carried out by a former research student in this laboratory [10].

3.7.1 Control Hardware

The IEEE CAMAC standard [9] defines a common dataway to which a number of instruments can be interfaced. CAMAC modules are slotted in a crate (Standard Engineering Corporation PCS 1410), with a backplane providing both data communication and power lines to each module. A common dataway of 24 read/write lines is used to transmit data to and from the units. Other data lines direct commands from the software on a Dell system 325 PC to the correct module or part of a module, and transmit acknowledgement of receipt of these commands or requests for attention to the microcomputer. The units used for controlling the experimental timings and acquiring data are shown in Figure 3.6.

Station one of the CAMAC crate was occupied by an IBM 1331/Turbo PC Interface module. This was connected to the PC by a 3 m long interconnection
Figure 3-3: Schematic of CAMAC based experimental control system.
cable and an IBM 1331 personality card. The module accepted commands from the computer to control the experiment, passing these on to the appropriate modules in the crate as well as passing recorded data down the dataway to the PC.

Experimental timing control was performed using two different pulse delay generators (PDG) which provided the trigger pulses to the experimental hardware. These were a Kinetics Systems 3655 8 channel PDG and a LeCroy 4222 4 channel PDG. The former could produce 200 ns FWHM TTL pulses with microsecond accuracy. This was triggered by an instruction through the dataway from the Dell PC, where the instructions were entered via the keyboard. The minimum possible interval between pulses from successive channels was 1 \( \mu \)s with a minimum jitter of around 1 ns. This unit was used to provide trigger pulses for the pulsed molecular beam valve, the CO\(_2\) laser, the excimer laser "charge on demand", and the LeCroy 4222.

The Le Croy 4222 PDG could generate four 100 ns TTL pulses of 1 ns accuracy. Each channel of the 4222 was independent, and could be used in any order or simultaneously. The jitter between pulses was less than 170 ps. Therefore, this module was used to trigger devices requiring precise timing control such as the time at which the ionisation laser fire and the transient digitiser were triggered.

The pulses from both PDGs did not produce enough current to drive 50 ohm loads over the long coaxial cables required in this experiment. The signals were also too narrow to trigger some of the external devices which had trigger thresholds of greater than 10 V, or required pulses of greater than 200 ns FWHM. The outputs from the PDGs had therefore to be boosted using a custom-built 8 channel line driver unit, housed in a NIM bin, which produced either 5 V, 50 \( \mu \)s or 15 V, 10 \( \mu \)s pulses [3].

### 3.7.2 Transient Digitiser

The arrival of ions at the microchannel plate detector following amplification generated a series of negative-going waveforms. Digitisation of this signal was achieved using a fast sampling Joerger TR200 transient digitiser (TD). This unit featured
Chapter 3. L²TOF Mass Spectrometry: Instrumentation

8 bit resolution, a range of 512 mV and a maximum sampling rate of 200 MHz. The module was completely programmable and the digitising rate was set using the programming software on the PC. Input offset adjustment was set using a front panel trimpot and could be monitored using an offset test point. In order to monitor the data being converted during a run a high speed digital to analog converter was provided on the front panel. This took the output of the data being latched from the ADC and generated an output signal that tracked the input being supplied.

After the TD was armed by a CAMAC dataway command, the input signal was continuously sampled and stored. This data was constantly overwritten, until the module received an external stop trigger pulse, when the module performed the required number of post-trigger samples. In these experiments, the following 2048 bytes constituted the mass spectral data.

As the maximum record length was 2Kb, only ca. 10 μs portions of the mass spectra could be viewed using the highest resolution sampling rate of 200 MHz. Flight-times could be of the order of 100 μs for many of the molecules examined. Therefore, in order to record a complete mass spectrum for each laser shot a slower sampling rate (25 or 50 MHz) was used. This resulted in a loss of spectral resolution. Under these conditions it was also possible that the digitiser could sample ion signals only on the wings, missing the peak of the signal, and thus the ion signals may also have appeared weaker than their true intensity. In order to utilise the higher sampling rates, the effective mass window for the TD could be moved in time by increasing the delay between the trigger pulse to the ionising laser and the TD stop trigger. This, however, meant mass spectra were recorded which did not encompass the complete mass range.

3.7.3 Control Software

The software was written by A. M. Butler [10], using the C high level programming language together with some assembler. This package controlled experimental timings and acquired data via the CAMAC interface.
A constant repetition rate was used in these experiments in order to prevent thermal lensing of the Nd<sup>3+</sup>:YAG rods, which detunes the laser. This was accomplished by employing the interrupt mechanism of the PC. This ensures that changes made to experimental parameters were executed during the dead time between shots. The interrupts were operated at twice the experimental repetition rates. On alternative interrupts the software toggled between routines for controlling the experiment and processing the data, known as TIC and TOC, respectively. The TIC routine armed the digitiser, loaded the time delays and triggered the KS 3655 initialising the experimental cycle. A typical timing sequence is shown in Figure 3.7. The TOC function was executed after a TIC to read data from the transient digitiser. This data was then inverted to a positive-going waveform and summed with that from previous shots.

### 3.6 Data Acquisition Modes

There were three basic modes in which the experiment could be run. The simplest operational procedure was that used to record TOF mass spectra. These spectra were displayed on the PC monitor as soon as the data was obtained. Successive single shot spectra could be accumulated before the screen was refreshed, or alternatively, the mass spectra could be displayed individually as they were summed. Typically, the mass spectra were summed until a satisfactory signal-to-noise ratio was obtained. The display in this mode showed autoscaled intensities (normalised to the most intense peak) on the vertical axis and a time labelled horizontal axis. The spectra could be re-calibrated to show a horizontal scale in mass units.

Acquisition of TOF mass spectra required the optimisation of several timing delays. Some of these, such as the time delay between triggering and firing the lasers were accurately known. However, the delay between firing the desorption laser and firing the ionisation laser could only be estimated crudely, similarly the delay between the trigger pulses to the pulsed molecular beam valve and the desorption laser. These delays could only be determined accurately by experiment.
Using Nd:YAG Laser.

Using ArF Excimer Laser.

Figure 3-7: Typical trigger pulse timing set-up for L²TOFMS experiments. All times in microseconds.
In the timescan mode it was possible to scan one trigger pulse in time relative to the others in the timing sequence whilst monitoring the signal in a particular mass channel. The time channels for a particular mass were established by running a non-optimised mass spectrum – these signals were obtained by loading new delay values into the PDGs until a signal was found. Up to ten different mass peaks could be monitored with the time scan facility while a particular time delay was scanned. The display showed the changing ion signal as a function of time delay. Two examples are shown in Figure 3.8 and 3.9. In Figure 3.8 the distribution of desorbed material as it arrives in the ionisation region is displayed by simply scanning the time at which the ionisation laser fires relative to the fixed desorption laser time, shown here for the laser desorption of carbazole from a stainless steel probe. The peak of this distribution represents the optimum timing for the ionising laser, i.e. the point at which the maximum photoion signal could be obtained. In the case of weak or noisy signals, it is possible to scan over the defined delay range repetitively to build up an appreciable signal intensity. Figure 3.9 shows a timescan spectrum obtained for aniline. This was seeded into the He molecular beam from a stainless steel reservoir. In this case no desorbing laser was used. The profile shown represents the accumulation of four consecutive scans. A time delay of 60 μs was used prior to initiating the scan. The actual onset, or time-of-arrival for the pulsed beam, is therefore the UV delay time given on the x-axis plus this delay time of 60 μs. The time-of-arrival measured in this way is a measure of the time taken for the desorbed material to reach the ionisation region. This particular mode of operation was used both to optimise the experimental parameters, without the complication of laser desorption, and to determine the temporal profile of the molecular beam.

The third data acquisition mode available was a frequency scan. This operated on a similar principle to the time scan facility. Firstly the time channels for a mass peak of interest were obtained and loaded into the signal definition register. Details regarding the use of a dye laser could be entered into the frequency scanning routine and a number of single shot spectra accumulated at each designated frequency. This mode of operation was used to record the [2+1] resonance enhanced
Figure 3–8: Desorption profile of carbazole obtained using timescan mode.

Figure 3–9: Temporal profile of the aniline seeded He molecular beam obtained using timescan mode.
Figure 3.10: L$_2$TOF [2+1] resonant photoionisation spectrum of the atomic lead $6p7p^3P_0 \rightarrow 6p^2^3P_0$ transition.

multiphoton ionisation spectra for the atomic resonance of lead shown in Figure 3.10 [11].

An EMG 201MSC, thyratron-switched excimer laser pumping a FL3002E dye laser [3] was used to perform this experiment. When the scan was started, the laser grating was moved under computer control while the changing mass spectrum or frequency scan was displayed on the PC screen. The scan shown if Figure 3.10 covered the range from 450.32 nm to 450.27 nm in increments of $5 \times 10^{-4}$ nm. Radiation over these wavelengths was generated using coumarin 47 laser dye (Exciton). This frequency facility scan was used rarely in this work due to the shot-to-shot instability in the desorption yield. This instability could be observed most clearly when operating under frequency scan conditions but using a fixed ionising frequency. Such a procedure maps the signal yield on desorbing from different locations on the sample probe. An example of this is shown in Figure 3.11, which clearly show the difficulties in obtaining shot-to-shot stability when a sample of perylene is doped onto the sample probe from solution. It is clear that
Figure 3-11: Fixed frequency scan of perylene demonstrating positionisation signal instability when sample is deposited onto the square faced sample probe slot from solution. Accumulation of sample material at both ends of the slot is clearly apparent.

during evaporation of the solvent the solute sample material accumulates at the opposing ends of the slot. The positionisation signal obtained in the central region of the slot is sporadic and subject to significant fluctuations.

A better method of sample preparation, for the purposes of obtaining a more stable signal is to mix the sample of interest into a tacky paste with glycerol and then press this firmly into the slot as a thick layer. This sample can then be subjected to repeated passes of the desorption laser over the sample and provides much greater desorption stability as reflected in the positionisation signals. This is shown for three consecutive passes over a carbazole sample in Figure 3.12. The third pass generates much lower intensity signals. It is possible that on this last pass the loose material present on the surface has been removed and the remaining material is more tightly bound to the bulk.
Figure 3-12: Fixed frequency scan of carbazole, demonstrating postionisation signal stability on consecutive passes of the sample. The sample was prepared as a tacky paste in glycerol and pressed firmly into the square face slotted sample probe.
Bibliography


Chapter 4

A Survey into the Analytical Applications of L²TOFMS

4.1 Introduction

In the previous chapter the practical features of the L²TOFMS technique as used in Edinburgh were discussed at length. In this chapter a survey of the studies carried out using L²TOFMS since its development in 1986 is given in order to provide an overview of the application of this technique and to set these studies in context with the work to be presented later.

The development of L²TOFMS as a mass spectrometric and spectroscopic technique is principally the work of three other research chemists, Schlag, Lubman and Zare. Two variations on the same basic methodology have been employed. The essential difference between these approaches concerns whether a pulsed molecular beam is used (as in the apparatus described in Chapter 3) to entrain and transport the laser desorbed material into the ionisation region, or whether, alternatively, no molecular beam entrainment is employed. Each methodology has its own advantages and disadvantages. The work of Schlag and Lubman has generally employed molecular beam entrainment whilst in that of Zare no mechanism of beam entrainment is used.

In 1985, Schlag's group [1] first demonstrated the ability of the two-stage laser desorption laser photoionisation technique to produce soft ionisation mass spectra of small biological molecules. This was followed by their first of many studies of biomolecules in the gas phase. Employing a combination of pulsed infrared
laser desorption, supersonic molecular beam cooling and ionisation with a tunable frequency-doubled dye laser, they recorded the mass spectra of native chlorophylls in a reflectron TOF mass spectrometer [2]. These ground-breaking papers were followed by a series of further investigations into the gas-phase mass spectrometry of a variety of biomolecules [3,4,5,6,7]. Around this time, Lubman and coworkers developed a similar instrument and published the mass spectra of several polynuclear aromatic hydrocarbons using pulsed CO$_2$ laser desorption followed by 266 nm laser photoionisation, using a linear TOF mass spectrometer [8]. In their paper they also reported the jet-cooled mass spectra of several small biological molecules. Lubman, like Schiag, went on to investigate the application of this two-stage technique to a wide range of biologically interesting molecules. Along with producing wavelength-selective soft ionisation mass spectra, these groups have demonstrated the use of higher ionisation laser fluences, generating hard ionisation mass spectra with structurally significant fragmentation patterns.

An important goal for many workers in this particular field of research has been the development of a truly two-dimensional analytical methodology. Mass spectrometry has, therefore, been combined with jet-cooled vibronic spectroscopy to enable highly selective detection of target molecules. Levy and coworkers pioneered the recording of vibronic spectra of labile organic molecules using R2PI [9, 10]. They, along with Schlag and Lubman, adopted the technique of laser desorption for the vaporisation of organic molecules. However, a disc source/entrainment faceplate system was used to obtain efficient cooling in the supersonic molecular beam. Using such an approach Lubman et al. [11,12] have obtained analytical R2PI spectra for a variety of biological systems and have used the gas-phase absorption characteristics of the sample to discriminate between isomeric molecules or components in complex mixtures.

In 1987, Zare’s group reported the development of a similar two-stage laser desorption/ionisation instrument. However, in their instrument they dispensed with the need for molecular beam entrainment by simply ionising the desorbed material directly above the surface of the substrate [13]. Their initial investigations were also directed towards biomolecules. However, in this case the aim was
to demonstrate the ability of the technique to sensitively and quantitatively determine the mass spectra of large organic systems. Research into the applications of the technique has, therefore, developed in two parallel directions. Molecular beam entrainment is an inefficient process resulting in a loss of detection sensitivity. However, the desorption of sample molecules into the throat of a supersonic expansion can translationally, rotationally and, to a lesser extent vibrationally, cool the gas phase molecules. This cooling enables photoionisation spectra of organic species to be recorded and the information from such spectra to be subsequently used for wavelength selective REMPI mass spectrometry. Thus, a requirement for high sensitivity and more accurate quantitation means dispensing with the entrainment, whilst a desire for highly wavelength selective mass spectrometry means that entrainment/jet-cooling in a molecular beam must be retained and improved.

An important differentiation must be made here between the technique of L^2TOFMS and that of matrix assisted laser desorption ionisation (MALDI). As mentioned in Chapter 2, MALDI relies on the use of a UV absorbing matrix in order to aid desorption and facilitate ionisation. In the two-stage technique - the subject of this thesis - a matrix is not a critical component as regards the ionisation process. However, in some experiments described later, matrices are used in order to enhance desorption efficiencies or to modify the desorption conditions. By modifying desorption conditions in this manner it is possible to investigate the thermal processes which may be occurring during the desorption process. Generally, however, no matrices are used in the sample preparation. The presence of matrices in sample preparation will be indicated where appropriate. It is also important to note that, excepting the imaging experiments described in Section 4.7, the samples are not moved during the execution of an experiment. This means that spectra are generated as a consequence of repeated desorption laser shots impinging on the same area of sample and substrate. A variety of different substrates have been used as sample probes. Briefly, Schlag's group have generally used a stainless steel sample probe and Lubman's group a Macor ceramic rod. Zare's group have used two different approaches, either depositing the sample on the surface of a glass
cup or on a Macor ceramic platter. No detailed study has been performed, to date, in order to determine which of the various substrates provides the optimum conditions for IR laser desorption.

The following chapter describes further the various areas of research in which L²TOFMS has been used to date. These are divided into various sections based on the broad molecular type of the particular target analytes. This review, along with the experimental work described later, demonstrates the potential utility of L²TOFMS and permits comparison, in later chapters, with more established mass spectrometric techniques.

4.2 Small Organic and Biological Analytes

Small organic and biologically important analytes have been a rich source of studies using L²TOFMS. The first study of biomolecules was carried out by Schlag's group [1], as mentioned previously. They followed this with an extensive series of publications dedicated to the L²TOF mass spectrometry of biomolecules [2,3,4,5,6,14,15,16,17,18]. The samples examined have included native chlorophylls, porphyrins [3], and phenylthiohydantoin (PTH) derivatised amino acids [5] which are the final derivatives of the Edman degradation method for sequencing proteins and peptides [19]. Grotemeyer et al. [5] recorded the mass spectra of all the derivatised amino acids, under both soft and hard ionising conditions, using 266 nm laser ionisation. Using this two-photon excitation scheme, it was found that the PTH-leucine and PTH-isoleucine isomers could be distinguished by their photofragmentation patterns. The L²TOF mass spectra of PTH amino acids were also reported elsewhere in the literature [13,20]; Zare's group used the soft ionisation mass spectra of PTH-serine and PTH-threonine to demonstrate that even delicate molecules are desorbed with relatively low internal temperatures. These two molecules were desorbed intact even though they are known to lose water on heating to above 400K.
In the case of the free amino acids, it was found that by tuning the ionisation lasers to the optimum wavelength, not only is the signal-to-noise ratio improved, but the non-aromatic amino acids, histidine and arginine, could be investigated [5]. Arginine is of particular interest as it is extremely polar and is one of the strongest organic acids. Its mass spectrum was measured via a non-resonant ionisation scheme using 250 nm excitation. Under soft-ionisation conditions, the mass spectrum exhibited a strong molecular ion peak at 174 amu, with the only other peaks consisting of one at 157 amu (the base peak), due to loss of NH$_3$ from the molecular ion, and another at mass 116 amu. This was the first observation of the arginine molecular ion by any mass spectroscopic technique. In preliminary experiments following the construction of the instrument developed in Edinburgh, the photoionisation mass spectra for a number of laser desorbed polycyclic aromatic hydrocarbons and free aromatic amino acids were obtained under similar soft ionisation conditions [21].

Tembreull and Lubman [22] have recorded the mass spectra of several classes of important biological molecules. They used a CO$_2$ laser to desorb from a Macor ceramic support. Laser photoionisation was performed using a variety of fixed frequencies – 280, 266, 245 and 222 nm. Clinically important catecholamines and their metabolites, which are neurotransmitters, are extremely labile. However, soft ionisation mass spectra were recorded for these molecules at either 266 nm or 280 nm. The L$^2$TOF mass spectra of indoleamines, tricyclic phenothiazine based neuroleptic drugs and purine bases were also recorded with minimal fragmentation. Similarly, the mass spectra of several water soluble vitamins were recorded. Thiamine is of specific interest as it is a chloride salt, unlike the other compounds investigated. It is thought to be desorbed as a neutral salt; this is then dissociated to the ionic form which subsequently undergoes fragmentation. Following this work Tembreull and Lubman obtained R2PI electronic absorption spectra for a number of small molecules [11], which were incompletely cooled by the jet entrainment procedure. The effect of peripheral substituents on the position of the origin of $\pi - \pi^*$ transitions were investigated for catecholamines, indoles and amino acid
derivatives. The shift in the position of the origin band was seen to depend on the electron-withdrawing or electron-releasing properties of the substituents.

Li and Lubman [12] subsequently made simple modifications to their apparatus in order to obtain enhanced cooling of mixtures of several laser desorbed indole and catechol derivatives: biogenic indoles serve as important metabolites for biological processes in the human body. The increase in vibrational cooling obtained was seen to increase the degree of spectroscopic discrimination available. Despite structural similarities between the compounds studied, R2PI spectroscopy was shown to be a sensitive probe of subtle differences in molecular structure. An example used to test the limitations of the technique was a mixture of tryptamine (mass 160) and tryptophol (mass 161). Although further limited by the $^{13}$C fingerprint of tryptamine, an optical discrimination of 1:100 of tryptamine in tryptophol was obtained by tuning the ionisation laser wavelength to $\lambda = 286.3$ nm, the strongest resonance in the tryptamine spectrum. A discrimination factor of 1:105 was obtained for tryptamine in tyramine at this wavelength. If, however, the origin band of the molecule of interest is at a shorter wavelength than the origin bands for the other components present in the mixture, the optical discrimination is generally lower. This is due to the background absorption from these other components into higher lying electronic transitions above their $S_1-S_0$ origin bands.

Saccharose has also been examined using L$_2$TOFMS [7]; this is a classical example of a compound which does not give molecular ions upon electron impact ionisation. However, using non resonant MPI, at $\lambda = 252$ nm, the molecular ion of saccharose is clearly detectable. More recently, Dey et al. [23] have investigated molecular beams of polyenes using CO$_2$ laser desorption and resonantly-enhanced ionisation at 250 nm. Polyenes are pigments that are found in many biochemical systems, including those involved in vision and photosynthesis. Retinal and $\beta$-carotene were examined in a polyethylene and platinum matrix; in each case strong molecular ion signals were observed under soft ionisation conditions.

L$_2$TOFMS has also been used to obtain the mass spectra of indoleamines, PAHs, catecholamines, peptides and pharmaceuticals by desorbing the target species directly from silica gel TLC plates [24]. No signal is generated which cor-
responded to the silica gel matrix. More recently, Rogers *et al.* [25] have reported the L<sup>2</sup>TOFMS of caffeine and theophylline obtained by infra-red laser desorption directly from TLC plates. This group have also published the mass spectra of a number of indole derivatives and larger indole containing alkaloids [26]. A further publication outlines some “anomalous” features of the laser desorption laser ionisation mass spectrum of 3-indoleacrylic acid [27]. These findings are explained as being the result of a gas-phase Diels-Alder [4+2] cycloaddition. A wide range of similar molecules have recently been investigated by Langridge-Smith *et al.* [28].

Finally, a novel method of presenting organic samples for laser desorption, followed by laser postionisation, has been introduced by Becker *et al.* [29]. This involves CO<sub>2</sub> laser desorption of organic compounds from frozen aqueous solutions. The concept behind these experiments is that rapid matrix vaporisation results in the entrainment of solute molecules in the solvent plume. The internal temperature of molecules volatilised in this process is low enough, because of collisional cooling, to ensure that even extremely labile molecules will remain intact on desorption. This was first demonstrated for a tripeptide in a frozen aqueous solution. More recently this approach has been used for the determination of phenol contaminants in drinking water [30].

### 4.3 Peptide Analysis

As a result of the desire of mass spectrometrists to perform gas-phase sequencing of peptides, thereby augmenting or by-passing the time-consuming Edman degradation procedures, a large number of early papers were dedicated to this topic. The analysis of PTH derivatised amino acids has been discussed in the previous section. In 1986, Schlag’s group first reported the MPI mass spectra of a derivatised tripeptide [3] under soft, hard and partially hard ionising conditions. Fragmentation was observed at both the N- and C- termini; electron impact gives fragmentation only at the C terminus, whilst for complete sequence elucidation degradation at both ends is essential. This was soon followed by the MPI mass
spectrum of an unprotected decapeptide, angiotensin I [4]. By pumping a \( \pi-\pi^* \) transition, via an absorption of a tyrosine residue at \( \lambda = 271.3 \) nm, a soft ionisation mass spectrum was obtained showing exclusively the angiotensin I molecular ion. By increasing the ionisation laser power density, a number of fragmentation products were obtained, which allowed deduction of the full peptide sequence; again fragmentation occurs from both the C-terminus and the N-terminus.

Tembreull and Lubman reported the study of several tryptophan, tyrosine and phenylalanine based di- and tri- peptides, using infra-red laser desorption into a supersonic beam of CO\(_2\) with subsequent ionisation using either 266 nm or 280 nm radiation [31]. In most cases, mass spectra were obtained exhibiting a strong molecular ion signal or characteristic fragment ions such as (M-OH)+ or (M-COOH)+. However, no molecular ion was seen at all for many phenylalanine containing peptides. Similar behaviour was later noted with respect to several pentapeptides [20]. Leucine and methionine enkephalin were examined using 266 nm excitation to promote R2PI through the \( \pi-\pi^* \) transition. At the fluences used, extensive fragmentation was observed along with a substantial molecular ion peak.

The lack of observable molecular ion for some phenylalanine containing peptides prompted Walter et al. [14] to reinvestigate these samples. Using a tunable, rather than a fixed frequency ionisation laser, and by manually tuning to optimal wavelengths for different peptides they observed strong molecular ions with minimal loss of hydroxyl or water. The optimum wavelength for phenylalanine-containing species was previously found to be near the \( S_1-S_0 \) origin band transition for toluene, at 272.7 nm [6]. The exact value was found to depend on the other amino acid substituents in the peptide. Electron withdrawing groups, e.g. glycine and lysine shift the optimal wavelength to the blue, and electron releasing groups, e.g. a second aromatic tryptophan, shift the optimal wavelength to the red [10, 32]. As expected, the optimum wavelengths for tyrosine and tryptophan containing peptides were red-shifted relative to 270 nm. It was also demonstrated that by increasing the ionising laser fluence, reverse dipeptides such as phenylalanine-leucine and leucine-phenylalanine can be distinguished by the different features in their mass spectrum.
Li and Lubman [32] have used n-carbobenzoxy(CBZ)-derivatisation to provide an absorption centre at 266 nm for a number of small peptides. Under medium hard ionisation conditions, they were able to utilise small, but significant differences in the laser-induced fragmentation patterns to distinguish isomeric peptides containing leucine and isoleucine residues. They followed this work with an R2PI spectroscopic study of several tyrosine dipeptides [33]. Although no sharp features were observed, with spectral contours being ca. 1.5 nm FWHM, the shapes of the contours were distinctive, allowing discrimination between isomeric “reverse” peptides. The lack of spectral structure appeared to be due to the large number of possible conformers for the dipeptide, which are not resolved under these experimental conditions. The dipeptides with tyrosine on the C-terminal end and those with the tyrosine on the N-terminal gave different spectra. At the peak absorption wavelengths, the L²TOF mass spectra showed exclusive molecular ion production, but at much lower power densities than had been required for non-resonant ionisation at 266 nm or 280 nm.

Grotemeyer and Schlag [15], meanwhile, showed that using L²TOFMS, it was possible to make mass spectrometric measurements on mixtures containing hydrophilic and hydrophobic peptides. This demonstrated that the yield of neutral molecules by IR laser desorption does not depend on the hydrophilicity of the specific samples. Further investigations have been carried out regarding the nature of thermal decomposition processes operative during the desorption stage. The presence of the (M-17)⁺ and (M-18)⁺ fragment ions, with varying degrees of intensity, in many peptide L²TOF mass spectra was noted [16,34]. Changing the matrix for desorption was found to modify the rate of thermal surface reactions, and therefore could be used to determine whether the signals are from desorption or MPI fragmentation. The mass spectrum of leucine-tryptophan at λ = 286.5 nm gave a ratio of M⁺:(M-18)⁺ of ca. 2:1. Addition to the sample of 1 mg of Na₃PO₄ per 1 mg of peptide altered this ratio to 1:1.2. On the other hand, addition of 1 mg of glucose per mg of peptide almost completely suppresses the (M-18)⁺ ion. The presence of sugars is thought to keep the surface temperature low both during
and after irradiation. Also, pyrolysis of sugars, which is endothermic leads to an excess of water, which helps drive peptide dehydration in the other direction.

These investigations were followed up by Li and Lubman [35] in order to examine the decomposition products that are produced in the laser desorption of oligopeptides. In order to understand these mass spectra it is crucial to know under what conditions thermal decomposition occurs and what products are formed. Li and Lubman showed that one of the thermal decomposition products is a 2,5-diketopiperazine which is formed by the cyclisation of the amino acids in linear oligopeptides. Furthermore, it was shown that the formation of this product can be controlled by varying the sample thickness.

Beavis et al. [34,36] have also investigated the effects of a number of organic and inorganic matrices on the L²TOFMS yield of peptides. Briefly, inorganic matrices which are infrared absorbers reduce the photoion yield from dipeptides by decreasing the desorption efficiency. Those which are infrared transparent helped to increase the yield, although dipeptides with polar sidechains did not show an improved M⁺ yield, and organic semi-transparent sugars suppressed pyrolysis. In general, it appears that the more transparent the matrix, the better the yield of neutral molecules. Recently Li's, group [37] have confirmed the previous assertions [35] that the (M-18)⁺ ion is generated via thermal decomposition to a cyclodipeptide with elimination of water during laser desorption. Also, they demonstrated that dipeptides having an N-terminal aromatic group generate (M-16)⁺ and (M-17)⁺ as well. These, however, were shown to be formed due to fragmentation during MPI, from the elimination of an amino radical and an ammonia neutral from the molecular ion.

Kinsel et al. [38] have investigated the mass spectra of peptides using ultraviolet desorbing radiation (λ = 266 nm), rather than the more established infrared radiation (λ = 10.6 μm). The high energy photons vaporised intact molecules. However, a variety of structurally significant neutral fragments were observed in their L²TOF mass spectra. It was postulated that these species were generated in the desorption process. Postionisation of these neutral fragments provided sufficient sequence-specific information to enable the amino acid sequence of the
peptide to be deduced. This work was followed by a more detailed study in an attempt to confirm that the neutral fragments are formed in the desorption process, rather than as a result of fragmentation of the molecular ion [39]. They concluded that it was experimentally difficult to distinguish between fragments formed in the desorption process or in the postionisation process, and suggested further experiments which would clarify this point.

In an effort to demonstrate the sequencing ability of L²TOFMS, a number of medium-large peptides, with a molecular weight of over 1000 amu, have been studied [7]. The complete sequence of substance P (molecular weight = 1346 amu) has been unambiguously deduced from its medium-hard ionisation mass spectrum. Similarly, angiotensin I, II and III have been successfully sequenced [15], along with gramicidin S (molecular weight = 1140 amu) [40]. The largest molecule to have been successfully studied with this technique is bovine insulin (molecular weight = 5729 amu) [6]. By tuning the ionisation laser to 272.7 nm to excite the $\pi-\pi^*$ transition of the phenylalanine or tyrosine residues in this molecule, a soft ionisation mass spectrum has been recorded containing only two other significant species due to breakage of the two disulphide bridges in the insulin molecular ion.

The most recent investigations concerning peptide analysis have involved a comparison between single-photon (photon energy = 10.5 eV) ionisation and multiphoton ionisation (MPI) [41]. Single-photon ionisation requires the use of vacuum ultraviolet radiation (VUV). The use of VUV means that the target molecules no longer require a UV chromophore. Di- and tri- peptides containing glycine, alanine, leucine and proline were examined. Single-photon ionisation (SPI) produced abundant molecular ions in all cases, along with significant amounts of fragmentation. It is also apparent that the non-specific SPI generates high intensity peaks corresponding to the carrier gas and background contaminants. SPI and MPI were compared for tryptophan containing peptides of up to four amino acid residues. The smaller peptides showed similar fragmentation patterns, but for larger peptides significant differences in the fragmentation patterns were observed.
4.4 Nucleosides and Nucleotides

Nucleosides pose a mass spectrometric challenge, as they are polar and thermally labile. Li and Lubman [42] used CO₂ laser desorption with 266 nm laser ionisation to produce mass spectra of a variety of nucleosides and free bases. Adenine, cytosine and guanine readily yield soft and hard ionisation mass spectra. However, no spectrum was observed for uracil at this ionising wavelength. This can be attributed to the high ionisation potential (IP = 9.5 eV) of uracil. Two photon absorption at 266 nm is not sufficient to exceed this ionisation threshold. Adenosine, guanosine and cytosine were also investigated, along with various modified nucleosides. All gave mass spectra with strong molecular ions and no cationization. In addition, a principal fragment due to the free base was observed. The molecular ion peak, along with the characteristic free base fragment, can be used to distinguish between ribose and deoxyribose sugars in the nucleoside. As before, uridine and many of its derivatives cannot be ionised at 266 nm. However, on addition of -NH₂, an electron releasing group, the ionisation potential is reduced and a mass spectrum can be obtained. These experiments also demonstrated the ability to distinguish, through fragmentation, isomeric methyl-substituted nucleosides.

Lindner et al. [17] have investigated both protected nucleosides and protected nucleotides. The wavelength used for ionisation was optimised for each molecule in the region around 250 nm in order to maximise the ionisation yield. They used 5'-OH-protected deoxynucleosides (where the adenine, guanine, cytosine and thymine exocyclic amine groups are protected), 5'-OH protected 3'-phosphate protected deoxynucleotides and 3'-phosphate protected deoxynucleotides. A large molecular ion signal was found to be common to all the nucleobase-containing compounds, with the most significant fragmentation occurring at the C₅-O-protecting group bond and the glycosidic bond between sugar and nucleobase. In addition, cleavage of the phosphate ester bond is found to play an important role in the fragmentation of nucleotides. These nucleosides absorb in the infrared region of the spectrum. Again, matrices were added to the sample during preparation in order to modify...
the desorption conditions. An infrared absorber, Na$_3$PO$_4$, was added which acts as a sink for the desorbing radiation. In this case, contrary to the behaviour of peptides, the desorption yield was observed to increase by the addition of this absorbing matrix.

The next development in this area was the analysis of dinucleotides by Lindner and Grotemeyer [18]. All sixteen combinations of isomeric protected dideoxyribonucleotides containing the phosphotriester linkage have been investigated. Molecular ions were seen at low ionising laser powers, whilst under hard ionising conditions, intense low mass fragments were observed which enable the nucleic base composition of the dideoxyribonucleotide to be established. Fragmentation was predominantly at the glycosidic bond between the sugar and nucleobase, and at the phosphate ester bond. The intensity of the 5' end fragments was observed to be larger than the 3' fragments, indicating the high absorption of the dimethoxytrityl (DMT) group, or the better charge stabilisation of the DMT group resulting in the 5' charge retention. It was further determined that by assigning the fragments it was possible to distinguish the isomeric dideoxyribonucleotides with L$^2$TOFMS.

4.5 Polymers and Additives

The use of L$^2$TOF mass spectrometry for the analysis of aromatic polymers was reported by Lustig and Lubman using CO$_2$ laser desorption followed by 266 nm laser photoionisation [43]. Three polystyrene samples were analysed, including two polymer blends, along with polycarbazole and polyamide. The polystyrene samples all showed the monomer molecular ion along with characteristic molecular ion fragments. This demonstrated the ability of the technique to selectively detect target aromatic polymers in aliphatic polymer blends. Similarly, the other samples showed predominantly the molecular ion of the monomer. A more complete study followed [44] of polystyrene (and blends), EPON epoxyresins and polyamides. Here, on examining a sample with an average molecular weight of 600 amu, a distribution of oligomers was observed along with a peak corresponding to the
anionic initiator and the end group butylstyrene. Similar mass spectra have been recorded elsewhere [45]. In Lubman’s publication [44], the authors assert that larger oligomers than \( n=1 \) are rarely observed with any significant intensity due to photo- or thermal decomposition of the bulk polymer during desorption. This conclusion is directly contradicted by recent work carried out in Edinburgh [46], where, using the same experimental methodology, distributions for polystyrene oligomers of up to mass 3800 amu have been observed.

The utility of \( \text{L}^2\text{TOFMS} \) for the analysis of polymer distributions above ca. mass 5000 amu remains in doubt, due to problems associated with the photoionisation of large organic molecules. This has recently become the subject of theoretical studies [47]. However, the ability to selectively detect aromatic species in complex aliphatic substrates is more certain. Recent work has been reported on the mass spectrometric analysis of rubber vulcanizates using \( \text{L}^2\text{TOFMS} \) without entrainment [48]. This included a comparison between the mass spectra obtained by postionising laser desorbed neutrals and analysis of ions produced directly by laser ablation. Raw rubbers are mixed with various additives which produce certain characteristics in the polymer. Using 308 nm radiation for desorption and 355 nm for ionisation, a few of the chosen sample additives were preferentially detected over the ubiquitous hydrocarbons in the rubber polymer. Upon changing the postionisation wavelength to 212 nm, the selectivity of analysis is diminished since radiation at this wavelength is absorbed by most of the additives and fragments characteristic of the bulk polymer. A variety of other postionisation laser wavelengths were used, and the spectra generated demonstrated that each one accentuates a different characteristic species in the vulcanizate.
4.6 Quantitation and Sensitivity Measurements

The technique of L<sup>2</sup>TOFMS has risen to prominence principally because it allows identification of complex molecules, rather than providing a method of measuring absolute or relative molecular concentrations. Similarly, until recently, little emphasis has been placed on investigating the ultimate sensitivity of the technique. These parameters are difficult to judge effectively, they are specific not only to individual instrument geometry, but also the experimental conditions. Sample preparation, desorption wavelength and ionisation wavelength will all have significant impact on the measured parameters. The absorption characteristics of the target molecules at the ionising laser wavelength has enormous impact on the detection efficiency, since each molecule will have a different ionisation cross-section at any specific wavelength.

Zare's group, having dispensed with molecular beam entrainment and transportation, report the highest detection sensitivities [13,49,50,51,52,53]. For example, a detection limit, at S/N = 2, of $4 \times 10^{-17}$ moles of protoporphyrin IX dimethylester was reported at 266 nm ionising wavelength [51]. Furthermore, the ion signal was demonstrated to be linear with surface coverage over five orders of magnitude, from nanomoles to sub-femtomoles. The amount desorbed was calculated on the basis of the amount of sample deposited on to the substrate, the new area exposed to each shot, and the assumption that all irradiated molecules were desorbed. Similarly, Zare's group have reported the detection limit of a prototype microanalytical L<sup>2</sup>TOFMS instrument to be ca. 8 femtomoles for the PAH coronene using 266 nm as the ionising wavelength. An investigation into the limits of detection obtained for a number of PAH molecules on the Edinburgh instrument is described in Section 5.3.

Zare's group have also examined the use of the technique for performing quantitative analysis of specific polynuclear aromatic hydrocarbons in meteoritic carbonaceous chondrites [50]. Quantitation was achieved by spiking the target samples with a series of standard additions, and then extrapolating a linear plot of
concentration versus intensity to zero intensity. This gives a value for the unspiked concentration of, in this specific case, phenanthrene in the meteorite.

Li and Lubman [12] have reported the detection limits for a number of indole derivatives, under soft ionisation conditions at the observed maxima in the jet-cooled R2PI spectra of these molecules. For example, a detection limit of ca. 6 femtomoles was obtained for indole-3-acetic acid, whilst they estimated that 2–3 nanomoles of sample was required to produce an optical spectrum over a 1.5 nm scan. Later studies [24] have shown that the same molecule can be desorbed quantifiably from TLC plates over four orders of magnitude, with detection limits (at 266 nm) in the low picomole range. An investigation of carbobenzoxy (CBZ)-oligopeptides [32] has shown that the apparent detection limit rises as the size of the peptide increases. The detection limit for CBZ-amino acids was typically 50-100 pg, whereas for CBZ-tripeptides a detection limit of 500 pg-1 ng was determined. As the peptide size increases further, the detection limit becomes higher still. The detection limit appears to be affected mainly by the laser-induced ionisation efficiency. The latter will be influenced by the absorption coefficient of the molecule, as mentioned earlier, and the competing radiationless processes. In nonrigid peptide chains there are many vibrational modes which can participate in rapid electronic-vibrational intramolecular radiationless relaxation, thus resulting in a decreased ionisation efficiency. This problem may yet be overcome by the use of picosecond or femtosecond laser excitation.

4.7 Microscopic Organic Analysis

Laser desorption laser multiphoton ionisation mass spectrometry has recently been developed as a microanalytical technique, where the aim has been the analysis of the organic constituents of particulates and imhomogeneous samples with high spatial resolution. Two groups, Zare's group at Stanford [53] and de Vries' group at IBM [54], are presently pioneering this area of research. The instruments differ primarily in the nature of the desorbing laser. The Stanford instrument can
achieve a spatial resolution of 40 μm using a pulsed CO₂ laser whilst in the IBM instrument a UV waveguide excimer laser is used to obtain a spatial resolution 1 μm. The former instrument has been used successfully for the analysis of organic constituents in meteorites whilst the latter instrument has been tested on well characterised samples adsorbed onto silicon chips. Most recently, Voumard et al. [55] have developed a similar instrument using CO₂ laser desorption with a spatial resolution on the order of tens of microns. This instrument is reported to have attomole detection sensitivity and has been applied to the spatial analysis of trace organic species and the detection of selected chemical compounds in complex mixtures. Specific examples given included rhodamine 6G deposited on a glass slide and the analysis of contaminants adsorbed on aerosol particulates. Techniques for the analysis of molecules on a microscopic scale are far less advanced at present than those available for elemental analysis. Future development of instruments which exploit this two-step laser desorption laser ionisation approach could find important application in the analysis of heterogeneous molecular samples such as plant and animal tissue, microfossils and integrated electronic circuits.

4.6 Concluding Remarks

It should be clear from the preceding discussion that laser desorption linked with laser postionisation is a powerful technique for mass spectrometric analysis of thermally labile molecules. The range of applications is steadily increasing to encompass microanalytical tasks, as well as an expanding range of molecular species. The advantages of MPI for selectively ionising molecules, along with providing unique structural information, has been proven. Similarly it has been demonstrated that molecules can be successfully desorbed from a variety of substrates. Thus, L²TOFMS can be seen to provide important capabilities for detection, identification, and structural analysis of molecules that cannot be readily studied by other mass spectrometric methods. However, experimental complexity, instrumentation costs and the limited range of molecules that can be probed using MPI have meant that the technique is far from being a standard laboratory analytical tool.
Although the efficacy of the technique has been demonstrated for a wide variety of different model systems, little work has been performed concerning the application of the technique to specific analytical problems. The following chapters describe work performed in Edinburgh on the home-built L²TOFMS instrument, as described in detail in Chapter 3. The research areas are divided into groupings of common molecular types. However, within these groups the advantages of the combined laser desorption laser photoionisation methodology will be highlighted with reference to particular application areas. The development of application areas also includes the study of 'real' systems, with discussion on the unique ability of this two-stage methodology to address such complex analytical problems.
Bibliography


Bibliography


[28] C. R. Redpath, A. C. Jones, P. R. R. Langridge-Smith, unpublished results


[45] J. Lindner, personal communication


Chapter 5

L^2 TOFMS of Polycyclic Aromatic Hydrocarbons (PAHs)

5.1 Introduction

Polycyclic aromatic compounds (PACs) are a complex class of condensed benzoid-ring compounds, the parent molecules of which are the polynuclear aromatic hydrocarbons (PAHs). Early studies of the parent PAHs led to the discovery of the first chemical carcinogen, dibenz[a,h]anthracene [1]. More recent studies have since led to the classification of many other PACs as potent animal carcinogens and/or mutagens [2,3]. Whilst there is currently no evidence that individual PACs cause cancer in humans, substantial evidence exists to suggest that synergistic effects among individual PACs render them hazardous constituents of complex hydrocarbon mixtures such as pitch, coal-tar, environmental tobacco smoke, oil shales and vehicle exhaust emissions [3,4,5,6]. PAHs are ubiquitous in the environment having widespread natural and anthropogenic sources [7]; human exposure to them, at some level, is therefore unavoidable. Until the beginning of the 20th century there existed a natural balance between the production and degradation of PAHs in the environment. However, increasing industrial development has meant that the natural balance has been disturbed and the accumulation rates of PAHs and PACs are constantly rising. In the light of this, PAHs have been classified as priority pollutants by the US Environmental Protection Agency (EPA).

Mass spectrometry has been used for the analysis of PACs since 1951 [8]. The hundreds of papers reporting the use of MS for qualitative and quantitative anal-
ysis of PACs, are reviewed elsewhere [5]. The extensive electron delocalisation of these molecules means that their electron impact (EI) mass spectra usually contain intense molecular ions and small fragment ions. Gas chromatography combined with mass spectrometry (GC-MS) is currently the most widespread analytical technique for the analysis of such compounds. Where this combination of techniques is unable to differentiate between isomeric species, chemical ionisation (CI) has been used in positive-ion and negative-ion modes. Recently, a variety of analytical techniques have been developed, in order to differentiate between PAC ring sizes and to identify specific PAH substituents and derivatives at trace levels of detection [9]. Novel approaches have included gas-chromatography linked to a variety of highly selective and sensitive instruments which utilise laser multiphoton excitation of the target analyte. These have included multiphoton ionisation Fourier-Transform mass spectrometry [10], multiphoton ionisation time-of-flight (TOF) mass spectrometry [11,12,13] and supercritical fluid injection mass spectrometry [14]. A multi-dimensional approach, involving the combination of several techniques - capillary column gas chromatography, resonance-enhanced multiphoton ionisation, time-of-flight mass spectrometry, laser induced fluorescence with flame ionisation detection - in a single instrument, has been employed by Dobson et al. [15] for the detection of a variety of PACs. However, all these techniques, along with EI and CI, require that the analyte species is present in the gas phase. The standard procedure is to feed the eluent from a gas chromatograph into the ion source. However, the analytical recovery of high molecular weight PACs using hyphenated GC techniques is often impeded by their low volatility and high affinity for the stationary phase. This problem has been addressed by the use of desorption techniques. Secondary ion mass spectrometry (SIMS) [16], has been used, with a variety of different substrates and matrices, yielding both molecular ions and adduct ion peaks. Fast atom bombardment [17] has been little used for the analysis of PAHs as they are not soluble in glycerol. Furthermore, FAB appears to work best for species which are reasonably polar. It can be useful if a functional group is added such that the compound becomes soluble in glycerol. Grigsby et al. [18] used FAB for the analysis of nitrogen containing compounds in fossil fuels. Laser desorption has also been used [19,20] for both positive and
negative ion analysis of PAHs. In positive-ion mode, the mass spectra resembled EI spectra, whilst in negative-ion mode mass peaks corresponding to the species \((M+H)^-\) and/or \((M-H)^-\) are prevalent. In negative-ion mode, however, most fragmentation products are \(C_n^-\) and \(C_nH^-\) clusters which contain little structural information. These desorption techniques enable the evaporation and ionisation of PAH analytes, but the ionisation process is nonselective and the mass spectral information from the analysis of complex mixtures is often obscured by matrix or fragmentation peaks.

The investigation of PAHs contained in environmental matrices poses further problems in terms of reliable chemical characterisation because of the occurrence of contamination in more than one medium, and the existence of a complex matrix of materials with a diverse range of physiochemical properties [21]. In order to determine contaminant PAHs in environmental matrices using the techniques mentioned above, extensive sample clean up operations are necessary. Extraction and separation procedures are both expensive and time consuming and, furthermore, can introduce problems with respect to solubility, storage, biotransformation and photodegradation. For the purpose of assessing compliance with environmental quality criteria, the initial screening of environmental samples requires a fast, selective fingerprinting technique which is able to circumvent the problems associated with complicated clean-up protocols. Considerable effort has been directed towards this goal. Novel techniques are presently under development which aim to enhance analyte extraction efficiencies, improve clean-up processes and enable the analysis of contaminants in situ. These include supercritical fluid extraction (SFE) [22], laser-excited fluorescence [23], thermal-desorption mass spectrometry (TE-MS) [24] and remote laser-induced fluorescence (RLIF) [25].

In this chapter, the application of \(L^2\)TOFMS to the analytical screening of contaminant or native PAHs directly from their environmental matrices is described. Firstly, the \(L^2\)TOF photoionisation mass spectra of a number of pure PAHs are presented along with a brief discussion concerning the effect of ionising laser parameters on the resulting mass spectra. This is followed by an appraisal of the instrumental limits of detection for these compounds using two different ionising
wavelengths. A description of some studies involving the use of L^2 TOFMS for the direct determination of PAHs from contaminated environmental matrices, specifically soils, lubricating oil, soot and coal-tar creosote, constitutes the latter half of the chapter. The advantages of this technique are then discussed along with a brief assessment of its future development as an environmental screening tool.

5.2 L^2 TOF Mass Spectra of Pure PAH Analytes

Characteristic L^2 TOF mass spectra obtained using 266 nm and 193 nm laser photoionisation for selected PAHs (coronene, perylene, pyrene, tetracene, pentacene) are shown in Figures 5.1 and 5.2 respectively. The choice of these wavelengths was determined by the fact that they were both readily available and both enable the efficient multiphoton ionisation of PAH molecules. The use of 266 nm radiation for the ionisation of PAHs has been reported previously [26,27,28]. High ionisation efficiencies at 266 nm and 193 nm can be attributed to two factors. It is known that in these regions of the spectrum, the PAHs often exhibit dense, almost continuous absorption. Furthermore, the ionisation potentials for this class of molecules decrease as the degree of conjugation increases due to resonance stabilisation of the molecular ion ground state. The vertical ionisation potentials of the PACs studied in this work are listed in Table 5.1, along with their skeletal molecular structures. These IP values indicate that, using 193 nm or 266 nm, the ionisation continuum is reached after the absorption of two photons. Thus, the ionisation mechanism involves a two-photon excitation scheme. All the spectra shown in Figures 5.1 and 5.2 have been normalised with respect to the molecular ion, and are plotted using relative intensity for the y-axis.

The purpose of these initial experiments was to demonstrate the ability of the L^2 TOFMS technique to generate simple, readily interpretable soft ionisation mass spectra for a variety of PAH compounds. To this end, the experimental parameters were adjusted during the examination of each PAH in order to obtain reasonably
Figure 5-1: L²TOF mass spectra of selected PAHs obtained using 266 nm laser photoionisation: a) coronene, b) perylene, c) pyrene, d) tetracene, e) pentacene.
<table>
<thead>
<tr>
<th>PAH</th>
<th>Mass (amu)</th>
<th>IP (eV)</th>
<th>Skeletal Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronene</td>
<td>300</td>
<td>7.36a</td>
<td><img src="image" alt="Coronene Structure" /></td>
</tr>
<tr>
<td>Perylene</td>
<td>252</td>
<td>7.00a</td>
<td><img src="image" alt="Perylene Structure" /></td>
</tr>
<tr>
<td>Pyrene</td>
<td>202</td>
<td>7.42b</td>
<td><img src="image" alt="Pyrene Structure" /></td>
</tr>
<tr>
<td>Tetracene</td>
<td>228</td>
<td>6.97c</td>
<td><img src="image" alt="Tetracene Structure" /></td>
</tr>
<tr>
<td>Pentacene</td>
<td>278</td>
<td>6.74a</td>
<td><img src="image" alt="Pentacene Structure" /></td>
</tr>
</tbody>
</table>

Table 5-1: Molecular weights, IP's, and skeletal structures of selected PAHs. (IP data: a - [29], b - [30])
resolved soft-ionisation mass spectra. The optimal ionising laser power density was different at each ionising wavelength. When using 193 nm laser photoionisation, the optimum power densities were in the region 0.1-1 MW cm\(^{-2}\) whilst at 266 nm the power densities were in the range 2-3 MW cm\(^{-2}\). The CO\(_2\) desorption laser was maintained at a constant laser power density of 22.5 MW cm\(^{-2}\) for the desorption of all the PAH compounds examined.

Sample preparation for each of the PAHs investigated involved depositing them from a dichloromethane solution into the slot of a square-faced sample rod. The sample probe was translated orthogonally to the desorption laser and the pulsed molecular beam, at a rate of 6.6\( \times \)10\(^{-3}\) mm s\(^{-1}\), exposing a fresh area of sample to each laser shot. The heterogeneity of the sample layer after solvent evaporation resulted in a poor shot-to-shot reproducibility in the photoion yield. The molecular ion intensity was observed to fluctuate by up to an order of magnitude between successive experimental cycles. In order to obtain reasonably reproducible ion intensities the data from twenty successive laser shots were accumulated when ionising at 193 nm, whilst two hundred shots were accumulated when ionising at 266 nm. Thus, approximately 5 nmoles of sample were used to generate the 193 nm mass spectra of coronene, perylene and pyrene, whilst between 50 and 80 nmoles of sample were required to generate the 266 nm mass spectra. At both photoionisation wavelengths a larger sample size was required to obtain the spectra of tetracene and pentacene shown in Figures 5.1 and 5.2. Approximately 30 nmoles were required for the 193 nm spectra compared to several hundred nmoles for the 266 nm mass spectra of these PAHs. The amount of sample used was estimated from the rate of rod translation, the laser spot size and the amount of sample on the probe. This calculation relied on two important assumptions. Firstly, that the covering of material in the sample slot was homogeneous, and secondly that all the target material was desorbed and removed in a single pass of the rod. The second assumption was demonstrably more reliable than the first, since a second pass of the desorption laser over the sample probe yielded no further ion signal.

The resolution (FWHM) of the instrument (m/\( \delta \)m) was typically ca. 500 at 193 nm and ca. 300 at 266 nm. The disparity between these values reflects the
Chapter 5. L²TOFMS of Polycyclic Aromatic Hydrocarbons (PAHs)

Figure 5-2: L²TOF mass spectra of selected PAHs obtained using 193 nm laser photoionisation. a) coronene, b) perylene, c) pyrene, d) tetracene, e) pentacene.
Figure 5-3: L²TOF mass spectrum of perylene obtained using 193 nm laser photoionisation. Resolution (m/Δm) = 1350.

different incident laser beam dimensions. At 193 nm, a 1.3 mm wide collimating slit was employed to define the laser beam geometry, whilst at 266 nm a collimating telescope was used to form a circular beam, 4 mm in diameter. The use of the larger incident laser beam width is accompanied by a loss in spatial resolution (see Section 2.4). In addition, the higher fluences used at 266 nm may induce increased space-charge effects, which further degrade the resolution of the spectrometer. The values for the resolution stated above do not represent the maximum resolution of the instrument. On optimising the experimental conditions using 193 nm photoionisation, the achievable resolution was improved to ca. 1350 in the mass spectrum of laser desorbed perylene. This spectrum is shown in Figure 5.3. However, it is important to appreciate the trade-off between resolution and instrument sensitivity.

The typical resolution, 300-500 (FWHM), represents a practical compromise between the need to differentiate between neighbouring peaks and the ability to detect small quantities of analyte. The critical parameters, external to the TOF instrumentation, are the spatial size of the incident ionising laser spot and the
ionising laser power density. Increasing both results in a decrease in mass spectral resolution whilst simultaneously increasing instrument sensitivity. To obtain a resolution of 1350, the 193 nm laser beam was collimated using 0.7 mm wide slit and had a power density of $6.6 \times 10^{-4}$ W cm$^{-2}$. The spectrum shown in Figure 5.3 was recorded using a 5 ns sampling rate on the transient digitiser in order to maximise the number of collected data points defining the ion packet width.

It is clear from both Figures 5.1 and 5.2 that simple, soft ionisation mass spectra can be obtained for all the PAH compounds examined. At 266 nm (see Figure 5.1) the spectra contain predominantly the molecular ion in all cases. Expansions of the molecular ion region are inset in each mass spectrum. These show that ion signals corresponding to $(M-n)^+$, where $n = 1,2,3,4...$ etc, are present as well as the molecular ion peak itself. However, little other fragmentation is observed to lower masses. These peaks arise from successive hydrogen loss from the molecular ion. (This is not observed for pentacene as the spectral resolution is insufficient to enable differentiation between neighbouring peaks.) This fragmentation is not a simple sequential process. The loss of even numbers of hydrogen atoms is clearly preferred to the loss of odd numbers of hydrogen atoms. This is reflected in the alternating intensity patterns observed in the mass spectra. Molecular hydrogen is assumed to be the neutral fragment resulting from $(M-2)^+$ formation [31]. When the heats of formation of $(M-1)^+$ and $(M-2)^+$ are calculated it is found that $\Delta H_f(M-1)^+ < \Delta H_f(M-2)^+$. This analysis provides a thermodynamic explanation for the preferential loss of hydrogen pairs as a hydrogen molecule. Further fragment ions are observed in the spectra in Figure 5.1 which correspond to the loss of $(C_2H_n)^+$, where $n = 1,2,3,4...$ etc. This is a common feature of all the PAH species examined here. However, these signals have negligible intensity when compared with the molecular ion peak, under soft-ionisation conditions. On detailed examination of the spectrum for pyrene (see Figure 5.1c) a further cluster of diminutive peaks can be seen. These correspond to the loss of a second $(C_2H_n)$ moiety. Peaks observed in the spectra between 140 and 143 amu can be attributed to background contaminant species present in the ion source.

The spectra obtained using 193 nm laser photoionisation, shown in Figure 5.2,
are similar to those obtained at 266 nm. Again, the molecular ions dominate the mass spectra, and are the base peaks in every case. Successive loss of hydrogen atoms from the molecular ion is again observed. However, the lower power densities employed in the work at 193 nm ($\leq 0.5 \text{ MW cm}^{-2}$) generally result in the loss of fewer hydrogen atoms than is observed in the spectra obtained using 266 nm laser photoionisation (2-3 MW cm$^{-2}$). For coronene, perylene and pyrene, no peaks corresponding to loss of ($C_2H_n$) were observed. Pentacene and tetracene, however, fragment by losing both ($C_2H_n$) and ($CH_n$) moieties.

An interesting feature in the mass spectra of the two cata-condensed PAHs, pentacene and tetracene, is the presence of peaks with masses which exceed the molecular weight of the target species. These peaks can be attributed to the formation of molecular adduct ions with the neutral ($C_2H_n$) and ($CH_n$) fragments. In addition, the mass spectra of both these molecules have an anomalous ($M+3)^+$ peak in their mass spectra. These peaks are far larger than anticipated from a consideration of the natural isotopic abundances of the constituent atoms, and are not observed with 266 nm mass spectra for any of the PAHs studied. This feature is most clearly observable in the mass spectrum of tetracene (see Figure 5.2d) and appears to be characteristic of photoionisation of these molecules at this wavelength. Table 5.2 compares the molecular ion intensities calculated using the natural isotopic abundance of $^{13}$C, with those measured directly from the mass spectra in Figure 5.2. For all the PAHs studied, the measured intensities are significantly larger than those calculated. Inspection of the measured intensities of the ($M+2)^+$ species highlights the fact that these are consistently larger than the vanishingly small calculated values. The values tabulated for pentacene and tetracene confirm the observation of anomalous ($M+3)^+$ ion formation. For these molecules the discrepancy between the listed values is between one and two orders of magnitude. The increased yield of protonated molecular ions can be attributed to facile proton transfer reactions occurring either in the gas phase or during the desorption process. Other peaks that are observed in the mass spectra shown in Figure 5.2 are all of relatively low intensity and lie in the low mass region. The most intense of these signals can be attributed to atomic aluminium, at 27 amu,
and iron at 56 amu. Aluminium is present as a result of the ablation of the nozzle faceplate and the iron is a consequence of the ablation of the stainless steel sample probe.

The mass spectra in Figure 5.4 show the soft and hard ionisation mass spectra of perylene obtained using 266 nm laser photoionisation. As expected, increasing the laser power density results in an increase in the degree of fragmentation observed in the mass spectrum. Using an incident laser power density of ca. 5 MW cm\(^{-2}\) resulted in the production of many fragment ions. These are characteristically clustered in \((C_nH_m)^+\) groups. The hard ionisation spectrum (see Figure 5.4b) reveals that fragmentation is able to proceed until an intense signal at 12 amu, corresponding to an isolated carbon ion is produced. The same behaviour is observed using 193 nm as the ionising wavelength. It is therefore clear that by judicious control over the ionising laser fluence we can obtain either simple mass spectra containing only the molecular ions of PAHs or mass spectra containing structurally significant fragmentation. This degree of control means that by selecting the optimal ionising laser fluence, it is possible to obtain relatively simple mass spectra of multicomponent PAHs mixtures in which each signal corresponds to the molecular mass of the PAH components.

The fragmentation pattern produced under hard ionisation conditions is characteristic of the analyte molecule. Such fragmentation patterns can sometimes be used to distinguish between structural isomers. Figures 5.5 and 5.6 show the mass spectra of the isomeric compounds, pyrene and fluoranthene, under increasingly severe ionisation conditions, i.e. from soft ionisation, through the onset of fragmentation, to hard ionisation. These spectra were recorded using 193 nm laser photoionisation and the mass spectral data obtained is summarised in Table 5.3. In previous work [26], it has been asserted that the different relative intensities of the \((M-C_2H)_n^+\) peak, under both soft and hard ionisation conditions, permitted discrimination between anthracene and phenanthrene isomers. In the spectra for fluoranthene and pyrene significant mass spectral differences are not immediately apparent. On inspection, even the relative intensities within each spectral feature are similar. The ability to distinguish between such isomers from their hard ioni-
Table 5-2: A comparison between relative mass spectral peak intensities measured from the mass spectra in Figure 5.2 and those calculated from the natural isotopic abundance of $^{13}$C.
Figure 5-4: L²TOF mass spectra of perylene using 266 nm laser photoionisation with incident laser power densities of a) 0.5 MW cm⁻² and b) 5 MW cm⁻².
sation fragmentation patterns is thus limited. In such cases, differentiation would require recourse to selective ionisation of either component on the basis of their different absorption spectra.

The controlled fragmentation experiments discussed above were performed using a thick sample pressed into the sample slot, along with a reduction in the CO$_2$ laser intensity. This resulted in improved shot-to-shot stability and allowed for multiple passes over the sample surface. On inspection of the molecular ions in Figures 5.4, 5.5 and 5.6 it can be seen that a concomitant effect of increasing the ionisation laser power density is a reduction in mass spectral resolution. For example, with fluoranthene, at the lowest laser power density of $< 0.8$ MW cm$^{-2}$ the resolution of the molecular ion mass peak was calculated to be ca. 400 whilst at a power density of 12 MW cm$^{-2}$ the resolution was only ca. 80. The principal peak broadening mechanism responsible for this effect is the coulombic explosion which results from the high concentration of ions formed simultaneously in the ionisation volume. The intensities of the molecular ions neighbouring $(M+H_n)^+$ and $(M-H_n)^+$ peaks are similarly broadened and overlay one another producing a broad unresolved band.

The L$^2$TOFMS technique is also capable of producing mass spectra of more polar polyaromatic species. To demonstrate this, a nitrogen containing heteroaromatic compound, carbazole, was examined. The resulting spectra, obtained using 193 nm laser photoionisation, are shown in Figure 5.7. The mass spectral data is summarised in Table 5.4. Again, at the highest ionising laser power densities, the molecular ion undergoes successive fragmentation producing, eventually, a strong signal corresponding to carbon ions. For carbazole, it can be seen that when using an ionising laser power density of 12 MW cm$^{-2}$ the molecular ion is no longer the base peak of the mass spectrum. In this case the most abundant species is at 36 amu. This species, C$_7^+$, is a noticeably stable fragment in all the hard ionisation mass spectra recorded. Close inspection of the spectrum shown in Figure 5.7c reveals a number of peaks not previously observed in the photofragmentation patterns of the previously examined PAHs. For example, in Figures 5.5 and 5.6 the fragment cluster corresponding to $(C_2H_n)^+$ contains only four intense mass
Figure 5-5: L²TOF mass spectra of pyrene using 193 nm laser photoionisation with incident power densities of a) ≤ 0.8 MW cm⁻², b) 1.7 MW cm⁻² and c) 12 MW cm⁻².
Figure 5-6: L^3TOFMS spectra of fluoranthene using 193 nm laser photoionisation with incident fluences of a) \(\leq 0.8\) MW cm\(^{-2}\), b) 1.7 MW cm\(^{-2}\) and c) 12 MW cm\(^{-2}\).
<table>
<thead>
<tr>
<th>PAH</th>
<th>Laser fluence</th>
<th>m/z (observed ions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrene (202 amu)</td>
<td>≤ 0.8</td>
<td>202(100) 201(50) 200(30)</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>202(100) 175(5) 151(5)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>202(100) 210(60) 200(18) 177(5) 176(8) 175(13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>153(4) 152(8) 151(12) 123(5) 122(8) 111(5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>110(8) 109(7) 108(3) 99(10) 98(20) 97(8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>87(11) 86(15) 85(18) 84(13) 77(4) 76(7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75(16) 74(30) 73(16) 72(6) 63(25) 62(16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>61(37) 60(30) 51(27) 50(35) 49(33) 48(28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>39(28) 38(35) 37(99) 36(100) 27(13) 26(10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25(12) 24(23) 13(5) 12(78)</td>
</tr>
<tr>
<td>Fluoranthene (202 amu)</td>
<td>≤ 0.8</td>
<td>203(25) 202(100) 201(59) 200(20)</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>202(100) 201(47) 200(26) 175(5) 151(5)</td>
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<tr>
<td></td>
<td>12</td>
<td>202(100) 201(73) 200(17) 175(15) 151(17) 122(11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>111(7) 110(10) 109(10) 108(5) 99(15) 98(28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>97(11) 87(18) 86(24) 85(27) 84(21) 77(8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>76(12) 75(22) 74(42) 73(24) 72(9) 63(39)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>62(23) 61(55) 60(45) 52(7) 51(28) 50(55)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>49(45) 48(45) 39(53) 38(49) 37(79) 36(80)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27(23) 26(16) 25(20) 24(50) 13(9) 12(78)</td>
</tr>
</tbody>
</table>

Table 5-3: L²TOFMS mass spectral peaks of pyrene and fluoranthene using 193 nm laser photoionisation (relative intensities in parentheses).
peaks. However, for carbazole, five peaks are apparent (excluding mass 23 for Na\(^+\)). This is further reflected in higher mass \((\text{C}_n\text{H}_m)^+\) clusters, where additional mass peaks are present at the high mass end. This feature is a consequence of the presence of the nitrogen heteroatom in carbazole. Therefore, it would appear that the observation of these "extra" mass peaks in the mass spectra of carbazole is a good indicator of the presence of a nitrogen heteroatom in the PAC. Fragmentation would appear to be a more facile process in carbazole, as compared with the PAHs. For carbazole, fragmentation is observed at a laser power density of 1.7 MW cm\(^{-2}\), see Figure 5.6b, whilst at the same ionising laser power density the PAHs exhibited only minimal fragmentation. At 23 and 56 amu, small peaks are apparent which can be attributed to sodium and iron cations respectively. Their presence is a result of the higher desorption laser power densities required to desorb carbazole compared to the PAH samples. This not only promotes complete removal of the sample but causes ablation of the stainless steel probe liberating these atomic species.

5.3 Limits of Detection (LOD) for PAH Analytes

As discussed in Section 4.5, the detection sensitivity has not been of prime concern in many of the previous investigations using L\(^2\)TOFMS. However, in order to assess the range of analytical application for this technique, the sensitivity of detection is an important consideration. The absolute limit of detection acts as a constraint on the nature of potential applications. When considering the limits of detection for L\(^2\)TOFMS it is important to note that the ion signal intensity is critically dependent on the choice of both the ionisation laser wavelength and power density. The electronic absorption spectrum of a particular molecular species is characteristic of that compound. At any ionising wavelength the ionisation cross-section will vary between two different species. The size of the ionisation cross-section depends upon whether the target molecules have strong or weak absorption at that wave-
Figure 5-7: L²TOF mass spectra of carbazole using 193 nm laser photoionisation with incident power densities of - a) ≤ 0.8 MW cm⁻², b) 1.7 MW cm⁻² and c) 12 MW cm⁻².
<table>
<thead>
<tr>
<th>PAH</th>
<th>Laser fluence MW cm(^{-2})</th>
<th>m/z (observed ions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbazole</td>
<td>0.8</td>
<td>168(21) 167(100) 166(59) 165(11) 143(10) 142(13) 141(10) 140(10)</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>167(100) 143(25) 142(30) 141(20) 140(20) 115(12) 114(8) 113(10) 90(6) 89(10) 88(7) 87(6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>86(5) 77(5) 76(5) 75(8) 74(10) 65(5) 64(8) 63(21) 62(11) 61(13) 60(6) 52(8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51(15) 50(13) 49(8) 39(40) 38(16) 37(23) 36(15)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>167(52) 143(14) 142(18) 141(13) 140(14) 115(8) 114(7) 113(8) 89(11) 88(7) 87(10) 86(9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>85(8) 84(5) 77(5) 76(6) 75(8) 74(12) 73(6) 65(6) 61(27) 60(20) 56(7) 52(10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51(23) 50(33) 49(24) 48(23) 40(8) 39(88) 38(45) 37(98) 36(100) 28(7) 27(25) 26(16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25(12) 24(23) 23(16) 13(6) 12(67)</td>
</tr>
</tbody>
</table>

Table 5-4: \(L^2\)TOFMS mass peaks of carbazole obtained using 193 nm laser photoionisation (relative intensities in parentheses).
length. Thus, the detection limits measured using fixed ionisation wavelengths are largely molecule specific. In selecting molecules to perform sensitivity measurements on further factors must be considered. To make realistic assessments of the limits of detection, molecules with low vapour pressures at room temperatures are required. It must be feasible to put small quantities of these materials on the sample probe without pumping them away in a $10^{-6}$ mbar vacuum. Higher mass PAHs prove to be ideal molecules for this purpose having low vapour pressures and relatively high ionisation efficiencies at 193 nm and 266 nm.

The sensitivity of detection was investigated for two pure PAH materials, coronene and perylene, using 266 nm laser photoionisation. In both cases, the desorption laser power was estimated to be ca. 22 MW cm$^{-2}$ and the 266 nm power density was estimated to be ca. 0.7 MW cm$^{-2}$. There experimental parameters were held constant throughout the experiments. Dichloromethane solutions of known concentration were used. Aliquots of these solutions were then applied to the sample slot, using a glass syringe, and the solvent allowed to evaporate. Successively lower sample loadings were used by reducing the number of aliquots added to the probe. At each sample loading, spectra were obtained by accumulating the signal from 1000 consecutive experiments. These were acquired as the sample probe was translated in front of the IR desorption laser. The area interrogated over the time taken to collect 1000 shots could therefore be calculated. Assuming that all the sample was retained in the sample slot, and that it was deposited in a homogeneous layer on the probe surface, it is possible to calculate the amount of material desorbed during the generation of each spectrum. A relatively large number of consecutive laser shots were accumulated in order to minimise signal variations caused by poor sample homogeneity.

Figures 5.8a and 5.8b show the mass spectra obtained using 266 nm laser photoionisation, for four successively reduced sample loadings, in the cases of coronene and perylene, respectively. The calculated amount of material desorbed during the collection of each 1000 shot spectrum is given on the spectra. Clearly, the signal-to-noise ratio becomes increasingly poor with lower sample dopings. A combination of poor sample coverage and ionisation laser power density fluctuations resulted in
signal instability at low sample concentrations. Therefore, the mass spectra shown for coronene and perylene at the lowest signal-to-noise levels, represent a practical detection limit for these two materials under these experimental conditions. It is, however, important to realise that for a homogeneous sample, the single shot detection limits obtained correspond to ca. 0.33 picomoles and ca. 3.3 picomoles for coronene and perylene, respectively.

These limits of detection for coronene and perylene represent an estimate of the amount of material that must be present in the source chamber in order to generate a mass spectrum. These are not absolute limits of detection for the instrument but provide a guide to the instrument sensitivity. For example, along with the laser wavelength and fluence, the instrument geometry will also have important ramifications with regards to sensitivity. The distance between the desorption and ionisation regions and the efficiency of the entrainment process both influence the instrument sensitivity.

It is possible to extrapolate backwards from the ion signal observed in the mass spectrum in order to obtain an estimate for the number of ions reaching the MCP detector. A 1000 shot mass spectrum obtained after desorption of approximately 500 nmoles of coronene, using 193 nm laser photoionisation, gave an integrated ion signal of 19100 counts. Since 1 count is equivalent to 2 mV, the average total ion signal per laser shot is $(19100 \times 2/1000)$ mV = 38.2 mV shot$^{-1}$. The signal cable is terminated through 50 $\Omega$, so assuming a detector amplification of ca. $10^6$, the current produced per shot was $(38.2 \times 10^{-9}/50) = 7.64 \times 10^{-10}$ Amps. Assuming an ion peak width of ca. 50 ns FWHM, the total charge on the detector is $(50 \times 10^{-9} \times 7.64 \times 10^{-10}) = 3.82 \times 10^{-17}$ Coulombs per shot. This is the equivalent 238 ions reaching the MCP per laser shot. Thus, less than 300 ions are detected for every $3 \times 10^{13}$ molecules desorbed per laser shot, i.e. only 1 in $10^{11}$ desorbed molecules are ionised and detected. Similar measurements performed elsewhere [27,32] agree with this analysis.

Obviously, even though the limits of detection are in the region of picomoles there is scope for improvements in the instrument sensitivity. For example, it is possible to increase the ionisation efficiency by increasing the power of the ioni-
Figure 5-8: 1000 shot L²TOF mass spectra obtained for a) coronene and b) perylene, using successively reduced sample probe loadings.
sation laser. However, a threshold is soon reached where fragmentation becomes important. The greatest losses occur during the entrainment and transportation of the laser desorbed molecules.

It is possible to extrapolate backwards from the number of ions detected in order to estimate the magnitude of the sensitivity losses sustained during the entrainment and transportation processes. If it is assumed that 300 ions are detected, the ionisation efficiency is 1% and the ion transmission is 10% then $3 \times 10^5$ molecules are present in the ionisation volume during the ionisation process. Now, if the pulsed molecular beam is assumed to be travelling at $1.7 \times 10^5$ cm s$^{-1}$, a pulsed molecular beam with a duration time of ca. 900 µs exists over 153 cm in space. However, the desorbed material is only seeded into ca. 20.4 cm of the beam, as can be seen from a typical timescan profile (see Figure 3.8). A 10 ns ionisation pulse with a spatial width of 0.1 cm samples only ca. 0.5% of the seeded molecules which cross the laser beam interaction region. Therefore, ca. $6 \times 10^7$ molecules can be considered to be present in the ionisation region. This is equivalent to sustaining a loss of ca. $10^6$ during the entrainment and transportation stage. (This argument assumes that all the target material is desorbed and that there are no losses due to nascent ion formation in the desorption stage.)

Clearly, the poor entrainment efficiency imposes severe restrictions on the instruments absolute limit of detection. Zare's group have developed an instrument which dispenses with the entrainment stage [33]; the desorbed neutrals are ionised directly above the sample surface. Using a recently modified version of this instrument they have recently reported zeptomole ($10^{-21}$) detection limits [34] for pure coronene. Similar modifications to the instrument used in the investigations described here could improve the instrument sensitivity by approximately five orders of magnitude.
5.4 Direct Determination of PAHs in Environmental Matrices

The following sections describe studies of the feasibility of L\(^2\)TOFMS to assay for PAH contaminants directly from their environmental matrices. In all cases the samples were examined without any extraction or pre-separation procedures being employed. They were all presented to the sample probe with a minimum of sample preparation. CO\(_2\) laser desorption was used to simultaneously evaporate all the components in the environmental mixture. R2PI was then exploited to selectively ionise only those components which had significant absorption at the chosen ionising wavelength. Aliphatic species were not present in the resulting mass spectra as these materials have no resonant absorption at either of the ionising wavelengths employed, 193 nm or 266 nm. Thus, simple, readily interpretable mass spectra were obtained from the analysis of extremely complex environmental matrices: contaminated soils, engine oil, coal soot and creosote. The rapid, pulsed mode of operation, simple sample presentation and selectivity of target analytes combine to make L\(^2\)TOFMS an effective screening tool.

5.4.1 Mass Spectra of Contaminated Soils

The soil samples used in this study were obtained from a contaminated former coal gasification plant and coal-tar distillery in the UK. Samples were taken from shallow trial pits (0.5 m depth), and consisted of three grab samples taken at 120 degrees from each other prior to being composited into approximately 5 kg total samples. These were air-dried at ambient temperatures in a forced draught. After the resulting aggregates were broken up, the entire sample was passed through a 10-mesh sieve to remove rock fragments. The fraction passing through was then homogenised by being riffled down to a 50 g subsample [35]. To ensure good sample coverage on the desorption probe the samples were ground into a fine powder, bound together with a drop of glycerol and applied as a homogeneous
paste to the slot in the probe. The sample surface exposed to the desorption laser beam was then dried with a dusting of alumina-type H.

Each of the six contaminated soil samples was examined using both 266 nm and 193 nm laser photoionisation. Typical mass spectra obtained for one of these samples, using 266 nm and 193 nm photoionisation, are shown in Figures 5.9a and 5.9b respectively. They represent the total accumulated ion signal obtained on summing 200 laser shots. It is important to note the absence of peaks corresponding to either the glycerol matrix or the soil itself. The spectra are simple and readily interpretable. The absence of signals derived from the soil in glycerol eliminates the need for the analysis of blank samples for comparative and subtraction purposes. These fingerprint mass spectra were obtained in consecutive experiments, and the entire preliminary screening of the six samples was completed within 90 minutes. Each 200 shot spectrum represents the total ion signal obtained on interrogating approximately 1 mg of the contaminated soil. Samples up to ca. 50 mg can be examined in one continuous scan of the probe slot. This requires the accumulation of 6000 consecutive shots but provides more consistently reproducible data concerning relative PAH concentrations in a sample.

PAH signals can be seen, in both Figures 5.9a and 5.9b, in the region between 100 and 400 amu. Signals at masses higher than 300 amu indicate the presence of PAHs with more than seven fused rings. In the low mass region, below 100 amu, the dominant signals are due to the presence of elemental cations, e.g. Na⁺ and K⁺. The source of the signal due to Al⁺ at 27 amu was the alumina on the surface of the sample matrix, whilst the peak due to Fe⁺ could be from either the soil sample itself or from ablation of the stainless steel probe. The PAH signal intensity is generally lower, over all masses, in the mass spectra obtained using 266 nm laser photoionisation. This means that some of the less intense mass peaks may not be observed at this wavelength, as is evidenced by less intense or missing high mass peaks (above 300 amu) in the spectrum in Figure 5.9a. Furthermore, the more energetic 193 nm photons are more efficient at ionising the metal species resulting in increased ion yield compared to photoionisation at 266 nm.

These soil samples had previously been investigated using GC-MS [35], follow-
Figure 5-9: L²TOF mass spectra of ca. 1 mg of contaminated soil obtained using a) 266 nm and b) 193 nm laser photoionisation.
Table 5-4: PAH assignment for the $L^2$ TOF mass spectrum of contaminated soils obtained using 193 nm laser photoionisation.

It was possible to assign these species to a number of the more intense peaks in the $L^2$ TOF mass spectra. For example, peaks at 178, 202, 228, 252 and 276 amu correspond to phenanthrene/anthracene, fluoranthene/pyrene, chrysene/benz[a]anthracene, benz[b or k]fluoranthene/benzo[a]pyrene, and indeno[1,2,3-cd]-pyrene/benzo[ghi]perylene. The peaks corresponding to these species are numbered in Figure 5.11 which, for clarity, shows an expansion of the PAH containing region of the mass spectrum.
Figure 5-10: PAH species previously determined present in contaminated soil by GC-MS following CH$_2$Cl$_2$ extraction.
Figure 5-11: Expansion of L²TOF mass spectrum of contaminated soil between 170 and 320 amu to show more clearly the resolved mass spectral peaks. Confirmed mass assignments from GC-MS are 1, 178, phenanthrene/anthracene; 2, 202, pyrene/fluoranthene; 3, 228, chrysene/benzo[a]anthracene; 4, 252, benzo[a]fluoranthene/benzo[a]pyrene; 5, 276, indeno[1,2,3-cd]pyrene/benzo[ghi]perylene.

obtained using 193 nm laser photoionisation. Possible assignments for all the major peaks in this spectrum, corresponding to skeleton parent PAHs, including those previously determined, are summarised in Table 5.5. As their identification is based only on molecular weight, only one or two representative compounds are indicated for each mass.

It is worth noting that although the presence of some of these species was confirmed by GC-MS analysis, they are not necessarily exclusively responsible for the ion signal at a particular mass; isomers cannot be differentiated directly from these photoionisation mass spectra. To fully distinguish the component species would require the use of spectroscopically selective ionisation of individual isomers, or recourse to more advanced tandem (MS/MS) TOF instrumentation. The exact assignment of higher mass PAHs is further complicated by the increasing number
of possible isomers that may be present. Other peaks in the spectrum which are separated by characteristic increments of 14 amu can be identified. These series of peaks originate from various skeletal unsubstituted PAHs, and indicate the presence of successively alkylated species where -H is successively replaced by -CH₃.

The mass spectra of pure PAHs, discussed in Section 5.2, that were obtained under soft ionisation conditions, show that although the molecular ion is the dominant mass peak, there are other less intense peaks commonly present which correspond to [M+1]+, [M-1]+ and [M-2]+ etc. These peaks could account for some of the intensity of the smaller mass peaks and for the presence of odd mass peaks in the spectra of the PAH containing soils (see Figure 5.11).

Differences in the relative abundances of the same compound in different soil samples can be estimated by comparing the signal intensities at a particular mass between two mass spectra. Relative signal intensities within a particular mass spectrum, however, do not directly reflect the relative concentrations of different species in the soil sample. This is because parent ion peak intensities are proportional not only to the concentration of a compound in the sample, but also to the ionisation cross-sections for different species at any particular ionising wavelength. Thus, for example, it is possible that the components pyrene/fluoranthene, corresponding to mass 202 amu, are not the most prevalent in the sample, but are the most efficiently ionised at the laser wavelength employed.

In earlier GC-MS work carried out on these samples, selected PAHs had been quantitatively determined as being present in concentrations between ca. 0.1 and several hundred ppm [35]. These concentrations are listed in Table 5.7 for one of the six soil samples. It is possible to determine the approximate concentrations of particular species using L²TOFMS. Quantitation was achieved using a series of standard additions. Six 50 mg samples of a particular soil sample were spiked with various amounts of phenanthrene. Figure 5.12 shows the 178 amu: 202 amu signal intensity ratio versus the amount of phenanthrene added for the soil sample with the composition tabulated in Table 5.6.

The ratio of peak heights was determined using the signal at 202 amu as an
<table>
<thead>
<tr>
<th>PAH</th>
<th>Mass (amu)</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>128</td>
<td>4.3</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>154</td>
<td>175.4</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>152</td>
<td>44.9</td>
</tr>
<tr>
<td>Fluorene</td>
<td>166</td>
<td>156.6</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>178</td>
<td>207.5</td>
</tr>
<tr>
<td>Anthracene</td>
<td>178</td>
<td>102.3</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>202</td>
<td>200.5</td>
</tr>
<tr>
<td>Pyrene</td>
<td>202</td>
<td>338.8</td>
</tr>
<tr>
<td>Chrysene</td>
<td>228</td>
<td>60.9</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>228</td>
<td>87.2</td>
</tr>
<tr>
<td>Benzo[b + h]fluoranthene</td>
<td>252</td>
<td>124.4</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>252</td>
<td>118.2</td>
</tr>
<tr>
<td>Indeno[123-cd]pyrene</td>
<td>276</td>
<td>NF</td>
</tr>
<tr>
<td>Dibenzo[a]anthracene</td>
<td>278</td>
<td>4.5</td>
</tr>
<tr>
<td>Benzo[ghi]perylene</td>
<td>276</td>
<td>200.3</td>
</tr>
</tbody>
</table>

Table 5-6: PAH concentrations determined for soil sample using GC-MS [35]. The combined concentration of phenanthrene and anthracene (309.8 ppm) can be compared with that obtained using L²TOFMS and the method of standard additions (see text). NF - determinand not found.
Figure 5-12: Plot of 178 amu : 202 amu signal intensity ratio versus spiked concentration of phenanthrene for a contaminated soil sample. Extrapolation to zero spiking concentration yields an approximate concentration of phenanthrene/anthracene contamination.
internal standard, rather than the absolute signal intensity of the 178 amu peak, in order to account for variations in the total amount of spiked sample applied to the probe. A least squares fit of these data points was extrapolated to zero spiking concentration to yield the concentration of phenanthrene/anthracene in an unspiked soil sample, as shown in Figure 5.12. The results obtained using this approach gave values for the total anthracene/phenanthrene concentration which are in reasonable agreement with those determined by GC-MS. For example, the value obtained from the data shown in Figure 5.12 is ca. 390 ppm (linear correlation coefficient, $R = 0.9756$) compared with the combined concentration of 309.8 ppm for anthracene and phenanthrene obtained via GC-MS (see Table 5.6). In comparing these two figures it is important to note that the extraction and separation processes required for sample preparation in the GC-MS study are difficult to quantify reliably and that the ionisation cross-sections for anthracene and phenanthrene have been assumed to be the same in the $L^2$TOFMS experiment.

5.4.2 Mass Spectra of Contaminated Engine Oils

The engine oil studied in these experiments was Castrol GTX lubricating oil. The engine oil was examined both as received and on removal from a car engine after approximately six months of average use. In each case the samples were prepared by mixing the oils into a paste with alumina-type H and then applying directly to the sample probe.

The mass spectrum obtained from the interrogation of a clean, unused engine oil sample is shown in Figure 5.13a. This was obtained using 193 nm laser photoionisation and represents the accumulation of data from 1000 laser shots. Several homologous series of peaks are immediately apparent. In the mass region between 30 amu and 180 amu several such series can be identified. In each series the peaks are separated by characteristic increments of 14 amu. For example, the peaks at 91, 105, 119, 133 amu and 117, 131, 145, 159, 173 amu constitute two such series. Similar series are observed above 250 amu.
Figure 5-13: L²TOF mass spectra of clean, unused engine oil obtained using 193 nm laser photoionisation; spectrum a) shows the entire mass range up to 380 amu, and spectrum b) an expansion of the region between 240 amu and 520 amu.
Figure 5.13b shows these higher homologues more clearly. No mass peaks with significant intensity are observed above 500 amu. The separation between peaks is again 14 amu, suggesting their assignment to a homologous series in which an aromatic head group is appended with a lengthening hydrocarbon chain (or chains). The peak separation within each of these features is 2 amu which may be accounted for by the hydrogenation of unsaturated alkene bonds in the hydrocarbon side chains. The lack of significant ion signals in the central region of the spectrum (see Figure 5.13a), and at lower masses, suggests that photofragmentation is minimal under the soft ionisation conditions employed and that the peaks correspond to the presence of individual molecular ions not fragmentation products.

The mass region between ca. 170 amu and 300 amu is where signals corresponding to PAH components would be expected to occur. In the case of the “clean” oil sample these species are not observed. They are, therefore, either not present or are present in concentrations below the limit of detection of the instrument (sub ppm). However, the presence of PAHs as combustion products in used engine oils is well known and consequently the disposal of such materials is of considerable environmental concern. The mass spectra we obtain, under identical conditions to those stated above, for a similar sample of used engine oil are shown in Figures 5.14a and 5.14b. There is an important difference between these spectra and those of the clean oil (see Figure 5.13a and 5.13b). The central region of the mass spectrum for the used oil sample is now dense with new features, as shown in Figure 5.14b. These new peaks can be tentatively identified as belonging to several series of successively alkylated parent PAHs where -H is replaced with -CH₃. For example, a series of alkylated phenanthrenes have been indicated by the arrows on the mass spectrum shown in Figure 5.14b. In Table 5.7 the most prominent series of peaks that can be identified in the spectrum are summarised together with an assignment of the parent PAHs.

It is clear that these fingerprint mass spectra yield analytically useful data, allowing rapid screening of lubricating oils for contaminant PAHs. Despite the presence of blank or background signals from the base oil, these contaminant species are easily observable, without requiring recourse to any extraction and
Figure 5-14: L²TOF mass spectra of contaminated, used engine oil obtained using 193 nm photoionisation. In spectrum a) a series of contaminants in the mass "window" in the spectrum shown in Figure 5.13a between 170 amu and 300 amu can be clearly identified. These are shown more clearly in the expanded spectrum b). The assignment of these peaks to a series of alkylated PAHs is indicated (see text).
<table>
<thead>
<tr>
<th>Mass (amu)</th>
<th>Assignment</th>
<th>Molecular Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>178</td>
<td>Phenanthrene/Anthracene</td>
<td>C_{14}H_{10}</td>
</tr>
<tr>
<td>192</td>
<td>C_1-Phenanthrene</td>
<td>C_{15}H_{12}</td>
</tr>
<tr>
<td>206</td>
<td>C_2-Phenanthrene</td>
<td>C_{16}H_{14}</td>
</tr>
<tr>
<td>220</td>
<td>C_3-Phenanthrene</td>
<td>C_{17}H_{16}</td>
</tr>
<tr>
<td>234</td>
<td>C_4-Phenanthrene</td>
<td>C_{18}H_{18}</td>
</tr>
<tr>
<td>248</td>
<td>C_5-Phenanthrene</td>
<td>C_{19}H_{20}</td>
</tr>
<tr>
<td>202</td>
<td>Pyrene/Fluoranthene</td>
<td>C_{18}H_{10}</td>
</tr>
<tr>
<td>216</td>
<td>C_1-Pyrene</td>
<td>C_{17}H_{12}</td>
</tr>
<tr>
<td>230</td>
<td>C_2-Pyrene</td>
<td>C_{18}H_{14}</td>
</tr>
<tr>
<td>244</td>
<td>C_3-Pyrene</td>
<td>C_{19}H_{16}</td>
</tr>
<tr>
<td>258</td>
<td>C_4-Pyrene</td>
<td>C_{20}H_{18}</td>
</tr>
<tr>
<td>252</td>
<td>Benzo[a]pyrene/Perylene</td>
<td>C_{20}H_{12}</td>
</tr>
<tr>
<td>266</td>
<td>C_1-BenzoPyrene</td>
<td>C_{21}H_{14}</td>
</tr>
<tr>
<td>276</td>
<td>Benzo[ghi]perylene</td>
<td>C_{22}H_{12}</td>
</tr>
</tbody>
</table>

Table 5-7: Peak assignments for L^2 TOF mass spectrum of contaminated engine oils obtained using 193 nm laser photoionisation.
separation procedures. Because these species are by-products formed during the active employment of lubricating oils, such a rapid screening technique may have application in the on-line monitoring of their development. In addition, the concentrations of these contaminants that are present may be obtained using the sample spiking methodology described in the previous section.

5.4.3 Mass Spectra of Coal Soot

The L²TOF mass spectrum for a sample of coal soot, bound in glycerol, is shown in Figures 5.15. This mass spectrum represents the accumulation of 500 laser shots. Again, it is possible to determine the presence of a number of unsubstituted PAHs. These constitute the main features of the mass spectrum. Such species have been observed previously in rice straw soot using a similar methodology [36]. The dominant signals found in the spectra obtained from rice straw soot were at 178, 202, 228, 252 amu. These mass peaks correspond to three, four and five-ring PAHs.

The distribution of PAHs in oil shale soot particles has also been the subject of a previous study using laser microprobe mass spectrometry (LAMMA) [38]. The positive ion LAMMA mass spectra contained features corresponding to both inorganic and organic components, rendering the assignment of some peaks ambiguous. However, here again a series of mass peaks were observed corresponding to parent PAHs. In the case of these oil shale soot particles, predominantly high mass components corresponding to five-ring PAHs and above, were formed. It is possible to assign parent isomer groups to the mass peaks observed in the spectrum shown in Figure 5.15. The peaks labelled in the mass spectrum at 178, 202, 228, 252 amu, correspond to phenanthrene, pyrene, chrysene, benzo[\textit{a}]pyrene and benzo[\textit{ghi}]perylene, or their respective isomers. The production of such PAHs is favoured by the high temperatures found in combustion processes [37]. This is confirmed by the strong signals corresponding to the higher molecular weight PAHs, although signals corresponding to PAHs with more than five rings are not observed. The contribution from odd mass peaks is very small in Figure 5.15,
suggesting that nitrogen heterocycles are either not present, or present only at low concentrations, in the soot matrix.

5.4.4 Mass Spectra of Creosote

Figure 5.16 shows a characteristic fingerprint mass spectrum of a coal-tar creosote obtained using L²TOFMS. Creosote is derived from either coal or coal-tar distillation; the creosote fraction being the material which distills over between 210 and 280 °C [39]. Acute toxicity and carcinogenicity associated with this material have obvious ramifications for human health [40].

Table 5.8 lists the predominant polycyclic aromatic hydrocarbons found in coal-tar creosote [41]. This list is incomplete, as creosote contains many phenolic (ca. 10%) and heterocyclic compounds (ca. 5%) in addition to the PAHs [42,43,41]. However, this simplified list highlights the correlation between anticipated and observed polyaromatic species. The mass spectrum shown in Figure 5.16 is dominated by the signals at 178 amu and 202 amu, corresponding to three-ring and four-ring PAHs respectively. However, this spectrum also contains peaks
corresponding to the masses of the majority of species listed in Table 5.8. The only species not accounted for are those of low mass, such as naphthalene and the methylnaphthalenes. These species are considerably more volatile than the higher mass PAHs and have probably been lost from the sample during pump-down of the desorption vacuum chamber. The persistence of naphthalenes in solid environmental samples is known to be relatively low because of its volatility and facility to undergo biotransformations (the majority of naphthalene in the environment is associated with the vapour phase [44]). This probably accounts for the absence of these well known contaminants from all the mass spectra shown so far. There are also other mass peaks present in Figure 5.16 which correspond to PAHs not listed in Table 5.8. The most intense of these are at 190, 218, 230 and 242 amu. These can be tentatively assigned to 4H-cyclopenta[def]phenanthrene, dimethyl-4H-cyclopenta[def]phenanthrenes, methylbenzofluorenes and methylchry-senes respectively, or their isomers. A further group of neutral components known to be present are the alkanes. However, these are not ionised at the UV laser wavelengths used in this study and therefore do not appear in the mass spectra.
<table>
<thead>
<tr>
<th>PAH</th>
<th>Relative % (by wt)</th>
<th>Mass (amu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>13</td>
<td>128</td>
</tr>
<tr>
<td>2-Methylnaphthalene</td>
<td>13</td>
<td>142</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>13</td>
<td>178</td>
</tr>
<tr>
<td>Anthracene</td>
<td>13</td>
<td>178</td>
</tr>
<tr>
<td>1-Methylnaphthalene</td>
<td>8</td>
<td>142</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>8</td>
<td>154</td>
</tr>
<tr>
<td>Fluorene</td>
<td>8</td>
<td>166</td>
</tr>
<tr>
<td>2,3-Dimethylnaphthalene</td>
<td>4</td>
<td>156</td>
</tr>
<tr>
<td>2,6-Dimethylnaphthalene</td>
<td>4</td>
<td>156</td>
</tr>
<tr>
<td>Acenapthene</td>
<td>4</td>
<td>154</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>4</td>
<td>202</td>
</tr>
<tr>
<td>Chrysene</td>
<td>2</td>
<td>228</td>
</tr>
<tr>
<td>Pyrene</td>
<td>2</td>
<td>202</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>1</td>
<td>208</td>
</tr>
<tr>
<td>2-Methylanthracene</td>
<td>1</td>
<td>192</td>
</tr>
<tr>
<td>2,3-Benzofluorene</td>
<td>1</td>
<td>216</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>1</td>
<td>252</td>
</tr>
</tbody>
</table>

Table 5-8: Predominant polycyclic aromatic hydrocarbons in coal tar creosote.
5.5 Concluding Remarks

The data presented above concerning the analysis of individual PAH analytes have demonstrated that laser desorption, with minimal decomposition, and laser ionisation with minimal fragmentation, provides simple mass spectra consisting of mainly parent ion peaks. The results show that temporal and spatial separation of the desorption and ionisation processes can circumvent many of the traditional problems which complicate mass spectra. The benefit of this degree of control in enabling the successful application of L$^2$TOFMS to the in situ analysis of PAHs in complex environmental matrices has also been demonstrated. The results contained in Section 5.4 show that L$^2$TOFMS can be used for rapid qualitative analysis of PAHs by direct interrogation of the host matrix. Such an approach to sample screening is both robust and flexible, featuring low detection limits for the target PAH materials. The simple sample preparation reduces sample extraction and handling difficulties. Furthermore, the instrument has been shown to be capable of handling a large throughput of real, dirty sample systems. This rapid screening capability is further complemented by the ability to perform semi-quantitative analysis on individual components or isomer groups.

L$^2$TOFMS should, therefore, prove to be a valuable addition to existing environmental screening methodologies [45]. The ability to provide fingerprint mass spectra of high molecular weight PAHs extends its range beyond that of GC-MS. In addition, as a tool for the examination of environmental contaminants themselves, e.g. oil, the mass spectra provide data concerning the presence of PAHs in such materials. Increasing concern about human exposure to PAHs means that future target matrices may include foodstuffs, aerosol condensates, food additives and roadside particulates. Simple modification of the instrument should also allow for more detailed analyses of PAHs in individual environmental particulates. This would require the use of laser mass microscopy, a microanalytical technique whereby a tightly focussed laser (≤ 50 μm) is used to effect desorption with high spatial resolution [34]. Such a technique interrogates only a small amount of the
sample and therefore requires a highly sensitive method for the detection of the desorbed species. As discussed in Section 5.3, the entrainment process used in the instrumental configuration described in this thesis is extremely inefficient and a significant limiting factor with respect to the instrument detection sensitivity. Therefore, in order to perform laser mass microscopy experiments, it would be beneficial to dispense with this process and ionise the desorbed molecules directly above the sample surface.

Although the technique has been shown to be very effective for the direct determination of PAH contaminants in environmental matrices, its wider application to environmental screening is still under investigation. PAHs are good target analytes for \textsuperscript{2}TOFMS as they have strong absorption at the 193 nm and 266 nm ionising wavelengths employed and are therefore readily ionised. It has been shown that the technique of laser desorption is capable of volatilising a wide variety of molecular species and this work has demonstrated the ability to desorb intact molecules directly from complex matrices. By judicious choice of the ionisation wavelength it is expected that the same methodology can also be extended to include the examination of a wide variety of potential pollutants including dioxins, halobenzenes and specialist chemical site contaminants.
Bibliography


Chapter 6

L²TOFMS of Porphyrins

6.1 Introduction

Porphyrins are of prime importance in a large variety of processes related to biology, biochemistry, catalysis and geology. Qualitative identification and characterisation of porphyrins, such as those involved in tumor localisation and treatment [1], or porphyrins that occur naturally in biological systems [2,3] and fossil fuels (geoporphyrins) [4,5] is often desirable. The identification of porphyrin materials deposited in tissues or excreted by an organism is of biomedical importance. Such information can be used to diagnose a variety of metabolic abnormalities [6]. Synthetic porphyrins have been used extensively to investigate single electron transfer reactions of natural biological processes [7] such as photosynthesis [8] and catalysis in artificial enzyme mimics [9]. Geoporphyrins are found in petroleum deposits and associated petroleum source rocks. Correlation between the patterns of porphyrins in these materials can, therefore, be used to enhance oil prospecting efficiency [10] by providing information on source, maturity, depositional environment, extent of biodegradation and relative migration distances. Petroporphyrins are organic compounds present in original source materials whose carbon skeletons have been preserved throughout the geological record. Molecules like porphyrins which reveal information about the transformation of a system through geological time are known as biomarkers [11]. Although metalloporphyrins were the first compounds of conclusive biological origin identified in petroleum [12], their use in oil-oil and oil-source rock correlation studies, maturation studies and in depositional environment reconstruction has been limited [10]. The reasons for this
include the fact that the porphyrin-biological precursor maturation process and the relationship between the depositional environment and porphyrin evolution is not fully understood. A major factor, however, has been the analytical difficulty of isolating and characterising the geoporphyrins.

Mass spectrometry has proved an important tool for the analysis of porphyrins. This is especially the case with respect to the more volatile and robust porphyrins which have been extensively investigated using electron impact (EI) ionisation mass spectrometry [13,14]. The EI mass spectra of porphyrins are usually characterised by a singly charged molecular ion as the base peak, and relatively abundant singly charged and doubly charged fragment ions. The fragments arise mainly from the cleavage of substituents \( \beta \) to the pyrrolic rings. There is generally no extensive cleavage of the macrocycle nucleus. Macrocycle fragmentation is necessary if the pyrrole sequence of the porphyrin and the location of the substituents on the individual pyrrole units are to be determined. For this reason Van Berkel et al. [15] used high pressure ammonia chemical ionisation (CI) to promote the formation of the reduced molecular species and tri-, di-, and monopyrrolic fragment ions. More recently the same group has used CI tandem mass spectrometry in order to simplify the reproduction and interpretation of the complex parent porphyrin CI spectra [16]. Tandem mass spectrometry has also been used for the analysis of geoporphynins isolated from Boscan crude oil by two different groups. Brodbelt et al. [17] used a combination of isobutane CI and argon collision induced dissociation (CID), whilst Johnson et al. [18] used EI followed by CID. A triple quadrupole mass spectrometer was employed in both cases. The added specificity of MS/MS enabled the individual parent porphyrins to be characterised by their CID fragmentation patterns.

Many biological and synthetic 'biological-type' porphyrins are not amenable to these types of mass spectrometric analysis. Such molecules often contain functionalised substituent groups that make them involatile or thermally labile. The analysis of these molecules requires the use of desorption techniques. Field desorption mass spectrometry has been useful in providing molecular weight information on a series of porphyrins [14] and has been used to study the hydration behaviour
of chlorophyll a [19]. $^{252}$Cf-plasma desorption has also been used to study a variety of porphyrin and porphyrin-like compounds, including chlorophyll a [20], geoporphyrins [21] and synthetic porphyrins [22]. Thermospray ionisation has been applied to the analysis of chlorophylls [23] in edible oils and electrospray ionisation, using a quadrupole mass spectrometer, has been employed for the analysis of a variety of porphyrins [24]. However, the most popular techniques for the analysis of porphyrins have been fast atom bombardment (FAB) and laser desorption Fourier transform (LD-FT) mass spectrometry. Barber et al. [25,26] first demonstrated the use of FAB for natural porphyrin derivatives, vitamin B$_{12}$ and related cobaltamines. Musselman has investigated hematoporphyrin derivatives [27] and more complex dimeric porphyrins [28], whilst Zhang et al. [29] have investigated substituted tetraphenyl porphyrins and their metal complexes. However, the limited stability of free-base porphyrins and metalloporphyrins in many FAB solvent matrices, along with problems posed by matrix interferences [30], and reduction and demetalation of the porphyrin macrocycle in the matrix [31] have hindered the extensive application of FAB to porphyrin characterisation. These problems are especially apparent in attempting to analyse complex porphyrin mixtures such as those isolated from geological samples [30]. The development of flow-FAB may overcome a number of these problems by enabling a wider range of solvent systems to be used and facilitating on-line coupling to a separation technique [32].

The combination of laser desorption with Fourier transform mass spectrometry has been widely used for the study of porphyrins. This technique has been applied to naturally occurring porphyrins such as chlorophyll a and b [33] and to the study of a number of synthetic porphyrins [34,35]. Nuwaysir and Wilkins [36] used LD-FT to investigate the XeCl excimer laser photodissociation of trapped porphyrins and metalloporphyrins as an alternative to collision-induced dissociation for structure analysis purposes. The strength of the LD-FTMS technique is that photodissociation/photoionisation can be induced in the laser desorbed species by using a second synchronised laser pulse. The mass spectra of tetraphenyl porphyrins attached to hydrocarbon spacers [37], vitamin B$_{12}$ and cobester [38] have recently been obtained using both direct laser desorption and laser desorption laser pho-
toionisation FTMS. The mass spectra of vitamin $B_{12}$ and cobester were obtained using matrix assisted laser desorption (MALD) in the FTMS instrument. This significantly reduced the amount of fragmentation resulting from the LD process, simplifying the mass spectra. The subsequent photodissociation of these species was proven to generate structurally significant data.

The following chapter describes the application of L$^2$TOFMS to the mass spectrometric analysis of a variety of porphyrins. The compounds selected for this study constitute representatives of a wide range of porphyrin types. The L$^2$TOF mass spectra of octaethylporphyrins (OEPs), a number of natural biological porphyrins and tetrphenylporphyrins (TPPs) using 193 nm laser photoionisation will be discussed in the following section. These encompass porphyrins which contain pyrrole substituents, meso-substituents, or are the unmetallated free-base species. The systematic study of these different porphyrins reveals a number of shared mass spectrometric features. This is followed by a description of the comparative studies performed on the metallotetraphenyporphyrins and metallo-octaethylporphyrins using both 193 nm and 266 nm laser photoionisation. These investigations show that there are characteristic differences in the photodissociation products observed at each ionisation wavelength. An analysis of the photophysical processes responsible for these differences is then described before concluding remarks concerning the potential contribution of L$^2$TOFMS to porphyrin analysis.

6.2 L$^2$TOFMS of Porphyrins Using 193 nm Laser Photoionisation

In order to demonstrate the ability of L$^2$TOFMS to produce simple, readily interpretable mass spectra, a variety of porphyrin molecules was investigated using 193 nm laser photoionisation. Both soft and hard-ionisation mass spectra were obtained at this wavelength demonstrating the ability of the technique to provide molecular weight information along with important structural information about the macrocycle ring substituents. The results of these experiments are presented
in the following sections, each section being devoted to groups of similar porphyrin types. All the samples investigated were purchased from Aldrich and used as received.

6.2.1 L^2 TOFMS of Octaethylporphyrins (OEPs)

The L^2 TOFMS experiments that were carried out on the octaethylporphyrins utilised the linear time-of-flight instrument described to in Chapter 3. Sample desorption was achieved using the 532 nm second harmonic output from a Nd:YAG laser rather than the more commonly used 10.6 μm radiation from a CO_2 laser. Exploratory studies using this desorption wavelength were observed to produce spectra, for a variety of molecules, which were essentially identical to those obtained when desorbing with IR radiation. The structures and masses of the octaethylporphyrins used in these experiments are summarised in Table 6.1.

For all the octaethylporphyrins investigated (OEP, CoOEP, CuOEP, NiOEP and ZnOEP), soft ionisation at 193 nm resulted in the production of molecular radical cations with minimal fragmentation. This is illustrated for OEP, CoOEP and NiOEP in Figures 6.1a, b and c respectively. The spectra show similar features to those observed in the EI mass spectra of substituted porphyrins [14]. The base peak in all three spectra corresponds to the mass of the target species molecular ion and the fragmentation observed at this low laser power density arises mainly from β cleavage of peripheral substituents on the pyrrolic rings. These fragment ions are thought to be resonance stabilised in a manner similar to those formed by benzylic cleavage of benzene derivatives [13,14]. Comparison of the EI mass spectra of related compounds has allowed the relative stability of the various side chains commonly found in porphyrins to be assessed. A methyl substituent has been found to be a more stable substituent than an ethyl group, strengthening the argument for a benzylic fission mechanism. The mass spectra obtained using 193 nm laser photoionisation are not, however, identical to their EI counterparts. EI mass spectra of substituted porphyrins show two distinct groups of ions: a high m/z group of singly-charged species as observed in the MPI mass spectra and a
### Table 6-1: The structures and masses of the octaethylporphyrins investigated using L^2 TOFMS.

<table>
<thead>
<tr>
<th>OEPs</th>
<th>Mass (amu)</th>
<th>M</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free-base OEP</td>
<td>534</td>
<td>H,H</td>
<td>Ethyl</td>
</tr>
<tr>
<td>Cobalt OEP</td>
<td>591</td>
<td>Co</td>
<td>Ethyl</td>
</tr>
<tr>
<td>Copper OEP</td>
<td>596</td>
<td>Cu</td>
<td>Ethyl</td>
</tr>
<tr>
<td>Nickel OEP</td>
<td>591</td>
<td>Ni</td>
<td>Ethyl</td>
</tr>
<tr>
<td>Zinc OEP</td>
<td>598</td>
<td>Zn</td>
<td>Ethyl</td>
</tr>
</tbody>
</table>
lower m/z group of doubly charged species. The doubly charged ions observed in the El mass spectra of porphyrins are of comparable intensity to the singly charged ions, although the doubly charged molecular ion is not necessarily the most intense peak of its group. Due to the differences between the MPI and El mechanisms (see Chapter 2) these doubly charged ions are not observed in the L²TOFMS experiments carried out here.

The singly-charged fragments observed in Figures 6.1a-6.1c all have intensities of less than 20% and correspond to the successive loss of methyl radicals. Under soft-ionisation conditions fragment ions resulting from the loss of successive methyl radicals are of declining intensity. In the three examples shown here the [M-4CH₃]⁺ ion is present to a vanishingly small extent. It is important to note that the mass spectra of the nickel and cobalt metallo-OEPs exhibit similar features to that of the free base octaethylporphyrin, i.e. they undergo β-cleavage whilst retaining the metal atom in the macrocycle. This is a characteristic feature of the L²TOF mass spectra of metalloporphyrins obtained using 193 nm laser photoionisation. Further investigations have demonstrated that the retention of the metal atom is influenced by the incident photoionisation wavelength. This is discussed in more detail for the tetraphenylporphyrins and octaethylporphyrins in Section 6.3.

Increasing the ionising laser intensity results in further fragmentation of the molecular ions as shown for OEP, CoOEP and NiOEP in Figures 6.2a-6.2c respectively. The side-chains can be seen to fragment by losing methyl radical species until all eight ethyl substituents have undergone benzylic cleavage at the β position. For example, the free-base octaethylporphyrin (see Figure 6.2a) has strong ion signals at masses 534, 519, 504, 489, 474, 459, 444, 429 and 414 amu. These masses correspond to [M-nCH₃]⁺, where n = 0-8. After the loss of eight methyl groups the intensity of further fragment ions falls away rapidly. In all three spectra fragment ions which correspond to the break-up of the macrocycle are of low intensity. There is, however, some evidence of such fragmentation occurring. A series of low intensity peaks between 120 amu and 320 amu, as shown in Figure 6.2c, may correspond to the fragment ions resulting from fragmentation of the aromatic nucleus. Again, as anticipated from EI mass spectra, the metal
Figure 6-1: L²TOF mass spectra of a) OEP, b) CoOEP and c) NiOEP obtained under soft ionisation conditions using 193 nm laser photoionisation.
atom is retained until the macrocycle itself begins to fragment. These features of the hard-ionisation mass spectra are in accord with the stability of the aromatic nucleus, which allows extensive delocalisation of the positive charge. This means that the porphyrin macrocycle forms a stable support structure, allowing for the detailed study of side-chain fragmentation.

It is clear from these examples that using L²TOFMS one can induce the fragmentation of the macrocycle substituents, in a controllable manner, to produce mass spectra with distinctive features. For the OEPs a methyl radical is the characteristic neutral fission product for both the free-base porphyrin and the metallo-OEPs. The factors which influence the generation of specific fragment ions are the stability of the various bonds in the molecule and the relative stability of the ionic and neutral fragments. When using MPI rather than El the cleavage of a particular bond may not be just a result of the simple competition between these two factors but can also be dependent on the outcome of photophysical processes occurring in an intermediate excited state. This wavelength dependence adds a further dimension to L²TOF mass spectra. However, photoelectron spectroscopy of the metallo OEPs [39] has shown that their ionisation potentials lie in the range 6.09-6.39 eV. A single 193 nm (6.42 eV) photon may therefore be sufficient to achieve ionisation. This provides a plausible explanation for the similarity between the photo-induced fragmentation and that resulting from El.

Porphyrins preferentially fragment in such a way as to give even-electron daughter ions [14]. Photoionisation of the free-base OEP at 193 nm creates radical molecular cations. The favoured fragmentation from this molecular ion is therefore loss of an odd number of radical species to leave a stable, even number of electrons on the fragment ion. Close inspection of the hard ionisation mass spectrum in Figure 6.2a confirms this observation. Although the intensity differences are less dramatic than those observed in El mass spectra, it is still apparent that the peak intensities alternate between successive fission, products corresponding to the loss of odd numbers of methyl radicals being the more intense. It may be anticipated that the introduction of a metal into the OEP will change the fragmentation behaviour. In the case of the free-base OEP, ionisation occurs by
Figure 6-2: $L^2$TOF mass spectra of a) OEP, b) CoOEP and c) NiOEP obtained under hard ionisation conditions using 193 nm laser photoionisation.
removal of one electron from the \( \pi \)-system and, as observed, fragmentation proceeds by preferential loss of odd numbers of methyl radicals. If the the electron deficiency of the \( \pi \) system can be avoided by the removal of an electron from the metal it might be expected that preferential loss of even numbers of radicals will dominate. However, if alkyl or heterosubstituents are present, the aromaticity of the macrocycle need not be disturbed if ionisation occurs either by removal of a nonbonding electron from a hetero-atom, or with concomitant fragmentation of the alkyl substituent [41]. Alkylporphyrins conform to the latter case, yielding the benzylic \(-\text{CH}_2^+\). In such cases the fragmentation behaviour of the metal complex will not differ greatly from that of the uncomplexed compounds when using EI or single photon ionisation. This can be clearly seen in Figure 6.2b for CoOEP, where, again, the peaks corresponding to loss of odd numbers of methyl radicals are more intense than their even numbered counterparts. One exception to this is the peak corresponding to \([M-8\text{CH}_3]^+\) which is more intense than its neighbour corresponding to \([M-7\text{CH}_3]^+\).

The alternation of peak intensity is not as clearly visible in Figure 6.2c, for NiOEP. In this case, at the same hard ionising laser power density, the base peak of the mass spectrum is no longer the molecular ion but corresponds to \([M-8\text{CH}_3]^+\). The abundance of all fragment ions is dependent on both their rate of production and their rate of decomposition. An increase in the stability of the ion will, therefore, affect the latter rate and give an increase in the abundance of that species. In the case of NiOEP, the stability of the \([M-8\text{CH}_3]^+\) ion, even though it contains an odd number of electrons, is sufficient to assure its longevity and therefore its dominance in the spectra. Such stability is also observed for the molecular ion species. This is demonstrated again in Figure 6.3 for ZnOEP. At successively higher laser power densities the fragmentation pattern changes from having vanishingly small low mass fragment intensities, through a stage where all eight major fragments have approximately equivalent intensity, to a point at which \([M-8\text{CH}_3]^+\) dominates the fission products. Throughout this process the intensity of the higher mass fragments remains approximately constant and below 30% of the \(M^+\) intensity. Clearly the fragmentation is tending towards the production
of a particularly stable ion and thus the multiple loss of substituents gains in importance whilst the formation of even electron electron species becomes less important.

The response of CuOEP under both soft- and hard-ionisation conditions was markedly different. These spectra are shown in Figures 6.4a and 6.4b respectively. In both cases the mass spectra show fragmentation corresponding to successive loss of methyl radicals, as seen previously for both the free-base and metalloOEPs. A series of fragments up to \([M-3\text{CH}_3]^+\) can be observed in both spectra at masses 582, 567 and 552 amu. However, there is an anomalously intense peak present at mass 534 amu. This appears to correspond to the loss of the coordinated metal atom from the centre of the porphyrin macrocycle giving \([M-\text{Cu}]^+\). In Figure 6.4a the two fragment peaks at lower masses than this have masses of 519 amu and 504 amu, which corresponds to the successive loss of methyl radicals from the \([M-\text{Cu}]^+\) moiety. Under hard ionisation conditions the lower mass fragment species become more difficult to assign confidently as the fragments from both the molecular ion and the \([M-\text{Cu}]^+\) species are superimposed on one another. Under the present experimental conditions, the mass resolution attainable is not sufficient to enable the differentiation between neighbouring mass peaks. Inspection of Figures 6.1 and 6.2 reveals that the metallo-OEPs fragment more easily at a particular power density than the free-base porphyrin. This increased peripheral fragmentation in the case of the metal complexes is in accord with charge withdrawal from the periphery of the molecule by charge localisation on the metal atom. This means that the peaks below mass 534 amu, in Figure 6.4b, are most probably dominated by fragment ions derived from the parent metalloporphyrin.

CuOEP is clearly anomalous in the series of porphyrins discussed in this section. The reasons for this unusual behaviour are not clear at present. CuOEP has a strong absorption feature in its vapour phase absorption spectrum at 532 nm [40] suggesting that the copper free porphyrin may be a product of photodissociation occurring in the vaporisation process. However, \(L^2\)TOFMS experiments performed using 10.6 \(\mu\)m from a CO\(_2\) laser to desorb the CuOEP also generated spectra where the \([M-\text{Cu}]^+\) peak was present with significant intensity. The loss of the
Figure 6-3: L²TOF mass spectra of ZnOEP obtained using 193 nm laser ionisation with increasing laser pulse energies; a) ca. 0.5 mJ/shot, b) ca. 3 mJ/shot and c) ca. 6 mJ/shot.
Chapter 6. \textit{L}^2\textit{TOFMS of Porphyrins}

Figure 3-4: \textit{L}^2\textit{TOF mass spectra of CuOEP obtained under a) soft and b) hard ionisation conditions using 193 nm laser photoionisation.}
copper atom must therefore originate from either thermal decomposition during the desorption stage, photodissociation specific to CuOEP at 193 nm, or possibly be due to the photoionisation of impurities in the sample. CuOEP has also been seen to lose the coordinated metal atom when using 248 nm laser photoionisation. Therefore, it seems most likely that the anomalous peak represents an impurity or is a product of thermal decomposition. Such behaviour is not ubiquitous in copper containing porphyrin molecules. For example, the L_2 TOF mass spectra of copper tetraphenylporphyrin show no such demetallation at either 193 nm or 248 nm. The mass spectrometry of the CuTPP is discussed in more detail in Section 6.2.3.

6.2.2 L_2 TOFMS of Biological-Type Porphyrins

L_2 TOFMS has also been used to determine the mass spectra of a number of natural, extracted porphyrins and biological porphyrin derivatives. These include etioporphyrin I dihydrobromide, hemin, hematin, hematoporphyrin IX, chlorophyll a and a coppered chlorophyllin trisodium salt. The structures and masses of these materials are summarised in Table 6.2. All these species were examined using the reflectron TOF instrument described in Chapter 3 and desorbed using IR radiation from the Alltec CO_2 laser. As noted in the previous section, the dominant fragmentation pathways for peripherally substituted porphyrins involve the sequential loss of the macrocycle side-groups and the preservation, under soft-ionisation conditions, of the porphyrin nucleus. The results presented in the following section confirm this thesis for a variety of different molecules using 193 nm as the photoionisation wavelength.

The resolution of the mass spectra obtained during these experiments was insufficient to resolve neighbouring masses above 100 amu. Therefore, as seen in the previous section, the major fission processes can be observed in the mass spectra, e.g. benzylic cleavage with the accompanying loss of a side-chain radical, but the complex hydrogen rearrangements which occur concurrently, such as the loss of hydrogen radicals from the molecular ion, are not individually resolvable.
### Table 6-2: The structures and masses of biological-type porphyrins investigated using L²TOFMS.

For details concerning the entries marked with an asterisk refer to the complete chlorophyll structure shown in Figure 6.11a.

<table>
<thead>
<tr>
<th>Porphyrin</th>
<th>Mass (amu)</th>
<th>M</th>
<th>C</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>R₅</th>
<th>R₆</th>
<th>R₇</th>
<th>R₈</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etioporphyrin I (2HBR)</td>
<td>640</td>
<td>H,H</td>
<td>H</td>
<td>Methyl</td>
<td>Ethyl</td>
<td>Methyl</td>
<td>Ethyl</td>
<td>Methyl</td>
<td>Ethyl</td>
<td>Methyl</td>
<td>Ethyl</td>
</tr>
<tr>
<td>Hematoporphyrin</td>
<td>656</td>
<td>H,H</td>
<td>H</td>
<td>Methyl</td>
<td>CHOCH₃</td>
<td>Methyl</td>
<td>CH₃CH₂CO₂H</td>
<td>CH₂CH₂CO₂H</td>
<td>Methyl</td>
<td>Methyl</td>
<td>CH₃CHOH</td>
</tr>
<tr>
<td>Hemin</td>
<td>651</td>
<td>FeCl</td>
<td>H</td>
<td>Methyl</td>
<td>CH=CH₂</td>
<td>Methyl</td>
<td>CH₃CH₂CO₂H</td>
<td>CH₂CH₂CO₂H</td>
<td>Methyl</td>
<td>Methyl</td>
<td>CH=CH₂</td>
</tr>
<tr>
<td>Hematin</td>
<td>633</td>
<td>FeOH</td>
<td>H</td>
<td>Methyl</td>
<td>CH=CH₂</td>
<td>Methyl</td>
<td>CH₃CH₂CO₂H</td>
<td>CH₂CH₂CO₂H</td>
<td>Methyl</td>
<td>Methyl</td>
<td>CH=CH₂</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>893</td>
<td>Mg</td>
<td>o</td>
<td>Methyl</td>
<td>CH₃CH₃</td>
<td>Methyl</td>
<td>o</td>
<td>CH₂CH₂CO₂Phytol</td>
<td>Methyl</td>
<td>Methyl</td>
<td>CH=CH₂</td>
</tr>
<tr>
<td>Chlorophyllin</td>
<td>722</td>
<td>Cu</td>
<td>CH₂CO₂Na</td>
<td>Methyl</td>
<td>CH₃CH₃</td>
<td>Methyl</td>
<td>CO₂Na</td>
<td>CH₂CH₂CO₂Na</td>
<td>Methyl</td>
<td>Methyl</td>
<td>CH=CH₂</td>
</tr>
</tbody>
</table>
In the spectra presented here, these are contained within the broad, unresolved envelopes associated with the loss of larger fragments. The following discussion is therefore primarily concerned with the fragmentation processes which result in the loss of species larger than hydrogen, and reference to the facile hydrogen reactions will be made only where this has clearly affected the position of the mass spectral peak. All the reported masses were determined at the signal maxima. The mass spectrum of each molecule investigated is discussed in detail independently and the principal fragments observed in the L$^2$TOFMS spectra of these molecules are summarised at the end of this section in Table 6.3.

**Etioporphyrin**

As mentioned in the introduction, porphyrins are widely used as indicators of sedimentary maturity [42,43]. For example, the ratio between the concentrations of etioporphyrin (ETIO) and desoxophylloerythroetioporphyrin (DPEP) has been used as a sensitive test of source rock maturity [42]. In the light of this, L$^2$TOFMS was used to investigate the mass spectrometry of etioporphyrin I dihydrobromide. The soft ionisation L$^2$TOF mass spectrum of this is shown in Figure 6.5a. The base peak of the spectrum is at mass 478 amu and corresponds to the mass of the molecular ion without the two HBr groups. At this ionising laser power density there are only three other peaks of interest present, at mass 663, 448, and 433 amu. These correspond to the loss of successive methyl groups from the macrocycle sidechains. Etioporphyrin has both ethyl and methyl side-chains. It is most probable, from considerations of substituent stability, that these fission products are the result of benzylic cleavage of the ethyl group as discussed earlier for the OEPs. The hard ionisation mass spectrum is shown in Figure 6.5b. Under the experimental conditions employed here there are more fragment peaks present than in the soft ionisation mass spectrum. Their production is anticipated from the previous experiments performed on the OEPs. There is, however, a notable difference between the spectrum in Figure 6.5b and those obtained for the OEPs under hard ionisation conditions (Figures 6.2a, 6.2b and 6.2c). For etioporphyrin, even under hard ionisation conditions, the fragmentation of substituent groups re-
Figure 6-5: L^2 TOF mass spectra of etioporphyrin I dihydrobromide obtained under a) soft and b) hard ionisation conditions using 193 nm laser photoionisation.

Figure 6-5 shows the mass spectrum of etioporphyrin obtained under partially hard ionising conditions with an enlarged mass scale up to 1000 amu. In the central region of this spectrum are the peaks corresponding to the molecular ion and its fragments. However, another peak is observed at higher mass. This peak at

results in peaks of diminishing intensity. The strongest peak in the spectrum is that which corresponds to the loss of a single methyl group. It would therefore appear that the fragmentations do not lead to an especially stable structure such as that found at [M-8CH₃]^+ in the OEPs. The structure corresponding to [M-4CH₃]^+ would appear to confer no such stability in the case of etioporphyrin.
mass 956 amu corresponds to the etioporphyrin dimer. Both porphyrins and metalloporphyrins are known to aggregate in solution [44]. In free-base porphyrins the aggregation is a result of $\pi$-$\pi$ interaction in the absence of strongly aggregating substituents. In metalloporphyrins, these $\pi$-$\pi$ forces are often exceeded in magnitude by metal-ligand coordination interactions. In the case of ETIO the dimer is held together by a $\pi$-$\pi$ interaction. It is presently unclear at what point in the mass spectrometric procedure the dimerisation occurs. The aggregation could feasibly be occurring during desorption and entrainment, or as a result of an ion-molecule reaction in the ionisation region. However, the ETIO dimer is most probably due to desorbed molecules aggregating during their entrainment by the supersonic molecular beam. Dimer formation is most likely here, as the molecular beam will cause some cooling of the desorbed molecules in the region where the three body collision rate is sufficiently high for clustering to occur. Aniline clusters are also observed to be present in the molecular beam, as shown in Figure 6.6. Aniline was seeded into the molecular beam from a reservoir upstream of the nozzle and used as an internal calibrant. The peaks corresponding to the monomer, dimer and trimer aniline clusters, at masses 93, 186 and 279 amu respectively, are labelled on the spectrum.

**Hemin, hematin and hematoporphyrin**

The pyrrole pigments constitute the most abundant colouring materials in natural systems. Hemin, hematin and hematoporphyrin IX are all naturally occurring porphyrins. Hematoporphyrin IX is a free-base porphyrin, a photosensitiser and a precursor to the pigments in blood. Hemin and hematin are both heme derivatives. The heme group is the active centre of an important class of electron carriers called cytochromes; it consists of a porphyrin ring chelated to an iron atom. As the oxidation state of iron may be either +2 or +3 they are able to act as redox intermediates in electron transfer reactions. Thus, heme derivatives are present in mitochondria and take part in the reverse process of respiration by determining the ability of haemoglobin to bind oxygen. Such species are also responsible for blood’s distinctive red colour.
Figure 6-3: $L^2$TOF mass spectrum of etioporphyrin I dihydrobromide obtained under partially hard ionisation conditions using 193 nm laser photoionisation. The species labelled with an A correspond to aniline and its clusters.

Figures 6.7a and 6.7b show the soft and partially hard ionisation mass spectra of hematoporphyrin IX obtained using 193 nm laser photoionisation. In Figure 6.7a, there are three major peaks at 598, 580 and 562 amu. The first of these corresponds to the molecular ion, whilst the latter two correspond to successive loss of water from the molecular ion. These peaks are most probably a result of the dehydration of the secondary alcohol substituents present on the pyrrolic groups. Similar fragmentation has been observed in EI studies [14] and has more recently been reported using electrospray ionisation (ES) [24]. It is also possible, however, that some dehydration is associated with the loss of water from one of the two acid groups concurrent with the formation of an anhydride. Previous EI studies have not been able to determine whether this dehydration is thermally induced in the source or a facile fragmentation resulting from the ionisation process. The $L^2$TOFMS experiment, however, furnishes evidence which suggests that the dehydration is a product of the desorption process rather than fragmentation resulting from photoionisation. This is discussed below.

The spectrum in Figure 6.7a is plotted with an expanded mass scale, from 0 to
Figure 6-7: L^3TOF mass spectra of hematoporphyrin IX obtained under a) soft and b) hard ionisation conditions using 193 nm laser photoionisation. The species labelled with an A corresponds to the aniline molecular ion.
1300 amu, in order to show that hematoporphyrin IX, like etioporphyrin discussed above, forms molecular dimers during the L^2TOFMS mass spectrometric procedure. The bonding is, once again, likely to be a result of π-π interaction between the porphyrin macrocycles, although the polar sidechains may also influence the nature of the interaction in this case. The three high mass peaks are centered at mass 1124, 1142 and 1160 amu. These correspond to the species \([M-2H_2O]_2\), \([M-H_2O]_2[H-2H_2O]\) and \([M-H_2O]_3\), respectively. Such species could be formed in either the entrainment process or in the ionisation region. As discussed above the most probable process in which they are generated is that of supersonic molecular beam entrainment. It can therefore be postulated that the dehydration is a result of thermal processes operative in the desorption stage. This accounts for the presence of three intense signals in the molecular ion region even under soft ionising conditions. On reducing the ionising laser intensity the intensity of all three peaks is diminished, confirming that they are not fragments of the molecular ion. Three other peaks are observed in the spectrum at mass 93, 186 and 279 amu; these correspond to aniline monomers, dimer and trimer.

Further evidence for the thermal dehydration of the hematoporphyrin molecule can be seen in the partially hard ionisation mass spectrum in Figure 6.7b. Under these ionisation conditions, a number of new, intense ion peaks can be observed in the mass spectrum. Close inspection reveals that two clusters of peaks, at 521, 539, 503, and 462, 440, 422 amu, are present whose intensities mimic those of the 598, 580 and 562 amu peaks. This suggests that the fragments are derived independently from the three molecular species in the soft ionisation mass spectrum, confirming the presence of the latter as neutral species in the ionisation region. The dehydration is not, therefore, a result of the stepwise fragmentation of the molecular ion but of neutral thermal dissociation during evaporation. The fragment ions which are a result of the photofragmentation of the three molecular ions are due to the successive loss of \([CH_2CO_2H]\) radicals from the carboxylic acid sidechains. This is the familiar benzylic cleavage \(β\) to the porphyrin macrocycle. The fragmentation pathways which are apparent from Figure 6.7b are summarised schematically in Figure 6.8.
Figure 6–8: Scheme summarising the principal fragmentation products obtained from the hematoporphyrin IX molecular ion using 193 nm laser photoionisation. All masses shown are in amu.
Hemin and hematin are structurally similar to hematoporphyrin. The most significant differences are, firstly, that the secondary alcohol groups are replaced by unsaturated ethene substituents and, secondly, that the free base porphyrin hydrogen atoms are replaced with a coordinated iron atom. The Fe(III) is ligated to a chlorine atom in hemin and to a hydroxy group in hematin (see Table 6.2). These structural similarities result in similar L²TOF mass spectra. Figures 6.9a and 6.9b show the soft and partially hard photoionisation mass spectra of hemin, respectively. Under soft ionisation conditions, the mass spectrum consists of two principal peaks at 651 amu and 616 amu. These correspond to the molecular ion and to the \([M-Cl]^+\) ion, respectively. Hematin shows the same fragmentation. However, loss of the -OH group on the coordinated Fe(III) appears to be a more facile process in this case and the molecular ion is of negligible intensity. Both molecules undergo further fragmentation from the porphyrin residue of mass 616 amu. The fragmentation pathways for this species under partially hard ionising conditions are shown schematically in Figure 6.10. The two highest mass fragments at 571 amu and 557 amu correspond to the species \([M-Cl-COOH]^+\) and \([M-Cl-CH_2COOH]^+\), respectively. The peaks observed at 526 amu and 498 amu correspond to the loss of either 2[COOH] or 2[CH_2COOH] and the peak at 512 amu corresponds to the loss of [CH_2COOH] from one of the acid side-groups and [COOH] from the other. Lower mass fragments therefore correspond to the partial or complete loss of the remaining side groups. As the vinyl side group has been determined by EI to be one of the most stable substituents, it is probable that the methyl substituents are lost first.

Chlorophyll a

Chlorophylls are porphyrins which are found widely in natural systems. The chlorophyll ring system is a porphyrin in which a double bond on one of the pyrrole rings has been reduced. A fused cyclopentanone is also present. The four nitrogens of the porphyrin ring are chelated to a magnesium atom and the reduced pyrrole ring has a phytol substituent consisting of a highly hydrophobic 20-carbon alcohol esterified to an acid side chain (see Table 6.2). The complete structure
Chapter 6. L²TOFMS of Porphyrins

Figure 6-9: L²TOF mass spectra of hemin obtained under a) soft and b) hard ionisation conditions using 193 nm laser photoionisation.
Figure 6-10: Scheme summarising the principal fragmentation products obtained from the hemin molecular ion using 193 nm laser photoionisation.
Chapter 6. \( L^2 \)TOFMS of Porphyrins

of chlorophyll a is shown in Figure 6.11a. Chlorophyll a is the principal photoreceptor in the chloroplasts of plant material. The photosynthetic unit consists of two parts: the light harvesting complex and the reaction centre. The light harvesting complex consists of an array of chlorophyll molecules and other pigments which efficiently absorb radiation. The reaction centre consists of chlorophyll pigment which undergoes photochemical electron transfer. The energy harvested by antenna chlorophylls in the light harvesting complex is conducted to a single reaction centre by electronic energy transfer during photosynthesis. As chlorophylls absorb light in the far red region it is the main source of green pigment in living vegetation. The proprietary chlorophyll a used to produce the mass spectra discussed here was produced by extraction from spinach.

Intact chlorophylls have been investigated previously using \(^{252}\)Cf-plasma desorption [45], field desorption [19], electron impact [46] and laser desorption mass spectrometry [47]. However, these techniques produced only quasi-molecular ions and adduct ions and no intact molecular ions. Figures 6.11a and 6.11b show the soft ionisation and partially hard ionisation mass spectra of chlorophyll a, deposited from a methanolic solution, using 193 nm laser photoionisation. The soft ionisation mass spectrum, Figure 6.11a, clearly demonstrates the ability of \( L^2 \)TOFMS to investigate fragile biomolecules. It contains only two mass spectral peaks of significant intensity. The base peak of the spectrum at 893 amu corresponds to the molecular ion of chlorophyll a. The main fragment is the result of the loss of the phytol side-chain which, with hydrogen attachment, leads to the peak at 615 amu. There are also two other less intense peaks in the vicinity of the molecular ion. The peak at 908 amu can be assigned to the molecular ion of 10-hydroxychlorophyll a, which is a well known oxidation product formed during the extraction procedure [48]. The origin of the peak at 862 amu is not as clear, but can be assigned to the loss of \([\text{CH}_3\text{O}]\) from the ester side-chain on ring 5, the fused cyclopentanone species. Two further low intensity peaks are present at 583 amu and 539 amu. The fragment at 583 amu can be assigned to the loss of \([\text{CH}_3\text{O}+\text{H}]\) from the 615 amu fragment or loss of phytol from the species at mass 862 amu. At present, the origin of the peak at 539 amu is unclear. No fragmentation of the
Chlorophyll a has previously been examined using L²TOFMS [49] employing CO₂ laser desorption but ionising with 275 nm photons. The fragmentation obtained under soft ionising conditions using this wavelength was essentially the same as that reported above. However, when using 275 nm, an intense peak was observed at 870 amu which corresponds to the molecular weight of phaeophytin a. The structure of this species is shown in Figure 6.12. This molecule is essentially the free-base precursor to chlorophyll a, where the central magnesium atom has been replaced by two hydrogen atoms. Grotemeyer et al. [50] postulate that the formation of such a species may occur in either the work-up procedure or during the vaporisation step. A comparison with the data in Figure 6.11a, where no such peak is present, would suggest that the loss of the central metal atom is in fact an ionising wavelength dependent phenomenon. Using 193 nm as the ionising laser wavelength no peak is observed at 870 amu, whilst when using 275 nm the metal atom is lost. The identity of the phaeophytin a was confirmed by Grotemeyer et al. using higher resolving power to identify the isotopic fingerprint of this species. This wavelength dependent demetallation of the metalloporphyrins is previously unreported or misinterpreted in the literature. In the present study further examples of such behaviour, where demetallation competes with stepwise fragmentation of the molecular ion have been determined. These are discussed more fully in Section 6.3.

By increasing the ionisation laser power, the number of fragments observed in the mass spectrum of chlorophyll a is increased. A partially hard ionisation mass spectrum is shown in Figure 6.11b. The principal fragmentation pathways are shown schematically in Figure 6.12. Under partially hard ionisation conditions the molecular ion peak is no longer the base peak in the spectrum. In Figure 6.11b the base peak is at 482 amu. This peak can be explained by the loss of [CH₃OOC] from ring 5 along with benzylic cleavages at ring 4 and ring 2 for the phytol ester and the ethyl substituents, respectively. Such benzylic cleavages are anticipated to be dominant over competing fragmentation pathways in the light of the results.
Figure 6-11: $L^2$TOF mass spectra of chlorophyll a obtained under a) soft and b) partially hard ionisation conditions using 193 nm laser photoionisation.
discussed previously. The decomposition pattern below 482 amu is consistent with the further loss of aliphatic side-groups, as the peaks are separated by either 14 or 15 mass units. Fragments with masses below 370 amu may correspond to either the fragmentation of the macrocycle or the partial loss of the fused cyclopentanone ring 5.

The largest peak, after that at 482 amu, is at 615 amu. This is the principal fragment observed previously in the soft ionisation mass spectrum (Figure 6.11a). These two species are clearly the most stable products resulting from the stepwise fragmentation of the molecular ion. The fragment at 862 amu and the 10-hydroxychlorophyll a species at 908 amu are also present in the partially hard ionisation mass spectrum. There is also a low intensity peak at 834 amu which corresponds to the complete loss of the ester side-chain, \([\text{CH}_3\text{OOC}]\), from ring 5 of the molecular ion. The fragmentation apparent between the intense peaks at 615 amu and 482 amu can be assigned to products from the fission of either aliphatic side-chains or ester groups from the 615 amu moiety. The moderately intense peak at 279 amu is not associated with the target chlorophyll a molecule but is an aniline trimer. Only the mass range from 200 amu to 1000 amu is shown in Figure 11a as the lower mass region is dominated by aniline, its dimer and their fragments.

**Chlorophyllin**

Figure 6.13 shows the L²TOF mass spectrum of the trisodium salt of coppered chlorophyllin under partially hard conditions. This compound is different from the others discussed in this section in that the side-chains are not acids or esters but the sodium salts of these functionalities. If the sodium counter ions were to dissociate from the molecule during the desorption process, leaving an ionic macrocycle, then no signals would be observed in the mass spectrum as they would be unable to penetrate the fixed field of the extraction region. However, it is clear from Figure 6.13 that this is not the case, since a number of peaks can be observed in the mass region between 400 amu and 650 amu which correspond to fragments of the chlorophyllin molecular ion. In contrast to the chlorophyll a mass spectra, there
Figure 6-12: Scheme summarising the principal fragmentation products obtained from the chlorophyll a molecular ion using 193 nm laser photoionisation.
Figure 6-13: L²TOF mass spectra of the trisodium salt of coppered chlorophyllin obtained under partially hard ionisation conditions.

There is no evidence of the molecular ion peak at 722 amu. The spectrum is normalised to the intensity of the largest chlorophyllin fragment at 495 amu. This is not the base peak of the mass spectrum. The true base peak is at mass 23 amu which corresponds to the sodium cation. Other intense signals in this low mass region are at 39, 93, 186 and 279 amu, corresponding to the potassium cation, aniline, aniline dimer and aniline trimer respectively.

The main fragmentation pathways, along with the molecular structure, are shown schematically in Figure 6.14. The most important fission products can be assigned to the loss of part or all of the carboxylate salt groups. A loss of three $[\text{NaO}_2\text{C}]$ groups would result in the species found at mass 522 amu, whilst the loss of two such species from the meso position on the porphyrin ring and at ring 3, along with $\beta$ benzylic cleavage at ring 4 would generate the fragment of mass 507 amu. Meso substituents are recognised through EI studies [14] as undergoing different fragmentation compared to those on the periphery of the porphyrin pyrrolic groups. For example, peripheral formyl groups hardly participate in fragmentation whilst meso-formyl groups lose CO to an appreciable extent. It is therefore possible that the base peak at 495 amu is a product of the following substituent
fragmentations: the loss of \([\text{Na}_2\text{CCH}_2]\) from ring 4, the loss of \([\text{Na}_2\text{C}]\) from ring 3 and the loss of \([\text{CH}_2\text{CO}_2\text{Na}]\) from the meso-substituent with back transfer of two hydrogen atoms. Similar behaviour has been reported previously [51] and was shown to occur more readily when the neighbouring pyrrolic carbons carry ester side chains. That this even electron product species at 495 amu is relatively stable is evidenced by its presence in the spectrum as the base peak.

Fragment ions with masses below 495 amu correspond to the successive loss of alkyl substituents from the porphyrin macrocycle. The first of these is likely to result from benzylic cleavage at the \(\beta\) position of the ethyl substituent, on the basis of group stability considerations, followed by the loss of the four methyl substituents. These fragment peaks are observed with gradually diminishing intensity at masses 480, 465, 450 and 435 amu. The origin of the peaks at masses above 522 amu are, at present, not readily interpretable. It would seem likely, however, that they are the result of either cleavage or rearrangement reactions involving the carboxylate salt groups. Attempts to record softer mass spectra resulted only in an intensity reduction for all peaks. No molecular ion was seen, even under conditions of threshold ionising laser power density. This suggests that at least some of the fragmentation processes may be the result of thermal decomposition in the desorption process.

The mass spectral fragments corresponding to the loss of all or part of the porphyrin substituents are summarised in Table 6.3 for all the molecules discussed in this section under partially hard ionisation conditions. Fragments corresponding to the break-up of the macrocycle are not listed. It can be concluded from this investigation of a number of peripherally substituted biological porphyrins that at 193 nm the fragmentation pathways observed parallel that seen in EI mass spectrometry. The principal loss mechanisms include the loss of any ligand coordinated to the metal other than the porphyrin macrocycle, \(\beta\)-cleavage of the peripheral substituents and the retention of the chelated metal. Furthermore, it has been observed that fragmentation is a much more facile process in metallated porphyrins than in the free-base species. It is clear from the results summarised
Figure 6-14: Scheme summarising the principal fragmentation products obtained from the trisodium salt of coppered chlorophyllin using 193 nm laser photoionisation.
in Table 6.3 that specific structural information about the nature of peripheral substituents can be obtained using L²TOFMS.

6.2.3 L²TOFMS of Tetraphenylporphyrins (TPPs)

The free-base and metallotetraphenylporphyrins (TPPs) discussed in the following section were investigated using the reflectron TOF instrument described in Chapter 3. The Alltec CO₂ laser was used for desorption and the 193 nm ionising radiation was generated using the ArF line of the Lumenics excimer laser fitted with stable, rather than unstable, resonator optics. The structures and masses of the compounds investigated are summarised in Table 6.4. These compounds differ from the porphyrins discussed previously in this chapter in that all four substituents are located symmetrically about the porphyrin nucleus at the meso positions, not at the peripheral pyrrolic sites.

For all the TPPs investigated (TPP, NiTPP, ZnTPP, CoTPP, CuTPP and VOTPP), soft ionisation of the laser desorbed molecules using 193 nm photons resulted in a mass spectrum containing an intense peak in the molecular ion region and minimal fragmentation. This is illustrated for TPP, CoTPP and NiTPP in Figures 6.15a, 6.15b and 6.16c, respectively. These spectra are shown only over the mass region containing the principal fragments. Photoelectron spectroscopy of the TPPs [52] has shown that their ionisation potentials lie in the range 6.3-6.5eV. A single 193 nm (6.42 eV) photon may therefore be sufficient to achieve ionisation. In the L²TOFMS experiments it is difficult to determine reliably the dependence of ion intensity on laser power density because of signal fluctuations arising from the desorption process. However, an approximate value of 1.4±0.2 has been obtained for the power density dependence. This value, whilst not conclusive, suggests that a (1+1), rather than a single photon excitation scheme is operative.

Increasing the ionising laser intensity caused increased photofragmentation of the molecular ion as shown in Figures 6.16a, 6.16b and 6.16c for the free-base TPP, CoTPP and NiTPP, respectively. The predominant fragment species are associated with the loss of one or two phenyl groups from the meso positions on
Table 6-3: Composition of principal fragment ions produced by L²TOFMS using 193 nm laser photoionisation for a variety biological porphyrins.

<table>
<thead>
<tr>
<th>Etioporphyrin I Dihydrobromide</th>
<th>Hematin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (amu)</td>
<td>Fragment Composition</td>
</tr>
<tr>
<td>478</td>
<td>[M-2HBr]</td>
</tr>
<tr>
<td>463</td>
<td>[M-CH₃]</td>
</tr>
<tr>
<td>448</td>
<td>[M-2(CH₃)]</td>
</tr>
<tr>
<td>433</td>
<td>[M-3(CH₃)]</td>
</tr>
<tr>
<td>418</td>
<td>[M-4(CH₃)]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hematoporphyrin IX</th>
<th>Chlorophyll a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (amu)</td>
<td>Fragment Composition</td>
</tr>
<tr>
<td>598</td>
<td>[M]</td>
</tr>
<tr>
<td>580</td>
<td>[M-H₂O] = X</td>
</tr>
<tr>
<td>562</td>
<td>[M-2(H₂O)] = Y</td>
</tr>
<tr>
<td>539</td>
<td>[M-CH₂CO₂H]</td>
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<td>521</td>
<td>[X-CH₂CO₂H]</td>
</tr>
<tr>
<td>503</td>
<td>[Y-CH₂CO₂H]</td>
</tr>
<tr>
<td>479</td>
<td>[M-2(CH₂CO₂H)]</td>
</tr>
<tr>
<td>462</td>
<td>[X-2(CH₂CO₂H)]</td>
</tr>
<tr>
<td>444</td>
<td>[Y-2(CH₂CO₂H)]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hemin</th>
<th>Coppered Chlorophyllin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (amu)</td>
<td>Fragment Composition</td>
</tr>
<tr>
<td>651</td>
<td>[M]</td>
</tr>
<tr>
<td>616</td>
<td>[M-Cl]</td>
</tr>
<tr>
<td>571</td>
<td>[M-Cl-COOH]</td>
</tr>
<tr>
<td>557</td>
<td>[M-Cl-CHCO₂H]</td>
</tr>
<tr>
<td>526</td>
<td>[M-2(CO₂H)]</td>
</tr>
<tr>
<td>512</td>
<td>[M-CH₂CO₂H-CO₂H]</td>
</tr>
<tr>
<td>498</td>
<td>[M-2(CH₂CO₂H)]</td>
</tr>
<tr>
<td>Porphyrins</td>
<td>Mass (amu)</td>
</tr>
<tr>
<td>-----------------------</td>
<td>------------</td>
</tr>
<tr>
<td>TPP</td>
<td>614</td>
</tr>
<tr>
<td>NickelTpp</td>
<td>671</td>
</tr>
<tr>
<td>ZincTPP</td>
<td>677</td>
</tr>
<tr>
<td>CobaltTPP</td>
<td>671</td>
</tr>
<tr>
<td>CopperTPP</td>
<td>675</td>
</tr>
<tr>
<td>VanadylTPP</td>
<td>679</td>
</tr>
<tr>
<td>Tetramethoxyphenylporphyrin</td>
<td>824</td>
</tr>
</tbody>
</table>

Table 6–4: The structures and masses of the tetraphenylporphyrins (and a tetramethoxyphenyl derivative) investigated using $L^2$TOFMS,
Figure 6-15: L²TOF mass spectra of a) TPP, b) CoTPP and c) NiTPP obtained under soft ionisation conditions using 193 nm laser photoionisation.
Figure 6-16: L²TOF mass spectra of a) TPP, b) CoTPP and c) NiTPP obtained under hard ionisation conditions using 193 nm laser photoionisation.
the porphyrin nucleus. Even at a very high power density, no peak is observed which corresponds to the loss of a third phenyl group. This fragmentation pattern is characteristic of all the TPP compounds examined using 193 nm laser photoionisation. Under both soft and hard ionising conditions, the metalloTPPs are found to retain their chelated metal atom. (CoTPP has a minor peak at 614 amu which may correspond to the replacement of the central metal atom with two hydrogens to give free-base TPP. This is clearly not the principal fragmentation pathway as at a high laser power density its intensity is not appreciably increased.) The retention of the metal atom by CuTPP is in direct contrast to the behaviour of the CuOEP molecule as discussed earlier. CuTPP has been shown to retain its coordinated metal atom even when desorbing with 532 nm radiation, thereby confirming that the loss of Cu from CuOEP is a feature particular to that molecule.

Although the resolution of the mass spectra is insufficient to enable the differentiation of neighbouring mass peaks, it can be seen that there are important differences between the mass spectra obtained for free-base TPP and its metal counterparts. TPP itself fragments preferentially by losing a single phenyl substituent from the molecular ion giving a peak at 537 amu, see Figure 6.15a. It is likely that a degree of hydrogen loss is associated with this fragmentation but is concealed under the unresolved peak envelope. Under hard ionisation conditions, see Figure 6.16a, the molecular ion peak remains the most intense whilst other fragment peaks can be observed, which correspond to the loss of a further phenyl group, at 460 amu and further losses of fragment species from the molecular ion. Fragment ions to lower mass than the [M-2Ph]+ daughter ion consist of a series of peaks separated by 12 or 13 mass units. This sequence of fragmentations results in the generation of low mass ions. These correspond to the complete fragmentation of the porphyrin nucleus.

The metalloTPPs appear to show similar fragmentation patterns to that of TPP. On closer inspection, however, it is apparent that the peak maxima for these molecules are generally shifted from their expected molecular weight values. For example, in the soft ionisation spectra of CoTPP and NiTPP the "molecular ion" peak maxima were measured to be at 669 amu and 670 amu, respectively.
These masses are one or two units lower than the calculated molecular weights of the target compounds. It has previously been reported from both EI studies [53] and laser desorption Fourier transform mass spectrometry [36] that metalloTPP chelates exhibit fragmentation patterns which include the loss of up to 8H from [M]+, the most intense of these fragments being those which correspond to the loss of even numbers of radicals. This is in contrast to free-base TPP and the OEP. The loss of so many radicals can be explained by considering the stability of the product ion. In the case of the metalloTPPs, the \( \pi \)-electrons of the phenyl groups are interactive with the porphyrin \( \pi \) electron system, and together form a larger composite electron system with a stable number of electrons (26 from porphyrin and 24 from the phenyl groups) totalling 50 in all. This number conforms to the criteria for a conjugated Huckel ring system, expressed as 4S+2. Such a system may be expected to be very stable. All the stable, singly charged ions are likewise stabilised by containing a composite macrocyclic ring with an effective number of \( \pi \) electrons expressible as 4S+2. Meot-Ner et al. [41] suggest that when a complexed metal atom is present an odd number of electrons in the [M]+ \( \pi \)-system can be avoided by the removal of a non-bonding electron from the metal. The metalloTPP molecular ions therefore retain 4S+2 electrons and the subsequent fragmentation will preferentially involve the loss of even numbers of radical species. Furthermore, whenever possible, a new stable ion will be formed which is super-stable. A super-stable number of electrons is expressed by 4S+2 where S = 4r+2 (e.g. r = 1, 4S+2 = 26 for porphyrins). In the metalloTPP EI mass spectra some of the more stable ions fit this rule, such as [M-8H]+. The consequence of such facile loss of hydrogen on the L\(^2\)TOF mass spectra is a shift in the peak maxima to lower masses than anticipated. If it were possible to fully resolve the cluster of peaks around the molecular ion in Figures 6.15a and 6.15b, a series of peaks corresponding to the loss of hydrogens from the molecular ion would be apparent.

A further distinction between TPP and its metal derivatives is apparent on closer inspection of the major fragment peaks observed under both soft and hard ionisation conditions. Clearly, the fragmentation associated with loss of phenyl groups is more facile in the case of the metal containing molecules. In fact,
situ metal attachment to free-base porphyrins during laser desorption has been suggested as a useful analytical strategy, designed to enhance photodissociation [36]. As suggested for the molecular ion, the fragment peak envelopes are likely to contain a number of different species corresponding to simultaneous H loss. In the soft ionisation spectra for CoTPP and NiTPP, Figures 6.15b and 6.15c, the peak maxima of the two most intense fragments correspond to [M-Phenyl-H]+ and [M-2Phenyl]+, measured as their difference from the molecular ion peak maximum. In the case of the hard ionisation mass spectra in Figures 6.16b and 6.16c, the fragment peak maxima correspond to [M-Phenyl-3H]+ and [M-2Phenyl-2H]+. Again, this is what would be expected on the basis of the EI mass spectra, the loss of even numbers of fragment radicals conforms to the assumptions of Meot-Ner et al. A number of these major stable product ions can be explained by the super-stable π-electron argument outlined above. For example, [M-Phenyl-H]+ contains 42 electrons in accordance with 4S+2 = 42 when r = 2.

The L²TOF mass spectrum of ZnTPP obtained using hard photoionisation conditions is shown in Figure 6.17. This mass spectrum includes the whole mass range covered by ZnTPP and its fragments. Under these ionising conditions fragmentation proceeds down to a peak at 12 amu corresponding to [C]+. It is important to note that the relative intensities of the principal fragments, [M-Phenyl-3H]+ and [M-2Phenyl-2H]+, with respect to that of the molecular ion remain constant under different hard ionisation conditions. If [M-Phenyl-3H]+ were a precursor to the [M-2Phenyl-2H]+ species, then one would expect to see a relative change in intensity between these peaks and the molecular ion. As this is not the case, it would appear that these fragments are not formed in a consecutive process, although the loss of [Phenyl-3H]+ is obviously a more facile process. The structure of the ions resulting from the fragmentation of the macrocyclic ring are not known at present. However, such fragmentation appears to be characteristic of the hard ionisation mass spectra of the porphyrin nuclei and has been seen previously for the OEPs and etioporphyrin.

It is clear from the results discussed above that free-base and metalloTPPs produce L²TOF mass spectra which are similar in nature to those obtained using
Figure 6-17: L$^2$TOF mass spectrum of ZnTPP obtained under hard ionisation conditions using 193 nm laser photoionisation.

EI. The principal differences being that no doubly charged ions are generated using 193 nm, photoionisation and at high laser power densities the porphyrin nucleus undergoes complete dissociation. The final example in this section is the methoxy derivative of the iron chloride TPP. The structure and mass of this compound are shown in Table 6.4. Soft and hard ionisation mass spectra, obtained under the same conditions employed for the TPPs, are shown in Figures 6.18a and 6.18b, respectively. The fragmentation pattern observed here is more complicated than for the TPPs due to the involvement of both the Cl and methoxy substituents. In the soft ionisation mass spectrum, the base peak corresponds to the intact molecular ion, and the principal fragmentation pathway involves the loss of the chlorine atom coordinated to the central iron atom. These are also the most intense peaks in the hard ionisation mass spectrum. In EI studies the molecular ion is found to be the most intense peak and the major fragmentation pathway proceeds as [M-OMe]$^+$, [M-Phenyl-OMe]$^+$ and [M-Phenyl-2OMe]$^+$, etc [53]. Such a progression is not as readily identified in Figure 6.18b. However, the major peaks do correspond to fragmentation of the molecular ion. The peaks at 610 amu and 547 amu correspond to the fragments [M-2PhenylOMe]$^+$ and [M-2Phenyl-4OMe]$^+$.
Figure 6-18: L²TOF mass spectra of 5,10,15,20 tetrakis (4-methoxyphenyl) 21H,23H porphine iron (III) chloride under a) soft and b) partially hard ionisation conditions using 193 nm laser photoionisation.
respectively. A series of low intensity peaks above 610 amu appear to be due to fragmentation of the [M-Cl]⁺ species and correspond to species of the type [M-Cl-Phenyl-OMe]⁺ with successive loss of Me and OMe. The resolution is insufficient to enable identify the neighbouring peaks, but it is likely that these also involve back transfer of hydrogen to the ionic fragment. The single, anomalous peak at 279 amu is due to the aniline trimer.

### 6.3 Laser Photodissociation of Metalloporphyrins

The previous sections have described the detailed analysis of the L²TOF mass spectra of a variety of substituted porphyrins. It has been found that photoionisation using a wavelength of 193 nm is capable of almost universally ionising porphyrin molecules. At this ionising wavelength the porphyrin molecules appear to undergo molecular photoionisation followed by photofragmentation. Only metallo compounds coordinated to ligands other than the macrocycle, or porphyrins with readily dehydrated substituents, may not conform to this description. In the case of the teraphenylporphyrins and the octaethyl porphyrins it is clear from their soft and hard ionisation mass spectra that photoionisation precedes photofragmentation. Such behaviour has been defined as “Class A” by Gedanken et al. [54] (see Chapter 2 for more details). However, a molecule can also exhibit “Class B” behaviour in its photochemistry. In this case, photodissociation of the molecule in its excited intermediate state precedes photoionisation of the neutral fragments. In the following sections it will be shown that, as a result of their diverse photophysical properties, the metallotetraphenylporphyrins and the metallo-octaethylporphyrins can exhibit both classes (Class A and Class B) of photochemical behaviour.

As a result of their low volatility, the photophysics and photochemistry of metalloporphyrins has only been rarely studied in the gas phase. The only extensive study of the gas-phase spectroscopy of metalloporphyrins is that of Edwards et al. who investigated the absorption spectra (from 200 nm to 800 nm)
of tetraphenylporphyrins [55], octaethylporphyrins and etioporphyrins [56]. The fluorescence excitation spectra and excited state lifetimes of free-base, Zn- and Mg-tetraphenylporphyrin have also been investigated under jet-cooled conditions by Even et al. [57]. The flexibility of the \textit{L}^2\textit{TOFMS} methodology means that it is possible to laser desorb a series of intact, neutral metalloporphyrrin molecules and investigate their photodissociation and photoionisation using different ionising wavelengths. In the work described in the following section a comparison is presented of the mass spectra obtained using 266 nm or 248 nm laser photoionisation with those resulting from 193 nm laser photoionisation.

6.3.1 \textit{L}^2\textit{TOFMS} of Metallotetraphenylporphyrins using 266 nm Laser Photoionisation.

As described previously, for all the metallotetraphenylporphyrins investigated (NiTPP, ZnTPP, CoTPP, CuTPP and VOTPP), soft ionisation at 193 nm results in the production of molecular radical cations with little or no fragmentation (see Figure 6.15), whilst increasing the ionising laser intensity causes increased photofragmentation (see Figure 6.16). This photodissociation of the molecular radical cation results in the loss of successive phenyl substituents and the metal is retained in the macrocycle. Photoionisation of CuTPP and ZnTPP at 266 nm produced similar results. Figure 6.19a shows the soft ionisation mass spectrum obtained for CuTPP. It can be seen that the molecular ion is produced exclusively. Increasing the ionising laser intensity results in fragmentation associated with successive loss of phenyl groups, as illustrated in Figure 6.19b.

However, the behaviour of CoTPP, NiTPP and VOTPP following 266 nm photoionisation is markedly different. As shown in Figures 6.20a, 6.20b and 6.20c, the mass spectra of these latter metallotetraphenylporphyrins, under soft ionisation conditions, show an intense fragment ion peak due to loss of the metal (or metal plus oxygen in the case of VOTPP) from the macrocycle, whilst the phenyl substituents are retained. This metal-free fragment ion is the base peak in each case, with the molecular ion appearing only weakly. Loss of the central metal atom appears to be
accompanied by the addition of 2Hs resulting in a peak at 614 amu corresponding to the free-base TPP radical cation. This fragmentation was observed even at threshold irradiances, the intact molecular ion peak being the first to vanish at lower laser intensities.

Increasing the 266 nm ionising laser intensity had the effect of slightly increasing the molecular ion intensity, relative to that of the metal-free fragment, in the case of CoTPP and NiTPP. This effect is much more pronounced in the case of VOTPP as can be seen in Figures 6.21a, 6.21b and 6.21c. At an ionising laser power density of ca. 0.2 MW cm\(^{-2}\) the VOTPP molecular ion peak is less than 30% of the intensity of the free-base fragment ion. Increasing the laser power density to ca. 0.6 MW cm\(^{-2}\) results in an increase in the molecular ion intensity to ca. 60% of the fragment intensity as seen in Figure 6.21b. A further increase in the ionising laser power density to ca. 3.6 MW cm\(^{-2}\) produces a molecular ion signal which is now the base peak in the spectrum, see Figure 6.21c. In this latter spectrum a number of other fragments are apparent. These correspond to the loss of phenyl groups from the molecular ion, or loss of the phenyl groups and the oxygen ligand from the chelated vanadium metal.

As the ionisation potentials of the metalloTPPs lie in the range 6.3 - 6.5 eV, it is clear that the absorption of two 266 nm (4.6eV) photons is required to effect ionisation. The results obtained on photoionising these species at 266 nm can be explained in terms of the competition between Class A and Class B behaviour in the intermediate state after the absorption of a single photon. As demonstrated in Figure 6.19 for CuTPP, Class A behaviour is dominant for Cu and ZnTPP at 266 nm. Morris and Johnson [58] have reported previously that 266 nm photoionisation results in molecular ion formation, as observed here. However, they also reported that NiTPP and CoTPP display similar molecular ionisation, although no mass spectra for these molecules were presented. Their conclusions in this regard are in direct conflict with the results of this study. Figures 6.20 and 6.21 show that for these molecules, neutral dissociation, followed by photoionisation, or Class B behaviour, generates a dominant metal-free fragment in their mass spectra. This process clearly dominates over Class A molecular ionisation, since the molecular
Figure 6-19: \textsuperscript{L}_2\textsuperscript{TOF} mass spectra of copper TPP under a) soft and b) hard ionisation conditions obtained using 266 nm laser photoionisation.
Figure 3-20: L^2 TOF mass spectra of a) CoTPP, b) NiTPP and c) VOTPP obtained using 266 nm laser photoionisation
ions are present at only low intensities. The competition between the two classes of behaviour is most obvious in the case of VOTPP. At low ionising laser power densities this molecule behaves as a Class B system. As the incident ionising laser power density is increased the rate of absorption of a second photon by the intermediate state, to form the molecular ion, becomes competitive with the rate of photochemical relaxation and the products of both processes are clearly observable in the mass spectrum. Metal-ligand multiphoton dissociation (Class B behaviour of the excited, neutral metalloTPPs) has been observed in other organometallic species, such as metal carbonyls [54] and metalloccenes [59]. Photodissociation of the metalloTPP radical cation, however, follows a completely different pathway, resulting in the consecutive loss of phenyl substituents and retention of the coordinated metal atom.

The tendency of the metalloTPPs to show either Class A or Class B behaviour when irradiated at 266 nm can be related to their known photophysical properties. The metalloporphyrins investigated here can be divided into two groups: the diamagnetic molecules, ZnTPP and NiTPP, and the paramagnetic molecules, CoTPP, CuTPP and VOTPP. In the latter, paramagnetic systems, coupling of the single unpaired d electron of the transition metal to the singlet and triplet \( ^2 \pi^* \) states of the porphyrin macrocycle perturbs the spin multiplicity of these states. This gives rise to singdoublet \( (^2S) \), tripdoublet \( (^2T) \) and tripquartet states \( (^4T) \) [60]. Intersystem crossing from the singdoublet to the tripdoublet manifolds is spin-allowed and occurs on the picosecond or subpicosecond timescale. For example, the \( ^2S_1 \) state of CuTPP is known to decay to the tripdoublet manifold in less than 350 fs [61]. The photophysical properties of the metal containing tetraphenylporphyrins are, therefore, a result of two principal factors; heavy atom induced spin-orbit coupling, which enhances the rates of intersystem crossing, and the stronger paramagnetic perturbation of the spin multiplicity of the excited intermediate states.

The diamagnetic molecule ZnTPP is one of the few fluorescent TPPs. One-photon excitation at 266 nm populates a high lying vibronic level in the singlet manifold. Rapid (picosecond or subpicosecond) intramolecular conversion of elec-
Figure 6-21: $L^2$TOF mass spectra of VOTPP obtained using 266 nm laser photoionisation of power density a) 0.2 MW cm$^{-2}$, b) 0.6 MW cm$^{-2}$ and c) 3.6 MW cm$^{-2}$.
tronic to vibrational energy can then occur to the $S_1$ electronic state, which has an energy of 2.17 eV [57] and, therefore, can be readily ionised by a single 266 nm (4.66 eV) photon. This sequence of events is illustrated in Figure 6.22a. ZnTPP thus undergoes exclusively Class A photoionisation when using 266 nm laser photoionisation. This is similar to the behaviour of the free-base TPP. The $S_1$ electronic state of this molecule has an energy of 1.94 eV and a fluorescence lifetime of 11 ns [57], and shows molecular ionisation followed by photodissociation of the molecular ion at 266 nm. The paramagnetic molecule CuTPP also shows Class A behaviour. In this case, however, the behaviour can be related to the photophysics of its tripmultiplet states which are populated by rapid intersystem crossing from the photoexcited singlet state. CuTPP has a long lived $^4T_1$ state which is luminescent; the lifetime of this state has been measured in solution-phase studies to be 600 µs at 77 K and 90 ns at room temperature [62]. The $^4T_1$ state is populated by rapid intersystem crossing from the $^2T_1$ state which has a lifetime of 450 ps at room temperature and lies only 600 cm$^{-1}$ above $^4T_1$. The Class A behaviour exhibited by CuTPP at 266 nm is consistent with the population of the long lived $^4T_1$ intermediate state, following single-photon excitation, and the subsequent single photon ionisation of this excited state. This implies that the energy of the $^4T_1$ state lies within 4.66 eV of the ionisation threshold which, for an IP of ca. 6.5 eV, gives an estimated excitation energy of ca. 1.8 eV in the gas phase. This value is consistent with the measured solution-phase excitation energy of 1.66 eV [62].

The diamagnetic molecule NiTPP and the paramagnetic molecule CoTPP and VOTPP do not exhibit Class A photophysical behaviour. As described above, these undergo photofragmentation prior to ionisation of the neutral fragments. In contrast to the behaviour of ZnTPP, the diamagnetic molecule NiTPP is non-fluorescent. In its $S_1$ state it undergoes rapid radiationless decay ($\tau = 10$ ps) to a low-lying (d,d) state which then decays rapidly to the ground state in 250 ps [63]. In NiTPP, therefore, 266 nm single-photon excitation is followed by rapid relaxation to a high vibrational level of the electronic ground state which cannot be photoionised by absorption of a second photon. The absorption of further photons and the subsequent rapid intramolecular conversion of electronic to vibrational en-
Figure 6-22: Schematic representation of the photophysical processes involved in: a) Class A photoionisation of ZnTPP at 266 nm. Absorption of two 266 nm photons results in formation of the molecular ion [ZnTPP]^+. b) Class B photoionisation of NiTPP at 266 nm. Photoexcitation is followed by rapid intramolecular conversion of electronic to vibrational energy to produce a vibrationally hot ground state species, [NiTPP]^+; dissociation followed by photoionisation yields the metal-free fragment ion [TPP]^+. 
Energy leads to the observed neutral photodissociation. This is shown schematically in Figure 6.22b. There appears to be no information in the literature on the macrocycle-metal dissociation energies for the metalloporphyrins. However, data available for other complexes [64] suggests that the metal-nitrogen bond energies are in the order of 1.5 eV, implying that at least two 266 nm photons are required to dissociate the metal from the macrocycle. A further two photons are then required to photoionise the macrocycle fragment. The absence of any significant intensity of the metal ions in our mass spectra suggests that the photoionisation cross-sections of the porphyrin macrocycle is considerably larger than that of the atomic metal at this wavelength.

The paramagnetic molecule CoTPP was also shown to exhibit Class B behaviour, as exemplified by NiTPP. However, as for CuTPP, photoionisation is related to the photophysics of its tripmultiplet states. Unlike CuTPP, CoTPP does not luminesce; population of the tripmultiplet manifold is followed by radiationless decay to the ground state in \(< 35 \text{ ps}\) [65]. This rapid decay is mediated by a low-lying \((\pi,d)\) charge transfer state. The Class B photodissociation behaviour of CoTPP is thus analogous with that of NiTPP.

Finally, VOTPP displays both Class A and Class B behaviour in competition with each other. There appears to be little known about the photophysics of VOTPP apart from the fact it is luminescent. The spectra shown in Figure 6.21 suggest that the excitation energy of the \(2T_1\) state is more than 4.66 eV below the ionisation threshold. This means that at low laser intensities there is a greater probability of intersystem crossing from the tripdoublet to the tripquartet occurring than absorption of a further photon followed by neutral dissociation, Class B behaviour, predominates. At higher laser intensities it would appear that up-pumping from the \(2T_1\) state can compete with intersystem crossing and, therefore, molecular ionisation is observed along with neutral photodissociation.

The question remains as to why Class A behaviour is observed exclusively at 193 nm. As mentioned previously, this is what would be expected if the photoionisation were a single photon process. However, our rudimentary power dependence measurements for CuTPP suggested a \((1+1)\) photon process. If ionisation at 193
nm is a two photon process, the observed behaviour suggests that the excited intermediate state is long lived. However, the one-photon excitation energy is expected to be close to the ionisation threshold. It therefore seems likely that the intermediate state would be a long lived Rydberg state. Recent investigation of the dynamics of Rydberg states of aromatic molecules, such as benzene and phenanthrene, have shown that high energy states close to the ionisation threshold can have lifetimes of several hundred nanoseconds [66], although lower-lying states (4000-10 000 cm\(^{-1}\) below the ionisation threshold) exhibit sub-picosecond radiationless decay [67].

6.3.2 \(L^2\)TOFMS of Metallo-Octaethylporphyrins using 248 nm Laser Photoionisation.

The results presented and discussed in the previous section clearly demonstrate that the inclusion of a metal atom in the macrocycle of tetraphenylporphyrins can have considerable impact on the photophysical and photochemical properties of the molecule. This behaviour is manifested in the diverse photochemistry exhibited following 266 nm photoionisation. Some metalloTPPs conform to Class A behaviour whilst others conform to Class B behaviour, or exhibit competing mechanisms. The results of similar experiments, using metalloOEPs as the target molecules, are described in this section. These studies involved a comparison between the nonlinear photochemistry observed following 193 nm and 248 nm photoexcitation. The results of these studies indicate that the influence of the chelated metals on the resulting mass spectra has similar characteristic features to those observed for the metalloTPPs.

As described previously, for all the metalloOEPs, excepting CuOEP, soft ionisation at 193 nm results in the production of molecular radical cations with little or no fragmentation, see Figure 6.1, whilst an increase in the ionising laser intensity causes increased photofragmentation, see Figure 6.2. This photodissociation of the molecular radical cation results in the successive loss of methyl substituents, via a benzylic cleavage, and the metal is retained in the macrocycle. The observa-
Chapter 6. \( L^2 \text{TOFMS of Porphyrins} \)

The ionisation of this exclusively Class A behaviour at 193 nm is what would be anticipated if the photoionisation of the metalloOEPs were a single photon process. The ionisation potentials of the metalloOEPs have been shown to be lower than those of the metalloTPPs, lying in the range 6.09-6.39 eV [39]. This suggests that photoionisation of the metalloOEPs occurs after the absorption of only one 193 nm (6.4 eV) photon.

Photoionisation at 248 nm is a two-photon process. The results obtained for the metalloTPPs would suggest that, for ZnTPP and CuTPP, Class A behaviour would be dominant at 248 nm, leading to the formation of the molecular ion and subsequent photolysis by the successive loss of methyl groups. Figure 6.23 shows the \( L^2 \text{TOF mass spectrum of ZnTPP obtained using 248 nm laser photoionisation.} \) It can be clearly seen that in this case formation of the ZnOEP molecular ion is the predominant process followed by benzylic cleavage of the ethyl substituents, resulting in a series of peaks separated by 15 amu. This behaviour can be explained in a similar manner to that of ZnTPP. One-photon excitation of ZnOEP populates a high lying vibronic level in the singlet manifold. Rapid (picosecond or subpicosecond) intramolecular conversion, of electronic to vibrational energy, then occurs until the \( S_1 \) state is reached. Absorption of a further 248 nm photon by the excited molecule results in photoionisation. ZnOEP thus undergoes exclusively Class A photoionisation at 248 nm. Similarly, free-base OEP shows molecular ionisation under the same conditions.

The behaviour of CuOEP is anomalous when photoionised using either 193 nm or 248 nm radiation. At neither wavelength does it exhibit strict Class A characteristics. The \( L^2 \text{TOF mass spectrum obtained using 248 nm laser photoionisation is shown in Figure 6.24.} \) It is similar to the spectrum obtained at 193 nm shown in Figure 6.4. The dominant peak corresponds to the molecular ion at 596 amu. To lower mass are three smaller peaks which correspond to the characteristic loss of methyl groups following benzylic cleavages of the ethyl substituents. If these were the only spectral features it would be possible to assert that CuOEP does conform to the anticipated Class A behaviour. However, there is an intense fragment peak present which corresponds to the species [M-Cu+2H]+ together with other peaks.
representative of subsequent benzylic cleavages of this species. This is in constrast to the behaviour observed for CuTPP and must derive from an alternative process. As discussed in Section 6.2.1, the loss of the copper atom must originate from either thermal decomposition during the desorption process or photoionisation of free-base impurities present in the sample.

The L²TOF mass spectra of NiOEP and CoOEP obtained using 248 nm laser photoionisation are shown in Figures 6.25a and 6.25b, respectively. In order to obtain these spectra the ionising laser power density was increased by approximately an order of magnitude above that required to produce the spectra shown in Figures 6.23 and 6.24. Along with the need for significantly higher ionising laser power density the resulting spectra are clearly different from those obtained for both Zn- and CuOEP. The spectra contain only small signals due to the molecular ion, and the base peak in each case corresponds to the loss of seven or eight methyl groups from the parent molecule. This photochemical behaviour is different, not only from that exhibited by Zn- and CuOEP, but is also different to that anticipated from the spectra of Ni and CoTPP. Inspection of the mass spectra in Figure 6.25, and consideration of the extreme ionisation conditions used, reveals that there
is no facile molecular radical cation formation, followed by photofragmentation, i.e. these molecules do not readily conform to Class A behaviour. However, it is also apparent that their photochemistry does not involve photoabsorption, followed by rapid intramolecular conversion via a (d,d) state of the metal, leading to neutral photodissociation of the metal and macrocycle, i.e. it is not directly analogous to the behaviour of Ni- and CoTPP. The spectra obtained do provide further evidence that the nature of a chelated metal atom does have a critical effect on the photochemistry of a porphyrin macrocycle. At present, a satisfactory interpretation of the 248 nm photoionisation behaviour of Ni- and CoOEPs has not been possible. One explanation may be that intramolecular relaxation, via excited states located at the metal atom, lead to successive photon absorption by the neutral molecule. In the case of the metalloOEPs, however, the lowest energy dissociation mechanism involves benzylic cleavage of the peripheral ethyl groups, rather than the loss of the metal species. (In the case of the metallo TPPs the substituents will be more stable as they are an integral part of the porphyrin π-system.) Therefore, multiple photon absorption leading to photoionisation can only compete effectively at a high incident laser power density, under which condi-

Figure 6-24: $L^2$TOF mass spectrum of CuOEP obtained using 248 nm laser photoionisation.
tions the most prevalent species in the gas phase are the neutral photodissociation products \([M-nCH_3]\). The high intensity of fragments corresponding to the loss of seven or eight methyl groups from the ethyl substituents can be explained by the enhanced stability of these species.

6.4 Concluding Remarks

The use of \( L^2 \text{TOFMS} \) for the mass analysis of a variety of porphyrins has been shown to be of considerable analytical utility. The technique allows a much wider range of porphyrins to be analysed than is possible with conventional EI or CI techniques. The data presented have unequivocally demonstrated that fragile biological porphyrins can be desorbed as intact neutral molecules and subsequently ionised with minimal fragmentation of the molecular ion. This results in the production of simple, readily interpretable mass spectra. On photoionising with 193 nm laser radiation the mass spectra obtained have been shown to be similar, with some exceptions, to those produced using EI. However, by increasing the ionising laser power density mass spectra have been generated which contain structurally significant data. This has been shown to involve principally fragmentation of the peripheral substituents of the porphyrin macrocycle, although further increases in the ionising laser power density lead to the characteristic fragmentation of the porphyrin nucleus itself. Such fragmentation is not observed in EI experiments; high energy electron impact ionisation preferentially leads to the formation of multiply charged species. A comparison of the two techniques in the light of this information highlights the increased analytical use of “ladder-switching” MPI over conventional EI for the investigation of free-base or metalloporphyrins. A further advantage of the technique is a consequence of the direct desorption of analyte molecules. By avoiding the use of matrices to assist desorption it is possible to ionise the intact vapor phase molecules without producing any significant artifacts resulting from adduct formation.
Figure 6-5: \(L^2\)TOF mass spectra of a) NiOEP and b) CoOEP obtained using 248 nm laser photoionisation.
Perhaps the most striking results presented in this chapter concern the consequence of using alternative ionising laser wavelengths on the nature of the resultant mass spectra. In the case of the metalloTPPs and the metallo-OEPs, changing the ionising laser wavelength from 193 nm to 266 nm completely changes the nature of the mass spectrum. Depending on the precise nature of the coordinated metal atom, it has been found that photodissociation of the neutral gas-phase molecules can occur resulting in the expulsion of the coordinated metal itself. A more detailed investigation of these results has shown that this phenomena is the result of subtly different photophysical processes occurring in the different metallo species. These results confirm that L²TOFMS is a tool capable of probing the photophysics of involatile molecules such as the porphyrins. The wavelength specific nature of such photofragmentation phenomena also has important analytical ramifications. For example, mass spectra which contain both a molecular ion peak and a fragment ion peak, corresponding to the loss of the coordinated metal, deliver direct information about the nature of the coordinated metal atom. Increasing the ionising laser power density to promote further fragmentation of these daughter ions can subsequently reveal information concerning the nature of the peripheral ring substituents.

Future studies of porphyrins using L²TOFMS will require an improvement in the resolving power of the instrument to enable the precise nature of hydrogen loss reactions and rearrangements to be identified. Furthermore, an important advance would be to perform tandem TOF experiments to determine the pathways for some of the more complex fragmentation processes. As described in Chapter 5, from the perspective of analytical utility, the instrumentation is robust enough to perform the analysis of complex systems. Specific uses of L²TOFMS for porphyrin analysis could therefore benefit from the exploitation of wavelength selective ionisation. This would enable specific target porphyrins to be assayed for directly from their host matrices. For example, a future goal may involve the direct determination of etioporphyrins in oil shales as part of an integrated oil-source rock correlation study.
Bibliography


Chapter 7

L²TOFMS of Dyes

7.1 Introduction

Dyestuffs are of considerable environmental and commercial interest because of their use as colourants in a wide variety of products such as textiles, paper, foodstuffs, leather and gasoline. Also, synthetic precursors, by-products and degradation products of aromatic dyes could be potential health hazards owing to their toxicity and/or carcinogenicity. Wastes generated from dyeing or pigmentation processes are of great environmental interest due to their possible health effects and the inability of waste treatment processes to remove such dyestuffs. Some dye compounds can be converted to carcinogens such as napthylamines [1,2], substituted phenylamines, or benzidine analogs [3,4,5]. However, aromatic dyestuffs had until the 1970s largely eluded characterisation by mass spectrometry. Commercial dyestuffs not only constitute a wide variety of structural types but also contain impurities, which can include homologous compounds and related synthetic precursors. Thus, complementary information from various analytical techniques, including mass spectrometry, is often required for the unambiguous identification of organic dyes. [6]

Before the advent of field desorption and electrospray ionisation [7], [8], mass spectrometric characterisation of dyestuffs, using electron impact (EI) ionisation, was only possible on materials which were thermally volatile or stable. The early mass spectrometric studies of dyestuffs relied on the use of chemical derivatisations [9] to give volatile neutral compounds, or were concerned with the analysis
of pyrolysis products [9]. The high source temperatures necessary for these experiments tended to enhance thermal degradation and thus reduce molecular ion abundances.

The development of desorption/ionisation techniques has permitted the mass spectrometric characterisation of ionic and non-volatile dyes. Field desorption (FD) experiments have been performed by a number of groups [10,11,12,13]. However, the lack of structurally significant peaks and the inability to desorb direct from chromatographic media (used in the separation of dye components) led to Cooks et al. [14] to report the use of secondary ion mass spectrometry (SIMS) and Monaghan et al. [16], [17] to report the use of fast atom bombardment (FAB) for the identification of dyes. These techniques involve single step desorption/ionisation and often lead to spectra containing complicated fragmentation patterns with little evidence of a molecular ion. More recently, thermospray ionisation LC-MS has been used [18] to analyse a series of dyes belonging to different chemical classes. One of the drawbacks of thermospray ionisation is that one obtains mainly molecular and adduct ions which do not provide enough information for the structural elucidation of dyes of unknown structure. Yinon et al. [19] overcame this problem using particle beam liquid chromatography electron impact (PB-LC-EI) mass spectrometry and were able to characterise a series of commercial dyes using the structural information available in the EI fragmentation patterns. It is a clear disadvantage that the aforementioned techniques, individually, do not have the ability to provide both molecular weight and structural information.

The following chapter describes the results of a comparative study of the mass spectra of a range of laser-desorbed, untreated dyestuffs at two different fixed laser photoionisation wavelengths, namely 193 nm and 266 nm. The data reveal differences, in some cases considerable, in the fragmentation pathways and detection sensitivities at these two wavelengths. All the mass spectra presented were obtained using the reflectron time-of-flight instrument along with CO₂ laser desorption. The dyestuffs examined in this study were all used as received, without any preseparation or purification. The samples were prepared by depositing them
on the surface of a 6 mm diameter stainless steel rod in solution and allowing the solvent to evaporate. The ionising laser power densities, at both 193 nm and 266 nm, were generally maintained at relatively low levels in order to maximise the intensity of the molecular (or in some cases pseudo molecular) ions and to avoid further photon absorption which could lead to fragmentation. The power densities were maintained at $2.4 \times 10^5 \text{ W cm}^{-2}$ for the studies at 193 nm, and at $5 \times 10^5 \text{ W cm}^{-2}$ for those at 266 nm. The hard ionisation mass spectra generated at 266 nm were obtained by increasing the laser power density to $5 \times 10^6 \text{ W cm}^{-2}$.

To simplify the correlation between structure and spectral features, the dyes that were studied can be classified into four groups. Those of class I contain the azo functionality, class II represents the phthalocyanines, class III are the anthraquinone dyes, and class IV are coumarin type dyestuffs. The structures of the dyestuffs examined are presented in Table 7.1. In the case of the azo dyestuffs, comparative mass spectra were obtained for a series of pure azobenzene derivatives. The relevant structural and mass data for these species are given later in the chapter where appropriate.

7.2 \textbf{L}^2\text{TOFMS of Azo Dyestuffs}

7.2.1 High-Purity Azo Dyes

Azo dyes constitute one of the most important classes of synthetic colouring materials. Commercial azo dyes are generally sold with less than 50% dye content. However, it was possible to obtain a sample of Disperse Red 1 of 95% purity from Aldrich. The samples of low dye content are discussed in the next section.

Mass spectra of azobenzenes have been reported previously using EI and SIMS, whilst Wang and Wang [21] have reported the photolytic behaviour of some azo pyridone disperse dyes on polyester substrates. In the case of electron impact ionisation [20], fragmentation patterns characteristic of substituted 4-amino-4' nitroazobenzenes include partial or complete loss of the amine side chains, loss of
### Table 7-1: Structural classification of dyestuffs.

<table>
<thead>
<tr>
<th>Class I</th>
<th>Structure</th>
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<tbody>
<tr>
<td>Disperse Red 1</td>
<td></td>
</tr>
<tr>
<td>( R_1 = N(CH_2CH_3)(CH_2CH_2OH) )</td>
<td></td>
</tr>
<tr>
<td>( R_2 = NO_2; R_3, R_4 = H )</td>
<td></td>
</tr>
<tr>
<td>Disperse Orange 1</td>
<td></td>
</tr>
<tr>
<td>( R_1 = NHPh; R_2 = NO_2 )</td>
<td></td>
</tr>
<tr>
<td>( R_3, R_4 = H )</td>
<td></td>
</tr>
<tr>
<td>Disperse Orange 3</td>
<td></td>
</tr>
<tr>
<td>( R_1 = NH_2; R_2 = NO_2 )</td>
<td></td>
</tr>
<tr>
<td>( R_3, R_4 = H )</td>
<td></td>
</tr>
<tr>
<td>Disperse Yellow 3</td>
<td></td>
</tr>
<tr>
<td>( R_1 = H; R_2 = NHCOCH_3 )</td>
<td></td>
</tr>
<tr>
<td>( R_3 = OH; R_4 = CH_3 )</td>
<td></td>
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</tbody>
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<table>
<thead>
<tr>
<th>Class II</th>
<th>Structure</th>
</tr>
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<tbody>
<tr>
<td>Phthalocyanines</td>
<td></td>
</tr>
<tr>
<td>( M = Mg, Co, Zn, Pb, 2Li, HH )</td>
<td></td>
</tr>
<tr>
<td>( R_1, R_2, R_3, R_4 = H )</td>
<td></td>
</tr>
<tr>
<td>Alcian Blue 8GX</td>
<td></td>
</tr>
<tr>
<td>( M = Cu )</td>
<td></td>
</tr>
<tr>
<td>( R_1, R_2, R_3, R_4 = CH_2SC[N(CH_3)_2]_2Cl )</td>
<td></td>
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<tr>
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<th>Structure</th>
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<tbody>
<tr>
<td>Disperse Blue 1</td>
<td></td>
</tr>
<tr>
<td>( R_1, R_2, R_3, R_4 = NH_2 )</td>
<td></td>
</tr>
<tr>
<td>( R_5 = H )</td>
<td></td>
</tr>
<tr>
<td>Disperse Orange 11</td>
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</tr>
<tr>
<td>( R_1, R_2, R_3 = H; R_4 = NH_2 )</td>
<td></td>
</tr>
<tr>
<td>( R_5 = CH_3 )</td>
<td></td>
</tr>
<tr>
<td>Basic Blue 47</td>
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<tr>
<td>( R_1, R_2 = H; R_3 = NH_2 )</td>
<td></td>
</tr>
<tr>
<td>( R_4 = NHCH_2CH_2N(CH_3)_2 )</td>
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</table>

<table>
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<tbody>
<tr>
<td>Coumarin 47</td>
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<tr>
<td>( R_1 = CH_3 )</td>
<td></td>
</tr>
<tr>
<td>( R_2 = N(C_2H_5)_2 )</td>
<td></td>
</tr>
<tr>
<td>Coumarin 152a</td>
<td></td>
</tr>
<tr>
<td>( R_1 = CF_3 )</td>
<td></td>
</tr>
<tr>
<td>( R_2 = N(C_2H_5)_2 )</td>
<td></td>
</tr>
<tr>
<td>Coumarin 102</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>( R_2 = )</td>
<td></td>
</tr>
</tbody>
</table>
-NO from the NO₂ substituent and the cleavage of the N-phenyl bonds adjacent to the azo linkage. It should be noted that where the phenyl substituents differ on either side of the N=N double bond the species retaining the positive charge is the amine fragment. More recently Yinon et al. [19] observed, using EI ionisation, the cleavage of the double bond between the two nitrogen atoms with transfer of two hydrogen atoms to form an amine, along with cleavage of the azo C-N bonds either side of the azo linkage. Similarly, the SIMS spectra [14] of azo dyes exhibit extensive fragmentation of the parent dye molecules, involving direct cleavage of the C-N bonds as well as cleavage of the N=N bond. Photoreductive cleavage of the azo bond has also been observed by Wang et al. [21], resulting from ultraviolet degradation of azo pyridone dyes on polyester substrates. In these latter cases the dissociation of the N=N bond is seen to be an important fragmentation pathway under a number of different conditions.

Figures 7.1a and 7.1b show the photoionisation mass spectra of Disperse Red 1 at 266 nm and 193 nm, respectively. The mass spectrum obtained at 266 nm is simple in appearance, containing a single strong peak at 180 amu. This fragment peak is the result of photolytic cleavage of the azo linkage followed by double hydrogen transfer yielding the amine positive ion. This fragmentation occurs under soft ionising conditions. Attenuation of the UV photoionisation laser intensity reduces the size of this peak but does not lead to any increase in the intensity of a molecular ion signal. In marked contrast, the spectrum following photoionisation at 193 nm radiation contains a number of strong ion peaks including that corresponding to the molecular ion hydrogen adduct. It can be seen in Figure 7.1b that there is no peak which corresponds to azo cleavage and that, therefore, irradiation at 193 nm does not lead to dissociation of the azo bond. In this case the predominant pathway for fragmentation is dissociation or complete loss of the aromatic side groups. The base peak at 284 amu corresponds to the species [M-NO]⁺. This species is a characteristic dissociation product of aromatic nitro compounds. There is a large enthalpic advantage of ca. 2eV for the rearrangement and loss of NO versus simple cleavage loss of NO₂. This arises from the much greater resonance stabilisation inherent in PheO⁺ over that of Phe⁺. A similar mass spectrum to that observed
at 193 nm is produced by single-stage laser desorption at 337 nm followed by mass analysis of the nascent desorbed ions [15]. The proposed fragmentation pathways are shown in Figure 7.2.

The fragmentation pattern observed at 193 nm can be explained in terms of the conventional multiphoton fragmentation mechanism, i.e. initial formation of a molecular ion which undergoes subsequent fragmentation following the absorption of further photons. Although, even here, the base peak is not replaced by the molecular ion peak under conditions of reduced ionising laser intensity. This suggests that the fragment [M-NO]+ is formed concomitantly with ionisation. The ionisation potential (IP) of Direct Red 1 is not known exactly. Even so, assuming that ionisation is a two photon process at 193 nm, the excess energy available above the IP, following the absorption of two photons would appear to be sufficient to facilitate this fragmentation. A different mechanism, which leads to exclusive cleavage of the azo bond, appears to be operating at 266 nm. It is well known that azobenzenes undergo trans-cis photoisomerisation about the azo linkage following excitation to low lying excited singlet electronic states [22]. Photoisomerisation is believed to proceed via an excited state potential surface which exhibits a minimum at the perpendicular configuration (i.e. midway between the trans and cis configurations) [23]. The ground state potential surface, of course, has a maximum at this value of the torsional coordinate (φ = 90°), corresponding to the top of the barrier between trans and cis configurations. It is proposed that the photodissociative ionisation observed at 266 nm is directly related to photoisomerisation, in that it proceeds via the same one-photon excited intermediate state. As only one of the possible product fragments is observed for Disperse Red 1, at 180 amu, it seems likely that dissociation occurs after ionisation. That is, the intermediate excited state absorbs a further photon producing an unstable molecular ion, which dissociates at the azo bond to give an ionic fragment and a neutral fragment. If dissociation was occurring in the neutral, excited intermediate state, it would be expected that both neutral fragments would be ionised and observed in the mass spectrum. Inspection of Figure 7.1a shows this is not the case for Disperse Red 1.

In order to confirm whether or not such a phenomenon is characteristic of azo
Figure 7-1: L²TOF mass spectra of Disperse Red 1 using a) 266 nm and b) 193 nm laser photoionisation.
Figure 7-2: Schematic representation of the principal fragmentation pathways resulting from either 266 nm or 193 nm laser photoionisation of Disperse Red 1.
molecules in general, and not simply specific to Disperse Red 1, a number of pure azobenzene derivatives were investigated using 266 nm laser photoionisation. As many of these materials were considerably more volatile than Disperse Red 1, they were mixed into a glycerol and alumina paste prior to investigation to prevent their evaporation, on standing under high vacuum, in the desorption chamber. The materials examined under these conditions were azobenzene (MW = 182 amu), methyl red (crystals) (MW = 269 amu), 4-phenylazoaniline (MW = 197 amu) and 4-phenylazophenol (MW = 198 amu). The mass spectra obtained using 266 nm laser photoionisation for these species are shown in Figure 7.3a, 7.3b, 7.3c and 7.3d, respectively. In each case the base peak of the mass spectrum corresponds to a product of N=N bond dissociation followed by double hydrogen addition. The L^2TOF mass spectrum obtained for azobenzene, shown in Figure 7.3a, is dominated by a peak at 93 amu which corresponds to the presence of aniline, as anticipated from the azo bond cleavage observed in Disperse Red 1. Similar species constitute the base peak of the other spectra shown in Figures 7.3. For methyl red, 4-phenylazoaniline and 4-phenylazophenol the base peaks are at 136, 93 and 93 amu, respectively. These dissociation pathways are summarised in Figure 7.4.

A number of other peaks are also present in the mass spectra shown in Figures 7.3. In the case of azobenzene there is a small peak at 182 amu which corresponds to the mass of the molecular ion. Also observable is a peak at 154 amu which corresponds to the mass of the species formed when N_2 is expelled from the molecule leaving a [Phenyl-Phenyl]^+ species. The loss of such small stable molecules can be highly favorable on an energetic basis. As the N_2 group is not an end group its loss must be accompanied by a cyclic rearrangement. In order to undergo such a rearrangement the configuration of the azo molecule must be cis with respect to the two phenyl groups. The structure of this species is given below in Figure 7.5. This requires the normally trans-azobenzene to undergo a trans-cis photoisomerisation reaction about the azo linkage. As mentioned previously, absorption of a 266 nm photon is suspected to promote such an isomerisation as a precursor to the cleavage of the azo bond. It may be expected therefore that azo bond cleavage and loss of nitrogen are competitive processes in the energetic molecular ion. Such species
Figure 7-3: \( L^2 \)TOF mass spectra of a) azobenzene, b) methyl red, c) 4-phenylazoanaline and d) 4-phenylazophenol, obtained using 266 nm laser photoionisation.
Figure 7-4: Schematic representations of the principal fragmentation pathways observed for azobenzene, methyl red, 4-phenylazoaniline and 4-phenylazophenol following 266 nm laser photoionisation.
Figure 7-5: Postulated cyclic transition state required to enable loss of N₂ from cis-azobenzene.

are not observed using 193 nm laser photoionisation since absorption of 193 nm photons does not excite the molecule to the same intermediate state. Loss of N₂ is also observed for both 4-phenylazoaniline and 4-phenylazophenol, resulting in the signals observed at 169 amu and 170 amu. A further species present in all the spectra shown in Figure 7.3 is that at mass 66 amu. This ion is a product of the fragmentation of the aniline species of mass 93 amu, involving the loss of a neutral HCN, and is often accompanied by loss of a further hydrogen atom producing the accompanying peak at 65 amu. Two other moderately intense peaks at 167 amu and 169 amu are also observable in Figure 7.3a. The origin of these species is not at present fully understood. However, it is possible that they correspond to the presence of impurities in the sample.

Methyl red crystals, as noted above, clearly demonstrate the characteristic N=N cleavage when using 266 nm laser photoionisation, resulting in a peak at 136 amu. At 137 amu there is also a less intense signal which may correspond to the mass of the other half of the cleaved molecule. The predominant fragment contains the tertiary amine side-group. Other fragment peaks observed in the mass spectrum, see Figure 7.3b, are all derived from these species. Successive loss of N, NH and NH₂ result in peaks at 122, 121 and 120 amu, whilst complete loss of the amine side-chain would result in the ion at 93 amu. No molecular ion was
observed. Similarly, no molecular ions were observed in the mass spectra of 4-phenylazoaniline and 4-phenylazophenol. However, in these spectra, see Figures 7.3c and 7.3d, peaks at 108 amu and 109 amu are present along with the base peaks at 93 amu, corresponding to both the products which result from azo bond cleavage. There are a number of possible explanations for this behaviour. The most straightforward one being that the ionising laser intensity is sufficient to ionise the neutral fragment resulting from the dissociation of the molecular ion. However, on this basis one might expect to see both the fragments in the mass spectra of Disperse Red 1. Alternatively, one might see both fragments because they compete effectively with one another to retain the charge from the molecular ion. If this were the case the relative peak heights would be related to the ability of the two species to stabilise the ionic charge. It is also possible, however, that the molecule undergoes fragmentation in its neutral excited intermediate state, the two resulting neutral fragments then undergoing multiphoton ionisation. If this latter mechanism is operative it should be possible, by increasing the ionising laser power density, to promote further photophysical processes, such as MPI, which can compete with dissociation and thereby modify the appearance of the mass spectrum.

The results obtained for the pure azobenzene derivatives at 266 nm appear to confirm that azo bond cleavage is a phenomenon characteristic of the wider generic group of azo molecules. This can therefore be used as a specific analytical probe to identify the presence of the azo functionality. It is clear from the mass spectra of Disperse Red 1 shown previously in Figures 7.1a and 7.1b that the L²TOF mass spectra obtained at 266 nm and 193 nm provide complementary diagnostic information characteristic of the dye studied. The mass spectral peaks obtained using this approach are compared with those obtained using electron impact ionisation in Table 7.2. It is readily apparent that less complicated spectra are obtained using L²TOFMS. At 193 nm a more intense peak for the molecular ion is obtained, along with a higher mass base peak, than is found in the EI mass spectra. At 266 nm the spectrum is somewhat simpler, being dominated by a single intense fragment peak characteristic of the sample. The two spectra,
Chapter 7. $L^2$TOFMS of Dyes

<table>
<thead>
<tr>
<th>Technique</th>
<th>MW (amu)</th>
<th>Mass of observed ions (amu)</th>
</tr>
</thead>
<tbody>
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<td>PB EI*</td>
<td>314</td>
<td>314(2); 297(2); 283(34); 267(11); 253(19); 237(8); 207(9); 180(15); 168(18); 149(15); 147(18); 133(100); 120(49); 108(63); 105(55); 103(47)</td>
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<tr>
<td>LD-MPI(193nm)</td>
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<td>315(23); 285(25); 284(100); 269(10); 255(12); 179(15); 133(42); 105(26)</td>
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<td>LD-MPI(266nm)</td>
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<td>181(20); 180(100); 151(10); 149(10); 136(5)</td>
</tr>
</tbody>
</table>

* - peaks reported in reference [19]

Table 7-2: Comparison of PB EI (Particle Beam Electron Impact) mass spectral peaks and photoionisation mass spectral peaks for Disperse Red 1 (Relative intensities in parentheses)
obtained using the same methodology, and altering only the ionising wavelength, provide a relatively simple way of characterising azo dyes.

7.2.2 Low Purity Azo Dyes

As with the studies on Disperse Red 1 discussed in the previous section, low purity dyes were analysed using both 266 nm and 193 nm ionising wavelengths. Since the purity levels in the commercial dyestuffs are, in some cases, as low as 15% one would expect more complicated mass spectra as a result. However, by reference to the mass spectral features observed in the case of both the pure azo dye, Disperse Red 1, and the azobenzene derivatives, it is possible to interpret the photoionisation mass spectra of these impure dyestuffs. Thus the L²TOFMS methodology can provide an analytical technique capable of direct identification of dyestuffs in complex or "dirty" samples.

Studies at 266 nm

At 266 nm the photoionisation mass spectrum of Disperse Orange 1, under soft ionising conditions, has a base peak at 169 amu, along with a number of other strong ion peaks between 100 and 200 amu, see Table 7.3. This spectrum is shown in Figure 7.6. If the anticipated azo bond cleavage were to occur one would expect a signal at 184 amu, which is indeed apparent; loss of 15 amu (-NH) from this leaves the fragment base peak at 169 amu. The other peaks in this region of the spectrum are not as easily assignable to the target dye molecule. In order to determine the origin of these other peaks the photoionisation mass spectra of Disperse Orange 1 at desorbing laser power densities of 5 MW cm⁻² and 500 MW cm⁻² were recorded. Increasing the desorption laser power density results in a decrease in the signal intensity by at least 50%. This is most likely due to either reduced penetration of the desorbed sample into the jet, or the formation of shock waves which disrupt the jet flow. However, a change in the relative intensities of the ion peaks is also apparent.
Table 7-3: L²TOFMS mass spectral peaks of class I dyes using 266 nm laser photoionisation.

If the desorption conditions are altered from optimum, the peaks at 169 amu and 184 amu are noticeably reduced in intensity relative to the other strong peaks in the spectrum. Each component of the sample mixture will have a different vapour pressure and thus a different effective desorption cross-section. Therefore, the observed difference in this dependence of peak intensities on the desorption laser power density indicates that the peaks at 169 amu and 184 amu originate from a different desorbed molecule than do the other ion peaks. A comparison with the spectra of the other low purity dyes, see Table 7.3, reveals that these other strong peaks, at 180, 164, 150, 137, 124 and 108 amu, are ubiquitous and can therefore be attributed to other species present in the dye samples supplied.

The fragmentation scheme shown in Figure 7.7 shows the photolytic cleavage and subsequent ion fragmentation for Disperse Orange 1 and Disperse Orange 3, as determined from the 266 nm photoionisation mass spectra. Figure 7.8 shows the mass spectrum obtained for Disperse Yellow 3. The base peak in this spectrum is at 180 amu and corresponds to an impurity molecule, as observed in the mass spectra of the other impure dyes. Direct cleavage of the azo bond in Disperse Yellow 3, followed by aniline formation, would lead to a mass peak at 150 amu. A peak at this mass is present in the spectrum, shown in Figure 7.8, but it is also present in all the other spectra of these impure dyes. A component of the observed

<table>
<thead>
<tr>
<th>Dye</th>
<th>Mass (amu)</th>
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<tr>
<td>Disperse Red 1</td>
<td>180⁹</td>
</tr>
<tr>
<td>Disperse Orange 1</td>
<td>184⁹ 180 169⁹ 164 150 137 124 108</td>
</tr>
<tr>
<td>Disperse Orange 3</td>
<td>180 164 150 137 124 108⁹</td>
</tr>
<tr>
<td>Disperse Yellow 3</td>
<td>230 180 170⁹ 164 150 137 124 108</td>
</tr>
</tbody>
</table>

a - mass peaks corresponding to photolytic cleavage of the azo bond.
Figure 7-3: L²TOF mass spectrum of Disperse Orange 1 produced by 266 nm laser photoionisation.

peak at 150 amu could be representative of an azo cleavage product but this is not an unequivocally characteristic peak. However, there are peaks at 170 amu and 171 amu which are indicative that this characteristic fragmentation occurs followed by formation of a sodium adduct ion. The fragmentation pathway is shown in Figure 7.9. The ability of certain species to form cation adducts is also observed in the spectra obtained using 193 nm photoionisation as discussed in the next section.

Studies at 193 nm

At 193 nm the photoionisation mass spectra of the low purity dyes show no indication of the azo bond cleavage observed at 266 nm. Instead, similar to the behaviour of the purified dye Disperse Red 1, there is a tendency to form molecular or molecular adduct ions and, at higher power densities, subsequent fragmentation of these species is observed. Figures 7.10a and 7.10b show the photoionisation mass spectra of Disperse Orange 1 and Disperse Orange 3, respectively. The masses and relative intensities of the strong peaks in the spectra are presented together in Table 7.4.
Figure 7-7: Schematic representations of the principal fragmentation pathways resulting from 266 nm laser photoionisation of Disperse Orange 1 and Disperse Orange 3.
For both dyes the spectra are simpler than those obtained at 266 nm, exhibiting just two strong peaks. The base peak in both cases is at 23 amu corresponding to the presence of sodium ions.

The ionisation potential of atomic sodium is 5.12 eV, thus at 193 nm with an associated photon energy of 6.4 eV, ionisation of sodium is a one-photon process, whereas at 266 nm (4.6 eV) this is a two-photon process. The second strong peak observed in the 193 nm photoionisation mass spectra of these dyes corresponds to the sodium adduct of the parent molecule. The obvious excess of sodium ions in the ionisation region, and the absence of any molecular ion suggests that the most likely mechanism for ionisation at 193 nm involves cation attachment. Although the ionisation potentials of these dyestuffs are unknown they are certainly expected to be greater than 6.4 eV, so that photoionisation at both 193 nm and 266 nm will be two-photon processes. Therefore at 193 nm, one-photon ionisation of sodium predominates over two-photon ionisation of the dye molecule. This contrasts with the situation at 266 nm, where sodium ion formation and molecular ion formation are both two-photon processes. If cation attachment were also the primary ionisation mechanism at 266 nm one would expect to see molecular ion
Chapter 7. \textit{L}^2\textit{TOFMS of Dyes}

Figure 7-9: Schematic representation of the principal fragmentation pathways resulting from 266 nm laser photoionisation of Disperse Yellow 3.
<table>
<thead>
<tr>
<th>Dye</th>
<th>MW (amu)</th>
<th>Mass of observed ions (amu)</th>
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</thead>
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<td>Disperse Red 1</td>
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<td>315(23); 284(25); 283(100); 269(10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>255(12); 179(15); 133(42); 105(26)</td>
</tr>
<tr>
<td>Disperse Orange 1</td>
<td>318</td>
<td>342(5); 341(20); 191(6); 23(100)</td>
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<tr>
<td>Disperse Orange 3</td>
<td>242</td>
<td>266(10); 265(42); 23(100)</td>
</tr>
<tr>
<td>Disperse Yellow 3</td>
<td>269</td>
<td>293(8); 292(40); 291(20); 270(28);</td>
</tr>
<tr>
<td></td>
<td></td>
<td>202(11); 144(15); 107(8); 23(100)</td>
</tr>
</tbody>
</table>

Table 7-4: L²TOFMS mass spectral peaks of class I dyes obtained using 193 nm laser photoionisation (Relative intensities in parentheses).
adducts; these are not present in the spectra of any of the systems examined at 266 nm. There is, however, evidence for fragment sodium adduct formation using 266 nm as the ionising wavelength as has been observed in the case of Disperse Yellow 3.

Figure 7.11 shows the 193 nm photoionisation mass spectrum of Disperse Yellow 3. This differs from the spectra for Disperse Orange 1 and Disperse Orange 3 observed at 193 nm. It is apparent that, in addition to the sodium and molecular adduct ion, there is a signal present at 269 amu corresponding to the singly charged molecular ion. The adduct peak is stronger than that due to the molecular ion, but the presence of the latter peak suggests that either direct photoionisation competes effectively with cation attachment in this instance, see Figure 7.12, or the concentration of sodium ions in the source region is lower than for the Disperse Orange samples. Further fragmentation includes loss of the amide side chains and cleavage of the N=N double bond followed by sodium cation attachment.

The phenomenon of sodium cation adduct formation may have utility with respect to the detection of other azo dye molecules which do not readily photoionise. In order to demonstrate this a number of experiments were performed in which a pure dye material was mixed, in equimolar concentrations, with an impure dyestuff, Disperse Orange 3 (95% dye content), which is known to give high intensity sodium adduct peaks in its 193 nm photoionisation mass spectrum. This mixture was then applied to the sample probe in the usual way. Figure 7.13 shows the L²TOF mass spectra obtained on photoionising at 193 nm mixtures of Disperse Orange 3 with Disperse Red 1 (Fig. 7.13a), methyl red (Fig. 7.13b), 4-phenylazoaniline (Fig. 7.13c) and 4-phenylazophenol (Fig. 7.13d), respectively. In Figure 7.13a, there are three peaks observable of significant intensity. The most intense of these is at 337 amu and corresponds to the sodium molecular adduct ion of Disperse Red. Clearly the sodium impurity contained in the Disperse Orange 3 dyestuff is able to become attached to the Disperse Red 1 molecule as well as to the Disperse Orange 3 molecule. No molecular ion of Disperse Red 1 is observed indicating that cation attachment is a more favorable ionisation mechanism than multiphoton absorption. The peak observed at 307 amu corresponds to the loss
Figure 7-10: L$^2$TOF mass spectra of a) Disperse Orange 1 and b) Disperse Orange 3 using 193 nm laser photoionisation.
Figure 7-11: L^2 TOF mass spectrum of Disperse Yellow 3 produced by 193 nm photoionisation.

Disperse Yellow 3

(269 amu)

\[
\begin{array}{c}
  \text{CH}_3\text{CNH}-\text{C}_6\text{H}_4-N=\text{N}-\text{C}_6\text{H}_4-\text{HO} \\
  \text{CH}_3 \\
\end{array}
\]

193 nm

\[
\begin{array}{c}
  \text{CH}_3\text{CNH}-\text{C}_6\text{H}_4-N=\text{N}-\text{C}_6\text{H}_4-\text{HO} \\
  \text{CH}_3 \\
\end{array}
\]

\[
\begin{array}{c}
  \text{M+Na}^+ \\
  292 \text{ amu} \\
\end{array}
\]

Figure 7-12: Schematic representation of the principal fragmentation reactions resulting from 193 nm laser photoionisation of Disperse Yellow 3.
of NO from the sodium adduct ion and the much less intense peak at 265 amu corresponds to the expected [M+Na]⁺ peak for Disperse Orange 3. The spectrum is only plotted above 100 amu as the lower mass region is dominated by an intense ion peak which corresponds to the presence of sodium.

Figure 7.13b shows the mass spectrum of Disperse Orange 3 mixed with methyl red crystals. As for Disperse Red 1, the spectrum is very simple. In this case only four peaks of significant intensity are observable. The base peak, at 270 amu, corresponds to the hydrogen adduct of the molecular ion. To higher mass there is a further peak at 292 amu which corresponds to the methyl red sodium adduct ion, with simultaneous loss of a single hydrogen atom. The only other peaks present in the mass spectrum correspond to fragments derived from the molecular ion. At 135 amu is a low intensity signal corresponding to the cleavage of the N=N bond with either charge retention on the carboxylic acid substituted fragment, or charge retention on the amine fragment accompanied by addition of a hydrogen atom. The peak at 120 amu conforms to the product resulting from the cleavage of the phenyl-N bond, adjacent to the azo bond, with the tertiary amine substituted phenyl species retaining the charge. In this spectrum, no sodium adduct ion is observed for the Disperse Orange 3.

Figures 7.13c and 7.13d both show similar features to those discussed above. In each case, a hydrogen adduct of the molecular ion of the pure material can be observed along with a sodium-molecular adduct ion minus a hydrogen atom. Also, both spectra have small peaks present reflecting the presence of the Disperse Orange 3 sodium adduct. These experiments suggest that the addition of sodium containing mixtures into the desorption sample mix may provide a useful mechanism for the ionisation of molecules which do not readily ionise at the chosen photoionisation wavelength. However, it is worth noting that preliminary investigations into this possibility have revealed that such cation additions are molecule specific. Different molecules appear to have a different affinity for sodium attachment. Attempts to replicate these experiments on the azo molecules with alternative materials, e.g. purine and pyrimidine bases, yielded no sodium adduct ions of the target molecules when prepared for desorption in a mixture with Disperse Or-
ange 3. Nevertheless, the experiments described above would suggest that cation attachment may be useful for the ionisation of some problematic target materials.

### 7.3 L²TOFMS of Phthalocyanine Dyes

Phthalocyanine dyes constitute an important group of synthetic colourants. They are closely related in structure to the porphyrin class of pigments, such as the chlorophylls and hemins, whose L²TOF mass spectra were presented in Chapter 6. Phthalocyanine pigments have previously been investigated using a heated probe to introduce the materials into an EI mass spectrometer [24,25,26,27]. However, high source and probe temperatures (≥ 350°C) are necessary, and the spectra of certain substituted phthalocyanines have proved difficult to obtain. Games et al. [11] attempted to overcome this problem by utilising field desorption. They suggested that the minimal fragmentation observed in field desorption spectra made the technique ideal for the rapid analysis of phthalocyanine mixtures.

Six different phthalocyanines were used in the investigations described here: magnesium, cobalt, lead, dilithium and zinc phthalocyanine, along with the free-base phthalocyanine. These materials were deposited on the slotted stainless steel probe from solutions in chloroform and desorbed using the CO₂ laser. All the target pigments were examined using 193 nm along with either 266 nm or 248 nm laser photoionisation. The dye content of the samples used varied from between 80% and 93%.

Figures 7.14a and 7.14b show the soft photoionisation mass spectra obtained for magnesium phthalocyanine at 193 nm and 266 nm, respectively. A similar mass spectrum was also obtained on using 248 nm laser photoionisation. Magnesium phthalocyanine has a large absorption cross section at both these ionisation wavelengths. In each of the mass spectra, the molecular ion is clearly recognisable, together with an intense group of signals corresponding to the presence of atomic magnesium. This cluster of peaks exhibits the anticipated isotopic distribution at 24, 25 and 26 amu. A further peak in both mass spectra at 50 amu corresponds to
Chapter 7. \( L^2 \)TOFMS of Dyes

Figure 7-13: \( L^2 \)TOF mass spectra of azobenzene derivatives mixed with Disperse Orange 3 (95% dye content), a) Disperse Red 1, b) methyl red, c) 4-phenylazoaniline and d) 4-phenylazophenol, obtained using 193 nm laser photoionisation.
the species MgCN. The mass spectra obtained for the cobalt, lead, zinc, dilithium and free-base phthalocyanines, using 193 nm and 248 nm laser photoionisation, were essentially the same as those shown in Figure 7.14. In each case, the soft ionisation mass spectra contained predominantly the molecular ion species. The only other peaks observed with significant intensity were those corresponding to the mass of the coordinated metal species. Considering the purity of the samples used, it would appear likely that the metal species are present as impurities, although some component of the metal signal may be a result of dissociation from the phthalocyanine macrocycle. However, as no [M-metal]$^+$ ions are observed under these conditions it seems unlikely that neutral phthalocyanine is present in the ionisation region. Previous studies have noted that the phthalocyanines exhibit unusual thermal stability. In the light of this it seems unlikely that the presence of the metal species is a result of thermal dissociation in the desorption stage.

Previous electron impact studies have shown that the inclusion of a transition metal into the phthalocyanine ring system has little effect on the IP of the gaseous complexes. These lie in the region between 7.22 and 7.46 eV [11]. Therefore, photoionisation at 193 nm, 266 nm or 248 nm will proceed via (1+1) multiphoton absorption. Figures 7.15 and 7.16 show the mass spectra of magnesium and cobalt phthalocyanine, obtained under partially hard ionisation conditions, using 193 nm laser photoionisation. Absorption of photons by the molecular ion leads to the fragmentation of the phthalocyanine macrocycle. These mass spectra are plotted on an expanded scale in order to more clearly show the fragment ions observable to lower mass. The phthalocyanines have been determined to have much greater stability than that for any other compound of comparable size [28]. In order to produce the mass spectra shown here, the ionising laser beam was tightly focussed generating a laser power density of ca. 20 MWcm$^{-2}$. On supplying sufficient energy to promote fragmentation the macrocycle begins to disintegrate. The highest mass fragment observed in the case of magnesium phthalocyanine, at 279 amu, corresponds to just over half the mass of the macrocycle, with retention of the coordinated metal species. Much of the fragmentation observed can be predicted from previous electron impact studies [11].
Figure 7-14: $L^2$TOF mass spectra of magnesium phthalocyanine obtained using a) 193 nm and b) 266 nm laser photoionisation.
Table 7-5: Principal $L^2$TOFMS fragment peaks of phthalocyanine dyes obtained, under hard ionisation conditions, using 193 nm laser photoionisation.
The assignment of the principal fragments observed for magnesium and cobalt phthalocyanine, along with those observed for lead phthalocyanine, is given in Table 7.5. It is clear from consideration of these assignments that the coordinated metal is not immediately relinquished upon fragmentation of the macrocycle. For both the magnesium and cobalt containing molecules, a peak corresponding to \([1/4P+\text{metal}]^+\) is observed, at 152 amu and 186 amu respectively, presumably due to the charged phthalodinitrile residue with the metal retained. The free phthalodinitrile fragment, at 128 amu, is present in only low abundance. Further evidence that the metal species have a high affinity for certain fragment species is provided by the presence of a relatively intense peak corresponding to \([\text{MCN}]^+\) at 50 amu and 84 amu for magnesium and cobalt phthalocyanine, respectively. However, hard ionisation of lead phthalocyanine produces a slightly different type of mass spectrum. In this case the majority of fragments are lead free. The only ionic fragments which retain the metal correspond to the species \([\text{PbCN}]^+\) at mass 233 amu and the anticipated peak corresponding to \([1/4P+\text{lead}]^+\) at 335 amu. The fragments observed below 207 amu must clearly be lead free and may be expected to correspond to the metal-free fragments observed on fragmentation of the other metallo-phthalocyanines. The fragment peaks resulting from hard ionisation of the free-base phthalocyanine follow a similar pattern to those observed for the metal containing species. In this case, however, the principal fragments correspond to \([1/4P+\text{H}]^+\) at, 129 amu, and \([\text{C}_6\text{H}_4\text{CN}]^+\) at 103 amu. Thus, a hydrogen atom is retained by the fragment species in place of the metal species.

The compound Alcian Blue 8GX is a more complicated phthalocyanine containing at each phenyl ring position a sulphur containing chloride salt. These are added to the molecule in order to facilitate its incorporation into fabric fibres, but are removed during some stage of the dyeing process leaving copper phthalocyanine behind as a blue pigment. Figure 7.17 shows the mass spectrum obtained for this molecule following ionisation with 193 nm radiation. It is immediately obvious that no molecular ion peak is present at 1299 amu. The base peak in this spectrum occurs at 576 amu, which corresponds to the molecular ion of copper phthalocyanine, i.e. the side groups are lost. The peaks above 576 amu correspond
Chapter 7. $L^2$TOFMS of Dyes

Figure 7-15: $L^2$TOF mass spectrum of magnesium phthalocyanine obtained, under hard ionisation conditions, using 193 nm laser photoionisation.
Figure 7-16: L^2TOF mass spectrum of cobalt phthalocyanine obtained, under hard ionisation conditions, using 193 nm laser photoionisation.
Chapter 7. \( L^2 \)TOFMS of Dyes

281

Figure 7-17: \( L^2 \)TOF mass spectrum of Alcian Blue 8GX obtained using 193 nm laser photoionisation.

to sequential addition of \( \text{CH}_2 \) units, and further fragments involving parts of the side chains. No breakdown of the macrocycle itself is observed at the low laser fluences used in this experiment. To lower mass there are peaks at 63 amu and 65 amu corresponding to the two isotopes of atomic copper.

7.4 \( L^2 \)TOFMS of Anthraquinone and Coumarin Dyes

In contrast to the azo dyes, anthraquinone dyes, see Table 7.1, show no wavelength dependence with regard to fragmentation pathways. However, there are some differences in the ionisation cross-sections at the two wavelengths. It appears that the cross-section for ionisation at 266 nm of the anthraquinone moiety is smaller than that at 193 nm. This is seen in the trend towards decreasing photoion intensity in the mass spectra on going from Disperse Blue 1 to Basic Blue 47, culminating in the lack of any observable characteristic ion for Disperse Orange 11 at 266 nm. A similar though less dramatic trend is observed for ionisation
at 193 nm, although overall the spectra obtained using this wavelength are more intense than analogous 266 nm spectra. Figures 7.18a, 7.18b and 7.18c show the mass spectra obtained for the anthraquinone dyes Disperse Blue 1, Disperse Orange 11 and Basic Blue 47, under soft ionisation conditions, using 193 nm laser photoionisation. In each case, these spectra are relatively simple in appearance and all contain a signal representative of the target materials molecular ion. All three dyes were used as purchased with no preseparation procedures employed.

The Disperse Blue 1 sample used contained only 30% dye in a complex mixture of synthetic byproducts and precursor materials. In order to present the complete sample for analysis it proved necessary to apply the Disperse Blue dyestuff to the stainless steel sample probe as a slurry. This procedure was followed in order to prevent selective solvation of only a few components present in the dyestuff. Consequently, the mass spectrum shown in Figure 7.18a is dominated by a peak at 23 amu corresponding to sodium. Furthermore, as observed previously, in the case of the azo dyes, there is a significant ion signal, corresponding to a molecular-sodium adduct ion at 291 amu. No other peaks of significant intensity are observed.

The Disperse Orange 11 sample used contained 95% pure dye material. Its spectrum, see Figure 7.18b, unlike that of Disperse Blue 1, is dominated by a molecular ion peak 237 amu. No peak is present as a result of sodium ions being present and there is no molecular sodium adduct formation. There are, however, a number of ion signals present at lower masses with only low intensity. These source of these species could be either sample impurities or fragment ions resulting from the decomposition of the molecular ion. It is well known from EI mass spectrometry that the elimination of small stable species is a common fragmentation pathway for certain molecules. This is particularly so if the elimination results in the formation of a new bond between aromatic rings, thus increasing the stabilisation of the ionic product. Beynon et al. [30] first pointed out a common loss of CO from aromatic compounds such as quinones. It is, therefore, possible that the low intensity fragments at 209 amu and 181 amu are a result of such consecutive eliminations. However, there is no evidence for this in the mass spectra of the other anthraquinone dyes investigated here.
Figure 7.18c was obtained using a sample of Basic Blue 47 with only 45% dye content. This spectrum is slightly different to those observed previously for Disperse Blue 1 and Disperse Orange 11. Here, the molecular ion is not one of the most intense peaks. Instead, only a small peak at 372 amu is observed corresponding to the [M+H]^+ ion. The base peak is observed at 328 amu. This mass corresponds to the loss of [CH₂=NC₃]^+ from the molecular ion. This corresponds to familiar benzylic cleavage β to the benzene substituent on the anthraquinone molecule. Three other peaks are observable to lower masses, at 23, 58 and 77 amu. The peak at 23 amu indicates the presence of sodium in the sample, whilst 58 corresponds to [CH₂=N(CH₃)CH₃]^+ and the peak at 77 amu can be attributed to a phenyl moiety. The origin of these peaks is not clear. It is possible that they are either products resulting from the fragmentation of the molecular ion or are present as impurities in the sample.

Figures 7.19a and 7.19b show the L²TOFMS spectra obtained for Disperse Blue under soft and hard ionisation conditions respectively, using 266 nm laser photoionisation. As seen in the 193 nm photoionisation mass spectra, there is a strong Na⁺ signal, and even when ionising at 266 nm a significant contribution from the molecular-sodium adduct ion. In fact, in the present cases there are peaks present which suggest that [M+2Na]^+ ions are formed. It has proved possible to suppress the formation of Na⁺ peaks. By dissolving the sample material in chloroform, or alternative solvents, and allowing any undissolved material to settle, it was possible to partially discriminate between the molecular species and sodium containing components. There are further differences between the mass spectra shown in Figures 7.19a and 7.19b and that in Figure 7.18a. Principally, there are two peaks present, even under soft ionisation conditions, at 94 amu and 108 amu. It is not clear from where these fragments are derived. However, under hard ionisation conditions, fragmentation procedes from these two species to lower mass suggesting that these may be fragments of the target molecule. Under neither soft or hard ionisation conditions are significant peaks observed between the molecular ion and the peak at 108 amu. Close inspection reveals that, under hard ionisation conditions, fragmentation procedes to production of C⁺ ions.
Figure 7-18: L²TOF mass spectra of a) Disperse Blue 1, b) Disperse Orange 11, and c) Basic Blue 47 using 193 nm laser photoionisation.
Figure 7-19: $L^2$TOFMS spectra of Disperse Blue 1 at 266 nm under soft and hard ionising conditions. Ionising laser power densities are a) $0.25 \times 10^6$ Wcm$^{-2}$ and b) $1 \times 10^6$ Wcm$^{-2}$. 
Chapter 7. $L^2$TOFMS of Dyes

The $L^2$TOFMS spectra of coumarin dyes, obtained using 266 nm laser photionisation, are characterised by strong molecular ion signals in high abundances, usually seen as the base peak. The relative absorption efficiencies at the two trial wavelengths, 193 nm and 266 nm, are the reverse of those observed for the anthraquinones. For the three molecules examined it was not possible to obtain mass spectra using 193 nm laser photoionisation. Figures 6.20a, 6.20b and 6.20c show the mass spectra of three coumarin-type dye molecules, namely Coumarin 47, Coumarin 102 and Coumarin 152a, respectively, obtained using 266 nm as the ionising wavelength. These dyes were purchased as pure materials and therefore all the peaks observed in these mass spectra should be derived from the molecular ion. All three spectra contain base peaks which correspond to the molecular ions of the target materials, although, in all cases, further peaks are present which can be attributed to fragmentation of the molecular ions. In each of the spectra shown, peaks marked with an X correspond to background signals and are not derived from the target material. Figure 7.20a has only one further fragment peak at 216 amu. This corresponds to the loss of CH$_3$ from the molecular ion of Coumarin 47. Similarly, for Coumarin 102 a group of peaks around 229 amu, shown in Figure 7.20b, correspond to the partial loss of the tertiary amine substituent. The fragmentation of Coumarin 152a is more complex, even under soft ionisation conditions. Three intense fragment peaks are observable at 270, 231 and 216 amu. These correspond to [M-CH$_3$]$^+$, [M-N(CH$_3$)$_2$]$^+$ and [M-CF$_3$]$^+$, respectively. The fragmentation pathways are restricted to the loss, or partial loss, of the coumarin substituent groups and, therefore, aid the identification of the molecular ion.

7.5 Concluding Remarks

The involatile and thermally labile nature of many dyestuffs makes conventional mass spectrometry difficult. This work demonstrates the effectiveness of a two-stage desorption/ionisation methodology for the analysis of a wide range of neutral dye molecules with a wide variety of structural features. It has been shown that using the technique of $L^2$TOFMS one can produce relatively simple mass spectra.
Figure 7–20: $L^2$TOF mass spectra of a) Coumarin 47, b) Coumarin 102 and c) Coumarin 152a, using 266 nm laser photoionisation.
under soft ionisation conditions, often providing direct molecular weight information. Furthermore, in selected cases significant structural information can be obtained by either judicious selection of ionisation wavelength, or by increasing the ionising laser power density to induce fragmentation. Characteristic fragmentations of the pure class I dyes include photolytic cleavage of the azo linkage at 266 nm, whereas initial loss of the phenyl side groups or cleavage around the azo bond is more characteristic at 193 nm. Hence, by employing laser photoionisation at both 193 nm and 266 nm, complementary structural information can be obtained. The class II dyes provide simpler spectra and demonstrate the ability of \( L^2 \)TOFMS to provide structurally significant information under hard ionisation conditions. The class III and class IV dyes similarly show simple, readily interpretable mass spectra. It has also been determined that the choice of ionising laser wavelength is critical for different dyestuffs as their ionisation-cross sections differ at different incident wavelengths.

\( L^2 \)TOFMS has also been shown to be effective in the analysis of impure dyestuff mixtures. The spectra generated from these mixtures are often more complex than those obtained for the pure dye materials. This can be seen clearly with reference to the impure azo dyestuffs. In these cases, assignment of the ion signals to target dye molecules or impurity species can become problematic. Future investigations will require mechanisms for clarifying the results obtained from two-stage experiments. There are two obvious methods for doing this. Firstly, a programme of chromatographic separations could be followed prior to analysis of the separated fractions. The analysis could conceivably be performed by desorbing the analytes directly from the chromatographic medium. It should then be possible to identify both the dyes and non-dye components present in the dyestuffs. However, such separations are often difficult due to the presence of both organic compounds and inorganic salts in the dyestuff mixture. Time-consuming pre-separations also negate one of the principal advantages of \( L^2 \)TOFMS as an analytical technique, namely the identification of target materials directly from their natural matrices. A more sophisticated method of approaching this problem would be to perform tandem experiments. By selectively photodissociating primary ions it should be
possible to determine which of the ion signals present in a mass spectrum are derived from a higher mass species or are derived from different materials in the sample. The use of 193 nm laser photoionisation for the analysis of azo molecules has suggested an alternative mechanism for the simplification of mass spectra, namely sodium cation attachment.

The studies presented above demonstrate the ability of L\textsuperscript{2}TOFMS to characterise dyestuffs. Problems associated with their characterisation are of major interest to numerous commercial and industrial applications. The obvious next step in these studies would be to investigate the possibility of desorbing dyestuffs directly from their host matrices, e.g. fabrics. The ability to do such experiments would pre-empt the need for extraction and purification steps, which may chemically alter the target material. This idea has been the subject of preliminary studies which are described in the following chapter. However, rather than using dyes, which are only present in dyed fabrics at prohibitively low concentrations, the target systems investigated were a variety of both model and real mixtures used to approximate fabric staining agents.
Bibliography


Chapter 8

Application of L²TOFMS to Complex Systems

8.1 Introduction

The results presented in the previous three chapters have demonstrated that L²TOFMS is a valuable technique for the identification of involatile or thermally labile organic molecules. It has also been shown that the technique is capable, in specific circumstances, of assaying for specific components of complex mixtures. The principal objective of the work presented in this chapter was to demonstrate the feasibility of analysing model and real systems, of significant commercial or industrial interest, using laser desorption laser photoionisation TOF mass spectrometry. The experiments were concerned predominantly with the detection of materials which constitute "real world" staining agents. These were examined both from the standard stainless steel probe and from alternative supports such as organic substrates.

Stains are remarkably complicated chemical systems which rarely exist as single component entities. They represent, therefore, a complex problem for the analytical chemist. Lloyd and Adams [1] have reviewed the different categories of textile soiling and examined the ways in which such soiling can be removed. The five classes they describe range from simple coatings to mechanically entrapped or chemically adsorbed particles. These different classes become progressively more difficult to remove from their substrate. It is also important to recognise the heterogeneous nature of a stain. It is often the combined chemistry of the highly
coloured species along with the less visible components, which influence the nature of a stain's adherence to its host organic substrate. It is, therefore, important to find analytical techniques capable of investigating such heterogeneous adsorbed layers. Conventionally, analysis of a stain is preceded by a series of extraction steps designed to remove the target material from its host substrate. However, the very nature of these systems means they are, by definition, difficult to extract. Thus, extractions are often performed under relatively harsh conditions which may result in the chemical alteration of the target species.

Staining agents are predominantly involatile substances which are adsorbed or bound to the surface of a substrate. A typical example would be the adhesion of fruit extracts to natural or synthetic textile fibres. In order to characterise these species mass spectrometrically they must first be volatilised. As mentioned previously, several desorption methods have been developed to enable the mass spectrometric characterisation of materials adsorbed on surfaces. These include FAB, SIMS and LDMS. These surface bombardment techniques are, however, of questionable use when the molecules of interest are bound or adsorbed to an organic substrate. The only study of this type in the literature concerns the direct determination of surfactants on textiles using laser desorption Fourier transform mass spectrometry (LD-FTMS) [2]. In the case of fabrics, these methods generally result in mass spectra in which the adsorbate is indistinguishable from background ions due to the ablated products from the substrate, at low concentrations. These background ions from the substrate effectively determine the detection limit. Any attempt to improve the sensitivity is hindered by the non-selective nature of the ionisation process; it is impossible to remove background signals and retain those of the target species. In order to maximise the chance of characterising the adsorbates they must be present in high concentrations and the level of background substances, which generate ions, as low as possible. Furthermore, the ion yield from the direct desorption/ionisation techniques can vary considerably depending upon the nature of the substrate. Such matrix effects make comparative measurements and quantitation very difficult. These contraints severely restrict the application of the desorption techniques to complex, "real world" problems.
In order to successfully analyse complicated mixtures directly from their host organic substrates or matrices it is necessary to either selectively volatilise the components of interest or selectively ionise the desorbed (or liberated) target species. Previous work carried out in this group at Edinburgh [3] suggested that the use of $L^2$TOFMS might enable the intact desorption of molecules from a variety of substrates yielding characteristic mass spectra after selective or partially selective postionisation. In this earlier study, by separating the desorption and ionisation stages, simple mass spectra of a number of organic sunscreens were obtained after desorption from both stainless steel and cotton. The spectra contained peaks attributable only to the dopant species and were not complicated by any background spectra from the cotton substrate. Thus, these spectra were simpler to assign than those previously obtained using alternative mass spectrometric techniques such as FAB and SIMS [4]. In this chapter the results which have been obtained subsequent to these earlier experiments are discussed. The work is divided into six sections, five of which concern particular types of staining agent. Included in each section are mass spectra obtained following desorption of both model compounds and the complex mixtures. Also included are the mass spectra obtained on desorbing either the model compounds or adsorbed mixtures from various organic substrates. These results are followed by a consideration of the fate of the substrate material in a $L^2$TOFMS experiment. The concluding section contains a summary of the results obtained and an assessment of the feasibility of using $L^2$TOFMS for the general analysis of adsorbates directly from organic substrates.

8.2 Investigation of Complex Stain Systems

All the experiments described in the following section were performed using the reflectron TOFMS. The samples were prepared for deposition on the stainless steel probe either in solution or as slurries. The doped fabrics were generally prepared by depositing the target material onto the fabric in solution or soaking the fabric in the solution itself. Once prepared, the fabric samples were bound to the stainless
steel target using thin gauge nickel wire. Laser desorption was performed in all cases using the CO₂ laser and ionisation was achieved using one of the two readily available fixed frequency UV sources at 266 nm or 193 nm.

8.2.1 Adsorbed Dyestuffs

Dyestuffs can be considered as well-characterised staining agents. As discussed in Chapter 7 there have been many attempts to characterise pure dyestuffs using a variety of mass spectrometric techniques. However, their identification on fabrics has only been very recently demonstrated by Yinon’s group [5,6] who used a combination of solvent extraction followed by thermospray and LCMS to detect 10-100 ng of a variety of disperse dyes from single fibres of polyester, diacetate and cellulose acetate. However, it is questionable whether this technique can be used to study more labile target species such as substituted porphyrins. Also, since this approach requires extraction and separation procedures to be carried out prior to mass spectrometric analysis, a suitable means of extracting the dyestuffs must be available. However, in general, it may prove difficult to remove tightly bound reactive dyes without degrading the extracted dyestuff.

Two of the dyestuffs examined in Chapter 7 were used to assess the feasibility of using laser desorption to remove dye molecules intact from a host substrate: methyl red was desorbed from cotton and magnesium phthalocyanine was desorbed from cotton, polyester and nylon. The spectra obtained in each case are shown in Figures 8.1 and 8.2, respectively.

In both cases the samples were prepared by doping the fabrics with dilute solutions of the dyestuffs and then allowing the fabrics to dry in air. These nonionic dyestuffs were chosen initially to ensure that no strong chemical binding was operative which might reduce the efficiency of the desorption process. The spectrum shown in Figure 8.1 for methyl red desorbed from cotton was obtained using 266 nm laser photoionisation. This is similar to the spectrum obtained when desorbing directly from the stainless steel probe (see Figure 7.3b). Again, characteristic cleavage about the central azo bond is observed resulting in the base peak at 136
Chapter 8. Application of $L^2$TOFMS to Complex Systems

Figure 8-1: $L^2$TOF mass spectrum of methyl red obtained using 266 nm laser photoionisation after IR laser desorption from a cotton substrate.

Clearly there is no significant interference due to peaks attributable to the substrate. This makes the mass spectrum is readily interpretable.

Similar behaviour is observed in the case of magnesium phthalocyanine. Each of the three spectra shown in Figures 8.2 were obtained by dropping approximately the same amount of dyed solution onto squares of fabric. The target dye was mixed with an additional internal calibrant, tetraphenylporphyrin (TPP). Aniline is present in all three spectra. This was seeded into the molecular beam, via a reservoir behind the pulsed nozzle, and acted as an internal mass calibrant for the system. The mass spectrum in Figure 8.2a is that obtained on desorbing from polyester. It consists of four principal features, namely calibrant peaks at 93 amu and 614 amu, corresponding to aniline and TPP, the molecular ion of the magnesium phthalocyanine at 537 amu and a peak at 638 amu. This latter peak appears to be due to the formation of magnesium TPP. It is unclear at which point the magnesium adds to the TPP. This could be during desorption, ionisation, whilst present on the fabric surface or in solution. Once again there are no significant peaks present in the mass spectrum which correspond to fragments.
Figure 8-2: L²TOF mass spectra of magnesium phthalocyanine obtained using 266 nm laser photoionisation after IR laser desorption from a) polyester, b) nylon and c) cotton substrates.
of the substrate material. Similar mass spectra were observed on desorbing the mixture from both nylon (Fig. 8.2b) and cotton (Fig. 8.2c).

Although the three spectra for these different substrates are similar there are significant observable differences. Most importantly is the difference in the intensities of the dye ion signals, relative to that of the aniline, between the synthetic and cotton fibres. It is possible that this could be a consequence of increased dye-substrate interaction in the case of the cotton. Alternatively, it could be an indication of the distribution of dye molecules within the substrate. If the ion signal is solely a result of the desorption of molecules present on the surface of the fibres then these spectra suggest that dye penetration is greatest for the cotton substrate. The actual physical structure of the woven fabrics must be taken into account, along with a consideration of their behaviour under irradiation with high intensity infrared radiation. Polyester and cotton are both relatively thick fabric sheets. Their fibres are woven into bulky fabrics whilst the nylon fibres are extremely fine resulting in a sheer woven textile. Thus, it is possible that more adsorbed dyestuff can be liberated from the nylon substrate, as more of it is accessible to the incident desorption laser beam. However, this explanation does not account for the similarity between the spectra obtained from the polyester and nylon substrates. Perhaps a more useful explanation is that which takes into account the effect of high intensity radiation on the substrates themselves.

After irradiation the three fabric types are all adversely affected. The cotton substrate remains mainly intact but tends to become fragile and brittle with a shallow groove evident in its exposed surface. The two synthetic fibres melt and degrade. It is possible that the increased quantity of material volatilised, in the case of the synthetic fibres, causes disruption of the molecular beam and a concomitant reduction in the aniline signal intensity. For cotton, much less material appears to be ablated. Therefore, the disruption to the molecular beam may have been less significant allowing a larger proportion of the aniline seeded molecular beam to reach the ionisation region. At present, it is not clear which of the explanations proposed here is responsible for the observed differences in the mass
spectra. It is more than likely that each of the effects discussed above contributes to the observed behaviour.

Further experiments were also performed using fabrics which had been exposed to conventional dyeing processes under controlled conditions. These consisted of a variety of synthetic fabrics treated with several of the disperse dyes discussed in the previous chapter. In this case no signals were obtained for either the dyestuff or the organic substrate materials. It is puzzling why these dye species were not detected. One possibility is that by dyeing the fabrics in a colour fast process they are now so tightly bound to the fabric substrates that they cannot be easily removed by laser desorption. However, disperse dyes are not designed to be chemically bound to the fibres but are retained by a combination of weak coulombic attraction and mechanical entrapment [7]. A more plausible explanation is that the dye concentration loading in a formal dyeing process is considerably lower than that attained on doping concentrated solutions directly onto the fabric. Further work is required to enhance the instrumental sensitivity and to enable the detection of these compounds from dyed fabrics.  

8.2.2 Chlorophyll a

Chlorophyll a is the compound used to model grass stains. The mass spectra in Figure 8.3 show the mass spectrum of chlorophyll a obtained using 193 nm laser photoionisation after laser desorption from a stainless steel substrate (Fig. 8.3a) and from a cotton substrate (Fig. 8.3b). This latter mass spectrum was obtained under partially hard ionisation conditions in order to maximise the signal intensity observed. The mass spectra contain similar features. However, on close inspection there are a number of significant differences. Firstly, the overall signal intensities

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1Since the completion of the studies reported in this thesis similar dyestuffs have been successfully detected directly from their fabric substrates. The increase in sensitivity required to do this was achieved by dispensing with the molecular beam entrainment process and ionising the desorbed species directly above the sample probe.
in the mass spectrum obtained after desorption from the cotton substrate. This probably reflects the difficulty of removing all the adsorbed material from the bulky cotton substrate. This is similar to the arguments discussed above with respect to the adsorbed dyestuffs. Most noticeably, in Figure 8.3b, the peaks at 834 amu and 556 amu are substantially larger than their counterparts in Figure 8.3a, relative to the molecular ion signal.

It seems possible that the latter differences can be attributed to facile fragmentation reactions occurring during the desorption step which compete with intact desorption. A similar phenomenon was observed previously in the case of a 1,3-diphenyl-1,3-propanedione derivative doped onto a cotton substrate [8]. The molecular structure of this, along with those of the principal fragmentation products observed in the L²TOF mass spectrum of this compound, are given in Figure 8.4. It was observed that the degree of fragmentation apparent in the mass spectrum, after desorption from the cotton substrate, was substantially enhanced over desorption from a stainless steel probe. This led to the suggestion that a component of the fragmentation was associated with the desorption process. It was postulated that the two carbonyl functionalities were involved in interactions with the cotton substrate and that on subsequent IR laser interaction, the intramolecular bonds adjacent to the carbonyl group fracture in preference to the substrate-adsorbate bond. Similarly, although the spectrum for chlorophyll a is much more complicated, the new fragments may be attributable to fragmentation associated with the moieties adjacent to its carbonyl functionalities.

In the case of the peaks observed in Figure 8.3b, the peak at 834 amu can be attributed to the loss of the CH₃CO₂ group from the chlorophyll a ring 5 and that at 556 amu to the loss of this group along with the Phytyl or the loss of Phytyl-OCOCH₂ from the molecular ion (see chlorophyll a structure in Figure 8.3). It is reasonable to assume that the ester functionality would play an important role in binding to the fabric. This is quite likely to interact with the polar functionalities on the cotton fibres and bind the adsorbate to the substrate surface. Therefore, these results suggest that the chlorophyll a is in some way attached to the cotton
Figure 8-3: $L^2$TOF mass spectra of chlorophyll a obtained using 193 nm laser photoionisation after a) IR desorption from a stainless steel substrate and b) IR desorption from a cotton substrate.
substrate and that a facile fragmentation during the desorption competes effectively in this case with intact desorption.

Chlorophyll a was also used to assess the detection sensitivity of the technique when desorbing trace species from organic substrates. Chlorophyll a was doped onto a 4.6 cm² area of cotton fabric using dilute solutions with successively lower quantities. Again, partially hard ionisation conditions were used to maximise the detection sensitivity. Three spectra obtained using successively more dilute solutions to dope the fabric are shown in Figure 8.5a, 8.5b and 8.5c. Each of the spectra shown represents the accumulation of 500 consecutive shots. The amount of adsorbed material present in the fabric over the area covered by 500 shots was estimated, assuming that the doping process had resulted in an homogeneous coverage. It was calculated that the spectra shown in Figure 8.5a, 8.5b and 8.5c corresponded to the removal of ca. 11 nanomoles, ca. 3 nanomoles and ca. 600 picomoles of material, respectively. The last spectrum effectively represented the limit of detection for chlorophyll a desorbed from cotton under these conditions. It should be noted these figures correspond to the amount of material doped onto
the fabric to obtain the mass spectra shown. Much less material will be actually liberated from the fabric than is doped on. This is clearly apparent on inspection of the fabric after the desorption experiment has been performed since the remaining fabric retains a strong residual green colouration indicating the persistence of adsorbed chlorophyll a.

The most striking feature in the spectra shown in Figure 8.5 is the presence of a significant broad background signal. This is especially noticeable in Figure 8.5c. It is thought that this background is a result of ions created in the desorption step penetrating the field in the extraction region and reaching the detector. This could be a consequence of the open design of the ion source optics allowing ions to pass around the edges of the repeller plate. These nascent ions will have a wide range of kinetic energies and will therefore result in a broad spectral feature. A further interesting feature of the spectra is the variation in the 615 : 834 and 834 : 892 mass peak intensity ratios. All three spectra in Figure 8.5 were recorded under similar desorption and ionisation conditions. These ratio may therefore have been expected to remain constant as a function of sample loading. This is clearly not the case. The intensity of the peak at 615 amu and that due to the molecular mass decrease at a much greater rate than the peaks at 834 amu and 556 amu with decreased levels of doping. A possible explanation for this behaviour is that at low sample loadings the predominant signals observed are those which correspond to chlorophyll a molecules bound to the fibres. At higher concentrations, the samples are many layers thick and many of the adsorbed molecules will be unable to form any bonds with the cotton fibres. These will therefore be desorbed intact and give signals which correspond to those obtained on desorption from a stainless steel substrate.

8.2.3 Curcumin, Turmeric and Curry

A familiar source of fabric stains is the collection of spices gathered under the umbrella title of curry. The stain resulting from the absorption of these spices is generally a bright yellow which discolours to brown over time. The fact that curry
Figure 8-5: $L^2$TOF mass spectra of chlorophyll a obtained using 193 nm laser photoionisation after IR laser desorption from a cotton substrates. The amount of material required in the sample cloth to produce each spectrum was a) 11 nanomoles, b) 3 nanomoles and c) 600 picomoles.
Chapter 8. Application of L²TOFMS to Complex Systems

is a mixture of spice, including paprika, cumin, ginger, cinnamon and turmeric, and that each of these are complex mixtures of molecules, means that any analysis of a curry stain is a complicated problem. Previous research has identified the molecule curcumin as being one, if not the major, colouring agents responsible for the staining effect [1]. The mass spectrum of this molecule was examined from both a stainless steel probe and from cotton soaked in a curcumin solution. The same experiment was then repeated with a curry paste and turmeric. The samples of the curry were prepared as thick water based sludges and applied to the probe with a brush, whilst the turmeric was applied as a concentrated solution in acetone.

Curcumin

Figures 8.6a and 8.6b show the L²TOF mass spectra of curcumin using ionisation wavelengths at 193 nm and 266 nm, respectively. The structure of curcumin is also shown in the same figure. At 193 nm it can be seen that there is considerable fragmentation of the molecular ion, which is in fact one of the weakest peaks present in the mass spectrum. Subsequent loss of -OH and then loss of the aromatic side chains accounts for the peaks seen at 351 amu and 273 amu respectively. However, the peaks of greatest intensity are associated with the products of fragmentation about the methylene group, yielding peaks at 191 amu and 177 amu. The base peak at 148 amu is associated with loss from these fragments of \( \text{CH}_2\text{CO} \) or CO respectively. Reducing the laser fluence lowers the signal intensity across the whole mass range. Thus, the molecular ion is diminished to noise level before a reduction in the number of fragment ion peaks is observed. This fact suggests that the fragmentation about the central methylene unit competes successfully with molecular ion formation, i.e. it would appear that there is competition between MPI and neutral photodissociation in the intermediate state.

A far simpler spectrum was obtained using 266 nm laser photoionisation as shown in Figure 8.6b. Here, no molecular ion is observed but there is an easily identifiable base peak at 150 amu. This characteristic peak can be attributed to fragmentation of the molecular ion, leaving \( [\text{CH}_2\text{CHPh(OH)CH}_3]^+ \). Peaks at 190,
Figure 8-6: L²TOF mass spectra of curcumin obtained using a) 193 nm and b) 266 nm laser photoionisation.
177 and 164 amu correspond to fragmentation following fracture on either side of the methylene group. As with ionisation at 193 nm, lowering the ionising laser power density results in reduced intensity for all peaks and does not promote the growth of a molecular ion, as would be anticipated by the stepwise fragmentation mechanism (see Chapter 2).

As the spectrum obtained at 266 nm gave the simplest characteristic spectrum of curcumin, this wavelength was used to obtain a spectrum of curcumin desorbed directly from a cotton substrate. Figure 8.7 shows the spectrum obtained after doping a cotton sample with a 10g/l solution of curcumin in acetone. The peak at 150 amu is still apparent in the mass spectrum, although it is significantly weaker than in the undoped spectrum. Its intensity is lower than even that of peaks due to background pump oil, which are labelled with an X. Approximate detection limits were determined for laser desorption of curcumin from both a stainless steel probe and the doped cotton substrate using 266 nm laser photoionisation. The same procedure as outlined for the chlorophyll a measurements was used. Each mass spectrum collected represented the accumulation of 500 successive laser shots. The experiments were repeated with lower sample loadings until the intensity of the largest signal peak was approaching the background noise level. This yielded detection limits of ca. 20 nanomoles and ca. 300 nanomoles for desorption from stainless steel and cotton fabric respectively. The decrease in detection sensitivity upon changing from the stainless steel to the cotton substrate is perhaps not surprising as the doped sample will have penetrated into the bulk of the fibres and would be difficult to liberate completely. These detection limits are somewhat higher than those obtained for chlorophyll a desorbed from cotton. This probably reflects a non-optimal choice of ionisation wavelength. Increasing the detection sensitivity would require some knowledge of the gas-phase absorption spectrum of curcumin and recourse to a tunable dye laser as the ionising source.

Turmeric

The spice turmeric is principally used as a yellow colouring agent. It is made from the sun-dried roots of the tropical turmeric plant and is widely employed
Chapter 8. Application of $L^2$TOFMS to Complex Systems

Figure 8-7: $L^2$TOF mass spectrum of curcumin obtained using 266 nm laser photoionisation after IR laser desorption from a cotton substrate.

in commercial curry powders. It would be expected that any spectrum obtained from this mixture of chemicals will contain signals which correspond to the presence of curcumin. Its presence has been recently reported by Hiserodt et al. [9]. They used particle beam liquid chromatography-mass spectrometry (PB-LC-MS) to investigate the methanolic extract of turmeric, and determined the major component to be curcumin. Other species were also determined to be present. The $L^2$TOF mass spectra obtained using 193 nm and 266 nm laser photoionisation are shown in Figures 8.8a and 8.8b, respectively.

At 193 nm, the spectrum of turmeric contains an intense base peak at 39 amu corresponding to the presence of potassium in the sample, and peaks at 177, 148, 137 and 120 amu. These peaks are also present in the $L^2$TOF mass spectra of curcumin at 193 nm (see Figure 8.6a). Similarly, the 266 nm $L^2$TOF mass spectrum of turmeric has a base peak at 150 amu which is also a characteristic mass signal for curcumin at this wavelength. These results suggest that curcumin is present in sufficient concentration to enable $L^2$TOFMS to identify the curcumin component.
Figure 8-8: L$^2$TOF mass spectra of turmeric obtained using a) 193 nm and b) 266 nm laser photoionisation.
Figure 8.9: L^2TOF mass spectrum obtained using IR desorption of a curry mixture followed by 266 nm laser photoionisation.

Curry Powder

Curry powder, as mentioned above, is an immensely complex mixture. The curry powder used for the following experiments was mixed into a paste using water. Figure 8.9 shows the mass spectrum obtained after the desorption of thick layers of dried curry paste from a stainless steel probe using 266 nm laser photoionisation. The base peak of this spectrum is at 180 amu. The complexity of the curry mixture makes the assignment of this peak problematic. However, there are peaks present in this spectrum which correspond to those observed in the spectrum for curcumin at 266 nm, namely the peaks at 120 amu and 150 amu.

In order to approximate conditions which would constitute a real stain environment, an aqueous solution of curry powder was prepared. Cotton samples were immersed in this and then dried with a hot air blower. No solid particulates were left on the fabric. Essentially, only the water soluble components were present on or in the cotton. The spectrum that was obtained from this sample is shown in Figure 8.10. As before, signals attributable to the background oil are labelled with an X. The peaks at 150 amu and 180 amu were observed in the 266 nm L^2TOF
mass spectrum of curry desorbed directly from stainless steel. However, now the relative intensities are reversed, with the base peak in this case being at 150 amu, which appears likely to originate from curcumin. The peak at 211 amu, which is present in the spectrum of curry desorbed from stainless steel, is not present above the noise in the spectrum of curry desorbed from cotton.

One possibility is that the species responsible for the peaks at 180 amu and 211 amu are more difficult to desorb from cotton, but easier to desorb from stainless steel than curcumin. Alternatively, it is possible that curcumin has a greater affinity for the cotton substrate than the species giving rise to the 180 or 211 amu fingerprint, and is preferentially adsorbed from solution. This would result in curcumin being present in larger quantities on the cotton than the other species. Part of the stained fabric was then subjected to solvent extraction in acetone. The residue from this extraction was then investigated by desorption from the stainless steel sample probe. The resulting mass spectrum is shown in Figure 8.11.

Here the relative intensities of the 150 amu and 180 amu peaks have reverted to those obtained for analysis of the curry paste direct from the stainless steel probe.
It is also clear that the species responsible for the 211 amu peak is present on the fabric. There are a number of possibilities which can explain these observations. If the solubilities and extraction rates of all the components are assumed to be the same it suggests that curcumin is not as strongly bound to the fabric as the other observed components. Alternatively, the reappearance of the 180 amu and 211 amu peaks could simply be a consequence of their greater solubility in acetone compared with curcumin.

Finally, an aged sample of curry stained cotton was investigated under the same conditions. No new peaks were seen in the mass spectrum obtained for the sample upon ageing the stain for several days. The spectrum was essentially the same as that shown in Figure 8.10, but with a much reduced signal to noise ratio. This would suggest either that the sources of the observed peaks are changing chemically with time, or that they remain intact but become more firmly bound to the fabric fibres. The absence of new peaks to higher mass does not rule out the possibility of polymerisation, for example, of the adsorbates, but may simply indicate an inability to desorb and/or ionise the polymeric species with sufficient sensitivity.

Figure 8-11: L²TOF mass spectrum of curry residue, extracted from a cotton substrate with acetone, obtained using 266 nm laser photoionisation.
Figure 8-12: L²TOF mass spectrum of black tea residue obtained using 266 nm laser photoionisation.

8.2.4 Tea and Coffee

Tea

Tea is another complex mixture of organic species which can lead to the discolouration of fabrics by staining. The chemistry of tea is enormously complex, with different combinations of species being present in green, black and brewed tea. Table 8.1 shows some of the major species present in black tea along with their approximate concentrations [10]. Sainsbury's Keemun tea was used in the present investigations. A sample of tea was boiled with water and the resulting solution separated from the tea bulk by filtration. The resulting black tea mixture was concentrated by evaporation before being applied directly to the rod. The mass spectrum of the resulting mixture was recorded using 266 nm laser photoionisation and is shown in Figure 8.12.

No mass peaks were observed above 200 amu. The base peak is at 194 amu and can be attributed to the molecular ion of caffeine. This known to have a large ionisation cross-section at 266 nm. There is evidence for the presence of
<table>
<thead>
<tr>
<th>Component</th>
<th>Weight % of Dry Extract Solids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>7.56</td>
</tr>
<tr>
<td>Theobromine</td>
<td>0.69</td>
</tr>
<tr>
<td>Theophylline</td>
<td>0.25</td>
</tr>
<tr>
<td>(-)Epicatechin</td>
<td>1.21</td>
</tr>
<tr>
<td>(-)Epicatechin gallate</td>
<td>3.86</td>
</tr>
<tr>
<td>(-)Epigallocatechin gallate</td>
<td>4.63</td>
</tr>
<tr>
<td>(-)Epigallocatechin</td>
<td>1.09</td>
</tr>
<tr>
<td>Biaflavanols</td>
<td>Trace</td>
</tr>
<tr>
<td>Theaflavic acids</td>
<td>Trace</td>
</tr>
<tr>
<td>Thearubigens</td>
<td>2.63</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>1.15</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>0.21</td>
</tr>
<tr>
<td>Sugars</td>
<td>6.85</td>
</tr>
<tr>
<td>Pectin</td>
<td>0.16</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>4.17</td>
</tr>
<tr>
<td>Oxallic acid</td>
<td>1.5</td>
</tr>
<tr>
<td>Malonic acid</td>
<td>0.02</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>0.09</td>
</tr>
<tr>
<td>Malic acid</td>
<td>0.31</td>
</tr>
<tr>
<td>Aconitic acid</td>
<td>0.01</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.84</td>
</tr>
<tr>
<td>Lipids</td>
<td>4.79</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.83</td>
</tr>
<tr>
<td>Other minerals</td>
<td>4.70</td>
</tr>
<tr>
<td>Peptides</td>
<td>5.99</td>
</tr>
<tr>
<td>Theanine</td>
<td>3.57</td>
</tr>
<tr>
<td>Other amino acids</td>
<td>3.03</td>
</tr>
<tr>
<td>Flavonol glycosides</td>
<td>Trace</td>
</tr>
<tr>
<td>Aroma</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 8-1: Black tea beverage composition.
potassium in the mixture with a peak at 39 amu. Other peaks between these well characterised species are more difficult to unequivocally assign to specific species. It was anticipated that some evidence may have been found for larger polyphenolic species such as the theaflavins and thearubigens which are likely to be present in tea stains. A number of model polyphenolic compounds have previously been investigated in Edinburgh [11]. These are likely substructures for larger polyphenolic compounds present in the tea residue and are readily available as pure materials for investigation. These earlier studies have shown that it is possible to obtain simple soft ionisation mass spectra for gallic acid, methyl gallate and catechin using 266 nm laser photoionisation. In the tea residue these species are likely to be present in much lower quantities than were used in the preliminary studies of the pure compounds. However, peaks at 170 amu and 153 amu correspond to the characteristic signals obtained for gallic acid using 266 nm laser photoionisation. The structures corresponding to a number of the possible constituents of both the tea and the coffee residues are given as examples in Figure 8.13.

Coffee

The composition of coffee depends upon both the type of bean used and the treatment it subsequently received in processing [12]. Table 8.2 shows the full list of components suspected of being present in coffee. A high proportion of the aromatic compounds in coffee beans are phenolic and are presumably derived from the lignin and tannin of the cell structure. Again, as with tea, it is polyphenolic species that are suspected to be the main constituents in coffee stains. The polymerisation of small units such as catechol, or the condensation with other plant polyphenols, produces macrostructures which could be important in the formation of stains that are difficult to remove from fabrics.

For the purposes of our trial investigations, Nescafe instant coffee was boiled with water and then concentrated to a thick sludge. The residue was applied to the stainless steel probe in the normal fashion. Figure 8.14 shows the mass spectrum obtained using 266 nm laser photoionisation. Again, no peaks were observed at high mass, so it can be assumed that we are unable to detect any
Figure 8-13: Example structures of a variety of polyphenolic species thought to be present in tea/coffee residues.
<table>
<thead>
<tr>
<th>Components</th>
<th>Total %</th>
<th>Water Soluble %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>as amine acids</td>
<td>9.0</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Carbohydrates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>water insoluble</td>
<td>24.0</td>
<td>0.0</td>
</tr>
<tr>
<td>water soluble</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>sucrose</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Glucose, fructose</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Lipids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>esters, glycosides</td>
<td>2.0</td>
<td>some</td>
</tr>
<tr>
<td>Sterols</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>other lipids/waxes</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><strong>Volatile acids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formic acid</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Nonvolatile acids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic, oxalic</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>pyruvic and citric</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Alkaloids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Trigonelline</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Ash</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minerals</td>
<td>4.0</td>
<td>3.5</td>
</tr>
<tr>
<td><strong>Water</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Volatile aroma cmpds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Browning cmpds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35.0</td>
<td>7.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>27.5</td>
</tr>
</tbody>
</table>

Table 8-2: The percentage composition of roasted Arabica coffee.
of the larger polyphenols intact. The base peak, at 194 amu, corresponds to the expected presence of caffeine. Peaks at lower masses are again difficult to assign. The peak at 39 amu is again representative of potassium, whilst the other peaks may be attributable to some of the polyphenolic species present. For example, the signals at 110, 136 and 150 amu could be due to the presence of pyrocatechol, 3,4-dihydroxystyrene, 3-methoxy-4-hydroxystyrene, or their isomers, respectively. These structures are shown in Figure 8.13.

These molecules are of major importance with regard to stain formation. These types of molecule and associated compounds change from white to brown-tan on exposure to light and oxygen. This suggests that they are condensing or polymerising to become "condensed tannins". The latter species can have very large molecular weights and are not observed in the mass spectra. As mentioned previously, the inability to detect these species does not necessarily mean they are not present but may simply reflect our inability to desorb or ionise them with sufficient sensitivity.
Coffee and Tea Mixture

Investigation of coffee and tea residues adsorbed onto fabrics was performed using a mixture of the two concentrates. The first mass spectrum obtained was that after desorption from the stainless steel probe, as shown in Figure 8.15a. The resulting spectrum was simply a composite of the spectra obtained for the individual components. This procedure was repeated with a sample of knitted polyester which had been soaked in an aqueous solution of coffee and tea mixture before being air dried. The spectrum obtained is shown in Figure 8.15b.

There are a number of minor differences between the mass spectra. The peaks in Figure 8.15b are generally more intense, relative to the caffeine molecular ion signal, than those in Figure 8.15a. This may reflect a preferential adsorption of these species onto the polymer fibres from the aqueous mixture. This experiment was repeated using cotton as the target substrate. The resulting spectrum is shown in Figure 8.16. Most of the peaks seen previously are present. However, in this case the relative intensities of the peaks are more dramatically altered than in the case of desorption from polyester. Here, the base peak is no longer that corresponding to the caffeine component. It is possible that the change in the caffeine to phenolic signal intensity ratio could simply mean that the former species is more difficult to remove from the fabric. Further comparative experiments, using pure model compounds, are required to shed further light on these questions.

8.2.5 Malvin and Red Wine

The residual stains caused by the interaction of red wine constituents with fabrics are also thought to be associated with polyphenolic compounds [13]. Anthocyanins comprise a group of naturally-occuring pigments which are responsible for the blue, red, violet and magenta colouration of many species in the plant kingdom. In these experiments, malvin, whose structure is shown in Figure 8.17, was examined using 193 nm and 266 nm laser photoionisation, together with a red wine concentrate. Malvin was chosen as it has been shown to be a colouring agent component in red wine.
Figure 8-15: $L^2$TOF mass spectra of a tea/coffee mixture obtained using 266 nm laser photoionisation after desorption from a) the stainless steel probe and b) knitted polyester.
Figure 8-16: L²TOF mass spectrum of a tea/coffee mixture obtained using 266 nm laser photoionisation after IR laser desorption from a cotton substrate.

Figure 8-17: Molecular structure of malvin.
Figures 8.18a and 8.18b show the mass spectra obtained for malvin using 193 nm and 266 nm laser photoionisation respectively. At 193 nm, the only significant peak seen is at 329 amu. This corresponds to the loss of the chloride counter ion from the molecular structure, followed by cleavage of the glycosidic bond, giving loss of the sugar group but retaining the oxygen atom. A similar peak, though of lower intensity is also seen in the 266 nm photoionisation mass spectrum. Both ionising wavelengths give rise to only weak signals. This is most probably because these particular wavelengths are not optimal for this molecule. Sugars are also known to be quite fragile species under the influence of IR radiation. Therefore, it is also possible that a considerable amount of sample degrades during the desorption process.

Two other peaks were observed when using 266 nm laser photoionisation, at 301 and 353 amu. The former peak is likely to result from the complete loss of the sugar groups (including the oxygens in the glycosidic bonds) and partial loss of the methoxy groups from the molecular ion. The latter peak is probably due to the retention of two carbon atoms by the 329 amu fragment. The fact that one can observe any signals from this molecule is quite remarkable. Being essentially ionic in nature, with chloride present as a counter ion, one might expect the material to dissociate in the desorption stage. In this case, the organic species, being positively charged would be unable to enter the ion source and would not be observed. The fact that characteristic fragments are seen lends weight to the argument that there is decomposition of the material in the desorption stage, yielding neutral fragmentation products which can be subsequently postionised.

As the signals obtained on desorbing pure malvin from the stainless steel probe were weak, it proved difficult to obtain any signals at all when malvin was doped onto a piece of cotton. Figure 8.19 shows the mass spectrum obtained for desorption of malvin from a cotton substrate and photoionising at 266 nm. It is clearly different from the spectrum shown in Figure 8.18b, having weak diagnostic peaks at 126 amu and 198 amu. These appear to originate from the dimethoxyphenol
Figure 8-18: $L^2$TOF mass spectra of malvin obtained using a) 193 nm and b) 266 nm laser photoionisation.
Figure 8-19: $L^2$ TOF mass spectrum of malvin obtained using 266 nm laser photionisation after IR laser desorption from a cotton substrate.

part of the molecule. If this is indeed the case then it could indicate that the dimethoxyphenol part of the malvin molecule is not involved in binding to the cotton substrate. The fact that different fragments are observable further supports the argument that the major fragmentation is occurring in the desorption stage.

Red Wine

The experiments performed on malvin were repeated using Sainsbury's Minervois red wine. This was prepared by reducing the water and volatile content through boiling. The remaining sludge was then applied to the sample probe in the conventional manner. The wine was expected to contain derivatives of malvin or other related anthocyanins. If malvin itself is present at sufficient concentration it was anticipated that characteristic peaks might be observed in the spectrum for red wine. Figure 8.20 shows the mass spectrum obtained for the red wine concentrate using 266 nm laser photoionisation. A weak peak is observed in this spectrum at 353 amu, which is also found in the spectrum for malvin, along with a stronger
peak at 198 amu, which is observed in the spectrum for malvin desorbed from cotton. A number of other peaks are also present to lower mass with significant intensity. However, no assignment can be made at this time. Even though it is unlikely that a complete assignment of the ion signals observed can ever be made, it is possible that using L^2 TOFMS, fingerprint spectra could be recorded for various wines and used to identify them.

8.2.6 The Fate of the Substrate

One of the attractions of the technique of L^2 TOFMS is that no signals are present in the resulting mass spectra which correspond to fragment ions from the substrate. In all the experiments described above involving desorption from either cotton, nylon or polyester substrates, no such peaks were observed. However, the IR desorption process does have a visibly damaging effect on the substrates, removing material from the bulk, charring cotton and causing synthetic fibres to melt and degrade.
There are a number of explanations which can account for the absence of background peaks in the mass spectra. The most likely possibility is that any of the substrate fragments which are entrained and carried through to the ionisation region are not ionised. Multiphoton ionisation of molecules using mid-UV photons is inherently selective, a fact demonstrated by the fact that non-aromatic components of a sample mixture are not detected: their ionisation potentials are generally too high. It is possible that any fragments that do arrive in the ion source have no appreciable one-photon absorption at 266 nm or 193 nm and are therefore simply not ionised. Alternatively, Schlag and Levine [14] recently proposed that as the molecular weight increases molecules become increasingly difficult to ionise, where ionisation is defined as the formation of a radical cation by ejection of an electron rather than cationisation etc. It is possible, therefore, that large fragments are vaporised from the natural or synthetic polymer fibres and that these are difficult to ionise using multiphoton ionisation techniques. A further possibility is that the IR laser does not actually ablate and fragment the fabric fibres, but that charring or melting results in large sections of the affected area simply collapsing and being pumped away in the source chamber.

3.3 Concluding Remarks

In the work presented in this final chapter it has been shown that $L^2$TOFMS can be used to examine a series of model and real stains desorbed from both organic and metallic substrates. In all cases, simply using two fixed wavelengths for ionisation, it was possible to obtain characteristic ion signals for the model compounds investigated. Furthermore, in certain cases it was possible to identify key target species in situ from their complex mixtures. This facilitates the identification of certain constituent materials without requiring complex extraction and separation procedures to be performed prior to analysis.

In these investigations $L^2$TOFMS has proven capable of generating significant information directly from complex heterogenous systems. The simplicity of the
approach, and its potential for the rapid generation of information from a wide variety of systems makes the technique uniquely attractive for the analysis of materials adsorbed onto organic substrates. As has been shown, many of the mass spectra resulting from the examination of mixtures are complex. This reflects the nature of the heterogeneous systems being examined. The complexity of the resulting mass spectra, combined with an inability to account for some of the peaks observed in the spectra from analysis of the mixtures, e.g. wine and curry, suggests that additional strategies are required to more fully exploit the ability of L²TOFMS to assay for materials adsorbed onto organic substrates.

In order to simplify the mass spectra obtained by this method the nature of the ionisation source needs to be considered. It is clear from this brief investigation that the two ionisation wavelengths chosen for the study are not ideal. Any further work must be preceded by a wider examination of the ionising wavelengths in an attempt to find more suitable and effective photoionisation schemes. A comparative analysis of the ability of nonresonant schemes and resonant schemes is required. By choosing an ionisation scheme particular to a specific target material it should be possible to selectively ionise that component in preference to other constituents.

Another feature of the experiment which must be addressed is the overall detection sensitivity. In the studies described in this chapter the levels of textile doping were generally much in excess of those which would constitute a "real" stain. The remedial measures required here are closely linked to the search for more suitable ionising wavelengths. By making use of molecule specific resonant ionising wavelengths the efficiency of the ionisation process is enhanced along with the potential for selective ionisation.

A more sophisticated extension of the basic methodology would be to perform tandem time-of-flight experiments. In the case of complex mixtures this would allow target ions to be mass selected, thereby simplifying the spectrum. These species could subsequently be photodissociated enabling structural characterisation of the target species.

Perhaps the most interesting outcome of these experiments is that some of the
results provide further evidence to suggest that IR laser desorption can be used to indicate the degree of interaction between the adsorbed species and the organic substrate. This was found for both chlorophyll a and malvin. The results from the experiments on these molecules suggested that the degree of fragmentation undergone by a target molecule in the desorption process may be related to the functional site specificity of its binding to the substrate. If this proves to be the case for a wide range of substrate-adsorbate systems, then L²TOFMS could prove to be an invaluable tool for determining target functionalities for detergent manufacture. Also, it could prove useful for monitoring time-dependent chemical changes in situ on substrate surfaces, for example, the effects of UV bleaching on dyed fabrics or the colour change observable over time in a blood or wine stain from a burgundy or red to a dull brown. Surprisingly, at present very few techniques are available which can provide molecule specific information on the nature of these processes. Such an approach could also be very useful in the examination of other analytically challenging materials directly on organic substrates, for example, airborne pollutants on vegetation, trace contaminants on polymer films, cosmetic formulations on skin or hair and inks and waxes on paper products.
Bibliography


[8] K. F. Costello, personal communication


Chapter 9

L²TOFMS - Concluding Remarks

9.1 Thesis Summary

In this thesis the application of laser desorption laser photoionisation time-of-flight mass spectrometry (L²TOFMS) to the analysis of a variety of organic systems has been described. Chapters 1 to 4 contain an introduction to the technique, an outline of the underlying physical principle, a description of the L²TOFMS instrumentation employed as well as a summary of the previous areas of research in which the technique has been applied. The main body of the thesis, Chapters 5 to 8, contains a detailed description of the application of the technique in several new areas of research.

The experiments described in Chapter 5 are concerned with the use of L²TOFMS for the characterisation of polyaromatic hydrocarbons (PAHs). Mass spectra for a series of pure PAHs are presented under both soft and hard ionising conditions and the resulting fragmentation patterns discussed. Mass spectra for the molecule carbazole are also presented to demonstrate the feasibility of studying more polar polyaromatic species. This work was a preliminary to studies involving the direct determination of such analytes from contaminated matrices. These latter experiments involved the determination of PAHs following direct desorption from a variety of complex environmental matrices, namely contaminated soil, contaminated oil, soot and creosote wood preservative. In all cases, signals were obtained which corresponded to the presence of PAHs. These results demonstrate the potential of L²TOFMS as a fast, selective and sensitive method of screening for hazardous, contaminant PAHs directly from their host matrices.
In the experiments described in Chapter 6 the L\textsuperscript{2} TOF mass spectra for a variety of porphyrin species were examined. A series of biologically important porphyrins were investigated using 193 nm laser photoionisation. The fragmentation processes observed in their mass spectra were found to conform closely with those anticipated on the basis of EI mass spectra. Two series of synthetic metalloporphyrins were also investigated, the metaltetraphenylporphyrins and the metallo-octaethylporphyrins. The fragmentation patterns observed in their photoionisation mass spectra were found to be markedly dependent on the ionisation wavelength employed. The metaltetraphenylporphyrins exhibited both class A and class B photochemical behaviour. ZnTPP and CuTPP both exhibited class A behaviour, i.e. ionisation followed by fragmentation, whilst NiTPP and CoTPP both exhibited class B behaviour, i.e. dissociation followed by ionisation. VOTPP exhibited both class A and class B processes in parallel. These results were interpreted in terms of the known photochemistry of these molecules. A similar distinction between class A and class B behaviour was observed for the metallo-octaethylporphyrins. These results are discussed with reference to the tetraphenylporphyrins.

Chapter 7 describes the studies carried out on a series of commercial dyestuffs using L\textsuperscript{2} TOFMS. The materials examined can be divided into four classes; azo dyes, phthalocyanine dyes, anthraquinone dyes and coumarin dyes. It was demonstrated that L\textsuperscript{2} TOFMS was able to produce characteristic mass spectra for each of these classes of dye. For the azo dyestuffs, the mass spectra obtained on using either 193 nm or 266 nm laser photoionisation are found to be different. Photoionisation at 266 nm promotes a characteristic photoreductive cleavage of the azo bond. It is proposed that this photodissociative ionisation is directly related to photoisomerisation, in that it proceeds via the same one-photon excited state. That this behaviour is characteristic of the wider class of azo molecules was confirmed by recording mass spectra of a series of pure azobenzene derivatives, namely azobenzene, 4-azophenylaniline, 4-azophenylphenol and methyl red crystals. The mass spectra of the commercial dyestuffs obtained using 193 nm photoionisation showed no indication of azo bond cleavage. Here, molecular or sodium-molecular
adduct ions were the principal species observed. The data obtained for the other classes of dye are discussed with reference to their photofragmentation and relative ionisation efficiencies at 193 nm and 266 nm. The phthalocyanines gave simple L^2TOF mass spectra containing predominantly the molecular ion under soft ionisation conditions. The anthraquinone dyes had stronger absorption cross-sections at 193 nm, giving soft ionisation mass spectra with strong molecular ions, whilst mass spectra of the coumarin dyes could only be obtained using 266 nm laser photoionisation.

In Chapter 8 the results of the application of L^2TOFMS to more analytically complex systems are presented. The principal thrust of this work was concerned with the analysis of materials which constitute “real world” staining agents. A number of systems were examined using desorption from organic substrates such as cotton or nylon fibres. The results indicate that L^2TOFMS has unique potential for the investigation of complex, heterogeneous systems. Furthermore, the studies demonstrate that the technique is capable of examining such systems directly from analytically challenging matrices and substrates.

9.2 Advantages of L^2TOFMS

It is clear from the work presented in this thesis that L^2TOFMS is a very powerful technique for the analysis of nonvolatile or thermally labile organic molecules. IR laser desorption can be used to vaporise such molecules with minimal decomposition allowing their analysis by photoionisation time-of-flight mass spectrometry. The advantages of the two-step, laser desorption/laser ionisation approach favour its application to a wide variety of problematic analytical scenarios. For example, it can be used to assay for target species directly from complex matrices. This precludes the requirement for time-consuming extraction and preseparation procedures. The technique could, therefore, be used as a rapid screening tool. It has also been shown that it is possible to desorb intact neutrals directly from polymeric organic substrates. A more conventional approach would have been to extract the
target analyte and purify it before mass spectrometric analysis. However, using \( L^2 \text{TOFMS} \) for direct *in situ* analysis, no interfering signals are observed corresponding to the organic substrate. The resulting mass spectra are therefore not complicated by analytically irrelevant matrix peaks. The laser desorption process, in this instance, provides not only a tool for sample volatilisation but is also a means for sample extraction or separation.

The successful application of rival mass spectrometric techniques, using surface bombardment or sputtering such as (FAB, SIMS, PD, LD), is often highly dependent on the method of sample preparation and presentation. The presence or absence of cations in the sample, the use of liquid matrices or clean surfaces to support the sample, and the presentation of the sample as monolayers or in bulk all appear to be critical factors for these different techniques. For many applications surface bombardment techniques require specific matrices or particular methods of sample presentation to enable a mass spectrum to be obtained. The most widespread use of matrices is in matrix assisted laser desorption (MALD). Here the matrix serves two major functions, namely absorption of energy from the probe laser and isolation of the analyte species. For studies of large biopolymers a matrix is crucial in enabling both volatilisation and ionisation without degradation of the target species. Similarly, support matrices, such as glycerol, are routinely used in FAB to maintain the liquid nature of the sample. This can lead to poor sensitivity and unusually high background signals. In plasma desorption mass spectrometry, on the other hand, no matrices are required but it is desirable to make the sample layer as thin as possible. To enable such films to be prepared requires the use of electrospraying techniques [1].

In contrast to these relatively stringent preparative conditions, the preparation required for IR laser desorption followed by laser photoionisation is considerably more straightforward. In the experiments described earlier in this thesis the \( L^2 \text{TOFMS} \) technique has been used to analyse a wide variety of sample types. For example, in the case of pure samples, complete desorption has been possible on both thick (ca. 0.5 mm) and thin (< 0.1 mm) sample layers by varying the desorption laser power. Furthermore, thick samples of dirty systems have been
succesfully analysed following IR laser desorption. In the few cases where matrices have been used as part of the sample preparation this has been to enable them to be loaded onto the sample probe, rather than to specifically enhance the desorption yield. It would appear, therefore, that the nature of the matrix does not prejudice the generation of a mass spectrum when the neutrals are analysed via postionisation. The absence of significant matrix effects has also been observed when the neutrals generated in a static-SIMS experiment are postionised [2]. Here, the flux of the emitted neutrals can be largely invariant to radical changes in the matrix composition whilst the nascent ion yield may be dramatically effected.

As well as the advantage of little or no requirement for complex sample preparation in the case of two-step L²TOFMS, there is an element of selectivity which cannot be matched by other single stage desorption techniques. As discussed in Chapter 2, laser photoionisation can be a selective process, depending upon the characteristic electronic absorption spectrum of the target molecules. This means that, even where there is a considerable matrix presence, the mass spectra need not be complicated by intense matrix peaks. In techniques where desorption and ionisation proceed in a single step, e.g. FAB, the mass spectra can be rendered useless as the important data is masked by strong background signals due to the matrix.

L²TOFMS also provides a very sensitive method of characterising trace molecular species. With the instrument in its present configuration, detection limits in the region of femtomoles are possible for selected PAHs. This sensitivity has been improved upon by Zare et al. [3] who claim zeptomole ($10^{-21}$ moles) detection limits for coronene using an instrument which dispenses with molecular beam entrainment. Work performed on contaminated soils (see Chapter 5), has determined that the instrument described in this thesis is capable of detecting PAHs in a solid waste matrix with concentrations as low as < 10 ppm by weight. However, in all discussions concerning sensitivity, it is important to note that the absolute detection limits are both wavelength and molecule specific. Thus, L²TOFMS spectra do not provide direct information on the relative concentrations of components in a mixture. To extract such information from the photoionisation mass
spectrum more detailed information concerning the photoionisation cross-section of the analytes at a particular ionising wavelength is required.

In addition to its analytical applications, multiphoton absorption can be used to probe the gas-phase photochemistry of target species. This was seen in the experiments performed on both the metalloporphyrins (see Chapter 6) and the azo dyestuffs (see Chapter 7). In each case, the photofragmentation observed was critically dependent upon the wavelength of the incident photoionising radiation. This gave direct information concerning the characteristic photochemistry of the target molecules.

The advantages of the L²TOFMS two-stage methodology, as applied in this work can be, therefore, summarised as follows:

- in situ analysis of complex systems.
- desorption with minimal decomposition.
- semi-selective soft ionisation, simplifying the spectra of complex mixtures.
- low limits of detection.
- determination of photochemical properties.

### 9.3 Current Limitations of L²TOFMS

It is also worth noting here a number of the current limitations of the technique. At present, only molecules which contain suitable UV chromophores can be examined. Whilst this adds a degree of selectivity, it simultaneously limits the range of molecules which are amenable to this type of mass spectrometric analysis. This can be solved by the use of a VUV ionising source. In addition, the existing design of the desorption/entrainment pick-up source limits the degree of selectivity of the existing multiphoton ionisation schemes. An improved source, capable of inducing
a greater internal cooling of the desorbed molecules, would considerably increase the opportunities for selective resonant multiphoton ionisation to be employed.

As mentioned previously, with regards to an assessment of the instrumental limits of detection, the technique is difficult to use quantitatively. One fundamental problem in attempting to quantify any measurements is that calibration experiments themselves are difficult to perform. To be confident at femtomole levels that any sample applied to the probe is in a homogeneous layer is problematic in its own right. However, if a set of experimental conditions are maintained constant, and sample preparation is performed with care, it is possible to determine a lower limit of detection for a particular species. For many applications, a simple “yes or no” answer, indicating whether or not a specific parameter is above or below a tolerable level, may be more important than obtaining a quantitative result at the expense of large amounts of time.

Other limitations include the resolution of the reflectron TOF mass spectrometer and the upper mass limit of the technique. Some ideas on how the mass resolution can be improved were given in Chapter 2. As for the latter problem, it has been postulated that larger molecules become more difficult to ionise using multiphoton ionisation as they can more readily dissipate electronic energy into their many vibrations. However, it may be possible to overcome this problem by simply resorting to an alternative postionisation method. One option would be to use shorter ionising laser pulses, e.g. picosecond lasers, in order to promote ionisation over the competing relaxation processes. Alternatively, it may be possible to exploit gas-phase cation attachment. In Chapter 7 it was observed that sodium ions readily formed adduct species with a number of laser desorbed dye molecules. Doping a sample with sodium-, or other metal-, containing species, may enable the process of gas-phase cationisation to be used for the ionisation of problematic large molecules.

Finally, in its present form the technique generates only limited information concerning the location of target species on the probe surface since the probe can move in only one dimension. The sensitivity losses associated with the molecular beam entrainment prohibit the use of small desorption spot sizes thus limiting
the spatial resolution attainable. Furthermore, using laser desorption it is not possible to achieve the controlled surface removal that is possible with some bombardment techniques, such as static-SIMS. With the latter technique, control over the primary ion current means that surface depletion in the submonolayer and the multilayer range is possible for all types of materials. This proves difficult, when using laser radiation, for a variety of reasons including focus diameter, excitation depth and shot-to-shot laser stability.

9.4 L²TOFMS - Analytical Future

The future of L²TOFMS would appear to lie in the development of specialist instrumentation, based on the same principles, and dedicated to particular applications; for example, the fabrication of a compact L²TOFMS mass spectrometer for on-site screening of polyaromatic hydrocarbons at contaminated land sites. However, the various applications are still being investigated. These are being pursued in two principal directions, namely molecular beam spectroscopy of laser desorbed molecules for highly selective mass spectrometric analysis and laser mass microscopy.

Supersonic jet spectroscopy yields dramatically simplified optical spectra and enables selective R2PI to be exploited for the analysis of components of complex mixtures. The goal in these investigations is to utilise the high sensitivity and excellent selectivity to determine target components in untreated mixtures. For example, the ability to assay for bio-marker porphyrins directly from crude oils or oil shales would be a significant improvement on present strategies employed in the oilfield exploration industry. The development of the optically selective aspect of the technique has been the goal of a number of research groups [4,5,6,7]. However, a major problem has been the difficulties in maintaining a sufficiently stable yield of desorbed molecules for periods long enough to allow reproducible wavelength-scanned spectra to be recorded. A number of attempts have been made to address this problem. Examples include the preparation of thin films on metal blocks and
polymers [8], slurry matrices [9] and materials such as glycerol [10]. Movement of
the sample has also proved advantageous, allowing a clean surface to be exposed
to each laser shot, thus improving stability [11,12]. Most recently, a new polymer
matrix containing particulate silver has been developed [13]. These silver containing
matrices permit stable desorption to proceed for extended periods of time.

The most exciting development, however, is the introduction of laser mass mi-
croscopy. This exploits the high sensitivity of laser photoionisation time-of-flight
mass spectrometry and the high spatial resolution achievable (ca. 1-40 μm) by
tightly focussing the desorption laser. As mentioned in Chapter 4, three groups
have developed instruments for this purpose [14,15,16]. The demands of techno-
logical progress have provided the driving force for the evolution of high resolution
mass spectrometric probes. Surface phenomena such as adhesion, friction, corro-
sion, adsorption, wettability and biocompatibility have become important in such
diverse areas as catalysis, microelectronics, clinical analysis, polymer development
and the environmental sciences. As these phenomena are governed by the molecu-
lar structure of the surface layers it is not sufficient to obtain elemental information
alone. A technique with high sensitivity and good lateral resolution, which can
provide detailed molecular information is required. A natural extension of such
laser desorption probes is to develop the capability to perform 2-D mapping exper-
iments for surface adsorbates on a variety of substrates. Desorption-postionisation
methods are most suited to these experiments because of their extreme sensitivity
and the optical selectivity inherent in the ionisation process. Examples of mapping
applications could include the investigation of heterogeneous stains on organic fi-
bres, detergents on hair, oils on clean surfaces, PAH contaminants on aerosol
particulates, biomolecules in plant tissue and the characterisation of additives in
polymeric films.

The principal aim of the work described in this thesis has been to develop the
applications of the two-stage mass spectrometric technique, L²TOFMS. However,
as pointed out in Chapters 1 and 2, it is the ability of laser desorption to volatilise
fragile molecules with little or no decomposition which underpins the technique
as a whole. Laser desorption, therefore, may be exploited as a separation or
deposition technique in its own right. Material evaporated from one substrate and subsequently deposited on a different substrate can then be analysed by a variety of techniques other than mass spectrometry. Laser desorption transfer (LDT) is an example of this methodology. De Vries et al. [17] created a simple transfer device in which the laser desorption beam traverses a substrate that is transparent for the laser and that is mounted as close as possible to the sample. On desorption the sample molecules travel in straight lines to the unheated substrate where they are deposited. This technique can be applied when the analysis of the molecules in the sample is difficult, e.g. infrared spectroscopy where the background from the substrate can limit the sensitivity of detection of the overlayers. LDT can transform this problem from reflection IR analysis to transmission IR analysis. Similar experiments should be feasible using other techniques such as fluorescence and UV/VIS spectroscopy.

In conclusion, $L^2$TOFMS has been shown to be a powerful technique for the analysis of involatile or thermally labile materials. It has proved exceptionally effective in interrogating samples in complex matrices and samples deposited on organic matrices. Although its general application is restricted by the inherent complexity of the laser desorption and laser ionisation processes it is now becoming apparent, seven years after its conception, that the technique has a particularly important role to play in the investigation of complex systems as a valuable complement to more conventional analytical techniques.
Bibliography


342


Appendix A

Courses and Conferences Attended

In accordance with the regulations of the Department of Chemistry, University of Edinburgh I have attended the following courses during my period of study:

1. Lasers 1

2. Mass Spectrometry

3. Vued and Microemacs

4. Vuwriter

5. Unix 1 and 2

6. Fortran

7. Postgraduate Lectures

In addition I have attended the Laser Chemistry research group meetings, departmental seminars and joint Edinburgh-Glasgow laser chemistry group meetings.

I have also attended the following conferences:

   - poster presentation
Appendix A. Courses and Conferences Attended


3. The Biochemical Society Meeting No. 639, Manchester, 1991 - poster presentation


7. XXVIII Colloquium Spectroscopium Internationale, York, 1993 - oral presentation
Appendix B

Publications


A. C. Jones, M. J. Dale, M. R. Banks, I. Gosney, P. R. R. Langridge-Smith, "Delayed Ionisation of Laser Desorbed C$_{60}$ Molecules Following 193 nm Photoexcitation", Molecular Physics, 80, 583, (1993)


The mass spectra of a number of dye species have been investigated using two-step laser desorption mass spectrometry (L2TDMS). A pulsed CO₂ laser, wavelength 10.6 µm, was used to vaporize the samples on heated metal matrices which were photodesorbed using either 193- or 266-nm laser radiation. Four classes of dyes have been examined: azo, anthraquinone, phthalo-cyanine, and azomethine azo dyes. Significant differences were found in the fragmentation patterns and ionization efficiencies of these two laser wavelengths particularly for the azo dyes. The applicability of this mass spectrometric technique to the identification, characterization, and detection of aromatic dyes is discussed.

INTRODUCTION

Dyestuffs are of considerable environmental and commercial interest because of their use as colorants in a wide variety of products such as textiles, paper, foodstuffs, leather, and pharmaceuticals. Also, synthetic precursors, byproducts, and degradation products of aromatic dyes could be potential health hazards owing to their toxicity and/or carcinogenicity. However, aromatic dyestuffs had until the 1970s largely eluded mass spectrometry. Commercial dyestuffs not only constitute a wide variety of structural types but also contain impurities, which can include homologous compounds and related synthetic precursors. Thus, complementary information from various analytical techniques, including mass spectrometry, is often required for the unambiguous identification of organic dyes.¹

Before the advent of field desorption and electrospray, mass spectrometry was only possible on materials which were thermally volatile or stable. The early mass spectrometric studies of dyestuffs involved either the use of chemical reactions⁴ to give volatile neutral compounds or were concerned with the analysis of pyrolysis products.⁴ The high source temperatures necessary for these experiments tended to enhance thermal degradation and thus reduce molecular ion abundances.

The development of desorption/ionization methods has permitted the mass spectrometric characterization of ionic and nonvolatile dyes. Field desorption (FD) experiments have been performed by Winkler and Beckey,⁵ Jackson et al.,⁶ Matthias et al.,⁷ and McEwen et al.⁸ However, the lack of structurally significant peaks and the inability to desorb direct from chromatographic media (used in the separation of dye components) led Cooks et al.⁹ to report the use of secondary ion mass spectrometry (SIMS) and Monaghan et al.,¹⁰,¹¹ to report the use of fast atom bombardment (FAB) for the identification of dyes. These techniques involve single-step desorption/ionization and often lead to spectra containing complicated fragmentation patterns with little evidence of a molecular ion. More recently, thermospray ionization mass spectrometry (TSP/LC/MS) has been used¹² to analyze a series of dyes belonging to different chemical classes. One of the drawbacks of TSP/LC/MS is that one obtains mainly molecular and adduct ions which do not provide enough information for the structural elucidation of dyes of unknown structure. Yinon et al.,¹³ overcame this problem using particle beam liquid chromatography electron impact (PB/LC/EI) mass spectrometry¹⁴ and were able to characterize a series of commercial dyes, using the available structural information inherent in EI fragmentation patterns. It is clear disadvantage that the aforementioned techniques, individually, do not have the ability to provide both molecular weight and structural information.

The use of infrared (IR) laser desorption, using a CO₂ laser at 10.6 µm, coupled with MPI and time-of-flight mass spectrometry was first demonstrated by Schlag's group¹⁴ and followed by Tembreull and Lubman.¹⁵ They and others¹⁶-¹⁰ have subsequently demonstrated the effectiveness of this combination of techniques in the analysis of a wide range of molecular species. The great advantage of this approach lies...
Neutral molecules are desorbed from the sample probe using the pulsed output from a TBA CO₂ laser (Alltec 8564MS), which is capable of producing a maximum output of 600 mJ of 10.6 μm radiation in a 100-nsec pulse. The mass resolution, which is incident orthogonal to the molecular beam axis, is focused to a 2 × 2-mm square spot using a 300-mm focal length NaCl lens, giving a maximum power density of ca. 100 mW cm⁻². The sample rod is situated ca. 3 mm away from the molecular beam axis and ca. 3 mm downstream from the nozzle orifice. This ensures that the amount of desorbed material entrained in the supersonic molecular beam (4 atm of helium expanded through a 1-mm-diameter orifice, pulse duration 500 μs) is maximized. The molecular beam passes through a 2.5-mm-diameter collimating system into the source region of the mass spectrometer, where the entrained neutrals are ionized by two-photon absorption of either 193-nm (6.4 eV) or 266-nm (4.3 eV) photons. The distance from the nozzle to the point of ionization is ca. 300 mm.

Radiation at 193 nm is produced using the ArF line of a Lumonics T2-590T excimer laser fitted with unstable resonator optics, while 266 nm photons are generated using the fourth harmonic of a J-K 5Y750 Nd:YAG laser. Laser pulse energies were monitored using a Coherent 210 power meter and a Scientech 672 power meter at the 193- and 266-nm wavelengths, respectively. Generally, power densities were maintained at relatively low levels in order to minimize the intensity of background (or pseudo molecular) ions and to avoid further photon absorption by these ions, leading to subsequent fragmentation. Power densities were maintained at ca. 2.5 × 10⁸ W cm⁻² for the studies at 193 nm, and at ca. 5 × 10⁷ W cm⁻² for those at 266 nm. The hard ionization spectra generated at 266 nm were obtained by increasing the laser power density to 5 × 10⁷ W cm⁻². When using 266-nm radiation, the laser beam was collimated to a circular spot of area 0.1 cm². The 193-nm radiation was of sufficient intensity that it required no focusing and was simply apertured by a vertical slit to provide a rectangular beam of area 0.24 cm².

The ion source of the time-of-flight mass spectrometer consists of a Wiley–McLaren type two-stage acceleration field, followed by two sets of ion deflectors. The latter are used as a means of controlling the ion trajectories. The extraction optics are enclosed in a cryoshield in order to reduce background contamination. Ions are extracted with ca. 1085 eV of energy into the field-free drift region of a Mamin-type reflectron time-of-flight mass spectrometer. The drift region is 1.68 m in length, and the ion reflector is 121 mm in length, resulting in a typical time-of-flight of ca. 500 μs for an ion of mass 150 amu. Under the experimental conditions employed here, this translates to ca. 500. This reflects the use of a relatively large photoionization laser–molecular beam interaction region to enhance detection sensitivity under soft ionization conditions.

The ions are detected using a dual chevron-type microchannel plate (MCP) detector (R. M. Jordan) and the signal is further amplified by a factor of 10. Spectra are collected using a CAMAC-based data acquisition system, employing a transient digitizer with a maximum specification of 5-bit resolution (Jonger DSP2001) at a maximum sampling rate of 200 MHz. Custom software on a Dell System 325 microcomputer is used to control the experimental timing sequence via pulse delay generators and also to display and process the acquired data. The mass spectra reported here were typically obtained by accumulating 500 laser shots.

**RESULTS AND DISCUSSION**

**LTOFMS of Dyes.** To simplify the comparison between structure and spectral features, the dyes studied in the present work can be classified into four groups. These of class I contain the azo functionality, class II represent the anthraquinone group, class III are characterised by phthalocyanine ring, and class IV are coumarin type dyes. The structures of molecules examined are presented in Table I.

Table I. Structural Classification of Dye Molecules

class I

<table>
<thead>
<tr>
<th>Dye Type</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disperse Red 1</td>
<td>$R_1 = \text{N(CH}_2\text{CH}_3\text{CH}_2\text{OH})$, $R_2 = \text{NO}_2$, $R_3 = \text{H}$</td>
</tr>
<tr>
<td>Disperse Orange 1</td>
<td>$R_1 = \text{NHPh}$, $R_2 = \text{NO}_2$, $R_3 = \text{H}$</td>
</tr>
<tr>
<td>Disperse Orange 3</td>
<td>$R_1 = \text{NH}_2$, $R_2 = \text{NO}_2$, $R_3 = \text{H}$</td>
</tr>
<tr>
<td>Disperse Yellow 3</td>
<td>$R_1 = \text{H}$, $R_2 = \text{NHCOCH}_3$, $R_3 = \text{OH}$, $R_4 = \text{CR}_3$</td>
</tr>
</tbody>
</table>

class II

<table>
<thead>
<tr>
<th>Dye Type</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disperse Blue 1</td>
<td>$R_1, R_2, R_3, R_4 = \text{H}$, $R_5 = \text{H}$</td>
</tr>
<tr>
<td>Disperse Orange 11</td>
<td>$R_1 = \text{H}$, $R_2 = \text{NH}_2$, $R_3 = \text{H}$, $R_4 = \text{H}$</td>
</tr>
<tr>
<td>Basic Blue 47</td>
<td>$R_1, R_3 = \text{H}$, $R_2 = \text{N(C}_6\text{H}_4\text{N(CH}_3\text{)}_2$, $R_4 = \text{H}$</td>
</tr>
</tbody>
</table>

class III

<table>
<thead>
<tr>
<th>Dye Type</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium Phthalocyanine</td>
<td>$\text{M} = \text{Mg}$, $R_1, R_2, R_3, R_4 = \text{H}$</td>
</tr>
<tr>
<td>Alcian Blue 8GX</td>
<td>$\text{M} = \text{Cu}$, $R_1, R_2, R_3, R_4 = \text{CH}_2\text{SC(N(CH}_3\text{)}_2\text{C}_1\text{N}}$</td>
</tr>
</tbody>
</table>

class IV

<table>
<thead>
<tr>
<th>Dye Type</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coumarin 47</td>
<td>$R_1 = \text{CH}_3$, $R_2 = \text{H}$, $R_3 = \text{N(C}_6\text{H}_4\text{N(CH}_3\text{)}_2$, $R_4 = \text{H}$</td>
</tr>
<tr>
<td>Coumarin 102a</td>
<td>$R_1 = \text{CF}_3$, $R_2 = \text{H}$, $R_3 = \text{N(C}_6\text{H}_4\text{N(CH}_3\text{)}_2$, $R_4 = \text{H}$</td>
</tr>
<tr>
<td>Coumarin 102</td>
<td>$R_1 = \text{CH}_3$, $R_2 = \text{H}$, $R_3 = \text{N(C}_6\text{H}_4\text{N(CH}_3\text{)}_2$, $R_4 = \text{H}$</td>
</tr>
</tbody>
</table>

Class II (1): Disperse Red 1. Azo dyes constitute one of the most important classes of synthetic coloring materials. Commercial azo dyes are generally sold with less than 50% dye content. However, it was possible to obtain a sample of Disperse Red 1 of 95% purity from Aldrich. The samples of low dye content are discussed in the following section. Mass spectra of azobenzenes have been reported previously using EI and SIMS, while Wang et al.25 have reported the photolytic behavior of some azopyridone disperse dyes on polyester substrates. In these latter cases the dissociation of the N—N bond is seen to be an important fragmentation pathway under a number of different conditions. Figure 2a,b shows the photoionization mass spectra of Disperse Red 1 at 266 and 193 nm, respectively. The mass spectrum obtained at 266 nm is simple in appearance, containing a single strong peak at 180 amu. This fragment peak is the result of photolytic cleavage of the azo C—N bonds followed by double hydrogen transfer yielding the amino positive ion. This fragmentation occurs under soft ionizing conditions. Attenuation of the UV photoionization laser intensity reduces the size of this peak but does not lead to any increase in the intensity of a molecular ion signal. In marked contrast, the spectrum following photoionization at 193-nm radiation contains a number of strong ion peaks including that corresponding to the molecular ion hydrogen adduct. It can be seen in Figure 2b that there is no peak which corresponds to azo cleavage and that, therefore, irradiation at 193 nm does not stimulate dissociation of the azo linkage. In this case the predominant pathway for fragmentation is the dissociation or complete loss of the aromatic side groups. The base peak at 283 amu corresponds to partial loss of the amine side group. The proposed fragmentation pathways are shown in Scheme I.

Table II. Comparison of PE EI* (Particle Beam Electron Impact) Mass Spectral Peaks and Photoionization Mass Spectral Peaks for Disperse Red 1 (Relative Intensities in Parentheses)

<table>
<thead>
<tr>
<th>technique</th>
<th>mW</th>
<th>m/z observed ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE EIa</td>
<td>314</td>
<td>314 (2), 297 (2), 283 (34), 257 (11), 255 (19), 237 (6), 207 (9), 168 (15), 166 (18), 149 (15), 147 (18), 133 (100), 120 (69), 108 (53), 105 (55), 103 (47)</td>
</tr>
<tr>
<td>LD-MPI (193 nm)</td>
<td>314</td>
<td>315 (23), 284 (25), 283 (10), 269 (10), 255 (12), 179 (15), 133 (42), 105 (28)</td>
</tr>
<tr>
<td>LD-MPI (266 nm)</td>
<td>314</td>
<td>181 (30), 180 (100), 151 (10), 149 (10), 136 (5)</td>
</tr>
</tbody>
</table>

a Peaks reported in ref 13.

Table III. LTQ/FTMS Mass Spectral Peaks of Class II Dyes Using 266-nm Laser Photoionization

<table>
<thead>
<tr>
<th>dye</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disperse Red 1</td>
<td>180a</td>
</tr>
<tr>
<td>Disperse Orange 1</td>
<td>180a 169a 164 150 137 124 108</td>
</tr>
<tr>
<td>Disperse Orange 3</td>
<td>180</td>
</tr>
<tr>
<td>Disperse Yellow 3</td>
<td>230</td>
</tr>
</tbody>
</table>

* Mass peaks corresponding to photolytic cleavage of the exo bond.

Figure 3. LTQ/FTMS spectrum of Disperse Orange 1 produced by 266-nm photoionization.

Class II (iii): Low-Purity Azo Dyestuffs. As with the studies on Disperse Red 1 discussed in the previous section, low-purity dyestuffs were analyzed using both 266 and 193 nm as ionizing wavelengths. Since the purity levels in the commercial dyestuffs are, in some cases, as low as 15%, one would expect more complicated mass spectra as a result. However, by reference to the mass spectral features found for the pure azo dye, it is possible to interpret the photoionization mass spectra of these impure dyestuffs. Thus, the LTQ/FTMS methodology can provide an analytical technique capable of direct identification of dyestuff in complex or "dirty" samples. These samples were examined as supplied; no separation or preparative workup was employed prior to mass spectral analysis.

A study of 253 nm. At 253 nm the photoionization mass spectrum of Disperse Orange 1 under soft ionizing conditions has a base peak at 169 amu, along with a number of other strong ion peaks between 163 and 200 amu (see Table III). This spectrum is shown in Figure 3. If the anticipated exo bond cleavage were to occur in this case one would expect a signal corresponding to mass 164 amu, which is indeed apparent; loss of 15 amu (NE) from this leaves the fragment base peak at 159 amu. The other peaks in this region of the spectrum are not as easilyassignable to our target dye molecule. In order to determine the origin of these other

Thus a different effective desorption cross section. Therefore, the sample mixture will have a different vapor pressure and other strong peaks in the spectrum. Each component of conditions are altered from optimum the peaks at mass 169 which disrupt the jet flow. However, a change in the relative desorbed sample into the jet, or the formation of shock waves. This is most likely due to either reduced penetration of the results in a decrease in the signal intensity by at least 50%.

Increasing the desorption power density were obtained. Increasing the desorption power density peaks the photoionization mass spectra of Disperse Orange 1 at desorbing laser powers of 5 MW cm\(^{-2}\) and 500 MW cm\(^{-2}\) were obtained. Increasing the desorption power density results in a decrease in the signal intensity by at least 50%. This is most likely due to either reduced penetration of the desorbed sample into the jet, or the formation of shock waves which disrupt the jet flow. However, a change in the relative intensities of the ion peaks is also apparent. If the desorption conditions are altered from optimum the peaks at mass 169 and 184 amu are noticeably reduced in intensity relative to the other strong peaks in the spectrum. Each component of the sample mixture will have a different vapor pressure and thus a different effective desorption cross section. Therefore, the observed difference in this dependence of peak intensities on desorption laser fluence indicates that the ions of mass 169 and 184 amu originate from a different desorbed molecule than do the other ion peaks. A comparison with the spectra of the other low-purity dyes (see Table III) reveals that these other strong peaks, namely 180, 164, 150, 197, 124, and 108 amu, are ubiquitous and can therefore be attributed to other species present in the dye samples supplied.

Scheme II shows the photolytic cleavage and subsequent ion fragmentation for Disperse Orange 1 and Disperse Orange 3, as determined from their mass spectra. Figure 4 shows the mass spectra obtained for Disperse Yellow 3. The base peak in this spectrum is at mass 180 amu, corresponds to an impurity molecule as observed in the mass spectra of the other impure dyes. Direct cleavage of theazo bond in Disperse Yellow 3, followed by amine formation would lead to a mass peak at 180 amu. A peak at this mass is present in the spectrum, shown in Figure 6, but it is also present in all the other spectra of these impure dyes. A component of the observed peak at 180 amu could be representative of an azo cleavage product, but this is not an unequivocally characteristic peak. However, there are peaks at 170 and 171 amu which do indicate that this characteristic fragmentation occurs followed by formation of a sodium adduct ion. This is shown diagrammatically in Scheme III. The ability of certain species to form cation adducts is also observed in the spectra obtained using 193 nm discussed in the following section.

B. Studies at 193 nm. At 193 nm the photoionization mass spectra of the low-purity dyes show no tendency to undergo the azo link cleavage observed at 266 nm. Instead, similar to the behavior of the purified dye Disperse Red 1, there is a tendency to form molecular or molecular adduct ions and, at higher fluences, subsequent fragmentation of these species is observed. The masses and relative intensities of the strong peaks in the spectra are presented together in Table IV. Figure 5a,b shows the photoionization mass spectra of Disperse Orange 1 and Disperse Orange 3, respectively. In both cases the spectra are simpler than their counterparts at 266 nm exhibiting just two strong peaks. The base peak in both cases is at 266 amu corresponding to ionization of neutral sodium atoms. The ionization potential of atomic sodium is 5.12 eV, thus at 193 nm with an associated photon energy of 6.4 eV, ionization of sodium is a one-photon process whereas at 266 nm (4.6 eV) it is a two-photon process. The second strong peak in each case corresponds to the sodium adduct of the parent molecule. The obvious excess of sodium ions in the ionization region and the absence of any molecular ion suggests that at 193 nm the most likely mechanism for ionization is that of cation attachment. Although the ionization potentials of these dyestuffs are unknown they are certainly expected to be greater than 6.4 eV, so that photoionization at both 193 and 266 nm will be two-photon processes. Therefore at 193 nm, one-photon ionization of sodium predominates over two-photon ionization of the dye molecule. This contrasts with the situation at 266 nm where sodium ion formation and molecular ion formation are both.

### Table IV. LTOFMS Mass Spectral Peaks of Class I Dyes Using 193-nm Laser Photoionization (Relative Intensities in Parentheses)

<table>
<thead>
<tr>
<th>dye</th>
<th>MW</th>
<th>m/z observed ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disperse Red 1</td>
<td>314</td>
<td>315 (23), 284 (25), 283 (109), 269 (10), 255 (12), 179 (15), 133 (42), 105 (26)</td>
</tr>
<tr>
<td>Disperse Orange 1</td>
<td>318</td>
<td>342 (5), 341 (20), 341 (6), 23 (100)</td>
</tr>
<tr>
<td>Disperse Orange 3</td>
<td>242</td>
<td>266 (10), 266 (42), 23 (100)</td>
</tr>
<tr>
<td>Disperse Yellow 3</td>
<td>269</td>
<td>269 (3), 292 (40), 301 (20), 270 (28), 202 (11), 144 (15), 107 (5), 23 (100)</td>
</tr>
</tbody>
</table>

![Figure 4](image_url)
**Class III: Anthraquinone Dyes.** In contrast to the monoazo dyes, class II dyes (see Table I) show no wavelength dependence with regard to fragmentation pathways. However, there are some differences in the ionization cross sections between the two wavelengths. It appears that the cross section for ionization at 266 nm of the anthraquinone moiety is smaller than that at 193 nm. This is seen in the trend toward decreasing photofragment intensity in the mass spectra on going from Disperse Blue 1 to Basic Blue 47, culminating in the lack of any observable characteristic ion for Disperse Orange 11 at 266 nm. A similar though less dramatic trend is observed for ionization at 193 nm, although overall the spectra obtained using this wavelength are more intense than analogous 266-nm spectra. Figure 7a–c shows the mass spectra obtained for the anthraquinone dyes Disperse Blue 1, Disperse Orange 11, and Basic Blue 47 at 193 nm, respectively. Figure 8a,b shows the soft and hard ionization mass spectra respectively of Disperse Blue 1 at 266 nm. In both cases the mass spectra exhibit a very strong sodium peak at 23 amu, the applied target sample containing only 30% dye. However, both spectra also show a strong ion signal at 268 amu corresponding to the molecular ion of Disperse Blue 1. Smaller signals to higher mass are a result of sodium adduct ions corresponding to $\text{[M + Na]}^+$ and $\text{[M + 2Na]}^+$. The spectra are similar to those obtained using 193 nm as the ionizing wavelength, see Figure 7a. A relatively thick sample covering, of partially dissolved material, was used to obtain the mass spectrum shown in Figure 7a. If, however, a much thinner sample containing only a dye component which completely dissolves in the solvent, rather than a slurry, it is possible to suppress the sodium ion signal and sodium adduct signal. This is probably due to selective solvation of the non-cation-containing species. However, the solvated material in not sodium ions and increasing the desorption laser fluence has the effect of reionizing the sodium ion peaks, although obviously less intense than before. The mass spectra for Basic Blue 47 at both 193 and 266 nm show only a small sodium ion signal, the base peak in each case is due to the loss of the amino fragment from the amino side chain. A peak at 69 amu, which can be assigned to the fragment $\text{[(CH$_3$)$_2$NCH$_2$]}^+$ is also apparent in the spectra.

**Class III: Phthalocyanine Dyes.** Phthalocyanine dyes constitute an important group of synthetic colorants. They are closely related in structure to chlorophyll and hemin,
ANALYTICAL CHEMISTRY, VOL. 65, NO. 3, MARCH 15, 1993

Disperse Blue 193 nm.

(a) Disperse Blue 1 266 nm. "Soft"

(b) Disperse Blue 1 266 nm. "Hard"

(c) Basic Blue 47 193 nm.

Figure 7. LTOFMS spectra of (a) Disperse Blue 1, (b) Disperse Orange 11, and (c) Basic Blue 47 using 193-nm laser photolization.

Figure 8. LTOFMS spectra of Disperse Blue 1 at 266 nm under soft and hard ionizing conditions. Ionizing laser power densities are (a) 0.25 MW cm⁻² and (b) 1 MW cm⁻².

The base peak in this spectrum occurs at 576 amu, which corresponds to the molecular ion of copper phthalocyanine, i.e. the side groups are removed. The peaks above 576 amu correspond to sequential addition of CH₂ units, and further fragments involving parts of the side chains. No breakdown of the macrocycle itself is observed at the low laser fluences used in this experiment. To lower mass there are peaks at 63 and 65 amu corresponding to the two isotopes of atomic copper.

Class IV: Coumarin Dyes. The LTOFMS spectra of coumarin dyes are characterized by strong molecular ion signals in high abundances, usually seen as the base peak. Figure 11a–c shows the mass spectra of three coumarin type dye molecules: Coumarin 47, Coumarin 102, and Coumarin 152a, respectively, obtained using 266 nm as the ionizing wavelength. These were purchased as pure materials and therefore all the peaks observed in these mass spectra include, or are derived from the molecular ion. All the spectra shown in Figure 11 were obtained using a fluence of ca. 3.2 × 10⁵ W cm⁻² at 266 nm. The molecular ion peak is dominant in each case with fragment ions attributable to loss of part or all of the amino side chains also present. The low degree of fragmentation facilitates the identification of the molecular ion. The absorption cross section for 193-nm radiation is significantly smaller for coumarin dyes. Consequently, for the three examples shown here no mass spectra were recorded using this ionizing wavelength.

Detection Sensitivities. Detection limits for dye compounds analysed by mass spectrometry vary between compound classes and even within compound classes themselves. Using the LTOFMS methodology, the efficiency of ionization and thus the detection limit for a particular molecule is dependent on the choice of ionizing wavelength. The work
ANALYTICAL CHEMISTRY, VOL. 65, NO. 6, MARCH 15, 1993

Figure 9. LTQ/FTMS spectra of magnesium phthalocyanine using (a) 266-nm and (b) 193-nm laser photolization.

Figure 10. LTQ/FTMS spectrum of Alcian Blue 8GX produced by 193-nm photolization.

The presented work is limited, by design, to the use of non-tailored accessible, fixed wavelengths of ionizing radiation to demonstrate the utility of essentially non-resonant ionization processes for the detection of a wide range of dyestuffs. Under these experimental restrictions, the limits of detection for a selection of dyestuffs, using 193-nm ionizing radiation, were investigated. It was determined, without making any configurational changes to the apparatus, that detection limits varied from hundreds of nanograms for magnesium phthalocyanine to tens of micrograms for Disperse Red 1. The detection limits for the impure species (uncorrected for pure dye content) are 1–2 orders of magnitude lower than observed for Disperse Red 1. This is a result of the more favorable one-photon ionization of the sodium atom at 193 nm followed by adduct formation with the gas-phase dye molecule. Thus at 193 nm the impurities have an important role in the ion formation process itself. The two-photon absorption mechanism required for ion formation without the presence of a sodium-containing impurity would certainly mean worse detection limits in line with that found for Disperse Red 1. To ensure low detection limits without altering the machine geometry would require recourse to a dye laser: tuning the ionization laser to a strong electronic transition in the target molecule would substantially enhance the overall ionization efficiency. Recent work performed in Edinburgh on the LTQ/TOFMS analysis of polyyclic aromatic hydrocarbons using 193-nm ionizing radiation has demonstrated the potential for high-sensitivity analysis using the existing apparatus. In these studies limits of detection at picogram levels have been attained for perylene and coronene.

CONCLUSIONS

The involatile and thermally labile nature of many dyestuffs makes conventional mass spectrometry difficult. This work demonstrates the effectiveness of a two-stage desorption/ionization methodology for the analysis of a wide range of neutral dye molecules. An important advantage of L²TOFMS for mass spectral analysis of dyes is the ability of this technique to provide both molecular weight (under soft ionization conditions) and structural information (under hard ionization conditions or by changing the ionization wavelength) without any complicated sample workup. Preparation and presentation of the sample is straightforward; no sample preseparation is required. The use of CO₂ laser radiation facilitates the volatilization of a range of different species. By using two alternative fixed laser wavelengths for photoionization of ionizing radiation, namely 193 and 266 nm, spectra containing molecular ions or characteristic fragment or adduct ions are obtained for pure dye species.

Characteristic fragmentations of the pure class I dyes include photolytic cleavage of the azo linkage at 266 nm whereas initial loss of the phenyl side groups is more characteristic at 193 nm. Hence, by employing laser photoionization at both 193 and 266 nm complementary structural information can be obtained facilitating the identification of the target species. The class II dyes provide simpler spectra with molecular or adduct ions and also demonstrate the ability of L²TOFMS to provide structurally significant information under hard ionization conditions. Class III and IV dyes similarly show simple, readily interpretable and diagnostic mass spectra. Choice of wavelength is critical for the latter three dye classes as their ionization cross sections often vary widely at the different wavelengths.

L²TOFMS is similarly effective in the analysis of class I impure dyestuff mixtures. In these cases the mass spectra obtained at 266 nm are often more complex but still provide the relevant structural information. Using 193-nm laser photoionization, the mechanism of sodium cation attachment not only furnishes the molecular weight information but allows for enhanced detection sensitivities. Further applications of gas phase sodium cation attachment are at present being investigated.

ACKNOWLEDGMENT

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Application of Two-Step Laser Mass Spectrometry to the Analysis of Polynuclear Aromatic Hydrocarbons in Contaminated Soils

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Introduction

Contaminated site remediation is a multistage process that commences with an initial indication of contamination and is concluded with the long-term monitoring of remedial measures (1–3). Critical to the successful completion of the process is the informative nature of the site assessment data. This information ultimately determines the level of confidence with which risks to human health and the environment can be adequately assessed and managed (4–6).

Hydrocarbon-contaminated soils, such as those found at former manufactured gas plant sites, creosote wood-preserving plants, and petroleum refineries pose specific problems for reliable chemical characterization because of the presence of multimedia contamination, the existence of a complex matrix of individual contaminants exhibiting a diverse range of physiochemical properties, and the expensive analytical procedures often required (4, 7). Such sites have generated growing concern throughout industrialized countries because the chemical contaminants identified on-site almost invariably include the polynuclear aromatic hydrocarbons (PAHs), several high molecular weight analogues of which pose a toxicological hazard due to their documented carcinogenic activity (8).

Analytical techniques such as conventional gas chromatography-mass spectrometry (GC-MS) for hydrocarbon-contaminated soils require lengthy and involved sample cleanup procedures which can render them cost-prohibitive for extensive contaminated site assessment. Delay times between sampling in the field and the receipt of analytical data can result in sampling protocols being less focused than they would be if even a semiquantitative estimate of the extent of contamination were readily available. In acknowledgement of these difficulties, considerable effort has recently been focused at developing and validating field techniques capable of analyzing contaminants on-site without the need for sample cleanup. On-site screening and field techniques allow the rapid feedback of information to field personnel during the sampling procedure (9). Under certain sampling protocols, the screening of large numbers of samples prior to indicator compound(s) analysis will result in significant cost savings throughout the risk assessment and risk management process. Among the analytical methods currently under evaluation for on-site analysis are thermal desorption-gas chromatography-mass spectrometry (TE-GC-MS), ultraviolet fluorescence, and remote laser-induced fluorescence (RLIF) techniques (9–15).

In this paper, we describe initial investigations into the use of laser desorption laser photoionization time-of-flight mass spectrometry (L2TOFMS) for the direct determination of PAH analytes in solid waste matrices. This relatively new technique has potential as an on-site field screening tool, offering the ability to determine a wide variety of organics contained in a wide variety of matrices. It involves a two-step process in which the first laser desorbs primarily intact neutral molecules from the sample and the second laser causes "soft ionization" of selected classes of compounds which are identified by time-of-flight (TOF) mass spectrometry (16–18). This method of mass analysis, with its advantages of rapid, multiplexed data acquisition is ideally interfaced to the pulsed lasers used for sample desorption and photoionization. Previous work performed using this two-stage methodology has demonstrated its ability to determine analytically important organic compounds. These have included peptides (19), porphyrins (20), commercial dyestuffs (21), PAHs (22), and polymers (23). More recently, Kovalenko et al. have used a similar methodology to investigate the PAH components of selected meteorites (24). As a novel approach to sample screening, this circumvents many problems associated with more conventional analytical techniques, allowing determination of semivolatile, volatile, and thermally labile species without the need for extensive extraction and separation procedures. The results presented here demonstrate the capability of L2TOFMS to analyze PAHs in a rapid, highly selective, and sensitive manner directly from the contaminated soil matrix.

Experimental Section

Soils. The six soil samples used in this study were obtained from a contaminated former coal gasification plant and coal-tar distillery in the UK. Samples were taken from shallow trial pits (0.5 m depth) and consisted of three grab samples taken at 120° from each other prior to being composited into approximately 5-kg total samples. Samples were air-dried at ambient temperatures in a forced draught. After the resulting aggregates were broken up, the entire sample was passed through a 10-mesh sieve (≤2.00 mm), the fraction passing through was then homogenized by being riffled down to a 50-g subsample (25). Previous gas chromatography-mass spectrometry has determined the concentration of individual PAHs in these samples to range from 1 and 400 ppm.

Sample preparation for the L2TOFMS technique consisted of grinding the soils further into a fine powder, binding the particles together with a drop of glycerol, and applying the homogeneous paste to a 1.5 × 40 mm2 slot in the sample probe. The sample surface open to the desorption laser beam was then dried with a dusting of alumina-type H.

Instrumentation. A detailed account of the experimental apparatus has previously been reported (21). The instrument is composed essentially of three separate high vacuum chambers: the desorption chamber, ionization chamber, and a TOF mass spectrometer.
In the desorption chamber, the output of a pulsed CO₂ laser (Alltec 854M, 10.6 μm wavelength) is focused, using a NaCl lens, onto a stainless steel sample probe. The sample probe is translated orthogonally to the incident desorption beam, both extending the lifetime of the sample and exposing a fresh area of sample to each laser shot. The neutral molecules thus desorbed are entrained in a pulsed supersonic helium molecular beam and transported from the desorption chamber into the ionization chamber. Here, 193-nm photons (ArF line, Lumonics TE-960T laser) or 266-nm photons (fourth harmonic, JK HY750, Nd:YAG laser) are used to induce the multiphoton ionization (MPI) of the desorbed molecules. These ionizing wavelengths provide selective ionization of aromatic species over aliphatic components in a mixture of organic materials. Judicious control of the ionizing laser fluence, typically of the order ca. 2-5 × 10⁸ W cm⁻², provides "soft ionization" conditions so that the parent ions of the polynuclear aromatic compounds almost exclusively dominate the mass spectra.

The laser-generated photoions are mass separated in a home-built reflectron TOF mass spectrometer (resolution typically ca. 500) and detected by a dual chevron-type microchannel plate (MCP) detector. Experimental control and data acquisition are performed using a CAMAC-based system and interfaced to a Dell system 325 personal computer with in-house custom software. The entire experiment is performed at a repetition rate of 10 Hz. Typically, data from 200 laser shots were accumulated to enhance the overall signal to noise ratio. Using the present experimental configuration, the entire procedure, from subsample preparation to obtaining a mass spectrum, can be performed within a 10-min period.

Results and Discussion

Each of the six contaminated soil samples were examined using both 266-nm and 193-nm laser photoionization. The mass spectrum of one of the soil samples produced using 193-nm photoionization is shown in Figure 1, and an expansion of the region containing the PAH peaks is shown in Figure 2. Similar spectra were obtained for all the soil samples investigated. Figure 1 represents the total accumulated ion signal on summing of 200 laser shots. These fingerprint mass spectra were obtained in consecutive experiments; the entire preliminary screening of the samples was completed within 80 min. Under the prevailing experimental conditions, a 200-shot spectrum represented the total ion signal obtained on interrogating ca. 1 mg of soil. Samples of up to 50 mg can be interrogated in one continuous scan of the present sample probe. Examination of 50-mg quantities of material provides a better average PAH concentration and reduces subsampling errors.

The PAH signals are observed in the region between 100 and 400 amu. Signals at masses higher than 300 amu indicate the presence of PAHs with more than seven fused rings. Peaks at masses less than 100 amu are generally the result of ionization of elemental species, e.g., Na and K, or attributed to fragmentation of higher molecular weight species. This low mass region of the mass spectrum is dominated by a peak at 39 amu, corresponding to the atomic mass of potassium.

From Figure 2 it is possible to clearly identify strong ion signals which correspond to the masses of parent PAH skeletons. The dominant masses of 178, 202, 228, and 252 amu correspond to phenanthrene/anthracene, fluoranthene/pyrene, chrysene/benz[a]anthracene, benz[k]fluoranthene/benz[a]pyrene, and benz[k]fluoranthene/benz[a]pyrene, respectively. Other peaks of significant intensity correspond to further PAH compounds, e.g., 4H-cyclopenta[bc]chrysene at 183 amu and coronene at 350 amu. It is also possible to identify a series of peaks with mass separations of 14 amu, characteristic of a homologous series of successively alkylated PAHs, e.g., for pyrene/fluoranthene at 216 and 230 amu. This is shown more clearly in the insert in Figure 2. The absence of significant soil matrix peaks is striking. This eliminates the need for the analysis of blank samples. The use of ultraviolet (UV) radiation for ionization of the desorbed species means that photoionization mass spectra will only contain peaks from
organic species which contain a UV chromophore. There is further selectivity of detection in the specific choice of the wavelength of the ionizing radiation. Photoionization at 266 nm is well known to enhance the detection of this methodology were consistent with previously deter-
to that used by Hahn et al. (26). The results obtained by this methodology were consistent with previously determined concentrations obtained using gas chromatography-mass spectrometry. The results show clearly that the technique is able to detect PAHs contained in the soil matrices at the ppm level. Preliminary instrument characterization, using pure PAHs as analytes, showed minimum detection limits for a number of these species using 193-nm photoionization were in the subpicomole region. It is important to note that the parent ion peak intensities are not only proportional to the concentration of the compound in the samples but also depend on the overall ionization efficiency, which is largely determined by the absorption cross-sections for that compound at the laser wavelength employed. Furthermore, the different PAHs may have different desorption efficiencies. Therefore, the relative peak intensities do not necessarily directly reflect the relative concentrations of the different PAHs in the matrix.

These results demonstrate that L^2TOFMS is a fast, effective, and sensitive analytical tool for the direct determination of PAHs in complex solid environmental matrices. Furthermore, as a quantitative analytical tool for the prediction of PAH distributions in soils, L^2TOFMS could provide an effective alternative to more conventional approaches. Investigations are presently underway to determine the efficacy of the technique for the analysis of PAHs in a variety of different environmental media and to explore its potential as a rapid, on-site screening tool.

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Literature Cited

(8) U.S. Department of Health and Human Services Toxico-
logical Profile for Polyaromatic Hydrocarbons. TP-
(13) Mellone, A.; Smith, B. W.; Winefordner, J. D. Talanta 1993, 37, 111-118.

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Birch Reduction of $\text{C}_{60}$—a New Appraisal


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Birch Reduction of C₆₀—a New Appraisal


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Contrary to a previous report that Birch reduction of C₆₀ affords C₆₀H₃₆ as the principal product, laser desorption–laser photoionisation time-of-flight (L²TOF), laser desorption Fourier transform ion cyclotron resonance (FTICR), and liquid secondary ion mass spectrometry (LSIMS) show collectively that a mixture of polyhydrofullerenes, containing C₆₀H₁₈ through to C₆₀H₃₂ with a skewed distribution centred on C₆₀H₂₂ is formed, the discrepancy in results arising from the thermal lability of this mixture of polyhydrofullerenes when subjected to the elevated temperatures (>250 °C) required for mass spectroscopic studies using direct-insertion heated probes.

Subsequent to the discovery by Kratschmer et al.¹ of a method for the macroscopic synthesis of C₆₀ ¹, there has been an explosion of activity in fullerene research.² Our interest in the theory that a mixed population of the hydrides of the fullerenes is responsible for certain unexplained lines in the IR spectra observed from nebulae in the Galaxy,³ led us to carry out the Birch reduction of C₆₀. Hauffler et al.⁴ had already reported the isolation of an off-white compound of formula C₆₀H₃₃ as the principal product, albeit from the reduction of fullerite (a ca. 85:15 mixture of C₆₀ and C₇₀). They pointed out that this formula is consistent with a caged hydrocarbon in which twelve isolated double bonds remain, possibly in pentagonal rings. Such a structure is shown as 2 where the remaining sp² hybridised carbon atoms are shown as solid circles. We believe their conclusion to be misconceived.

The results reported here establish that the Birch reduction of C₆₀ gives rise to a mixture of polyhydrofullerenes containing C₆₀H₁₈ through to C₆₀H₃₅ with a skewed distribution centred on C₆₀H₂₂.

Pure C₆₀ (cf ref. 4) for use in our study was obtained by differential Soxhlet extraction of carbon-arc soot,⁵ followed by a novel preparative HPLC protocol using FullereneSep.⁶ For the Birch reduction,⁷ we used lithium metal added to a suspension of C₆₀ in liquid NH₃-tetrahydrofuran (THF) at -78 °C under argon, followed by addition of tert-butyl alcohol after 30 min. The product was isolated by normal work-up⁸ as a pale-yellow solid whose IR spectrum showed only C–H vibrations in the region above 2800 cm⁻¹.

Characterisation of the Birch product was initially carried out using laser desorption laser–photoionisation time-of-flight mass spectrometry (L²TOFMS). A pulsed TEA CO₂ laser (Alltec 854 MS) was used for desorption of the sample, deposited in the form of a toluene slurry, from a stainless steel probe; the resultant sample vapour was then entrained in a supersonic molecular beam of helium. Photoionisation of the desorbed neutrals was accomplished using the 193 nm output from a Lumonics TE-8617-4 excimer laser, operating on the ArF line. Fig. 1 shows the time-of-flight mass spectrum that was obtained. It is notable that the spectrum consists of just a single approximately symmetric feature, centred on mass 750 u. This feature, which has a FWHM of 7 u, is due to an unresolved distribution of hydrogenated products, ranging from 740 to 760 u, which cannot be resolved with the limited instrumental resolution of 300 (FWHM) available under the experimental conditions used. There is no evidence for the formation of a unique product (C₆₀H₃₆) as reported by Hauffler et al.⁴ Also there is no evidence of fragment peaks such as C₆₀⁺ under the soft ionisation conditions used. We believe, therefore, that this mass spectrum represents the nascent distribution of polyhydrofullerene products resulting from the Birch reduction.

Because the resolution of this time-of-flight mass spectrometer was insufficient to resolve the product distribution, the sample was then analysed by laser desorption Fourier transform ion cyclotron resonance (FTICR) mass spectrometry on a 3 Tesla Nicolet FTMS 2000 spectrometer, incorporating a Tachisto Model 216 CO₂ laser. Samples were prepared by depositing a toluene slurry of the sample onto a steel target. After evaporation of the solvent the target was exposed to pulses from the CO₂ laser. Ions were trapped at a potential of 0.2 V and, after a 10 s delay, were excited (1 kHz ms⁻¹, 200 kHz to 0, zero attenuation) and detected in...
the source side of the dual cell. A detection bandwidth of 100 kHz was employed and signals from 16 laser shots were averaged. Rotation of the target ensured that a fresh region of the target was exposed to each laser pulse. The final spectrum was obtained by Fourier transformation of 32k data points after 3-term Blackman Harris apodisation. The effective mass resolution was 4000 (FWHM). Fig. 2 shows the spectrum obtained. The principal feature in this spectrum is the prominent well-resolved series of peaks centred around the base peak at 753 u, corresponding to the distribution of polyhydrofullerenes. There is clear evidence of fragmentation as witnessed by the relatively strong peak due to C_{60}^*, and its ^{13}C isotope fingerprint. The weaker peaks between 720 and 730 u correspond to fragmentation to lower molecular weight polyhydrofullerenes. However, the overall intensity profile of the distribution of polyhydrofullerenes is very similar to the unresolved feature in the L^2 TOF mass spectrum (vide supra). Another striking feature of the product distribution shown in Fig. 2 is the pronounced even–odd intensity alternation, with the odd number mass peaks substantially more intense than the adjacent even number mass peaks. This result, at first sight, is at odds with the expected even distribution of products from the Birch reduction, which is generally thought to proceed by 1,4-hydrogen addition. We believe that this is due to facile proton attachment during the desorption process. The true experimental distribution of polyhydrofullerene products is also somewhat obscured by the very intense underlying ^{13}C isotope distribution.

In order conclusively to identify the nascent product distribution the sample was also characterised by liquid secondary ion mass spectrometry (LSIMS) using a Fisons VG Analytical ZAB2-T tandem mass spectrometer of BEBE geometry in which the first two sectors were utilised. Samples were prepared for analysis in a 3-nitrobenzyl alcohol (3-NOBA) matrix. A positive ion LSIMS experiment was carried out at an acceleration voltage of 8000 V with the caesium gun

Table 1 Experimental and calculated (Monte Carlo simulation) product distribution from Birch reduction of C_{60}

<table>
<thead>
<tr>
<th>Polyhydrofullerene composition</th>
<th>Experimental distribution</th>
<th>Monte Carlo distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{60}H_{24}</td>
<td>39 (24)</td>
<td>0.2</td>
</tr>
<tr>
<td>C_{60}H_{26}</td>
<td>53 (35)</td>
<td>3</td>
</tr>
<tr>
<td>C_{60}H_{28}</td>
<td>72 (57)</td>
<td>19</td>
</tr>
<tr>
<td>C_{60}H_{30}</td>
<td>96 (89)</td>
<td>53</td>
</tr>
<tr>
<td>C_{60}H_{32}</td>
<td>100 (100)</td>
<td>100</td>
</tr>
<tr>
<td>C_{60}H_{34}</td>
<td>71 (62)</td>
<td>98</td>
</tr>
<tr>
<td>C_{60}H_{36}</td>
<td>11 (10)</td>
<td>55</td>
</tr>
<tr>
<td>C_{60}H_{38}</td>
<td>(---)</td>
<td>14</td>
</tr>
<tr>
<td>C_{60}H_{40}</td>
<td>(---)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Derived from LSIMS data of Fig. 3 and (in parentheses) FTICRMS data of Fig. 2. Based on reduction of 20 000 molecules of C_{60}.

Fig. 2 Laser desorption Fourier transform ion cyclotron resonance mass spectrum of Birch-reduced C_{60}

Fig. 3 Positive ion liquid ion secondary ion mass spectrum of Birch-reduced C_{60} in 3-NOBA matrix
operating at 35 kV. The instrument was scanned from 2000 to 50 u at 10 seconds per decade. Fig. 3 shows the mass spectrum obtained at a resolution of 5000. An even-odd intensity distribution, similar to that observed in the FTICR mass spectrum (vide supra) is also apparent here; proton attachment is a commonly observed phenomenon in LSIMS. It is interesting to note the complete absence of any daughter ion peak in the spectrum obtained using this sample introduction technique due to C60+. The distribution of hydrogenated products can be clearly identified from this very high resolution spectrum. Deconvolution of the underlying 13C isotope fingerprint† yields the product distribution shown in column 2 of Table 1, which is in good agreement with model calculations (using Monte Carlo sampling) of the product distribution to be expected for 1,4-addition in the Birch reduction (vide infra).

Clearly these results are at odds with those of Haufler et al., who reported only two products, C60H16 as the major product, and C60H18 as a minor product. We believe that these conflicting observations can be attributed to the different mass spectrometric methods used for analysis. We have also recorded electron impact mass spectra (EIIMS) of the Birch product on a Kratos MS50TC double-focusing instrument. The sample was introduced using a heated direct-insertion probe into the El source which was initially heated to 145 °C. The sample was introduced using a heated direct-insertion probe on a Kratos MS50TC double-focusing instrument.

† Deconvolved data were obtained from the experimentally observed peak intensities, following background subtraction, by a least-squares fitting procedure incorporating the 13C natural isotope abundances up to the 13C4 12C56 isotopomer.

Fig. 5 Electron impact quadrupole (Ribermag R10-10C) mass spectra of Birch-reduced C60: (a) at 460°C; (b) at 460°C after 1 min; (c) at 560°C

‡ Fluorination of C60 has been reported by several groups with somewhat conflicting results, the origin of which may also partly lie in the method of sample introduction employed for their mass spectrometric characterisation.
satisfactorily for the observed polyhydrofullerene distribution.

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References

Delayed ionization of laser desorbed C$_{60}$ molecules following 193 nm photoexcitation

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Delayed ionization of C$_{60}$ on the microsecond timescale has been investigated following 193 nm excitation of infrared laser desorbed molecules. Biexponential decay of the delayed ion signal indicates the autoionization of two kinetically distinct superexcited states. The lifetimes of these states were determined to be 5 ± 1 μs and 0.8 ± 0.2 μs; their excitation energies are estimated to lie in the range 20–30 eV. These results are compared with the predictions of the Richardson–Dushman and Klots models of thermionic emission. The observation of a dependence of delayed ion intensity on desorption conditions is interpreted in terms of a thermally induced phase change during the desorption process.

1. Introduction

Following the discovery, by Krätschmer et al. [1] of a method for the macroscopic synthesis of C$_{60}$, there has been an explosion in fullerene research. One of the techniques which was applied to the early characterization of the fullerite produced by Krätschmer's carbon arc vaporization was laser desorption laser photoionization time-of-flight mass spectroscopy (L$_2$-TOFMS). An unexpected outcome of such experiments has been the observation of delayed ionization of C$_{60}$ on the microsecond timescale. Using L$_2$-TOFMS, Meijer and Bethune [2] produced the first direct mass spectrometric evidence that fullerite is composed principally of C$_{60}$ and C$_{70}$. The significance of the high mass tails on the C$_{60}$ and C$_{70}$ peaks in their mass spectra remained unrecognized, however, until the work of Campbell et al. [3], which revealed the occurrence of slow delayed ionization. In the latter experiments, fullerite was vaporized by a 248 nm excimer laser, producing C$_{60}$ and C$_{70}$ neutrals which were post-ionized using 308 nm radiation. Using a pulsed extraction field at a variable time delay after the ionizing laser pulse, the delayed production of C$_{60}$ and C$_{70}$ ions on the microsecond timescale was observed. The delayed ion signal exhibited a biexponential decay with time constants of 2.5 ± 0.5 μs and 15 ± 3 μs. Increasing the ionizing laser fluence from 200 μJ mm$^{-2}$ to 1 mJ mm$^{-2}$ led to the appearance of an additional exponential decay component with a lifetime of ~1 μs. Campbell et al. speculatively attributed this behaviour to the delayed ionization of neutral species by thermionic electron emission.

Unequivocal evidence that the delayed production of C$_{60}$ ions was due to delayed ionization of photoexcited neutrals was provided by Wurz and Lykke [4], who reported the observation of delayed photoelectrons. Their experiments were carried out using an effusive source of pure C$_{60}$ at 529°C with ionization over a range of laser
wavelengths (212.8, 266, 355, and 532 nm). Identical exponential tails were observed on the C\textsubscript{60} photoion and photoelectron signals in the respective time of arrival spectra. Again, biexponential decay of the delayed ion signal was reported, the decay constants of 2.4 ± 0.2 µs and 10 ± 0.5 µs were approximately the same at all photoexcitation wavelengths, and at high laser fluences a third component with a decay constant of 0.8 ± 0.3 µs appeared.

During L\textsuperscript{2}-TOFMS studies of fullerite and pure C\textsubscript{60}, we too have observed the peak tailing characteristic of delayed ionization. We report here the results of an investigation of this phenomenon following 193 nm photoexcitation of C\textsubscript{60} vaporized by pulsed CO\textsubscript{2} laser desorption. Despite the use of different vaporization conditions and shorter wavelength ionizing radiation, the delayed ionization kinetics observed are similar to those found in the above two studies. However, in addition, an infrared laser induced phase change in C\textsubscript{60} during the desorption process has been observed; this results in a dependence of the delayed ionization behaviour on desorption conditions, which demonstrates the role of thermal excitation in the delayed ionization process.

Thus, there is now considerable evidence that photoionization of C\textsubscript{60} proceeds via two channels: direct ionization and delayed ionization. Direct ionization occurs as a result of direct optical excitation of a single electron to an unbound state above the ionization potential. Delayed ions are produced by the autoionization of super-excited C\textsubscript{60} molecules which have internal energies greatly in excess of the ionization potential. In discussing the photoionization dynamics of C\textsubscript{60} we shall also use the term prompt ionization; we mean by this ionization which occurs within the duration of the ionizing laser pulse or which is indistinguishable from this within the time resolution of our experiments. Thus, prompt ionization covers both direct ionization and fast autoionization. We use the term delayed ionization to describe autoionization of photoexcited C\textsubscript{60} molecules which occurs on a timescale detectably longer than prompt ionization; in the present experiments, this means autoionization of excited states with lifetimes ≥ 500 ns.

2. Experimental

The experiments were carried out using a laser desorption laser photoionization molecular beam time-of-flight mass spectrometer system. The sample was desorbed from a stainless steel probe by a pulsed TEA CO\textsubscript{2} laser (Alltec 854MS) which produced 100 mJ of 10.6 µm radiation in a 100 ns pulse. The infrared beam was brought to a focus at the surface of the probe to give a peak power density of 0.25 MW mm\textsuperscript{-2} in a 2 mm × 2 mm square spot. The desorption probe consisted of a 6 mm diameter rod which was mounted vertically, beside the exit orifice of a pulsed supersonic nozzle (General Valve Corporation Series 9). The axis of the rod was mutually perpendicular to the molecular beam and the CO\textsubscript{2} laser beam, so that desorption took place from the cylindrical surface of the probe. Using a stepper motor driven screw mechanism, the sample rod could be rotated and axially translated simultaneously to expose a new area of sample to each pulse of the desorption laser. At a typical rotation speed of 30 deg s\textsuperscript{-1} a new sample area of 0.3 mm\textsuperscript{2} was presented to each shot.

The desorbed neutral molecules were entrained in a pulsed jet formed by free expansion of 3 bar helium through a 0.8 mm diameter nozzle with a valve open time of 600 µs. The molecular beam passed through a 2.5 mm diameter
collimating skimmer into the source region of the mass spectrometer; the distance from the nozzle to the point of ionization was approximately 300 mm. In the present experiments, photoionization was carried out with 193 nm (or, in a few cases, 248 nm) radiation from an ArF (or KrF) excimer laser (Lumonics TE-861T-4). The photoions were extracted by a Wiley–McLaren type [5] two stage acceleration field into the field-free drift region of a Mamyrin type [6] reflectron time-of-flight mass spectrometer. Ions were detected by a dual microchannel plate detector (R. M. Jordan) and the signal was processed by a Camac-based data acquisition system incorporating a Transiac DSP2001 transient digitizer with 8-bit resolution and a maximum sampling rate of 100 MHz. Time-of-flight spectra were displayed and processed by PC-based software which also controlled the experimental timing sequence. Data were accumulated over several hundred laser shots at a repetition rate of 10 Hz.

The C$_{60}$ samples used in this work were synthesized by contact arc vaporization of graphite rods in a helium atmosphere [1]. The resulting soot was extracted with toluene to yield fullerite (consisting predominantly of C$_{60}$ and C$_{70}$) from which pure C$_{60}$ was separated chromatographically [7]. The C$_{60}$ or fullerite samples were deposited onto the surface of the desorption probe from toluene solution.

3. Data analysis

The time dependence of the C$_{60}$ delayed ion signal was modelled by fitting an exponential function of the form

$$I(t) = \sum_{i=1}^{n} a_i \exp\left(-t/\tau_i\right)$$

(1)

to the experimental decay using an iterative least-squares procedure. For each data set, an attempt was made initially to fit the observed decay with a monoexponential function. In all cases where an inadequate monoexponential fit was obtained, it was found that the experimental data could be described well by a biexponential decay function. Fitting of the data was commenced at a point 300 ns after the maximum of the C$_{60}^+$ signal in order to exclude any contribution from the direct ion signal.

The difficulties in extracting physically meaningful parameters from multiexponential fits to real (i.e., imperfect) experimental data are well known [8] and have been borne in mind during the analysis and interpretation of the present results. There are also constraints imposed on the observable decay processes by the experimental technique. In addition to a lower limit of 500 ns on detectable decay times due to the time resolution, there is an upper limit due to the finite residence time of the excited neutral molecules in the acceleration region of the mass spectrometer. In our case, this is approximately 25 μs; therefore, delayed ionization events occurring at times greater than 25 μs after the excitation laser pulse will not be detected. There is another complication associated with delayed C$_{60}$ ion production downstream from the point where direct photoionization occurs: the further along the acceleration region delayed ionization occurs, the less kinetic energy the C$_{60}$ ions will acquire. This will lead to a distortion of the delayed ion signal at long times. This effect is expected to be compensated for largely by the reflectron and there is no obvious distortion apparent in the experimental data.
4. Results and discussion

4.1. Fullerite

The time-of-flight mass spectrum of fullerite produced by soft photoionization at 193 nm is shown in figure 1(a). The ionization potential of C\textsubscript{60} has been determined recently to be 7.58 eV [9]. Direct photoionization at 193 nm (6.4 eV) is, therefore, a two-photon process. The spectrum contains the C\textsubscript{60} and C\textsubscript{70} molecular ion peaks with no fragmentation apparent. At increased ionizing laser fluence (figure 1(b)) some fragmentation is induced and the delayed ionization process becomes apparent as a tailing on the C\textsubscript{60} and C\textsubscript{70} peaks. The doubling of the fragment ion peaks, which can be seen more clearly in the pure C\textsubscript{60} spectrum in figure 4, is due to dissociation of metastable C\textsubscript{60} ions in the first field-free region of the reflectron. These mass spectra were obtained by adjusting the reflector potentials to compensate partially for the difference in kinetic energy between daughter ions produced in the ionization region and those produced in the drift region. With the reflectron in fully compensating mode, as is the case under normal operating conditions, the two sets of fragment peaks are superimposed.

The ability to photoionize C\textsubscript{60} at 193 nm with little or no fragmentation is due to

![Figure 1](image_url)

**Figure 1.** Time-of-flight mass spectra of fullerite produced by photoionization with 193 nm laser radiation at fluences of (a) 35 \( \mu \)J mm\(^{-2} \) and (b) 60 \( \mu \)J mm\(^{-2} \).
a resonance enhancement at this wavelength. The resonant one-photon transition can be identified as the highest energy allowed $T_u \rightarrow A_g$ transition, which has been calculated [10] to occur at 197·4 nm and observed at 194·9 nm in solution [11]. At other ionization wavelengths, where such a resonance does not occur, much more extensive fragmentation is observed at comparable ionizing laser fluences. This is illustrated in figure 2(a), which shows the result of photoionizing fullerite using 248 nm radiation. In addition to the increased fragmentation, it can be seen that the delayed ion signal is much more prominent at this wavelength. At higher 248 nm laser fluence, as shown in figure 2(b), fragmentation is further increased and a more rapidly decaying component is discernible in the $C_{60}$ delayed ion signal.

The quality of the above decay data is insufficiently good to allow rigorous fitting of the time dependence of the $C_{60}$ delayed ion signal. However, approximate decay constants have been extracted which give an indication of the timescale of the decay processes. No attempt has been made to analyse the $C_{70}$ decay, as this is overlapped by the tail of the $C_{60}$ decay. In the case of 193 nm excitation, the $C_{60}$ delayed ion signal can be described adequately by a monoexponential decay function with a lifetime of approximately 4·$\mu$s. For 248 nm excitation, a monoexponential decay with a lifetime of approximately 7·$\mu$s is observed at low laser intensities; at higher
intensities, a second shorter component with lifetime < 1 μs appears. These results will be discussed further, below, in comparison with those obtained for pure C$_{60}$.

4.2. Pure C$_{60}$

On commencing these experiments, we became aware that the desorption properties of pure C$_{60}$ differed from those of the fullerite mixture: the pure C$_{60}$ appeared to be considerably more resistant to desorption, i.e., less volatile, than the impure material. In order to obtain reasonable signal intensities, it was necessary to reduce the speed of rotation of the probe rod so that the same area of sample was repeatedly irradiated by the desorption laser beam. As discussed below, this resulted in the desorption of internally hotter molecules from the pure material than from the impure fullerite. Experiments were generally carried out using sample rotation speeds of about 8 deg s$^{-1}$, considerably slower than the 'normal' speed of about 30 deg s$^{-1}$ which was used for the fullerite samples. Photoionization radiation at 193 nm was used throughout these measurements over a limited range of fluences of 20–65 μJ mm$^{-2}$. Typical photoion time of arrival spectra are shown in figure 3; they are qualitatively very similar to the 266 nm photoion spectra reported by Wurz and Lykke [4]. The fragmentation of C$_{60}^+$ μJ mm$^{-2}$ and delayed ionization of superexcited C$_{60}$, observed

![Figure 3. Time of arrival spectra of photoions produced by 193 nm ionization of pure C$_{60}$ at fluences of (a) 30 μJ mm$^{-2}$ and (b) 65 μJ mm$^{-2}$.](image-url)
under these experimental conditions, are discussed in the following two sections. We then consider the effect of desorption conditions on these two processes.

4.2.1. Fragmentation of $C_{60}^+$

The photoion spectra of pure $C_{60}$ (figure 3) show much greater fragmentation than was observed for fullerite under comparable ionization conditions (figure 1). The extent of fragmentation does not appear to depend strongly on ionizing laser fluence at the relatively low intensities used here. The intensity of each of the three fragment ions $C_{58}^+$, $C_{56}^+$ and $C_{54}^+$ relative to that of prompt $C_{60}^+$ appears to increase approximately linearly with fluence over the range $20-65 \mu J mm^{-2}$. Operating the reflectron in partially compensating mode reveals the occurrence of fragmentation in the first drift region as well as in the acceleration region, as shown in figure 4. Fragment ions formed in the acceleration region arise from dissociation processes occurring within 4 $\mu s$ of the ionizing laser pulse; fragmentation at later times, up to $\sim 50 \mu s$ after the laser pulse, will occur in the first drift region. The laser fluence dependence of the fragment ion intensity distribution ($C_{58}^+: C_{56}^+: C_{54}^+$, etc.) was the same for both series of peaks, suggesting that the same fragmentation mechanism is operating in both cases. Over the limited laser fluence range investigated, the relative intensities of the two series of fragment peaks remained constant, implying that short and long timescale dissociation processes largely result from absorption of the same number of photons by parent ions with different levels of internal energy.

In photofragmentation experiments on carbon cluster beams, O'Brien et al. [12] found that at least three photons of 193 nm radiation were needed to dissociate internally cold $C_{60}^+$ to $C_{58}^+$ on a timescale of about 3 $\mu s$. They also proposed that higher order fragmentation involves single step elimination from $C_{60}^+$ of a neutral fragment with four, six, eight or more carbon atoms. The $C_2$ binding energy has been determined by Radi et al. [13] to be about 4.6 eV ($eV \approx 1.60218 \times 10^{-19} J$), while results of a recent study by Yoo et al. [14] suggest it may be as high as 6–6.5 eV. The results of RRKM calculations presented in the latter paper predict that an internal energy of 24 eV or more is needed for dissociation of $C_{60}$ on the microsecond timescale for a $C_2$ binding energy of 4.6 eV.

![Figure 4](image)

Figure 4. Time-of-flight mass spectrum of $C_{60}^+$ photofragments recorded with the reflectron in partially compensating mode. The series of peaks displaced to higher mass is due to dissociation in the first drift region of the mass spectrometer.
The fragmentation observed for pure C\textsubscript{60} in the present work certainly is not consistent with the ladder switching mechanism of sequential photofragmentation [15], which would lead to a marked fluence dependence of the fragment ion distribution and requires fragmentation to occur within the duration of the laser pulse. Loss of C\textsubscript{2} on the nanosecond timescale is expected to require an internal energy of about 40 eV, equivalent to more than six 193 nm (6.4 eV) photons. The sequential loss of C\textsubscript{2} groups from internally hot fragment ions also seems implausible for multiple fragmentation within a few microseconds after the laser pulse. The observed fragmentation thus appears to be due principally to single step loss of C\textsubscript{2}, C\textsubscript{4}, C\textsubscript{6}, etc., from C\textsubscript{60}\textsuperscript{+}. The apparent ability of C\textsubscript{60} to dissociate by loss of small even-numbered carbon clusters which contain more than two carbon atoms may have important implications regarding the mechanism of the reverse process, nucleation.

Two-photon ionization of C\textsubscript{60} molecules with a vibrational energy of \( E_{\text{th}} \) will produce C\textsubscript{60}\textsuperscript{+} ions with internal energies up to a value of \( E_{\text{th}} + (2 \times 6.4) - 7.6 = E_{\text{th}} + 5.2 \text{ eV} \). Fragmentation to C\textsubscript{58}\textsuperscript{+} in the acceleration region then requires a minimum additional energy of 19.2 \( - (E_{\text{th}} - 5.2) = 14 - E_{\text{th}} \) eV, according to the work of O'Brien \textit{et al.} discussed above. An \( E_{\text{th}} \) of 1.2 eV would thus enable photodissociation of C\textsubscript{60}\textsuperscript{+} by absorption of a minimum of two 6.4 eV photons; for an \( E_{\text{th}} \) of 7.6 eV, a minimum of one photon would be required. On the basis of a mean vibrational wavenumber of 920 cm\textsuperscript{-1} for C\textsubscript{60} [16, 17], we estimate that a vibrational energy of 1.2 eV is equivalent to a temperature of about 460 K, while 7.6 eV is equivalent to about 1030 K. There is no straightforward way of determining the vibrational temperature of our entrained C\textsubscript{60} molecules. (In principle, information on the vibrational temperature could be obtained by R2PI spectroscopy, if the jet cooling were sufficient to give well resolved vibronic structure. But, on the basis of experience with smaller polyatomic molecules, we would expect to observe broad, structureless spectra under the present desorption/entrainment conditions.) However, the former temperature is certainly plausible and the latter may be possible for the rather involatile pure material. Wurz \textit{et al.} [18] estimated from velocity distribution measurements that the translational temperature of C\textsubscript{60} molecules desorbed with 308 nm laser radiation was 2300 ± 200 K. Thus, even allowing for some vibrational cooling in our experiments by entrainment in the helium beam, a vibrational temperature of 1030 K may be realistic. This would certainly account for the observation of considerable C\textsubscript{58}\textsuperscript{+} intensity at relatively low laser fluences and the low order fluence dependence of the fragment ion intensity. The enhanced fragmentation seen for pure C\textsubscript{60} samples, compared with the fullerite, is clearly consistent with the desorption of vibrationally hotter molecules from the less volatile pure material.

4.2.2. \textit{Delayed ionization of C\textsubscript{60}}

We will now consider the delayed ionization behaviour of pure C\textsubscript{60}, as exemplified in figure 3. It can be seen that the intensity of the delayed ion tail is, like the fragment ion intensities, considerably greater than for fullerite under similar ionization conditions. The time dependence of the delayed ion signal observed in this series of experiments could, in all cases, be modelled satisfactorily by a biexponential decay function with time constants of \( \tau_1 = 5 \pm 1 \text{ \mu s} \) and \( \tau_2 = 0.8 \pm 0.2 \text{ \mu s} \). None of the decays could be fitted by a monoexponential function. This is illustrated in figure 5, where the results of fitting mono- and biexponential functions to the 30 \( \mu \text{J mm}^{-2} \) data, shown in figure 3(a), are compared. The peak of the C\textsubscript{60} ion signal
Figure 5. Comparison of (a) monoexponential and (b) biexponential best fits to the delayed ionization data of figure 3(a).

is shown at zero time; fitting was commenced 300 ns after the peak in order to exclude the prompt ion signal. The value of the shorter decay time appeared to decrease at higher laser fluence, suggesting the onset of a third, faster process which cannot be resolved with the present time resolution.

These results indicate that, within the time window of the present measurements (see section 3 above), there are two kinetically distinct processes leading to delayed formation of $C_{60}^+$. We are thus observing the decay times of two different superexcited states of $C_{60}$ which have been populated by the absorption of different numbers of photons. In fact, each observed state must correspond to a distribution of internal energies, determined by the initial thermal population, which can be approximated by a single decay constant. For such long-lived superexcited states, it can be assumed that all electronic excitation has been dissipated into vibrational degrees of freedom, and the only two decay channels are delayed ionization and unimolecular dissociation. The relatively high delayed ion intensities indicate that ionization is occurring at a rate which is competitive with that of dissociation and, thus, the observed rate coefficients can be taken as good indicators of the magnitudes of the rate coefficients for delayed ionization. By taking the values of $1/\tau_1$ and $1/\tau_2$ as upper limits for the dissociation rates of the two excited states and assuming
that the dissociation kinetics of the neutral are similar to those of the positive ion, we can estimate the excitation energies of these two states. Thus, on the basis of the arguments set out above for $C_{60}^+$, we infer that the longer lived state has an internal energy in the region of 20 eV and is populated by absorption of three photons (or possibly two, if $E_{th}$ is high enough), while the shorter lived state lies 6-4 eV higher in energy and is populated by four (or possibly three) photon absorption.

The lower relative intensity of the delayed $C_{60}$ ion signal observed for fullerite samples at 193 nm (figure 1) is due to the lower vibrational temperature of the molecules desorbed from the more volatile material. In order to achieve the levels of internal energy estimated above, higher order photoexcitation processes are required so that delayed ionization does not compete favourably with two-photon direct ionization. The decay constant of about 4 μs observed in this case probably corresponds to a four-photon excited state which has comparable internal energy to the above $τ_1$ state. For excitation with 248 nm (5 eV) photons, even higher order absorptions will be involved in production of the observed decaying states. However, in the absence of a strong one-photon resonant absorption, the higher order photo-processes which lead to delayed ionization and ionic fragmentation are more competitive with direct two-photon ionization.

In molecular terms, delayed ionization is a consequence of the non-adiabatic coupling of vibrational and electronic degrees of freedom. The analogous process in extended solid state systems is the thermionic emission of electrons. Amrein et al. [19] have reported recently that the delayed ionization of small transition metal clusters can be modelled remarkably well by assuming the cluster to be a small fragment of the bulk and applying the Richardson—Dushman relationship [20, 21] for the rate of thermionic emission. Thermionic emission has also been invoked to explain the delayed production of multiply charged fragments following laser excitation of singly charged giant fullerene $(C_{100-60})$ ions [22]. There is already some evidence that the $C_{60}$ molecule displays quasi-solid-state electronic properties in that a giant plasmon resonance has been reported [23]. The plasmon resonance, which peaks at approximately 20 eV, was revealed in single photon ionization studies of gas phase $C_{60}$: autoionization via the plasmon excitation results in a large increase in the one-photon ionization efficiency in this energy region. This resonance, which is due to the collective excitation of valence electrons, was predicted theoretically by Bertsch et al. [24], who described it as a Mie-type plasmon in a conducting sphere of high charge density. There is also evidence from high resolution electron-energy-loss spectroscopy that the $C_{60}$ molecule can support collective resonances similar to those of graphite [25].

We now compare the present results with the predictions of the Richardson—Dushman relationship and the Klots model for thermionic emission from small particles [26]. The approach taken was to use the alternative models to predict the temperature, and hence internal energy, required to give emission on the same timescale as the observed delayed ionization. The rate coefficients determined from the decay of the delayed ion signal represent upper limits for the rates of delayed ionization. In view of the high relative intensity of the delayed ionization signal, we estimate that reasonable lower limits for the delayed ionization rate coefficients would be one tenth of the measured values. The Richardson—Dushman relationship for the thermionic emission current density, $J A^{-1} m^{-2}$, from a macroscopic elemental solid is:

$$J = AT^2 \exp \left( -\frac{\phi}{k_B T} \right),$$  (2)
where $A$ is the Richardson constant which has a value of $1.2 \times 10^6 \text{A m}^{-2} \text{K}^{-2}$, $\phi$ is the work function, $k_B$ is the Boltzmann constant and $T$ is the absolute temperature.

The Klots model [26] has been derived recently, specifically to describe thermionic emission from isolated aggregates of matter. It gives the following expression for the rate coefficient $k$:

$$k = \left(\frac{2k_BT_b}{h}\right)\left(\frac{Q_{\text{vib}}}{Q_{\text{vib}}^0}\right) \exp\left(-\frac{E_0}{k_BT_b}\right)\left\{2b/a_0 + 2(Q_{\text{surf}}\pi/4)^{1/2} + Q_{\text{surf}}\right\},$$

where $T_b$ is the isokinetic bath temperature, $E_0$ is the energy threshold for electron ejection, $b$ is the classical hard sphere collision radius, $a_0$ is the Bohr radius, $Q_{\text{vib}}^0$ and $Q_{\text{vib}}$ are the vibrational partition functions before and after emission and

$$Q_{\text{surf}} = \frac{2\mu b^2 k_B T_b}{(h/2\pi)^2},$$

where $\mu$ is the reduced mass, in this case equal essentially to that of the electron. For the present purposes, the ratio of the vibrational partition functions before and after emission has been approximated to unity.

The adiabatic ionization potential of $C_{60}$, 7.58 eV [9], was substituted for $\phi$ in equation (2) and for $E_0$ in equation (3) and the radius of $C_{60}$ was taken to be 0.355 nm [27]. The lower and upper limit values $T_1$ and $T_2$ of the temperature of $C_{60}$ required to give rate coefficients for thermionic emission of $2 \times 10^4 \text{s}^{-1}$ and $1.25 \times 10^6 \text{s}^{-1}$, respectively, were then determined for each model. The Richardson–Dushman relation predicts temperatures of 3590 K $\leq T_1$ $\leq$ 3980 K, equivalent to an internal energy (mean vibrational energy) range of $\sim$ 44–49 eV and 3900 K $\leq T_2$ $\leq$ 4350 K ($\sim$ 48–55 eV). The Klots model predicts somewhat lower temperatures of 3200 K $\leq T_1$ $\leq$ 3530 K ($\sim$ 38–43 eV) and 3450 K $\leq T_2$ $\leq$ 3840 K ($\sim$ 42–47 eV). Both predictions are far in excess of our estimated internal energies of $\sim$ 20 eV and $\sim$ 26 eV and seem much too high to be consistent with our observations. It is interesting to note, however, that substituting the work function of graphite, $\phi = 4.39 + (1.7 \times 10^{-4}T)$ [28], into the above expressions yields predicted temperatures which are much more in line with our estimated internal energy: 2290 K $\leq T_1$ $\leq$ 2580 K ($\sim$ 25–29 eV) and 2550 K $\leq T_2$ $\leq$ 2860 K ($\sim$ 28–33 eV) from the Richardson–Dushman relation and 1960 K $\leq T_1$ $\leq$ 2200 K ($\sim$ 20–23 eV) and 2150 K $\leq T_2$ $\leq$ 2420 K ($\sim$ 23–26 eV) from the Klots model. Bearing in mind that our estimated internal energies could easily be too low by 6–4 eV (one photon), the predictions for thermionic emission from graphitic clusters are remarkably close to the observed behaviour of $C_{60}$. It is not clear whether this is simply coincidental or whether it is a reflection of the electronic properties of $C_{60}$ at these very high internal energies, i.e. that the threshold energy for electron emission should be so much lower than the adiabatic ionization potential. Further elucidation of the mechanism of delayed ionization will require measurements under conditions of well defined internal energy which can be achieved only if both the thermal energy and the photoexcitation energy can be determined accurately.

The time dependence of the delayed ion signal in the present work is similar, both in its biexponential form and in the magnitude of the decay constants, to that reported by Campbell et al. [3] and that reported by Wurz and Lykke [4]. At first this seems surprising, since different vaporization methods and photoexcitation wavelengths have been used in the three investigations. However, the similarity becomes less surprising when one considers that the time window of observation is $\mu a_0 \approx 5.299 \times 10^{-11} \text{m}$. 

[6]
similar in all three experiments. Thus, we are all observing the decay of superexcited species with internal energies within a comparable, restricted range.

4.2.3. Dependence of delayed ionization and fragmentation on desorption conditions

During the course of these experiments, we were surprised to find that the intensities of delayed $C_{60}$ ions and fragment ions in the 193 nm photoion arrival time spectrum were markedly dependent on the rate of rotation of the desorption probe. This is illustrated in figure 6, which shows two spectra which were acquired at the same photoionizing laser fluence of 50 $\mu$J mm$^{-2}$ and using the same desorption laser power density, but at different sample rotation speeds. At the relatively high sample rotation speed of 30 deg s$^{-1}$, figure 6(b), the prompt ion signal dominates, and there is relatively little fragmentation or delayed ionization. At this rotation rate, the area of fresh sample exposed to each shot of the desorption laser is 0.3 mm$^2$, which constitutes 10% of the total desorption target area. At even higher rotation speeds, a weak prompt ion signal with negligible fragmentation and delayed ionization were observed; this closely resembled the $C_{60}$ spectrum produced by soft ionization of fullerite. As the sample rotation speed is decreased, the relative intensity of fragment ions and delayed $C_{60}$ ions increases, reaching a maximum for the static sample, figure 6(a). Irrespective of sample speed, the delayed ion signal consisted of a biexponential decay with the same time constants as above.

These observations can be explained in terms of a thermally induced phase change which has been reported [29] to result in a transition from initially amorphous $C_{60}$ to a polycrystalline form. The heat of sublimation of polycrystalline $C_{60}$ was found to be 167 kJ mol$^{-1}$, considerably higher than the value of 90 kJ mol$^{-1}$ for the amorphous material [30]. It appears that, in our experiments, infrared irradiation of the sample by the desorption laser induces such a phase change. We propose that when the desorption laser pulse strikes an area of fresh, amorphous sample, some material is vaporized from the surface layers but most of the sample is rendered polycrystalline and relatively involatile. This involatile material is then vaporized by repeated irradiation of the same sample area, resulting in the production of

![Figure 6](image_url)

Figure 6. Photoion arrival time spectra produced by ionization of pure $C_{60}$ by 193 nm laser radiation with a fluence of 50 $\mu$J mm$^{-2}$. Spectrum (a) was obtained following desorption from a stationary sample; spectrum (b) was obtained following desorption from a sample rotating at 30 deg s$^{-1}$. 
molecules which have higher internal temperatures than those produced from the amorphous material. The photoion spectra are recorded as a sum of several hundred laser shots. Thus, as the sample speed is varied there is a variation in the relative contributions of ‘hot’ molecules (from polycrystalline $\text{C}_{60}$) and ‘cold’ molecules (from amorphous $\text{C}_{60}$) to the integrated spectrum. Rapid rotation of the sample results in the production predominantly of ‘cold’ molecules which show negligible fragmentation and delayed ionization at this photoexcitation intensity; whereas desorption from a static sample leads to almost exclusive production of ‘hot’ molecules which are characterized by extensive fragmentation and delayed ionization. It was suggested above that the longer lived superexcited state could be populated by absorption of as few as two 6.4 eV photons if the thermal energy of the desorbed molecules were sufficiently high. The high relative intensity of the delayed ion signal from the static sample (figure 6(a)) suggests that this may indeed be the case. In the impure fullerite samples, the desorption laser-induced phase change does not appear to compete with vaporization of the amorphous material, so that relatively facile desorption of ‘cold’ molecules is observed.

These results demonstrate clearly the influence of thermal excitation on delayed ionization of $\text{C}_{60}$. This is somewhat at odds with the conclusion of Wurz and Lykke [4] that ‘...the initial temperature does not play an important role and the observed processes are initiated solely by the ionizing laser’. This conclusion was based on their observation of similar delayed ionization behaviour for $\text{C}_{60}$ from an effusive beam at 800 K and for laser-desorbed molecules at a translational temperature of 2000 K. However, they had adjusted the ionizing laser intensity to yield the same abundance of fragment ions in each case; they were thus producing excited species with similar levels of total internal energy, although with different contributions from thermal and optical excitation, at the different temperatures.

4.2.4. Photophysical considerations

The delayed ionization of $\text{C}_{60}$ is remarkable not only because the superexcited states involved are so long-lived, but also because they are so efficiently populated by multiphoton absorption in competition with prompt ionization. The processes of direct two-photon ionization at 193 nm and superexcited state formation by two-photon absorption are shown schematically in figure 7, taking into account what little is known of the photophysical properties of $\text{C}_{60}$. The lowest excited singlet state $S_1$ lies at about 2 eV [11, 31] and the lowest triplet state $T_1$ has been measured variously [11, 32–36] to lie in the range 1.4–1.8 eV. The ionization potential of $\text{C}_{60}$ has been determined by single photon ionization to be 7.58 eV [9]. If superexcited state formation is to compete with direct ionization, it is crucial that rapid intramolecular conversion of electronic to vibrational energy occurs at the intermediate, single photon excited level. In $\text{C}_{60}$, this relaxation will be mediated by intersystem crossing, which occurs with near unit quantum yield [31, 32]. The time evolution of the relaxed state is represented in figure 7 by the passage through vibronic states with decreasing levels of electronic energy. The lifetime of the lowest triplet state of $\text{C}_{60}$ at a vibronic energy of 4 eV is known to be 40 μs [36]. In the present case, the energy of the intermediate level is $(6.4 + E_{\text{th}})$ eV. For low values of $E_{\text{th}}$, therefore, relaxation will probably not proceed beyond the $T_1$ state within the duration of the excitation laser pulse. Absorption of the second photon will occur with the approximate conservation of vibrational energy (in accordance with the Franck–Condon principle). Thus, a one-photon transition from the vibronically excited $T_1$ state will
Figure 7. Schematic representation of the processes of direct and delayed ionization of C$_{60}$ by absorption of two 193 nm photons. S$_0$ is the ground electronic state, S$_1$ is the first excited singlet state and S$_n$ is the excited singlet state populated by absorption of one 193 nm photon. T$_1$ is the first triplet state and T$_n$ is the excited triplet state populated by one-photon transition from T$_1$. IP is the ionization potential.

populate a vibronic state with an electronic energy only 0.5 eV above the ionization potential. Very little electronic energy then has to be dissipated in order to preclude rapid autoionization. (For high values of $E_{th}$, intersystem crossing to the ground state may be sufficiently rapid to occur at the one-photon level. Then, absorption of a second photon will populate a state with electronic energy well below the ionization threshold.) Give the enormously high density of states at the two-photon excitation energy (10$^{38}$ cm$^{-1}$ at 13 eV [14]), there will be rapid thermalization to give an extremely vibrationally hot ground state species, C$_{60}$(e$^*$), which subsequently decays by ionization or dissociation. The important role of radiationless decay at the intermediate level in promoting population of the superexcited state is verified by the absence of delayed ionization following one-photon excitation above the ionization threshold: single photon excitation at 10.5 eV was found to produce exclusively prompt ionization [4].

Schlag et al. [37] have recently posed the question ‘do large molecules ionize?’. This was prompted by the observed decline in ionization efficiency with molecular size. They suggest that it may be inappropriate to describe the ionization of large molecules in terms of a direct process, since efficient thermalization of electronic energy will result in a predominantly thermal ionization mechanism. In the case of C$_{60}$, the thermalization of electronic energy at the expense of prompt ionization is manifested by the production of delayed ions through subsequent thermal ionization. If the timescale for delayed ionization of C$_{60}$ is typical of large molecular systems, then dissociation of the thermalized state must occur to the virtual exclusion of thermal ionization in most molecules. The occurrence of slow delayed ionization in C$_{60}$ is a consequence of the extraordinary stability of this species.

5. Conclusion

Delayed ionization of C$_{60}$ on the microsecond timescale has been observed following 193 nm excitation of infrared laser-desorbed molecules. Within the time window of the present experiments, (0.5 µs ≤ t ≤ 25 µs after the ionizing laser pulse), two kinetically distinct superexcited states of C$_{60}$ are observed to decay with the
production of delayed ions. The lifetimes of these states are $5 \pm 1 \mu$s and $0.8 \pm 0.2 \mu$s; their excitation energies are estimated to lie in the range 20–30 eV.

Both the Richardson–Dushman and the Klots models for thermionic emission appear to overestimate considerably the internal energy required for delayed ionization at the observed rates, when the ionization potential of C$_{60}$ is used as the threshold energy for electron emission. If instead the work function of graphite is used, the predictions are much closer to our estimated internal energies. This may be an indication that the electronic properties of C$_{60}$ at these high internal energies, and corresponding state densities, resemble more those of bulk graphite. There is already evidence of a similarity between the recently observed collective resonances of C$_{60}$ [23, 25] and the $\pi$ and $\sigma$ plasmons of the bulk. Further elucidation of the mechanism of delayed ionization awaits measurements under conditions of well defined internal energy.

A marked dependence of the delayed ion signal intensity on the desorption conditions is attributed to a thermally induced phase change of C$_{60}$, from an amorphous to a polycrystalline state, during the desorption process. The difference in internal temperatures of molecules desorbed from the two phases is manifested in the delayed ion signal. This effect demonstrates the role of thermal excitation in the delayed ionization process.

In C$_{60}$, the superexcited states which give rise to the observed delayed ionization are efficiently populated in competition with prompt ionization. This is attributed to rapid radiationless decay of electronic to vibrational energy at the one-photon excited level. The display of slow delayed ionization by C$_{60}$ (and C$_{70}$), but not by other large molecules, is a consequence of the high stability of the fullerenes which enables autoionization of vibrationally hot molecules to compete with dissociation. In less stable molecules, dissociation is expected to be the dominant decay process, so that thermalization of electronic energy will simply lead to a decrease in photo-ionization efficiency.

In some respects, C$_{60}$ represents an ideal system for studying cluster photophysics. In much of the current research on metal clusters, one of the major problems is the difficulty in characterization of cluster structure and hence internal temperature. In contrast, C$_{60}$ possesses a well defined, stable structure. Moreover, it is possible to excite the molecule efficiently to excess energies corresponding to state densities similar to those in smaller metal clusters. In the case of Nb$_7$, Ta$_7$ and W$_7$ [19], although the number of vibrational degrees of freedom is considerably smaller than in C$_{60}$, there are proportionally many more possible electronic terms. Thus, at total vibronic state densities sufficiently high to allow efficient electronic relaxation, the same phenomenon of delayed ionization is manifested in these systems which differ rather widely in size and chemical composition.

6. Postscript

After the preparation of this manuscript, Campbell et al. [38] published a second paper on the delayed ionization of C$_{60}$. In this publication, they compare their previously reported results (which, as detailed above, were obtained in experiments in which fullerite was vaporized using 248 nm laser radiation and post-ionized at 308 nm) with the Klots model for thermionic emission from clusters, using a value of 7.54 eV for the ionization potential of C$_{60}$. They assume the measured rate coefficients to be those of delayed ionization, neglecting the possibility of a competing
dissociation channel. As discussed above, the Klots model predicts that internal energies in the region of 50 eV are required to give rise to the observed rates. Campbell et al. [38] conclude that their results are in good agreement with the theoretical predictions, if it is assumed that eight photons of 248 nm radiation were absorbed during the desorption process, resulting in the production of \( \text{C}_60 \) molecules having an internal energy of about 44 eV prior to photoionization. As discussed in the present paper, the rate coefficients which we have determined are similar in magnitude to those reported by Campbell et al. [38], indicating that the decay of superexcited states with comparable levels of internal energy were being observed in both studies. However, since we were using infrared laser desorption at a wavelength of 10-6 μm (photon energy of 0.12 eV), it seems highly improbable that desorbed molecules with internal energy in excess of 40 eV were being produced in our experiments. The validity of the assumption upon which Campbell et al. [38] base the interpretation of their results appears to be questionable, therefore.

Following the submission of this manuscript, two further papers have been published which are relevant to this work. Wurz and Lykke [39] have published a more detailed account of their investigation of the delayed ionization of \( \text{C}_60 \); they also report the observation of photofragmentation of neutral \( \text{C}_60 \). Their delayed ionization experiments were carried out using an effusive beam of \( \text{C}_60 \) at a temperature of approximately 800 K and a range of photoexcitation wavelengths from 118–532 nm, corresponding to Nd\(^{3+}\) : YAG laser harmonics. Multiexponential decays are reported with decay constants in the range 120 ns–55 μs, which are taken to be the rate coefficients for delayed ionization. On the basis of the Klots model [26] of thermionic emission, it is inferred that the observed rate coefficients correspond to superexcited states with energies in the region of 50 eV and that, therefore, 10–20 laser photons are being absorbed by \( \text{C}_60 \) in order to populate these states. Thus, like Campbell et al. [38], Wurz and Lykke [39] have assumed the validity of a thermionic emission mechanism and interpreted their results accordingly. Further light has been shed on this matter in a recent publication, by Sandler, Lifshitz and Klots [40], on the kinetics of the dissociation and thermionic emission of \( \text{C}_60 \). On the basis of kinetic energy release measurements for the emission of \( \text{C}_2 \) from \( \text{C}_60 \), the microcanonical rate coefficients for the dissociation of neutral \( \text{C}_60 \) have been calculated and compared with the rate coefficients for thermionic emission of electrons from \( \text{C}_60 \). It is shown that the rate of dissociation greatly exceeds the rate of thermionic emission in the energy range 25–45 eV. At an energy of 30 eV, the rate coefficient for dissociation is found to be about \( 10^5 \text{ s}^{-1} \) while the rate coefficient for thermionic emission is only about \( 10^3 \text{ s}^{-1} \). This implies that a superexcited state of \( \text{C}_60 \) with a lifetime of 10 μs has an excitation energy of \( \leq 30 \text{ eV} \), and supports our view that the excitation energies of the states which we are observing to decay on the microsecond timescale are considerably lower than that required for the thermionic emission of electrons from \( \text{C}_60 \) molecules on this timescale. It follows from the results of Sandler et al. [40], that it is not valid to assume that the observed delayed ionization of \( \text{C}_60 \) conforms to a thermionic mechanism.

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References


Photoionisation and photodissociation of laser-vaporised metallotetraphenylporphyrins

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Abstract

The photoionisation and photodissociation processes induced by 193 and 266 nm excitation of a series of metallotetraphenylporphyrins (NiTPP, ZnTPP, CoTPP, CuTPP, and VOTPP) have been investigated, using laser-desorption laser-photoionisation time-of-flight mass spectrometry. At 193 nm, all of the molecules undergo molecular photoionisation followed by photofragmentation (class A behaviour). At 266 nm, ZnTPP and CuTPP exhibit class A behaviour, whereas NiTPP, CoTPP and VOTPP undergo neutral photodissociation followed by ionisation (class B behaviour). Neutral photodissociation results in cleavage of the coordinated metal from the macrocycle, whereas photodissociation of the molecular ion leads to loss of phenyl substituents from the macrocycle without loss of the metal atom.

1. Introduction

The photophysics of the low-lying electronic states of the metalloporphyrins has been studied widely in solution phase experiments. The interest in the electronic properties of these molecules arises from their central role in the electron transfer photoreactions of photosynthesis and their potential applications in catalytic solar energy conversion and photodynamic cancer therapy. The metalloporphyrins are involatile and have been little studied in the gas phase; their intrinsic photophysics and photochemistry, particularly following excitation to higher energy states, remain largely unexplored. The only extensive study of the gas-phase spectroscopy of metalloporphyrins is that of Edwards et al. who investigated the absorption spectra (from 200 to 800 nm) of tetraphenylporphyrins [1], octaethylporphyrins and etioporphyrins [2], as summarised in Gouterman's review [3]. The fluorescence excitation spectra and excited state lifetimes of free-base-, Zn- and Mg-tetraphenylporphyrin have been investigated under jet-cooled conditions by Even et al. [4]. Metalloporphyrins can be readily vaporised, without thermal decomposition, by pulsed infrared laser desorption. We have used this technique in combination with laser photoionisation time-of-flight mass spectrometry to investigate the photoionisation and photodissociation of a series of metallotetraphenylporphyrins excited at two UV wavelengths, 193 and 266 nm. This work has revealed a photodissociation channel which leads to demetallation of the neutral molecule. In contrast, photodissociation of the molecular radical cation results in loss of phenyl substituents, while the coordinated metal is retained.

The nonlinear photochemistry which may be induced by the interaction of an intense laser pulse with a molecule has been discussed in detail by Gedanken et al. [5]. They have defined two general categories

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of photochemical behaviour, class A and class B, which may be observed in multiphoton ionisation experiments. The molecule may be photoionised to form the molecular ion which may then undergo photofragmentation; this is class A behaviour. Alternatively, photodissociation of the molecule may occur, followed by photoionisation of the neutral fragments; this is class B behaviour. The present work shows that the metallotetraphenylporphyrins, as a result of their diverse photophysical properties, exhibit both type A and type B photochemical behaviour.

2. Experimental

Co(II)-, Cu(II)-, Ni(II)-, Zn(II)- and V(IV)O-tetraphenylporphyrin (hereafter referred to as CoTPP, CuTPP, NiTPP, ZnTPP and VOTPP) were purchased from Aldrich Chemical Company Ltd. and used without further purification. The laser-desorption laser-photoionisation time-of-flight mass spectrometer system used in these experiments has been described in detail elsewhere \[6\]. The metalloTPP sample was deposited as a solid film, from chloroform solution, onto a stainless steel probe and vaporised by pulsed infrared laser desorption. The desorption laser was a TEA CO₂ laser (Alltec 854MS) which produced 100 mJ of 10.6 μm radiation in a 100 ns pulse. The desorbed molecules were entrained in a pulsed free jet of helium, formed by expanding 4 bar of He through a 0.8 mm diameter pulsed nozzle (General Valve Corporation Series 9) with a valve open time of 600 μs. The molecular beam travelled into the source region of a time-of-flight mass spectrometer where the entrained neutrals were photoionised by laser radiation of wavelength 193 nm (ArF excimer laser) or 266 nm (Nd:YAG laser fourth harmonic). The mass-resolved ion signal was processed by a CAMAC-based data acquisition system and time-of-flight mass spectra were accumulated and displayed on a Dell PC. Data were accumulated, typically, over 500 laser shots at a repetition rate of 10 Hz.

In some experiments, aniline vapour was seeded into the molecular beam in order to produce aniline clusters for mass calibration purposes. The mass spectra recorded under these conditions showed no evidence of complex formation between the metal-tetraphenylporphyrins and aniline. Under the entrainment conditions used, the jet-cooling of the desorbed metalloTPP molecules was probably insufficient to sustain such complexation.

3. Results

3.1. 193 nm photoionisation

For all of the metallotetraphenylporphyrins investigated (CoTPP, CuTPP, NiTPP, VOTPP and ZnTPP), soft ionisation at 193 nm results in the production of molecular radical cations with little or no fragmentation. This is illustrated for CuTPP and NiTPP in Figs. 1a and 2a, respectively. Increasing the ionising laser intensity causes photofragmentation of the molecular ion, as shown in Figs. 1b and 2b. The predominant fragment species correspond to loss of one and two phenyl groups from the porphyrin nucleus, with the metal atom being retained in the macrocycle. Loss of the phenyl groups is accompanied
3.2. 266 nm photoionisation

Photoionisation of CuTPP and ZnTPP at 266 nm gave similar results to those observed with 193 nm ionisation. For these two molecules, soft ionisation at 266 nm yields the molecular ion almost exclusively, while increased ionising laser intensity results in fragmentation by loss of one and two phenyl groups, as illustrated for CuTPP in Fig. 3a. Free-base tetraphenylporphyrin (H₂TPP) also shows molecular ionisation at both wavelengths. However, the behaviour of CoTPP, NiTPP and VOTPP following 266 nm photoionisation is markedly different. As shown in Figs. 3b, 3c and 4a, under soft ionisation condi-

by loss of a number of hydrogen atoms, as has been observed previously in laser desorption Fourier-transform mass spectra [7] and electron impact mass spectra [8]. Minor fragment peaks, which appear to be due to loss of CH₂ groups from the major fragment ions, are also present in the hard ionisation mass spectra. A detailed account of the 193 nm photofragmentation of metalloporphyrin radical cations will be presented in a forthcoming publication [9]. Photoelectron spectroscopy of the metalloTPPs [10] has shown that their ionisation potentials lie in the range 6.3–6.5 eV. A single 193 nm (6.42 eV) photon might be just sufficient to achieve ionisation, therefore. In the present experiments it is difficult to determine reliably the dependence of ion intensity on laser fluence because of signal fluctuations arising in the desorption process. However, such measurements for CuTPP indicate that the order of the photoionisation process is 1.4±0.2. Although not conclusive, this suggests that (1+1) photoionisation, rather than single photon ionisation, is operative.

Fig. 2. Time-of-flight mass spectra of NiTPP following 193 nm photoionisation under (a) soft ionisation and (b) hard ionisa-
tion conditions. [M]⁺ indicates the molecular ion and Ph = C₆H₅.

Fig. 3. Time-of-flight mass spectra following 266 nm photoionisa-
tion of (a) CuTPP under hard ionisation conditions, (b) CoTPP under soft ionisation conditions and (c) NiTPP under soft ionisation conditions. [M]⁺ indicates the molecular ion and Ph = C₆H₅.
sections, the mass spectrum of each of these metalloporphyrins shows an intense fragment ion peak due to loss of the metal (metal plus O ligand in the case of VOTPP) from the macrocycle, with the phenyl substituents being retained. This metal-free fragment ion is the base peak in each case, with the parent molecular ion appearing only weakly. Loss of the central metal atom is accompanied by addition of 2H to give the free-base TPP radical cation. It must be stressed that this fragmentation process was observed even when extremely low ionising laser intensities were used. For CoTPP and NiTPPP, increasing the ionising laser intensity was observed to give a small increase in the intensity of the parent molecular ion relative to the metal-free fragment ion. In the case of VOTPP, this effect is much more pronounced as can be seen in Fig. 4. At an ionising laser power density of \( \approx 5 \) kW mm\(^{-2}\), see Fig. 4a, the VOTPP molecular ion peak is weak compared with the \([\text{TPP}]+\) fragment ion intensity. Increasing the laser power density to \( \approx 20 \) kW mm\(^{-2}\) results in an increase in the molecular ion intensity to \( \approx 60\%\) of the \([\text{TPP}]+\) intensity, Fig. 4b. Further increase of the laser power density to \( \approx 100 \) kW mm\(^{-2}\), Fig. 4c, produces a molecular ion signal with intensity slightly greater than that of the metal-free fragment. In the latter mass spectrum, fragment ion peaks due to the loss of phenyl groups and of the oxygen ligand from the molecular ion, without loss of vanadium, can also be identified as shown.

4. Discussion

The photoionisation and photofragmentation of the metalloTPPs induced by 193 nm laser radiation conforms with class A behaviour (as defined above). Under soft ionisation conditions the intact molecular ion is produced, while at increased laser power densities photofragmentation of the molecular ion occurs with the loss of one or two phenyl groups. Tandem mass spectrometry has shown previously that 308 nm photodissociation of Fe-, Mn- and Cr-TPP cations proceeds by the successive loss of two phenyl groups [71]. Similar fragmentation has also been observed in electron impact mass spectra of the metalloTPPs [8].

Photoionisation of the metallotetraphenylporphyrins at 266 nm is a two-photon process and the results we obtain at this wavelength can be explained in terms of competition between class A and class B mechanisms at the one-photon resonance level. For CuTPP and ZnTPP, class A behaviour is completely dominant at 266 nm, leading to formation of the molecular ion and its subsequent photolysis by loss of two phenyl groups, as observed at 193 nm. However, for CoTPP, NiTPP and VOTPP, neutral photodissociation followed by photoionisation (class B) results in the appearance of the metal-free fragment ion in the mass spectra. The formation of this fragment ion at even very low ionising laser fluences is strongly
characteristic of class B behaviour. The latter process dominates in CoTPP and NiTPP, with the molecular ion only appearing weakly in the mass spectra. Morris and Johnston [11] have reported previously that 266 nm photoionisation of CuTPP results in molecular ion formation, as has been observed here. However, they also reported that NiTPP and CoTPP display similar molecular ionisation, although they presented no mass spectra for these molecules in their paper. This observation is clearly at odds with the present results; this disagreement may be due to the use of much higher laser power densities by Morris and Johnston than in the present work. The competition between the class A and B processes is clearly apparent in the case of VOTPP. At low ionising laser fluence this molecule behaves as a class B system. At higher laser fluences, the rate of absorption of a second photon by the intermediate state, to form the molecular ion, becomes competitive with the rate of photochemical relaxation and the products of both processes are observed in the photoionisation mass spectrum. It is apparent from the above results that photodissociation of the neutral metalloporphyrin molecule results in cleavage of the coordinated metal atom from the macrocycle. Similar metal–ligand multiphoton dissociation has been observed in other organometallics, such as metal carboxyls [5,12, and references therein] and metalloccenes [13]. In contrast, photodissociation of the metalloTPP radical cation results in loss of phenyl substituents from the macrocycle, while the coordinated metal is retained.

The differing propensities for class A versus class B behaviour which are displayed by the different metalloTPPs at 266 nm can be related to the known photophysical properties of these molecules. The metalloporphyrins investigated here can be divided into two groups: the diamagnetic molecules, ZnTPP and NiTPP, and the paramagnetic molecules, CoTPP, CuTPP and VOTPP. In the latter, paramagnetic systems, coupling of the single unpaired d electron of the metal to the singlet and triplet ππ* states of the porphyrin macrocycle gives rise to singlet (S), triplet (T) and triquartet states (4T) [14]. Intersystem crossing from the singlet to triplet manifolds is spin allowed and occurs on the picosecond or subpicosecond timescale. For example, the S state of CuTPP is known to decay to the triquartet manifold in less than 350 fs [15].

Consider first the diamagnetic molecules, ZnTPP and NiTPP. ZnTPP is one of the few fluorescent TPPs. From the jet spectroscopic studies of Even et al. [4], it is known that the S state of ZnTPP lies at 17490 cm⁻¹ (2.17 eV) and the S state lies at ≈ 25150 cm⁻¹ (3.12 eV). Solution-phase measurements have shown that internal conversion from S to S occurs with a lifetime of 4 ps. Linewidth measurements under jet-cooled conditions [4] also indicated picosecond decay of the S state. Under jet-cooled conditions, the fluorescence lifetime of the S state is 3 ns [4]; the dominant decay channel is intersystem crossing which occurs with a quantum yield of ≈ 0.9 [17]. For the porphyrins, the lifetime of the S state has been found to be essentially independent of excess vibrational energy [4,18]. This invariance of fluorescence lifetime with respect to vibrational excitation in large molecules, such as the porphyrins, is a consequence of extensive intrastate anharmonic mixing which results in the vibrational energy being distributed over a large number of modes.

On the basis of the above knowledge of the photophysical properties of ZnTPP, the observation of class A photoionisation behaviour at 266 nm can be accounted for as follows. One-photon excitation of ZnTPP at 266 nm will populate a high-lying vibronic level in the singlet manifold, some 12000 cm⁻¹ above the S origin. Intramolecular conversion of electronic to vibrational energy will then occur on the picosecond timescale until the S electronic state, which has a lifetime of 3 ns, is reached. The latter state has an electronic energy of 2.17 eV and, therefore, can be ionised by one photon of 266 nm (4.66 eV) radiation. This sequence of events is illustrated schematically in Fig. 5a. Thus, the ZnTPP molecular ion is produced via a (1 + 1) photon process. Similar arguments apply to H₂TPP which has an S energy of 1.94 eV and a fluorescence lifetime of 11 ns [4] and shows molecular ionisation at 266 nm.

Unlike ZnTPP, NiTPP is non-fluorescent; its S state undergoes rapid radiationless decay (τ = 10 ps) to a low-lying (d, d) state which then decays rapidly to the ground state in 250 ps [19]. Thus, as illustrated in Fig. 5b, 266 nm one-photon excitation of NiTPP will be followed by rapid relaxation to a high vibrational level of the electronic ground state which cannot be photoionised by absorption of a second 266
Fig. 5. Schematic representation of the photophysical processes involved in: (a) Class A photoionisation of ZnTPP at 266 nm. Absorption of two 266 nm photons results in formation of the molecular ion \([\text{ZnTPP}]^+\). (b) Class B photoionisation of NiTPP at 266 nm. Photoexcitation is followed by rapid intramolecular conversion of electronic to vibrational energy to produce a vibrationally hot ground state species, \([\text{NiTPP}]^*\); dissociation followed by photoionisation yields the metal-free fragment ion \([\text{TPP}]^-\).

nm photon. The absorption of further photons and the subsequent rapid intramolecular conversion of electronic to vibrational energy leads to the observed neutral photodissociation. There appears to be no information available in the literature on the macrocycle-metal dissociation energy for the metalloporphyrins. However, data available for other complexes [20] suggest metal–nitrogen bond energies to be of the order of 1.5 eV, implying that at least two 266 nm photons would be needed to dissociate the metal from the macrocycle. A further two photons would then be required to photoionise the macrocyclic fragment. The absence of any significant intensity of metal ions in our mass spectra suggests that the photoionisation cross section of the porphyrin macrocycle is considerably larger than that of the atomic metal, at this wavelength.

The 266 nm photoionisation behaviour of the paramagnetic molecules, CuTPP, CoTPP and VOTPP, can be related to the photophysics of their tripmultiplet states which are populated by rapid intersystem crossing from the photoexcited singlet manifold. CuTPP has a long-lived \(^4\)T\(_1\) state which is luminescent; the lifetime of this state has been measured in solution-phase studies to be 600 μs at 77 K and 90 ns at room temperature [21]. The \(^4\)T\(_1\) state is populated by rapid intersystem crossing from the \(^2\)T\(_1\) state which has a lifetime of 450 ps at room temperature and lies only 600 cm\(^{-1}\) above \(^4\)T\(_1\). The class A behaviour exhibited by CuTPP at 266 nm is consistent with the population of the long-lived \(^2\)T\(_1\) intermediate state following one-photon excitation and the subsequent one-photon ionisation of this excited state. This implies that the energy of the \(^4\)T\(_1\) state lies within 4.66 eV of the ionisation threshold which, for an IP of ≈6.5 eV, gives an estimated excitation energy of ≈1.8 eV in the gas phase. This is consistent with the measured solution-phase excitation energy of 1.7 eV [21]. Unlike CuTPP, CoTPP does not luminesce; population of the tripultiplet manifold is followed by radiationless decay to the ground state in <35 ps [22]. This rapid decay is mediated by a low-lying (π, d) charge transfer state. The class B photodissociation behaviour of CoTPP is thus analogous with that of NiTPP, discussed above. Finally, we come to VOTPP which displays competing class A and class B processes. There appears to be little known about the photophysics of VOTPP apart from the fact that it is luminescent. The present observations suggest that the excitation energy of the \(^2\)T\(_1\) state lies within 4.66 eV of the ionisation threshold, while the \(^4\)T\(_1\) state is more than 4.66 eV below the ionisation threshold. Thus, at low laser intensities the probability of intersystem crossing from the \(^2\)T\(_1\) to the \(^4\)T\(_1\) state exceeds that of absorption of a second photon and neutral photodissociation predominates. At high laser intensities, up-pumping from the \(^2\)T\(_1\) state to the ionisation continuum is able to compete with intersystem crossing to the \(^4\)T\(_1\) state and molecular ionisation is observed in parallel with neutral photodissociation.

The observation of exclusively class A behaviour at 193 nm is what would be expected if photoionisation of the metalloTPPs at this wavelength were a single photon process. However, we have no direct evidence for one-photon ionisation. On the contrary, our power dependence measurements for CuTPP suggest a (1+1) photon process. If 193 nm photoionisation is, in fact, a two-photon process, the observed behaviour suggests that the one-photon excited state is long-lived. The one-photon excitation energy is expected to be close to the ionisation threshold and it seems likely, therefore, that the intermediate state would be a long-lived Rydberg state. Recent investigations of the dynamics of Rydberg
states of aromatic molecules, such as benzene and phenanthrene, have shown that high energy states close to the ionisation threshold can indeed have lifetimes of several hundreds of nanoseconds [23], although lower-lying states (4000–10000 cm⁻¹ below the ionisation threshold) exhibit sub-picosecond radiationless decay [24].

5. Conclusion

The metallotetraphenylporphyrins constitute a group of molecules which exhibits both class A and class B photochemical behaviour on multiphoton excitation. Class A behaviour, i.e. ionisation followed by fragmentation, is observed if one-photon excitation populates a long-lived (nanosecond or supra-nanosecond) excited electronic state which is high enough in energy to be single-photon ionised. Class B behaviour, i.e. dissociation followed by ionisation, is observed if one-photon excitation is followed by rapid (picosecond or sub-picosecond) non-radiative relaxation to an electronic state which is too low in energy to be single-photon ionised. If single-photon ionisation and relaxation of the one-photon excited state occur at comparable rates, as for example in VOTPP following 266 nm excitation, then both class A and class B processes are observed in parallel. Multiphoton dissociation of the neutral metallotetraphenylporphyrin molecule results in cleavage of the metal atom from the macrocycle and thus resembles the metal–ligand photodissociation which occurs in other organometallic compounds. Photodissociation of the metalloTPP radical cation does not, however, induce metal–ligand bond breakage but results in cleavage of phenyl substituents from the macrocycle.

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References