I declare that this thesis has been composed by myself and that the work is entirely my own,

G.R. Laird
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

by

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The history of chromatography is described from Tswett's first experiments at the turn of the century to the sophistication of modern high performance liquid chromatography. The renaissance of liquid chromatography in the late sixties as a result of the development of chromatographic theory is discussed.

The thermodynamics of the chromatographic process are outlined. Brief descriptions of the techniques of adsorption chromatography, liquid/liquid partition chromatography, ion-exchange chromatography, gel permeation chromatography and ion-pair chromatography are given.

The development of theories of peak dispersion is discussed. The "random walk" model of peak dispersion, developed by Giddings, is outlined with particular emphasis on the isolation of three independently contributing terms to the overall peak dispersion.

Studies of the variation in performance of Sherisorb alumina as a function of packing technique and column geometry are reported. The variation in performance with column geometry is related to the infinite diameter effect introduced by Knox and Parcher.

The separation of aromatic sulphonic acids is reported using a novel form of ion-pair chromatography in which the hexadecyltrimethylammonium ion is employed. Ion-pair chromatography was performed using two chromatographic supports: Partisil, an irregular porous silica and SAS, a chemically bonded stationary phase material produced by the Wolfson Liquid Chromatography Unit. Separations of some
commercially important sulphonated food dyes and their intermediates are presented.

A new equation is presented which relates the analysis time required for a separation in terms of the available pressure, the resolution required, the quality of the packing and to the properties of the solute and eluent. This equation is used to quantify the advantages of using very small particles in H.P.L.C. and this is illustrated by an experimental study of the effect of particle diameter on the efficiencies of columns packed with 6, 7.5, 10 and 20 micron Spherisorb alumina.
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CHAPTER 1

Historical Survey

Credit for the discovery of the chromatographic process is generally given to Michael Twsett, a Russian botanist (1). About 1903 he separated some coloured plant pigments by washing them through a column containing calcium carbonate using petroleum ether. He observed that several coloured bands appeared in the column due to the differential migration of different pigments. He named the process chromatography and the resultant separation a chromatogram.

However Twsett's results were largely ignored until the technique was rediscovered in 1931 by Kuhn, Lederer and Winterstein (2). The technique was used throughout the 30's but it was not until 1941 that the first major advance since Twsett was published.

In this now classic paper, Martin and Synge (3) pointed the way to many of the advances which were subsequently made in chromatographic technique. The paper introduced the concept of partition chromatography as opposed to adsorption chromatography which had been used previously. They used silica as an inactive support for an aqueous stationary phase over which they passed a chloroform/butanol eluent in order to separate some protein hydrosates. The faster diffusion into and out of the stationary phase increased the efficiency of the separation to a much greater extent than could be achieved by the countercurrent extraction technique used previously.

By analogy with countercurrent extraction and fractional distillation they developed their well known "theoretical plate"
theory of column efficiency, which although it is now out of favour, still gives some insight into the chromatographic process. They foresaw the advantages of using small particles and high pressures which have only been achieved experimentally in the last decade. They realised also the potential of using a gas as the mobile phase which led to the development of gas chromatography first demonstrated in 1952 (4). In the late 50's and 60's gas chromatography was refined into a sophisticated automated analytical technique with exceptional efficiency and sensitivity.

Parallel to the development of gas chromatography, interest in liquid chromatography was revived by the popularisation of paper and thin layer chromatography. The first report of the use of thin layers of adsorbent on glass plates was by two Russian workers, Izmailov and Shraiber in 1938 (5). In 1951 Kirchner, Miller and Keller (6) used the technique to separate terpenes in essential oils, but the credit for the popularisation of T.L.C. belongs to Stahl (7,8) who realised the importance of standardising the operating conditions. From 1958 to 60 Stahl demonstrated the wide applicability of the technique and by 1962 several hundred papers on the technique had been published.

The beginnings of paper chromatography can be traced to its use in the nineteenth century dye industry where the number of dyes produced could be determined by the number of concentric rings formed when the mother liquor was spotted onto filter paper. However the widespread use of paper chromatography developed from its usefulness as a support for the aqueous phase in the liquid/liquid partition
systems discovered by Martin and Synge.

Paper chromatography and T.L.C. were both faster and more efficient than "classical liquid column chromatography" with separation times as short as 30 minutes with T.L.C. The increased efficiency was due partly to the smaller particles which were used in T.L.C. and partly to the reproducibility of bed preparation. These open column techniques were ideally suited for small samples with nanogram quantities being detectable.

Meanwhile little progress had been made in column chromatography and the technique was still substantially the same as in Tswett's first experiments. The columns, typically 50 to 100 centimeters long and 2 to 10 centimeters in diameter, were packed with large (about 200 microns) porous particles. Typical separation times were of the order of hours or even days, while the separation efficiencies were low and strongly dependent on operator skill.

The renaissance in liquid column chromatography can be traced to the deeper theoretical understanding of the chromatographic process gained from gas chromatography. In particular the work of Giddings should be mentioned (9). Through his chromatographic theories based on the "random walk model" and later the "non-equilibrium theory" he realised that the critical parameter in peak dispersion was the ratio of the particle diameter to the diffusion coefficient of the solute in the mobile phase.

By using this parameter the linear eluent velocity can be "normalised" to take into account changes in the composition of the mobile phase and of the particle diameter. In particular both gas
and liquid eluents can be compared directly. If the plate height is also "normalised" by scaling to the particle diameter then all possible chromatographic packings and eluents may be compared directly by plotting the reduced plate height \( h = \frac{H}{d_p} \) as a function of the reduced eluent velocity \( v = \frac{u.d_p}{D_m} \).

The optimum column efficiency in both gas and liquid chromatography was predicted to occur at reduced velocities in the range 2 - 5. While this was already standard practice in gas chromatography with particle diameters of the order of 200 microns, the particle diameters in liquid chromatography had to be reduced by a factor of approximately 50 before optimum efficiencies could be achieved.

The requirements for high speed, high efficiency separations in liquid chromatography were therefore re-emphasised two decades after Martin and Synge's original statement "Thus the smallest H.E.T.P. should be obtainable by using very small particles and a high pressure difference across the length of the column."

In 1960 Hamilton (10) published a paper on ion-exchange chromatography in which he used small particles in order to reduce peak dispersion while amino acid analysis by ion-exchange had been developed by Spackman, Stein and Moore. (11) However these developments were slow to spread to other branches of liquid chromatography.

In the late sixties, liquid chromatographic performance was substantially improved by the introduction of pellicular packing materials. The idea of coating an impervious core with a thin layer of porous material in order to improve the mass transfer properties
had been first suggested by Golay (12). Forerunners in this field were Halasz (13) and Horvath (14) in ion-exchange and Kirkland (15,16) in liquid/liquid partition chromatography.

Kirkland's original "controlled surface porosity" beads were later improved by the use of a 1 - 2 micron layer of submicron silica microspheres as the surface coating. This material was marketed under the name of "Zipax" and was the best packing available in the early 70's. Zipax generated about twice as many theoretical plates as totally porous silicas of comparable particle diameter but suffered because of its low sample capacity. It was realised that the difference in performance between pellicular and totally porous materials would lessen as the particle size was reduced and this would obviate the need for pellicular supports but difficulties in producing and packing materials of less than 20 microns in diameter delayed their introduction until 1972. At this time efficiencies of the order to 20 effective plates per second were obtained by Kirkland and Majors by slurry packing 5 micron particles (18,19).

In Britain the introduction by Harwell of the Spherisorb range of spherical porous particles (particularly the alumina) was an important stepping stone towards higher chromatographic efficiency. 20 and 10 micron Spherisorb alumina could be dry packed in short "infinite diameter" columns to yield efficiencies of the order of 2500 to 4000 theoretical plates in ten centimeters. (20,21). The concept of the "infinite diameter column" introduced by Knox and Parcher (22),
is of paramount importance in modern high performance liquid chromatography and is the subject of chapter five.

Because of the short column lengths used for Spherisorb packings the pressure requirements were very low, often less than 500 pounds per square inch. This emphasised the misunderstanding by many early workers that high pressures would be necessary if the packing of microspheres was undertaken. The theoretical optimum particle size in liquid chromatography is dependant on the peak resolution required, on the analysis time and on the pressure available, but for most separations 3 - 5 micron particles should produce optimum performance. Dry packing could not be used for particles with diameters below 10 microns and therefore slurry packing methods had to be developed. This is discussed in detail in chapter seven.

At the time of writing, the use of 5 micron particles is becoming standard practice and the efficiencies which are being obtained are close to the optimum which can be expected from theory. The emphasis in H.P.L.C. will therefore swing back to developments in hardware, particularly the detection systems.

In the last decade we have seen a complete transformation in liquid chromatography in terms of both speed and efficiency such that L.C now compares favourably with G.C. in all respects except solute detection where both the sensitivity and "universality" of detectors require further improvement.
CHAPTER 2

Thermodynamics of liquid chromatography

2.1 Resolution in chromatography

The resolution of two solutes in chromatography requires that the zone centres are separated by a distance comparable with the width of the zones.

In elution chromatography the resolution, $R_s$, is defined as:

$$R_s = \frac{t_{R2} - t_{R1}}{\frac{1}{2}(w_2 + w_1)}$$

where $t_R$ is the retention time of the solute maxima, $w$ is the base width of the eluted peak in units of time and the subscripts 1 and 2 refer to two closely eluted peaks. For adequate resolution $R_s$ must have a value greater than unity.

The achievement of separation is dependent firstly on the differential migration of zone centres which is controlled by the distribution coefficients of the solutes between the mobile and stationary phases. That is the separation of zone centres is controlled by thermodynamic considerations.

The disengagement of zones is achieved by limiting the dispersion of the solutes. Zone dispersion in analytical chromatographic separations is governed by kinetic considerations and is treated in detail in chapter 3.

The attainment of resolution is therefore a combination of both kinetic and thermodynamic factors. This distinction can be expressed mathematically using equation 2.1 if the assumption is made that for
two closely spaced peaks, \( w_2 \) is approximately equal to \( w_1 \).

By mathematical manipulation equation 2.1 can then be rearranged to give:

\[
Rs = \frac{1}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{K'_2 - \frac{1}{2}}{1 + K'_2} \cdot N^2
\]

where \( K'_2 \) is the capacity factor of the second peak, \( \alpha = K'_2 / K'_1 \) is termed the selectivity and \( N \) is the number of theoretical plates in the column. The first two terms are purely thermodynamic while the number of theoretical plates is normally controlled only by kinetic considerations.

2.2 Theory of solute retention in liquid chromatography

Retention in H.P.L.C. is usually characterised by the solute capacity factor defined as:

\[
K' = \frac{\text{amount of solute in the stationary phase}}{\text{amount of solute in the mobile phase}}
\]

\[
= \frac{a_s}{a_m}
\]

The capacity factor is simply related to the equilibrium partition coefficient of the solute, \( K \), by the ratio of the "volumes" of the mobile and stationary phases, ie.

\[
K' = \frac{c_s \cdot V_s}{c_m \cdot V_m} = K \cdot \frac{V_s}{V_m}
\]

In adsorption chromatography, \( V_s \) is the volume of a monolayer of mobile phase molecules. (see section 2.3).
The relative rate of migration of the solute down a column is given by:

\[ R = \frac{\text{Velocity of solute zone}}{\text{Velocity of solvent}} \]

R can be simply related to the capacity factor by the following argument. Since the solute molecules only move down the column when they are in the mobile phase, R is equal to the fraction of time spent by an average molecule in the mobile phase. For a large number of molecules this is equal to the fraction of solute molecules in the mobile phase at equilibrium, i.e.

\[ R = \frac{a_m}{a_m + a_s} \]

By dividing numerator and denominator by \( a_m \) we obtain the important relationship,

\[ R = \frac{1}{1 + K'} \]

It should be noted that R is ideally equal to the RF value in laminar chromatographic techniques, though a correction factor is often necessary to take into account the "thinning out" of the solvent cross section at the solvent front.

For elution chromatography,

\[ R = \frac{u_R}{u_o} = \frac{L.t_o}{t_{R'\cdot L}} = \frac{t_o}{t_R} \]
where \( u_0 \) is the linear solvent velocity, \( u_R \) is the solute velocity, 
\( L \) is the column length, \( t_R \) is the time taken to elute the solute peak maxima and \( t_o \) is the corresponding time for an unretained peak.

From equation 2.4,

\[
K' = \frac{1}{R} - 1
\]

\[
= \frac{t_R - t_o}{t_o}
\]

2.5

\( K' \) can therefore be conveniently measured from the elution chromatogram.

The retention volume is simply related to the elution time by the equation:

\[
V_R = F \cdot t_R
\]

where \( V_R \) is the retention volume and \( F \) is the eluent flow rate. Hence,

\[
K' = \frac{(V_R - V_o) / F}{V_o / F}
\]

\[
= \frac{V_R - V_o}{V_o}
\]

\( V_o \), the elution volume of an unretained peak is identical to \( V_m \), the volume of mobile phase in the column, ie.

\[
V_R = V_m + K' \cdot V_m
\]

Finally using equation 2.3 we have:

\[
V_R = V_m + K \cdot V_S
\]

2.6
which is the fundamental retention equation for any chromatographic process.

One of the consequences of the technology developed for H.P.L.C. has been the unification of all branches of liquid chromatography. Thus adsorption, liquid/liquid partition, ion-exchange and gel permeation chromatography can all be used in a modern liquid chromatograph by simply changing the column and mobile phase. A brief description of these techniques is given below.

2.3 Adsorption Chromatography

The theoretical understanding of adsorption chromatography is more advanced than in any other branch of liquid chromatography. This understanding is largely due to the work of Snyder (23) who has also tabulated large amounts of experimental data.

The adsorbents primarily used in liquid chromatography are silica and alumina with the occasional use of magnesia being reported.

The structure of silica is now fairly well understood and because of its inertness to even the most labile solutes it is usually the first choice for an adsorbent. Commercial silica gels are manufactured by the acid gelation of sodium silicate. The surface area of the silica can be altered in manufacture by changing the pH or by the later application of heat and high pressure water vapour. Typical surface areas range from 50 to 400 m$^2$g$^{-1}$.

There is almost universal agreement that the active site for the adsorption of unsaturated or polar compounds is the surface hydroxyl group. A schematic representation of the silica surface is given in
figure 2.1.

On heating silica gel to 150 - 200 degrees centigrade all the adsorbed water is removed. Heating above this temperature results in the elimination of water at reactive groups. Above 400 degrees adjacent surfaces condense resulting in the loss of surface area. In order to condition the silica surface for adsorption chromatography 1 - 2 % w/w of adsorbed water is required to cover any very active sites which are present. If these sites are permitted to interact with the adsorbing solute molecule then the partition coefficient is not independent of the sample concentration and this leads to excessive peak broadening and tailing.

Chromatographic alumina is available with surface areas of 100 - 200 m$^2$. The active sites on the alumina surface are less well understood than those on silica. Surface hydroxyl groups are present but in contrast to silica, the chromatographic activity of the adsorbent increases with the progressive removal of these groups.

Snyder has suggested that there are three main adsorption sites:

(a) Acidic or electrophilic field sites which interact with solutes possessing regions of high electron density. This is the most common adsorption mechanism encountered.

(b) Basic or nucleophilic sites (probably oxide ions) which are responsible for the preferential adsorption of acids relative to other adsorbents.

(c) Electron acceptor (charge transfer) sites which form complexes with easily polarised aromatic molecules, e.g. naphthalene. The exact
nature of these sites is not yet known.

**Theory of adsorption chromatography**

The following treatment is essentially that of Snyder:

The basic mechanism of retention is a displacement of adsorbed mobile phase molecules by the adsorbing solute molecule, i.e.

\[ X_m + nS_a \underset{\text{adsorption}}{\overset{\text{desorption}}{\rightleftharpoons}} X_a + nS_m \]

where \( X \) and \( S \) are respectively the solute and solvent molecules and the subscripts \( m \) and \( a \) refer to molecules in the mobile and stationary phases respectively. The free energy change for this reaction is simply

\[ \Delta E = E_{xa} + nE_{sm} - E_{xm} - nE_{sa} \]

where \( \Delta E \) is a dimensionless free energy equal to \(-\Delta G / 2.3.R.T.\). This form is suggested by Snyder in order to simplify the determination of an equation to describe retention.

To a first approximation, particularly in weaker solvents where molecular interaction is usually non-specific, the solution energy terms are small in relation to the adsorption energy terms and in any case tend to cancel out. To a reasonable approximation therefore,

\[ \Delta E = E_{xa} - nE_{sa} \]

A further simplification results if the adsorption energy is factorised into separate contributions from the adsorbed molecules and the adsorbent surface.

ie.

\[ \Delta E_{sa} = f(S).f(A) \]
\[ \Delta E_{xa} = f(X).f(A) \]

\( f(A) \) is termed the surface activity function and is given the symbol \( \alpha \).
An adsorbent of standard activity is given an $\alpha$ value of unity. Hence,

$$\Delta E = \alpha f(x) - \alpha f(S)$$

$$= \alpha f(x, S)$$

When $\alpha = 1$, 

$$\Delta E = S^0_x - n S^0_s$$

where $S^0_x$ and $S^0_s$ are the standard adsorption energies of the solute and solvent molecules respectively.

Finally the eluent strength parameter $\varepsilon^0$ is defined as the adsorption energy of the solvent per unit area of a standard activity surface. 

ie.

$$S^0_s = \varepsilon^0 A_s$$

where $A_s$ is the area occupied by an adsorbed solvent molecule.

$n$, the number of solvent molecules displaced by one solute molecule, is equal to $A_x/A_s$. Therefore,

$$\Delta E = \alpha (S^0_x - n S^0_s)$$

$$= \alpha (S^0_x - A_x A_s \varepsilon^0)$$

The distribution coefficient for a solute in equilibrium with an adsorbent surface can be defined as,

$$K_{th} = \frac{N_{xa}}{N_{xm}}$$

where $N_{xa}$ and $N_{xm}$ are the mole fractions of solute in the stationary and mobile phases respectively. In analytical work the number of moles of solvent is very much greater than the moles of solute in both the mobile and adsorbed phases.
ie. \( n_{sa} \gg n_{xa}, \ n_{sm} \gg n_{xm} \)

therefore \( N_{xa} \approx n_{xa}/n_{sa}, \ N_{xm} \approx n_{xm}/n_{sm} \)

the partition coefficient is therefore given by

\[
K = \left( \frac{n_{xa}}{n_{xm}} \right) \cdot \left( \frac{n_{sm}}{n_{sa}} \right)
\]

\[
= K'. \frac{V_{m}}{V_{s}}
\]

Where \( V_{m} \) is the volume of the mobile phase in the column and \( V_{s} \) is the volume of an adsorbed monolayer of mobile phase molecules.

Since \( K \) is related to the free energy of partition by the equation,

\[
\Delta E = \log (K_{th})
\]

we can write an equation for \( K' \) in terms of the energy changes involved in adsorption and the "volumes" of the stationary and mobile phases. ie.

\[
\log (K') = \log \left( \frac{V_{s}}{V_{m}} \right) + \log (K_{th})
\]

\[
= \log \left( \frac{V_{s}}{V_{m}} \right) + \alpha (S_{x}^{o} - A_{x}.E^{o})
\]

Both \( S_{x}^{o} \) and \( A_{x} \) for a sample compound can be estimated by summing contributions from the functional groups comprising the molecule.

ie. \( A_{x} = \sum a_{i} \), and \( S_{x}^{o} = \sum q_{i}^{o} \)

Complications in the estimation of \( A_{x} \) result from two phenomena:

(1) For molecules with alkyl side chains, the chain is likely to be at least partially desorbed even in relatively weak solvents and hence the experimental \( A_{x} \) value may be less than the value calculated by a simple summation of \( a_{i} \).

(2) The adsorption sites on silica are more localised than on alumina and therefore correction terms must be added to the summation before
consistent experimental data can be obtained.

Similar problems arise in the estimation of group adsorption energies. In general when two groups i and j are introduced into a molecule, the adsorption energy is not simply the sum of the group adsorption energies but is modified by one or more of the following considerations.

1. If the two groups interact electronically the adsorption energies may be changed.

2. If the groups are bulky or are closely spaced, then steric interactions may hinder the interaction with the adsorbent surface.

3. In general, when a multifunctional molecule adsorbs onto a surface with discrete adsorption sites, maximum overlap of a group with an adsorbent site will only take place for one group, (generally the strongest adsorbing group). All other groups present will to a greater or lesser extent interact less strongly with the adsorbent sites than they would if they were the only (or strongest) adsorbing group in the molecule.

The decrease in adsorption energy caused by the inability of group i to adsorb locally due to the presence of a strongly adsorbing group k in the molecule is given by:

\[ f(Q_k) \cdot \sum_{i \neq k} Q_i \]

A good estimate of the standard adsorption energy of a multifunctional molecule is given by:

\[ S^0 = \sum Q_i - f(Q_k) \sum_{i \neq k} Q_i \]
The solvent strength parameter $\varepsilon^0$ has been measured for a large number of solvents and a list of these in ascending order is termed an eluotropic series. The precise value for each solvent depends on the nature of the adsorbent used but the general order remains almost unchanged. A few values of some commonly used solvents are given in table 2.1 for an alumina adsorbent.

**Table 2.1**

<table>
<thead>
<tr>
<th>Solvent</th>
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<tbody>
<tr>
<td>n-hexane</td>
<td>0.00</td>
</tr>
<tr>
<td>carbon tetrachloride</td>
<td>0.18</td>
</tr>
<tr>
<td>methylene chloride</td>
<td>0.42</td>
</tr>
<tr>
<td>ethyl acetate</td>
<td>0.58</td>
</tr>
<tr>
<td>acetonitrile</td>
<td>0.65</td>
</tr>
<tr>
<td>methanol</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Optimisation of solute capacity factors may require an eluent with an $\varepsilon^0$ value intermediate between two solvents or alternatively a solvent with a special selectivity for the solute may be required to be present in the eluent. In such cases a blend of two (or more) solvents whose values straddle the required $\varepsilon^0$ value may be necessary to obtain an optimum separation.

The general formula for calculating the $\varepsilon^0$ value of a blend of two solvents A and B such that $\varepsilon^0_B$ is greater than $\varepsilon^0_A$ is given by:
\[ \varepsilon_{AB} = \varepsilon_A + \log \left( \frac{n_b \cdot (\varepsilon_B - \varepsilon_A)}{N_b \cdot 10} + 1 - N_b \right) \]

where \( \varepsilon \) is the adsorbent activity function, \( N_b \) is the mole fraction of B in the mixture and \( n_b \) is the molecular area of solvent B.

Thus by summing contributions of adsorption energies and surface areas from all the groups within a complex molecule and choosing an eluent with an appropriate \( \varepsilon^0 \) value, an estimate of the net energy of adsorption can be made and hence an "expected" capacity factor may be calculated.

In adsorption chromatography, the water content of the adsorbent must be carefully controlled if reproducible capacity factors are to be obtained. Adsorbed water has the effect of reducing the surface area of the adsorbent available for solute adsorption and also decreases the adsorbent activity function (\( \alpha \)). Care must therefore be taken to ensure that the water content of the eluent remains constant and hence an equilibrium set up between the eluent and the adsorbent surface. This is a problem particularly with non-polar eluents where the water solubility is very small (20 - 100 ppm.). The water content of non-polar solvents is most easily controlled by blending the dry solvent with solvent which has been saturated with water by passage through a column of silica containing 30 % w/v of water. Dry solvents should never be used in adsorption chromatography since some water must be adsorbed onto the surface to block the most active adsorption sites. This ensures that a linear adsorption isotherm is obtained by making
the adsorption energies of the solute molecules more uniform.

Adsorption chromatography is most suited for separations of non-polar and moderately polar solutes where very high efficiencies and fast separation times can be achieved using microparticulate packings (18, 22) and low viscosity solvents (e.g. hexane, methylene chloride, acetonitrile).

For separations of polar compounds e.g. carboxylic acids, the eluent polarity must be greatly increased usually by the inclusion of water mixed for example with an alcohol and a non-polar solvent. In these cases the mechanism of retention is generally considered to be no longer adsorption but a partition system between the bulk eluent and a water rich stationary phase formed within the pores of the support by the passage of the eluent.

2.4 Liquid/liquid partition chromatography

Liquid/liquid partition chromatography is defined as a system in which a separation is achieved by passing a mobile phase over a stationary phase held mechanically within the pores of an inert support. The selectivity necessary for a separation can therefore be obtained by varying the composition of either the mobile or stationary phases. Experimentally however the selection of suitable phase pairs is rather limited by the requirement of mutual immiscibility which is necessary in order that a stable chromatographic system can be formed.

The thermodynamic criteria for equilibrium for a solute partitioned between two immiscible phases is that the activity for the solute be equal in the two phases, i.e.
where the subscripts $s$ and $m$ refer to the stationary and mobile phases respectively. The measured partition coefficient, $K$, is given by:

$$K = \frac{c_s}{c_m} = \frac{a_s/y_s}{a_m/y_m} = \frac{y_m}{y_s}$$

where $y_s$ and $y_m$ are the solute activity coefficients in the mobile and stationary phases respectively.

The prediction of activity coefficients is however difficult and unreliable so that the choice of mobile phase / stationary phase pairs for partition chromatography is still largely empirical. Nevertheless, the following three approaches to predicting retention behaviour have been suggested:

(1) For members of a homologous series, the solution activity coefficients can be estimated from the expression -

$$\ln y_i = a + b n_i$$

where $a$ and $b$ are empirical constants and $n$ is the number of carbons in the $i^{th}$ homologue.

The observed partition coefficient is therefore given by:

$$\ln (K_i) = \ln (y_{im}) - \ln (y_{is})$$

$$= a_{im} - a_{is} + (b_{im} - b_{is}) n_i$$

$$= A + B n_i$$
Therefore providing B is sufficiently large in relation to A, partition chromatography is ideally suited to the separation of members of a homologous series. This is borne out in practice where compounds differing only in the carbon number of side chains, while difficult to separate by adsorption chromatography, are relatively easily separated in partition systems.

An empirical approach to the selection of suitable phase pairs for a partition system on the basis that "like dissolves like" can be put on a "semi-quantitative" basis by the use of the Hildebrand solubility parameter, \((\delta)\). Defined as the "energy of vapourisation per millilitre of pure substance", it gives an indication of the polarity of a solvent. \((35)\) For example hexane which is non-polar has a solubility parameter of 7.3 while water has a value of 21.

Further refinement of the idea of a solubility parameter enables it to be split up into four terms, \((\delta_d, \delta_o, \delta_a, \delta_h)\) representing respectively, the dispersion solubility parameter, the orientation or polar solubility parameter, the proton acceptor solubility parameter and finally the proton donor solubility parameter. In order to separate a mixture, a stationary phase with a strong affinity for the solute is chosen. The mobile phase volume is very much greater than that of the stationary phase so that in order to obtain reasonable retention \((1<K<10)\) the solubility of the sample must be considerably less in the mobile phase.

An initial guess is usually made for the mobile phase composition. If retention is too strong then using the tabulated solubility parameters the mobile phase strength is increased until the sample components are
within the optimum range of capacity factors.

Once the magnitude of retention is established, if the resolution of the sample components is unsatisfactory, then the selectivity may be changed by varying the mobile phase composition while keeping the total solubility parameter constant. Good selectivity for two closely related compounds is expected when strong specific interactions between solute and solvent molecules are operative, therefore mobile phase components should be chosen which are as selective as possible. For example if closely related phenols are to be separated then solvents with large proton donating or accepting capability are likely to be most selective.

(3) The use of critical temperature data for a mixture of solute and solvent has been suggested. The critical temperature is defined as that temperature above which the two components exist in a single phase. It is therefore a measure of the mutual solubility of the two compounds, a high value of $T_c$ is indicative of low solubility while a low value indicates good miscibility.

The main use of these tables is to indicate solvents which are likely to be selective in their solubility for two closely related sample components. For example the critical temperature of some glycols in acetonitrile are:

<table>
<thead>
<tr>
<th>sample</th>
<th>$T_c$ in acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethylene glycol</td>
<td>- 13.5</td>
</tr>
<tr>
<td>propylene glycol</td>
<td>0.0</td>
</tr>
<tr>
<td>trimethylene glycol</td>
<td>- 6</td>
</tr>
</tbody>
</table>
Acetonitrile is therefore likely to be a good mobile phase component for the separation of glycols.

**Experimental**

The column packing for partition chromatography should be completely inert and serve only as a support for the partitioning stationary phase. In practice however the mechanism of retention is often a mixture of partition and adsorption. In order that the adsorption effects are as small as possible the surface area of the support should be as small as is practical. For some years the pellicular materials, particularly Zipax\(^R\) were dominant in the field of high efficiency partition chromatography, but now low surface area microparticulate supports are likely to take over.

The stationary phase can be coated onto the support in two ways:

1. The support is slurried in a solution of stationary phase in a volatile solvent. The solvent is then slowly evaporated leaving a uniform coating of the stationary phase on the support. The support is left free flowing and can be easily dry packed.

2. For slurry packed columns the stationary phase is usually added after the column has been packed. This so called "dynamic coating" method is performed by passing a solution of the stationary phase through the column and then changing to a mobile phase which precipitates the stationary phase onto the column.

In order for the retention in partition chromatography to remain constant it is vital that the volume of stationary phase does not change by the passage of the mobile phase. To ensure that no bleeding
of the stationary phase occurs the mobile phase must be pre-saturated with stationary phase by passing it through a pre-column containing the stationary phase deposited on a porous support. (Usually a cheaper material and of sufficiently large particle size so as not to present a significant resistance to the solvent flow). For accurate work it is also important that the column is thermostated.

A relatively recent development in liquid chromatography has been the production of stationary phases which are chemically bonded to the support (usually silica). The mechanism of retention in many of these systems cannot be explained in terms of simple partition into the chemically bonded phase. For example many polar compounds are retained on purely hydrocarbon stationary phases. The explanation of retention in these cases appears to be that one component is extracted from the mobile phase to form a mixed stationary phase eg. acetonitrile can be extracted from a water/acetonitrile mobile phase.

The whole question as to whether chemically bonded phases can be treated as equivalent to bulk liquid phases is still not settled, but it is probably best to regard it as a separate branch of H.P.L.C.

2.5 Ion-exchange chromatography

Ion exchange chromatography is widely used in the field of inorganic and organic analysis for the separation of ionisable compounds. Perhaps its single most important role in the last decade has been its use in the analysis of complex samples of biological origin, eg. nucleic and amino acids.

Until recently most commercial ion-exchange materials were based
on synthetic resins into which ionisable groups had been introduced. The most common resins are made by the co-polymerisation of styrene and divinyl benzene to produce a porous matrix. The extent of cross-linking can be controlled by the relative proportions of styrene and divinyl benzene, (more cross linking being produced by higher concentrations of divinyl benzene). The degree of cross linking, defined as the weight per cent of divinyl benzene in the reaction mixture, is typically 4 to 8 per cent with the most popular types having a nominal 8 per cent.

It should be noted that the pore size of polystyrene resins are quite small and even moderately sized ions can be excluded. For example only about 50 per cent of \( \text{C}_{16} \text{H}_{33} \text{N}^{+}(\text{CH}_3)_3 \) ions can exchange with a typical 5 per cent cross linked cation exchanger.

For the separation of large molecules of biological origin polyacrylamide and carbohydrate polymers are often used because of their more open pore structure. However these gels are easily deformed so their use is limited to very low pressures.

The type of resin is named by the nature of the exchangeable ion (ie. a cation exchange material has an anionic group bonded to the resin) and by the convention of weak and strong acids and bases with regard to the bonded group.

The most common ion-exchange groups are:

- Strong cation-exchanger: \( R - \text{SO}_3^- \text{H}^+ \)
- Strong anion-exchanger: \( R' - \text{NH}_3^+ \text{OH}^- \)
- Weak cation-exchanger: \( R - \text{COOH} \)
- Weak anion-exchanger: \( R - \text{NH}_2 \)
Polystyrene resins can be easily sulphonated using concentrated sulphuric acid or chloro sulphuric acid to produce a cation exchanger with approximately one sulphonic acid moiety for each phenyl group of the polymer. Since the basic unit is then \( \text{C}_8\text{H}_8\text{SO}_3^- \) (M.W. = 184) there will be approximately \( \frac{1000}{184} = 5.5 \) milliequivalents of ion exchange capacity per gm. of resin. This is a typical value for commercial cation and anion exchange resins.

As with all liquid chromatographic techniques the efficiency of a separation in ion-exchange has been limited by the slow mass transfer of molecules within the support. This problem is particularly acute with ion-exchange resins, not only because of the narrowness of the pores but also because of the slow rate of diffusion of ions within the matrix due to the highly ordered structure of the water within the pores.

The use of pellicular materials where a layer of resin is physically adsorbed onto a solid core support has been advocated in order to obviate the mass transfer problem but with limited success, while greatly reducing the ion exchange capacity.

The use of 10 micron ion-exchange resins is now commonplace and reasonable efficiencies are attainable, however for high pressure, high efficiency chromatography it looks likely that bonded phase silica supports will take over from resins. All four types of ion exchange can be produced on a silica base by silanization and subsequent derivatisation, (24, 25, 26) See section, 2.7.
Theory of ion exchange

For an ion-exchanger where only ion-exchange processes are responsible for solute retention, we have an equilibrium between the solute ions in solution and solute ions bound to the resin or bonded phase. Thus for a cation exchanger with an eluent containing the counter ion Na\(^+\) we have the equilibrium:

\[
X_m^+ + Na_a^+ \rightleftharpoons Na_m^+ + X_a^+
\]

The equilibrium constant is therefore given by

\[
Keq = \frac{(Na_m^+)(X_v^+)}{(Na_a^+)(X_m^+)}
\]

As with all chromatographic techniques the measure of solute retention is the capacity factor \(K'\) which is proportional to the distribution coefficient of \(X^+\) between the mobile and stationary phases, i.e.

\[
K' \propto \frac{(X_v^+)}{(X_m^+)}
\]

\[
\propto \frac{Keq (Na_a^+)}{(Na_m^+)}
\]

Since the concentration of adsorbed sodium ions is approximately constant,

\[
K' \approx \frac{\text{const.}}{(Na_m^+)}
\]
The capacity factor in pure ion-exchange chromatography should therefore be inversely proportional to the ionic strength of the counter ion in the eluent. This is easily verified experimentally. Gradient elution in ion-exchange chromatography also frequently makes use of increasing ionic strength in order to separate complex mixtures.

The effects of pH are more complex. Strong ion-exchangers are fully ionised at all workable pHs while the ion exchange capacity of weak ion-exchangers can be controlled by varying the pH of the eluent. That is the ionisation of a weak cation exchanger can be suppressed by lowering the pH (usually in the range 2 - 5) while the ionisation of a weak anion exchanger can be decreased by increasing the pH.

For all ion-exchangers the retention of the solute ion can be varied by changing the pH around about the pK\textsubscript{a} value of the solute, i.e. by varying the degree of ionisation of the solute.

With a weak base run on a cation exchanger with a slightly alkaline eluent:

$$\text{BH}^+ + \text{H}_2\text{O} \rightleftharpoons \text{B}^- + \text{H}_3\text{O}^+$$

i.e. $K_a = \frac{(\text{B}^-)(\text{H}_3\text{O}^+)}{(\text{BH}^+)}$

The capacity factor is given by:

$$K' = \text{const.} \frac{(\text{BH}^+)}{(\text{BH}^+_m + \text{B}_m)}$$

(where pure ion-exchange is operating the base is only adsorbed when it is protonated).
Dividing numerator and denominator by \((BH^+)^n_m\) we get

\[
K' = \frac{\text{const.} \frac{(BH^+)}{(BH^+)^n_m}}{1 + \frac{(B_m)}{(BH^+)^n_m}}
\]

\[
= \frac{K''}{1 + \frac{K}{(H_2O^+)}
\]

where \(K''\) is the capacity factor of the base when it is fully ionised.

From this equation it can be seen that the retention of a weak base is increased by a lowering of the pH while at a pH equal to the \(pK_a\) value of the solute, the capacity factor will be 0.5 times \(K''\).

Similar arguments apply for a weak anion run on an anion-exchanger where a lowering of the pH in the region of the solute \(pK_a\) will reduce retention.

It should be noted that until now solute retention has been assumed to be wholly due to an ion-exchange mechanism. In fact both ion-exchange resins and the new bonded phase ion-exchangers are inherently lipophilic in nature and hence the retention of organic ions is often the result of ion-exchange and other mechanisms of retention operating in parallel.

2.6 Gel permeation chromatography

Gel permeation chromatography (G.P.C.) is a separation technique which is based solely on differences in the molecular sizes of the sample components. A G.P.C. packing consists of a highly porous gel whose pore size is chosen such that the access of sample molecules to the pore volume is restricted by their molecular size. The volume of
stationary phase available to each sample molecule is therefore dependant on its molecular size. For a molecule sufficiently small to penetrate into all the pores, the retention volume is equal to the total void volume of the column, $V_T$, while a large molecule, unable to enter any of the pores, is eluted with a retention volume, $V_M$, equal to the interstitial volume of the column. Between $V_T$ and $V_M$ there is a region of selective permeation where molecules can be separated according to their molecular size. This can be represented schematically by figure 2.2. From figure 2.2 it can be seen that for this particular support, compounds C and D can be separated but in order to separate A and B a support with a larger pore diameter is required, while a smaller pore diameter is required to separate E and F.

Using this example it is obvious that a column suitable for adequately resolving C and D which are of comparable molecular size is unable to separate compounds A to F which differ widely in molecular size. With reference to figure 2.2 it can be seen that an increase in the gradient of the graph of molecular size against elution volume would increase the range of molecular sizes subject to selective permeation, but would decrease the resolution of C and D. Thus a column suitable for resolving for instance individual oligimers would require a low gradient and hence a packing with very uniform pore diameters while a column for producing a molecular weight distribution of a polymer would require a steep gradient and hence a wider distribution of pore sizes. Of course high resolution and the separation of a wide range of molecular sizes can be achieved by using a number of columns connected.
in series.

Materials

As with ion-exchange chromatography, the modern trend is towards high pressure work where the packings must be resistant to deformation. Lightly cross linked resins are unsuitable for modern liquid chromatography although highly cross linked polystyrenes can be used with pressures of the order of a thousand pounds per square inch. These highly cross linked polystyrenes are only suitable for separating relatively small molecules (of the order of a few thousand molecular weight units) but they are useful for the separation of relatively small polar molecules where adsorption effects may well be present if a silica support was used. For high pressure work with large molecules (and consequently large pore sizes) porous glasses or silica are the preferred supports. Microparticulate silicas of 5 to 10 microns are now appearing commercially and are likely to markedly increase the speed and resolution of G.P.C..(27) Both silica and porous glass supports are available with fractionation ranges from under a thousand up to several million molecular weight units.

The interpretation of a G.P.C. chromatogram is in many ways simpler than in any other branch of modern liquid chromatography since the separation is based only on differences in molecular size. The simplest method of interpretation of a chromatogram is by reference to a calibration graph such as figure 2.2 which can be constructed by running calibration standards of known molecular weights and plotting molecular weight against the experimental retention volume. Where no calibration standards are available, the calculation of molecular size or more
accurately the molecular radius of gyration is required. However this is outwith the scope of this chapter.

**Theory of G.P.C.**

The capacity factor in G.P.C. is given by:

\[ K' = \frac{V_R - V_M}{V_M} \]

where \( V_M \) is the interstitial volume

\[ V_R = V_M + K'.V_M \]  \quad \text{(2.16)}

but \( K' = \frac{\text{amount of solute in the stationary phase}}{\text{amount of solute in the mobile phase}} \)

\[ = \frac{(\text{concentration of solute in the stationary phase}) \cdot (V_{fs})}{(\text{concentration of solute in the mobile phase}) \cdot (V_M)} \]

\[ = \frac{K \cdot V_{fs}}{V_M} \]  \quad \text{(2.17)}

where \( K \) is the partition coefficient between the stationary and mobile phases and \( V_{fs} \) is the pore volume accessible to the solute molecules.

Substituting for \( K' \), \( V_M \) in equation 2.16,

\[ V_R = V_M + K \cdot V_{fs} \]

Putting this into the classical form for elution chromatography,

\[ V_R = V_M + K^O \cdot V_s \]

where \( K^O = \frac{K \cdot V_{fs}}{V_s} \)
$K^o$ in pure Gel Permeation Chromatography can obviously range from zero for a totally excluded solute to a maximum value of one for a solute which can permeate into all the pores.

2.7 Chemically bonded stationary phases

Chemically bonded stationary phases are formed by the bonding of a suitable organic moiety to the surface of a porous support. Silica has been used almost exclusively, but alumina has also been successfully employed. (24,28) The surface of silica can be modified by reacting the silanol groups, which are slightly acidic in nature, with a variety of organic reagents. The following three methods are the most commonly used.

(1) Esterification of the surface silanol group by reaction with an alcohol (29), ie.

$$\equiv Si - OH + ROH \rightarrow \equiv Si - OR$$

Unfortunately bonded phases produced in this way are not suitable for use with eluents containing water or polar organic solvents because of the instability of the ether linkage to hydrolysis.

(2) Conversion of the surface silanol group to the chloride by reaction with thionyl chloride followed by treatment with a grignard or organolithium compound to give a direct bond to the silica surface.

ie. $$\equiv SiOH + SOCl_2 \rightarrow \equiv Si - Cl$$

$$\equiv SiCl + RMgCl \rightarrow \equiv Si - R$$

(3) Direct reaction of the surface silanol group with an organo-chlorosilane,

ie. $$\equiv Si - OH + R_2Si - Cl \rightarrow \equiv Si - O - SiR_3$$
Methods (2) and (3) both produce stable bonds which are resistant to hydrolysis within a range of pH from 3 to 10. The most commonly used stationary phase is octadecyl (ODS) now available from almost all HPLC suppliers. It is easily prepared by refluxing silica with octadecyldimethylchlorosilane in a dry organic solvent. Other phases produced include octyl, cyano, amino and pyridyl while the production of ion-exchange materials is achieved by introducing NR₃, NH₂, COOH and SO₃ groups into the bonded phase (24,25,26,31,32,33). The manufacture of stationary phases specially tailored to meet a difficult separation problem is also possible. In this context the production of optically active stationary phases to separate D and L isomers has been suggested.

**Efficiency**

The effect on column efficiency of any stationary phase is determined by two factors, the mass transfer within the phase and the uniformity of the retention mechanism.

Some of the early bonded phases were based on pellicular materials (eg. DuPonts Permaphases) and hence to obtain adequate "loading" the stationary phase was polymerised. This resulted in relatively low efficiencies due to the restricted diffusion within the polymeric stationary phase. In recent years the use of relatively high surface area totally porous microparticulate silicas as the base for chemically bonded materials permits high loading of stationary phase by the bonding of a monolayer of organic moieties to the silica surface. The efficiency of these bonded phase microparticulate materials properly...
produced is as good as and in some cases better than that of the original support material. (24)

In achieving high efficiencies the uniformity of the adsorbing surface is of paramount importance since any non-linearity of the adsorption isotherm has a deleterious effect on column efficiency and in the worst cases on peak symmetry. With most bonded phase materials there is still a significant proportion of the silica surface left unreacted. Although this can be partially remedied by further reaction of the residual silanol groups with a very reactive silanising reagent such as hexamethyldisilazane, interaction of the solute with the silica surface can still cause problems. Thus moderately polar organic molecules can be run on bonded phase materials with excellent efficiency, but efficiency is generally decreased somewhat for very polar molecules. For basic compounds interactions with the residual silanol groups can cause problems of peak asymmetry and often these will not run satisfactorily on bonded phases.

An important advantage which bonded phases have over liquid/liquid partition chromatography is that the mobile phase can be similar or widely dissimilar to the stationary phase in terms of polarity since there is no problem with stationary phase bleeding. Thus all bonded phases may be used with both organic and aqueous eluent systems. The stability of the bonded phase also allows the use of solvent gradients to separate complex mixtures.

By far the greatest impact of bonded phase materials has been their use in reversed phase chromatography, i.e. with a polar eluent, usually
a mixture of water and a polar organic solvent "modifier" such as an alcohol, acetone, acetonitrile, dioxan or THF. In this mode of operation compounds are separated according to their polarity. Aromatic hydrocarbons are strongly retained by the organic bonded phase and hence it is necessary to use an eluent rich in organic modifier in order to elute them while with fairly polar organic solutes an eluent containing predominantly water is required to achieve suitable retention. (36) As discussed in the next section strong organic acids and bases can be retained on bonded phase materials using an ion-pairing technique.

For weak acids and bases the degree of retention is very different for the ionised and unionised species. Consider a weak acid with an ionisation constant, $K_a$ given by -

$$K_a = \frac{(A^-)_m (H^+)_m}{(AH)_m}$$

At low pH the partition coefficient will be given by

$$K_u = \frac{(AH)_s}{(AH)_m}$$

while at high pH

$$K_i = \frac{(A^-)_s}{(A^-)_m}$$

where the subscripts $u$ and $i$ refer to the unionised and ionised states. At intermediate pHs in the vicinity of the solute $pK_a$ value, the molecule will be partly ionised and therefore the partition coefficient will be given by
\[ K = \frac{(A^-)_s + (AH)_s}{(A^-)_m + (AH)_m} \]

\[ = \frac{(A^-)_s + (AH)_s}{(AH)_m (1 + \frac{K_a}{[H^+]})} \]

ie. \[(1 + \frac{K_a}{[H^+]}) \cdot K = \frac{(A^-)_s}{(AH)_m} + K_u \]

\[ = K_i \cdot \left( \frac{K_a}{[H^+]} \right) + K_u \]

Finally since the capacity factors \( K', K'_i \) and \( K'_u \) are proportional to \( K, K_i \) and \( K_u \) the expression becomes,

\[ K' = \frac{K'_i \cdot \frac{K_a}{[H^+]}}{1 + \frac{K_a}{[H^+]}} + K'_u \]

Feeding in typical values for \( K_a = 10^{-4}, K'_i = 1 \) and \( K'_u = 4 \), the variation of solute capacity factor with pH can be plotted as shown in figure 2.3.

Hence varying the pH of the eluent within 1 unit of the pK_a value of the solute has a dramatic effect on its capacity factor and therefore provides a convenient and predictable means of optimising separations.

For complex mixtures of unionised molecules, weak acids/bases and strong acids/bases, separations can often be achieved by varying the pH and the concentrations of ion-pairing agents. For these reasons there is little doubt that reversed phase chemically bonded phase chromatography
is the most useful and versatile HPLC technique in use at present.

2.8 **Ion-pair chromatography**

It has long been established that ionic compounds can be extracted into organic solvents by complexing them with an ion of opposite charge which contains a lipophilic moiety. (37) For example anions may be extracted by pairing with quaternary ammonium ions (38) while cations may be extracted with organic acids (39).

Ion pairs consisting of two lipophilic ions are easily extracted into organic solvents of moderate solvating ability (eg. chloroform or methylene chloride) but even inorganic ions such as perchlorate (40) may be extracted into an organic phase containing a strong solvating compound such as a higher alcohol.

As with any partitioning system, ion-pairing can be made the basis of a chromatographic separation. This was first developed in about 1973 and has rapidly become an important H.P.L.C. technique. (41,42) Both normal and reversed phase ion-pair chromatography are now widely used.

*Normal phase ion-pair chromatography*  In this technique the support of diamataceous earth, cellulose or silica (43) is coated with an aqueous buffer containing the counter ion. Coating is usually applied using the dynamic coating method and for accurate work the eluent must be saturated with the stationary phase by passage through a pre-column. Thermostating of the column is often required for precise work.

The mobile phase is usually a moderately polar solvent (eg. chloroform) which may have its solvating properties enhanced by the inclusion of a
higher alcohol or diminished by "diluting" with for example hexane.

Since batch extraction procedures require that the paired ion is partitioned strongly into the organic phase the extraction conditions must be greatly moderated in order to achieve sufficient retention on the aqueous stationary phase. Also a high concentration of the pairing ion must be present in the stationary phase to provide adequate buffering of the system, otherwise the partitioning of the solute will be dependent on its concentration and hence lead to assymetric peaks. It follows therefore that the eluent must have a very much weaker ion-pair solvating ability for chromatography than for batch extraction.

The following equilibria apply in ion pair partition systems:

$$X^-_{\text{aq}} + P^+_{\text{aq}} \rightleftharpoons XP_{\text{org}}$$

The extraction coefficient is therefore given by,

$$E_{XP} = \frac{(XP)_{\text{org}}}{(X^-)_{\text{aq}} \cdot (P^+)_{\text{aq}}}$$

while the distribution coefficient of the solute between the mobile and stationary phases is given by,

$$D_{XP} = \frac{(XP)_{\text{org}}}{(X^-)_{\text{aq}}}$$

In normal phase chromatography the capacity factor will be given by

$$K' = \frac{V_s}{V_m \cdot D_{XP}}$$
\[
V_s = \frac{V_m \cdot E_{XP} \cdot (P^+)_{aq}}{m_{aq}}
\]

ie. the capacity factor is inversely proportional to the concentration of the pairing ion in the stationary phase.

The degree of retention can therefore be controlled by the concentration of the pairing ion in the stationary phase while the selectivity of the system (and of course also the retention) can be varied by changing the nature of the pairing ion and the composition of the mobile phase.

Normal phase ion-pair chromatography has been used to enhance the detection of solutes by pairing them with U.V. absorbing ions with high extinction coefficients. In this technique it is vital that the concentration of the pairing ion is insignificant in the organic phase in the absence of the solute. Suitable ions are picrate \((44)\) for the chromatography of cations and dimethylprotriptylenium for anions. \((41)\)

Sample preparation usually involves shaking an aqueous solution of the solute and the pairing ion with the mobile phase to extract the solute into the organic phase which is then injected onto the column.

Normal phase ion-pair chromatography has proved to be a versatile and selective technique for the separation of ionisable compounds. In addition existing data from batch extraction can be used to predict selectivity for a column separation.
Reversed phase ion-pair chromatography

The breakthrough in this technique came with the development of chemically bonded phases. The use of ODS has already been reported (45), but there is no reason why any bonded phase may not be used to modify the selectivity of a separation. The eluent usually consists of a mixture of methanol and water containing a suitable pairing ion. Aliphatic quaternary ammonium ions are used to pair with anions while aliphatic sulphonic acids are the most convenient pairing ion for cations. If U.V. detection is employed then of course the pairing ion which is present in the mobile phase must not adsorb at the monitoring wavelength.

Increased retention is favoured by increasing the concentration of the pairing ion in the mobile phase or by increasing its lipophicity, i.e. by increasing the length of the alkyl chain of the pairing ion. Since the pairing ion is in the mobile phase it is much easier to change the concentration or the nature of the pairing ion than in normal phase ion-pair partition chromatography. Also since the system is operating as a reverse phase 'adsorption' system, unionised compounds may also be retained. A separation of an ionised and an unionised solute may therefore be obtained by setting the conditions such that the unionised solute is conveniently retained \(1 < K' < 5\) with the ionised solute almost unretained. A suitable pairing ion is then added to the eluent and its effect on the retention of the ionised solute observed. The concentration or the lipophicity of the pairing ion can then be optimised to obtain the best separation of the two compounds.
A special case of reverse phase ion-pair chromatography is the use of "liquid ion-exchangers" such as trioctylamine (46). These liquid ion-exchangers even when ionised are virtually insoluble in water and can therefore form a stable stationary phase in a liquid/liquid partition system. With the passage of an acidic eluent such as perchloric acid, the stationary phase becomes ionised. The mechanism of solute retention is therefore displacement of the perchlorate ion by the solute ion in a manner analogous to ion-exchange chromatography.
CHAPTER 3

Kinetics of Chromatography

As stated in chapter 2 the achievement of zone separation in chromatography is dependent firstly on the separation of zone centres and secondly on limiting the dispersion of the zones such that they disengage. The separation of zone centres is a result of the differences between the thermodynamic partition coefficients of the two solutes, while the limiting of zone dispersion is achieved by controlling the kinetics of solute transfer within the column. This is determined by the structure of the support particles, the mechanism of retention, diffusion in the mobile and stationary phases and on the packing structure of the particles within a column.

The dispersion of a solute zone in chromatography is measured in terms of the number of "theoretical plates" the column contains. The height equivalent to a theoretical plate (H) originated from Martin and Synge who developed a theory of zone dispersion by analogy with fractional distillation and countercurrent extraction. Although this theory is no longer used by the modern chromatographer, the original terminology still survives. The most satisfactory definition of the plate height is now the rate of increase of zone variance with the distance migrated. i.e.

\[ H = \frac{d\sigma^2}{dz} \]

For a uniform column in liquid chromatography, the plate height is constant throughout the length of the column and therefore the plate
height is simply given by:

\[ H = \frac{\sigma^2}{L} \]  

3.1

The number of theoretical plates, \( N \), is given by:

\[ N = \frac{L}{H} = \frac{L^2}{\sigma_L^2} \]

In elution chromatography the concentration of solute is monitored as it elutes from the column as a function of the eluent volume. The elution volume for a solute with a capacity factor \( K' \) is given by

\[ V_R = V_m (1 + K') \]

\[ = L (1 + K') A \]

where \( A \) is the cross section of the column occupied by the mobile phase.

The standard deviation of the solute in volume units as it elutes from the column is given by:

\[ \sigma_v = \sigma_L (1 + K') A \]

therefore,

\[ N = \frac{V_R^2}{\sigma_v^2} \]

In modern liquid chromatography the concentration of the solute in the eluent is continuously monitored as a function of time on a chart recorder. Therefore, since the elution time is proportional to the elution volume for a constant flow rate,

\[ N = \frac{t_R^2}{\sigma_t^2} \]
The two most common approaches to the measurement of N are:

(a) Tangents are drawn to the points of inflection of the peak and the distance between their intersections with the baseline measured. This distance \((w)\) is equal to \(4.8\) for a Gaussian peak and hence the number of theoretical plates is given by:

\[
N = \frac{t_R^2}{w^2} \cdot 16
\]

(b) The width of the peak at half the peak height is measured. From the geometry of a Gaussian peak, the number of theoretical plates is given by:

\[
N = \frac{t_R^2}{w_{1/2}^2} \cdot 5.54
\]

3.1 Theoretical plate Model

This theory, which was first suggested by Martin and Synge (3), postulates that a chromatographic column can be divided up into a series of slices which they termed 'theoretical plates' by analogy with fractional distillation and countercurrent extraction. It is assumed that averaged over each plate the solute is in equilibrium between the stationary and mobile phases and that zone dispersion results from the flow of eluent to the next plate and the subsequent re-equilibration of the solute. The mathematical treatment of this theory results in a concentration profile described by the binomial coefficients. In the limit of a large number of steps this reduces to the familiar Gaussian curve.
There are very obvious deficiencies in the model. Firstly, longitudinal diffusion of the solute between plates is ignored. This is unacceptable in gas chromatography though until recently it was an acceptable assumption for liquid chromatography.

Secondly, it does not provide equations involving the diffusion coefficients of the solute in the mobile and stationary phases or the particle diameter of the packing. It cannot therefore predict the optimum conditions for high efficiency.

Thirdly, the physical division of the column into discrete plates is untenable, although this objection may be partly overcome by replacing the discontinuous flow of a plate volume by an infinitesimally small volume. This treatment (48) leads to a Poisson distribution for the solute concentration profile which again approximates to a Gaussian peak when a large enough number of equilibrium steps are taken.

In the limit of a large number of steps the concentration profile of the solute is given by:

$$c = c_0 \exp\left(-\frac{(v - v_R)^2}{2 \cdot \delta v^2}\right)$$

where $c$ is the concentration of the solute and $c_0$ is the concentration at the peak maximum.

3.2 Rate theories/van Deemter equation

One of the first attempts to develop a rate theory for chromatography was made by Wilson in 1940 (47). The treatment given by him considered the roles of diffusion and non-equilibrium. "The width of a band may increase because of diffusion or because the leading edge migrates too
rapidly on account of a low rate of adsorption or because the tailing edge of the band migrates too slowly on account of a low rate of desorption." He realised from this that either very fast or very slow flow rates would cause excessive zone dispersion and hence introduced the idea of optimum operating conditions.

In 1948 H.C. Thomas (49) made one of the most outstanding contributions in which he described the formulae from which the adsorption and desorption rates could be obtained from the experimental solute concentration curves of chromatography.

In 1952 Lapidus & Amundsen (50) developed a general equation for zone spreading which resulted from the finite rate of mass transfer and from longitudinal diffusion. Glueckauf in a series of papers first related chromatographic dispersion to particle size and diffusion coefficients. (51) This theory was extended by Klinkenburg and Sjenitzer and more specifically by van Deemter, Zuiderweg and Klinkenburg. (52). The formulating of the "van Deemter equation" was largely responsible for the rapid development of efficient gas chromatographic techniques.

Van Deemter equation

The van Deemter equation showed that the efficiency of a chromatographic column could be related to eddy diffusion, molecular diffusion and to mass transfer within the mobile and stationary phases. These three mechanisms were treated independently.

Eddy diffusion

Because of the complex structure of a chromatographic column solute
molecules traverse a tortuous route as they move down the column. Individual molecules will either move ahead of the zone centre or lag behind depending on the path they take. In the van Deember equation the variance in distance travelled by solute molecules in traversing a single layer of particles of diameter $dp$ is given as:

$$\sigma_{dp}^2 = 2.\lambda.dp^2$$

where $\lambda$ is the packing characterisation factor. For a column with $L/dp$ particle layers the total variance is given by:

$$\sigma_L^2 = 2.\lambda.Ldp$$

ie. the contribution to the overall plate height is given by:

$$H_{E.D.} = \frac{\sigma^2}{L} = 2.\lambda.dp$$

### Molecular diffusion in the mobile phase

The dispersion of the solute due to molecular diffusion is given by the Einstein equation:

$$\sigma_D^2 = 2.D_{m}t_m$$

where $t_m$ is the average time spent by a solute molecule in the mobile phase and $D_m$ is the solute diffusion coefficient in the mobile phase. $t_m$ is the same for all solutes and is simply given by:

$$t_m = t_o = \frac{L}{u}$$

where $u$ is the fluid velocity.

Substituting for $t_m$ in equation 3.6, and taking

$$H_D = \frac{\sigma_D^2}{L}$$

we have

$$H_D = 2.D_m/u.$$
For a packed column the dispersion is slightly reduced by the inclusion of a factor to take into account the complex flow pattern in a packed column.

\[ H_D = 2D_m \frac{\gamma_m}{u} \]

where \( \gamma_m \) is termed the tortuosity factor.

**Resistance to mass transfer in the stationary phase**

Inevitably in chromatography there is a failure to attain equilibrium of the solute concentration between the mobile and stationary phases due to the flow of the mobile phase. The molecules either fail to go into the stationary phase and then travel slightly ahead of the band or are slow in getting into the moving phase and so lag behind.

If \( F_m \) and \( F_s \) are the fractional plate volumes occupied by the mobile and stationary phases and \( C_s \) is the mass transfer coefficient, the material balance per unit cross sectional area is given by:

\[
\frac{dc_m}{dt} = -F_m u \frac{dc_m}{dz} + C_s (c_s - K c_m')
\]

\( c_s \) is out of equilibrium value of \( c_s' \), \( K = c_s' / c_m' \)

and in terms of the stationary phase by:

\[
\frac{dc_s}{dt} = C_s (K c_m' - c_s)
\]

Solution of these differential equations leads to the result that:

\[
H_M = \frac{8}{\pi^2} \frac{K' d_s^2 u}{(1 + K')^2 D_s}
\]

where \( d_s \) is the thickness of the film of stationary phase and \( D_s \)
is the diffusion coefficient of the solute in the stationary phase.

If these three mechanisms of solute dispersion are operating in parallel and independently of each other then the total variance is given by:

\[ \sigma^2_{TOT.} = \sigma^2_D + \sigma^2_{E.D.} + \sigma^2_M \]

ie.

\[ H_{TOT.} = H_D + H_{E.D.} + H_M \]

\[ = 2 \lambda \cdot \sigma_p + 2 \frac{\gamma}{m} \cdot \frac{D_m}{\mu} + 8 \frac{K'}{d_s^2} \frac{1}{\pi^2 (1 + K')^2} \]

Keulemans and Kwantes (53) gave the simple and now familiar form of the van Deemter equation as:

\[ H = A + \frac{B}{u} + C \cdot u \]

An important consequence of the form to the van Deemter equation is that the minimum plate height can be obtained by simple differentiation of the equation. The result is that a minimum plate height of

\[ H_{min.} = A + 2 \cdot (B \cdot C)^{0.5} \]

should be obtained with a fluid velocity of

\[ u_{min} = (B/C)^{0.5} \]

3.3 Random Walk Model

This theory was developed by Giddings and is treated in detail in his book "Dynamics of Chromatography" (9). This section essentially follows his treatment. The random walk model of chromatographic dispersion has the advantage over the more rigorous non-equilibrium theory that it can be easily visualised while the mathematics of the
treatment are fairly straightforward.

The random walk model uses the laws of statistics to treat the random movements of solute molecules within a chromatographic column. Two well known laws of statistics are used:

(1) If a large number of objects (molecules) started at exactly the same location, then after a series of random movements the concentration of these objects will develop a Gaussian distribution profile symmetrically about the starting point. The measure of this spread is the standard deviation $\delta$ and in the random walk model the value of $\delta$ may be calculated by the equation:

$$\delta = l.n^{3/2}$$  \hspace{1cm}  (3.11)

where $l$ is the distance a molecule is displaced with respect to the origin in a single random event and $n$ is the number of such displacements. Since the number of displacements is proportional to the time the molecule spends in the column and hence to the length of the column we can immediately deduce that:

$$\delta \propto L^{3/2}$$

i.e. $$\delta^2 = H.L$$

where $H$ is a constant, defined as the variance per unit length for a uniform column.

(2) The second result of statistics already given in this chapter is that for a number of independent random processes each of which alone produces a variance of $\delta_i^2$, the total variance of all the processes is given by:

$$\delta_{TOT}^2 = \sum \delta_i^2$$
The path traversed by a molecule as it migrates down a column is determined by random forces. These are ordinary molecular diffusion, the flow pattern effect called eddy diffusion and sorption/desorption kinetics. Giddings uses the laws of statistics to derive the total plate height of a column from these three phenomena.

**Longitudinal Molecular diffusion**

The molecular dispersion resulting from ordinary diffusion is given by the well known Einstein equation -

\[ \sigma_D^2 = 2D \cdot t_D \]

where \( \sigma_D^2 \) is the variance of the solute concentration profile and \( t_D \) is the total time the molecules are subject to diffusion. In liquid chromatography account has to be taken of diffusion in both the mobile and stationary phases.

For any solute the total time spent in the mobile phase is simply -

\[ t_o = \frac{L}{u} \]

where \( u \) is the eluent velocity. The variance due to diffusion of a solute in the mobile phase is therefore given by:

\[ \sigma^2 = 2D_m \cdot t_o = 2D_m \cdot \frac{L}{u} \]

where \( D_m \) is the solute diffusion coefficient in the mobile phase.

ie.

\[ H = 2D_m \cdot \frac{L}{u} \]

For a packed column the plate height due to longitudinal diffusion is reduced because of the complex structure of the packing. This structure has the effect of increasing the effective distance a molecule has to diffuse and may also restrict diffusion due to constrictions within the pore structure of the packing.
Therefore the plate height contribution for longitudinal diffusion in the mobile phase is given by,

$$H = 2 \gamma_m \cdot D_m / u$$

where $\gamma_m$ is termed the obstructive factor for molecular diffusion.

For a solute, the capacity factor is given by,

$$K' = a_s / a_m$$

Since the amount of solute present at a given time in a particular phase is proportional to the time spent by an average molecule in that phase we can write,

$$K' = t_s / t_o$$

where $t_s$ is the time the average molecule spends in the stationary phase. Dispersion in the stationary phase due to diffusion is therefore given by,

$$\sigma^2 = 2 D_s \cdot t_s = 2 D_s \cdot K' \cdot t_o$$

Therefore,

$$H = 2 D_s \cdot K' / u$$

Finally the plate height is again reduced by a factor $\gamma_s$ which represents the fact that diffusion in the stationary phase cannot take place in a direct unobstructed path. The plate height contribution due to diffusion in the stationary phase is therefore given by,

$$H = 2 \gamma_s \cdot D_s \cdot K' / u$$\hspace{1cm} 3.13$$

The relative importance of longitudinal diffusion in the mobile and stationary phases depends on the particular system under consideration. In gas chromatography longitudinal diffusion in the stationary phase can be safely ignored but in some L.C. techniques, particularly with well
The total plate height contribution due to longitudinal diffusion is therefore the sum of the stationary and mobile phase contributions and is given by:

\[ H = \frac{2(\gamma_m D_m + \gamma_s D_s K')}{u} \]

### Adsorption/desorption Kinetics

Complex adsorption-desorption kinetics have been treated successfully only by using the generalised non-equilibrium theory of chromatography, however the random walk model does indicate the nature and importance of the factors involved. In the simplest case the molecules adsorb and desorb in accordance with the laws of first order kinetics i.e. the rate of the process is proportional to the number of molecules able to react. For such a process we can define a rate constant or transition rate \( k \) which denotes the fraction of the available molecules reacting in one second.

The mean desorption time \( t_d \) of adsorbed molecules is then simply equal to the reciprocal of the transition rate constant \( k_d \). Similarly for adsorption, \( t_a = \frac{1}{k_a} \). Time constants rather than rate constants are used to develop the random walk theory but it should be remembered that the process is essentially controlled by rate constants with their usual dependence on temperature and bonding energy.

As the solute molecules migrate down the column alternate adsorption/desorption steps occur at random due to the erratic transfer of activating energy in and out of molecular bonds. In terms of the random
walk model, an adsorption step is a step backward with respect to the zone centre while a desorption step is a step forward. The total number of such steps is equal to the sum of the number of adsorption and desorption steps which is just twice the number of adsorption steps since each adsorption is followed by a desorption.

On average a molecule will remain a time $t_a$ in the mobile phase before adsorption and during this time will be travelling at an average velocity $u$. The distance covered is therefore $u \cdot t_a$. The number of adsorption steps performed in traversing a length $L$ is therefore $L/u \cdot t_a$ and the total number of steps ($n$) is given by:

$$n = 2 \cdot L/u \cdot t_a$$

The net length of each step is equal to the step length before re-adsorption minus the distance travelled by the zone centre in time $t_a$, i.e. the length of the step is given by:

$$l = u \cdot t_a - R \cdot u \cdot t_a$$

$$= (1 - R) \cdot u \cdot t_a$$

where $R$ is the relative migration rate of the solute with respect to the eluent velocity.

From equation 3.11, the variance for the adsorption/desorption process is given by:

$$\sigma^2 = l^2 \cdot n$$

$$= (1 - R)^2 \cdot u^2 \cdot t_a^2 \cdot 2L/u \cdot t_a$$

$$= 2(1 - R)^2 \cdot L \cdot u \cdot t_a$$

The plate height $H$ is therefore given by:
$$H = 2(1 - R)^2 \cdot u \cdot t_a$$  \hspace{1cm} (3.15)

It is generally more convenient to express $H$ in terms of the mean desorption time $t_d$. Using the fact that the capacity factor $K'$ is given by:

$$K' = \frac{t_d}{t_a}$$

and replacing $(1 - R)$ by $K'/(1 + K')$ in equation 3.15 we arrive at

$$H = 2u \cdot t_d \cdot K'/(1 + K')^2$$ \hspace{1cm} (3.16)

Although this equation for the plate height contribution from adsorption/desorption kinetics has been derived from the simple random walk treatment it is rigorously correct even for heterogeneous surfaces. It can be seen that the plate height contribution from adsorption/desorption kinetics is proportional to the eluent velocity $u$ and to the mean desorption time $t_d$, while the variation of $H$ with capacity factor is also important with a maximum value occurring at a capacity factor of one and a minimum value for solutes which are either very lightly or very strongly retained.

**Diffusion controlled kinetics**

Using the random walk model, diffusion in the stationary phase can be treated in exactly the same manner as adsorption/desorption kinetics to obtain equation 3.16. The major difference is in the nature of $t_d$. In kinetics controlled by diffusion within the stationary phase, $t_d$ can be identified with the time taken by a molecule to diffuse from the interior of a pool of stationary phase.

$t_d$ is therefore approximately the average time $t_D$ required to diffuse a distance $d$, ie.
where $d$ is related to the depth of the pool of stationary phase and $D_s$ is the diffusion coefficient of the solute in the stationary phase. When this expression for $t_d$ is substituted in equation 3.16 we find that the contribution to the plate height by diffusion within the stationary phase is given by:

$$H = u_d^2 K'/D_s (1 + K')^2$$  

3.17

We can see from this equation that the plate height contribution will be reduced by decreasing the depth of the stationary phase and by choosing liquids for the stationary phase which are not too viscous (ie. $D_s$ is not too small). Equation 3.17 has the correct form for diffusion controlled kinetics in the stationary phase but the more exact non-equilibrium theory shows that the equation should be written as,

$$H = q u_d^2 K'/D_s (1 + K')^2$$  

3.18

where the configuration factor $q$ is introduced to take account of the precise shape of the stationary phase pools. For a uniform film, $q$ has the value 2/3.

**Diffusion in the mobile phase**

Diffusion in the mobile phase is more complicated than in the stationary phase since it occurs in the very complex network of interconnected channels and void spaces. In addition the flow velocity varies over wide limits in moving across from one flow channel to the next. The fact that flow velocity varies in the direction of flow as well as perpendicular to it leads to the coupling of mobile phase
diffusion to eddy diffusion. This is discussed in the next section.

The random walk theory tells us that zone spreading occurs wherever high and low velocities exist side by side with molecules exchanging between them. In the mobile phase there is a wide variation of flow velocities within the column. Giddings has classified these into five types of velocity inequality.

1. Transchannel - velocity inequalities within the intersitial flow channels.
2. Transparticle - velocity inequalities between stagnant mobile phase and the surrounding moving mobile phase.
3. Short range interchannel.
4. Long range interchannel.
5. Transcolumn - different velocities exist near the walls and at the centre of a column. Therefore for equilibrium to exist there must be solute transfer on a column wide scale. The advent of "infinite diameter" columns in liquid chromatography as discussed in chapter 5 has meant that diffusion on this scale cannot occur within the time spent by the average solute molecule in the mobile phase. Hence for infinite diameter columns, the wall region and the column centre are essentially two separate columns as far as the solute is concerned.

If we assume that the diffusion distance needed to transfer a molecule from one velocity extreme to another is proportional to the
particle diameter, ie.

\[ d = \omega_\infty dp \]

where the constant \( \omega_\infty \) can be estimated for each of the five categories of flow inequality, then we can calculate a certain exchange time \( t_e \) using the Einstein equation:

\[ t_e = \frac{d^2}{2D_m} = \frac{\omega_\infty^2 dp^2}{2D_m} \quad 3.19 \]

where \( D_m \) is the solute diffusion coefficient in the mobile phase.

The length of the step, \( l \), is the net distance gained or lost with respect to the mean because of the time spent in one of the velocity extremes. This distance is given by \( \Delta u \cdot t_e \) where \( \Delta u \) is the deviation from the mean velocity. In general \( \Delta u \) will be some fraction of the mean velocity,

ie. \[ \Delta u = \omega_\beta u \]

\( \omega_\beta \) can again be estimated for all five velocity inequalities.

The step length is therefore given by:

\[ l = \omega_\beta u \cdot t_e \]

\( u \cdot t_e \) is the distance which a molecule is carried without severe velocity change and is given the symbol \( S \), ie.

\[ l = \omega_\beta S \]

The number of random steps taken is simply the number of paths of length \( S \) comprising the total migration distance. ie. \( n = L/S \).

Hence using the equations \( \beta^2 = l^2n \) and \( H = \beta^2/L \), the plate height contribution is given by:-
Giddings has estimated values of $w_1$ for each of the five velocity inequalities. These are listed in Table 3.1.

**Flow pattern and Eddy diffusion**

Eluent in a packed column flows so as to avoid obstacles along its path and follow the path of least resistance. The velocity may fluctuate over wide limits - in the narrow channels it is very slow while it is fastest in the open unrestricted ones. In addition the flow falls off to zero at the surface of a particle due to viscous drag.

The flow in a chromatography column is generally laminar in nature, i.e., the flow pattern is independent of the magnitude on the flow and of the nature of the mobile phase. The pattern of flow is dependent only on the structure of the support and hence is termed a "structural property".

A molecule in a fast stream path may move ahead of the zone centre until it is transferred out either by diffusion or by flow. In the classical theory of eddy diffusion only the flow mechanism is dealt with.

As with diffusion controlled kinetics the plate height contribution
is given by:

\[ H = \omega_p^2 S \]

However for eddy diffusion S is independent of the velocity of the mobile phase. Since a change in the particle diameter will not alter the structure of the packing it is obvious that the path length will increase in proportion to it, ie.

\[ S = \omega_\lambda dp \]

where \( \omega_\lambda \) is a structural parameter, near to unity in most cases.

Combining these two equations the resulting plate height is given by

\[ H = \omega_p^2 \omega_\lambda dp \]

For a given velocity inequality \( i \) this may be written:

\[ H = 2 \lambda_i dp \]

where

\[ \lambda_i = \omega_p^2 \omega_\lambda / 2 \]

Giddings has estimated \( \lambda_i \) for each of the five velocity inequalities discussed above and these are listed in table 3.1.

<table>
<thead>
<tr>
<th>Type of velocity inequality</th>
<th>( \omega_i )</th>
<th>( \lambda_i )</th>
<th>( \nu_\frac{1}{2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transchannel</td>
<td>0.01</td>
<td>0.5</td>
<td>( 10^2 )</td>
</tr>
<tr>
<td>Transparticle</td>
<td>0.1</td>
<td>( 10^4 )</td>
<td>( 2 \times 10^5 )</td>
</tr>
<tr>
<td>Short-range interchannel</td>
<td>0.5</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>Long-range interchannel</td>
<td>2</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Transcolumn</td>
<td>0.02-10</td>
<td>0.4-200</td>
<td>40</td>
</tr>
</tbody>
</table>
The Coupling Theory of Eddy Diffusion

In the previous two sections separate contributions to the overall plate height of a column were calculated for diffusion within the mobile phase and for eddy diffusion on the basis that each was independent of the other. In fact this is not so. As a molecule is migrating down a column its residence in a given stream path may be cut short either by molecular diffusion or eddy diffusion. The effect of this is to shorten the step length \( l \) while increasing the number of transitions \( n \) between velocity extremes. Since the plate height is proportional to \( l^2 \) but only to the first power of \( n \) the effect of the coupling of flow and diffusion is to reduce the plate height. The assumption therefore that \( H \) is the sum of all contributing mechanisms is invalid.

The down stream distance \( S \) which a molecule is carried in one of the velocity inequalities without interruption was shown to be the critical parameter for the spreading process. This quantity may be written as

\[
S = \frac{L}{n}
\]

since \( n \) such lengths make up the total migration distance \( L \). The total number of exchanges between velocity extremes \( n \) is equal to \( n_f + n_D \) where the subscripts \( f \) and \( D \) refer to transitions due to the flow and diffusion mechanisms respectively.

The path length \( S \) in the mobile phase will therefore be given by:

\[
S = \frac{L}{(n_f + n_D)}
\]

As before the plate height is given by:
\[ H = \omega_f^2 S \]
\[ = \frac{\omega_f^2 L}{(n_f + n_d)} \]
\[ = \frac{1}{\frac{n_f}{\omega_f^2 L} + \frac{n_d}{\omega_f^2 L}} \tag{3.25} \]

For eddy diffusion:
\[ n_f = \frac{L}{S_f} \]
Substituting for \( S_f \) from equation 3.22
\[ n_f = \frac{L}{\omega_x dp} \]

While for diffusion:
\[ n_d = \frac{L}{S_D} = \frac{L}{t_e u} \]
Substituting for \( t_e \) from equation 3.19
\[ n_d = \frac{2D_m L}{\omega_i^2 dp^2 u} \]

Finally substituting these values for \( n_f \) and \( n_d \) in equation 3.25 and simplifying we obtain:
\[ H = \frac{1}{\frac{1/2 \lambda_i dp}{1/\lambda_i dp} + \frac{D_m/\omega_i dp^2 u}{1/D_m/\omega_i dp^2 u}} \tag{3.26} \]

As we can see from equations 3.21 and 3.23 this can be simplified to give:
\[ H = \frac{1}{1/H_f + 1/H_D} \tag{3.27} \]

An equation of this type can be derived for all five types of velocity inequality resulting in a total plate height contribution from the coupling of eddy diffusion and molecular diffusion of -
This form of the coupling equation is at wide variance with the classical form of the equation:-

\[ H = H_f + H_D \]

This can be seen diagramatically by figure 3.1. Instead of the total plate height being the sum of the two contributing mechanisms, it has a value less than either \( H_f \) or \( H_D \) and in fact approaches the smaller of the two terms from below as the difference between them increases. At high eluent velocities the flow mechanism of exchange dominates. It is useful to know the transition velocity \( u_1 \) at which the plate height term reaches half of its limiting value. This provides an indication of the relative importance of \( H_f \) and \( H_D \) as a function of \( u \) since above \( u_1 \), \( H_f \) will be the dominant term and visa versa.

From equations 3.21 and 3.22 the ratio \( H_D/H_f \) is given by

\[
\frac{H_D}{H_f} = \frac{\omega_i \cdot dp \cdot u}{2 \cdot \lambda_i \cdot D_m}
\]

When \( H_D/H_f \) is equal to unity,

\[
u = u_{1/2} = 2 \cdot \frac{\lambda_i \cdot D_m}{\omega_i \cdot dp} \]

Giddings has defined the dimensionless quantity,

\[
v = u \cdot \frac{dp}{D_m}
\]

where \( v \) is termed the reduced eluent velocity. This quantity is of fundamental importance to the unification of the theories of dispersion.
of gas and liquid chromatography. If two systems have the same values of \( u \cdot \frac{dp}{D_m} \) the relative role played by diffusion and flow is the same in each even if the actual flow differs, as it may in gas and liquid chromatography, by ten thousand fold.

Equation 3.29 can now be rewritten in terms of reduced velocities as:

\[
v_{i_2} = 2 \cdot \frac{\lambda_1}{\omega_1}
\]

ie. the reduced transition velocity \( (v_{i_2}) \) is a dimensionless quantity dependent only on the structural factors \( \lambda_1 \) and \( \omega_1 \) and is independent of diffusivity and particle diameter. Thus \( v_{i_2} \) is a characteristic of a given column and is independent of the nature of the eluent. Estimated values of \( v_{i_2} \) for each of the five velocity inequalities are given in table 3.1.

If the plate height is also scaled to the particle diameter, ie. the reduced plate height \( (h) \) is given by \( h = H/d_p \), then equation 3.28 can be rewritten in terms of reduced parameters to yield:

\[
h = \sum_i \frac{1}{1/2 \cdot \lambda_i + 1/\omega_i \cdot v_i}
\]

**Summary of the random walk treatment**

By summing the plate height contributions due to longitudinal diffusion (equation 3.14), coupling of diffusion and eddy diffusion in the mobile phase (equation 3.28) and mass transfer in the stationary phase (equations 3.16 and 3.18), the overall plate height of a column can be expressed as:

\[
H = \frac{B}{u} + \sum_i \frac{1}{1/A_i' + 1/C_i' \cdot u} + C^* \cdot u
\]
By introducing reduced parameters this becomes,
\[ h = \frac{B}{v} + \sum_{i} \frac{1}{1/2 \lambda_i + 1/\omega_i v} + C.v \]  \hspace{1cm} 3.32

Unfortunately experimental agreement with the coupling term of equation 3.32 is poor but data can be reasonably well expressed by the modified expression:
\[ h = \frac{B}{v} + \frac{1}{A'' + 1/C''v^{n'}} + C.v \]

where \( n' \) is approximately 0.3. (54).

Over two orders of magnitude this may be further simplified to
\[ h = \frac{B}{v} + A.v^{0.33} + C.v \]  \hspace{1cm} 3.33

The exponent 0.33 has been evaluated experimentally from the plate height curves of unretained solutes. (17).

Knox has used equation 3.33 in a series of papers to evaluate the qualities of packing methods and materials and has determined values of A and C for various H.P.L.C. supports. (34,24,55). The parameter A indicates the "goodness of packing" of a column, values around unity imply that the material has been well packed while high values indicate that improved performance will be obtained if attention is paid to the packing procedure.

The parameter C is determined by the efficiency of mass transfer between the flowing and static regions of the column. Values range from near zero for the pellicular material Zipax (17) to 0.1 for materials with poor mass transfer characteristics.

3.4 Non-equilibrium theory

The generalised non-equilibrium theory is the most powerful yet
derived for the mathematical analysis of peak dispersion in chromatography. It is based on the treatment of gross processes of mass transfer while the random walk model is based on the statistical analysis of a large number of individual events. In contrast to the random walk model it is rigorously correct and is limited only by the closeness with which the idealised models used approximate the reality of a chromatographic column.

Unfortunately the mathematics of the treatment quickly become very complex particularly for the "generalised theory of non-equilibrium" which Giddings has used to derive mass transfer terms for different configurations of pore structure and stationary phase. The concept of non-equilibrium is however straightforward and it is useful just to outline the theory as it is applied to the simple situation of adsorption/desorption kinetics.

As a chromatographic zone is migrating down a column it is inevitable that the concentration profile in the stationary phase will deviate slightly from the equilibrium value due to the finite rate of equilibration. On the leading edge of the zone the concentration of the solute in the mobile phase will be slightly greater than the equilibrium value while on the trailing edge the concentration will be slightly less. True equilibrium will only occur at the maximum of the concentration profile. This situation is shown diagramatically in figure 3.2. An immediate consequence of this analysis of peak dispersion is that the relative solute velocity $R$ will be greater than the equilibrium value on the leaking edge of the peak and less than the equilibrium value on the trailing edge. In other words the zone continually broadens as it
migrates down the column. The distance $sz$ between the maximum of solute concentration in the mobile and stationary phases is a measure of the degree of non-equilibrium. The aim of increasing the efficiency of mass transfer is to reduce this distance and hence operate the column as close to equilibrium as possible.

The non-equilibrium theory is based on solute concentration and changes in concentration which result from the flow and kinetic processes of chromatography. Equilibrium departure $\xi_s$ and $\xi_m$ (referring to the stationary and mobile phase respectively) are introduced by the equations:

$$c_m = c^*_m (1 - \xi_m)$$
$$c_s = c^*_s (1 - \xi_s)$$

where $c_m$ and $c_s$ are the concentrations in the mobile and stationary phases respectively and $c^*_m$ and $c^*_s$ are their equilibrium values. It is clear that the equilibrium departure terms are not independent of each other since a deficit in one phase corresponds to an excess in the other. Giddings has derived the relationship that

$$\xi_s = -\xi_m R/(1 - R)$$

The rate of mass transfer for the mobile phase is denoted by:

$$s_m = (dc_m/dt)_{mass \ transfer}$$

which for adsorption/desorption kinetics is simply the rate of desorption minus the rate of adsorption, i.e.

$$s_m = k_d c_s - k_a c_m$$

where $k_d$ and $k_a$ are the apparent first order rate constants.
fact that at equilibrium,

$$s_m = 0 = k_d c_s^* - k_a c_m^*$$

and equation 3.34 the following expression can be derived for $s_m$ relating it to the equilibrium departure term $\xi_m$. 

$$s_m = - c_m^* (k_d + k_a) \xi_m$$  \hspace{1cm} 3.36

The net rate of increase of concentration in the mobile phase is given by:

$$\frac{\partial c_m}{\partial t} = (\frac{\partial c_m}{\partial t})_{\text{mass transfer}} + (\frac{\partial c_m}{\partial t})_{\text{flow}}$$  \hspace{1cm} 3.37

The first term is simply $s_m$ while the second term represents the gain of solute by flow.

$(\frac{\partial c_m}{\partial t})_{\text{flow}}$ is simply the velocity of the eluent ($u$) times the concentration gradient $\frac{\partial c_m}{\partial z}$. ie.

$$\frac{\partial c_m}{\partial t} = s_m - u \frac{\partial c_m}{\partial z}$$  \hspace{1cm} 3.38

where the minus sign denotes the fact that the concentration gradient is negative in the leading edge of the zone. In order to make this equation solvable the approximation has to be introduced that the concentrations are always very close to their equilibrium values. Since $c_m^*$ and $c_m$ are nearly equal, $\frac{\partial c_m}{\partial t}$ and $\frac{\partial c_m}{\partial z}$ may be replaced by $\frac{\partial c_m^*}{\partial t}$ and $\frac{\partial c_m^*}{\partial z}$ respectively. Using these substitutions in equation 3.38 and rearranging we find that:

$$s_m = \frac{\partial c_m^*}{\partial t} + u \frac{\partial c_m^*}{\partial z}$$  \hspace{1cm} 3.39

Since $c_m^*$ is a constant fraction $R$ of the total concentration $c$ in a given region no increase in $c_m^*$ can be achieved by transfer between the
two phases. The increase in $c_m$ is therefore due only to flow and is 
given by:

$$\frac{dc_m^*}{dt} = R \frac{dc}{dt} = R \left( \frac{dc_m}{dt} \right)_{\text{flow}}$$

$$= -R u \frac{dc_m}{dz}$$

Hence equation 3.39 can be simplified to:

$$s_m = (1 - R) u \frac{dc_m}{dz}$$

We now have two independent equations for $s_m$ (eqns 3.36 and 3.41) 
and therefore $s_m$ can be eliminated to give an expression for the 
equilibrium departure term $\xi_m$, ie.

$$\xi_m = -\frac{(1 - R) u}{k_d + k_a} \frac{1}{c_m} \frac{dc_m^*}{dz}$$

The relationship of the equilibrium departure term ($\xi_m$) to the 
spreading of the solute zone is arrived at by analogy with diffusion by 
the following argument. The solute flux $J$ is defined as equal to the 
concentration of solute, $c_m$, which will soon be carried through a cross 
section times the volume swept out by the flow in unit time. For a 
cross sectional area of unity this is just the concentration in the 
mobile phase times the eluent velocity, ie.

$$J = c_m u$$

$$= c_m^* u + c_m \xi_m u$$

$$= J^* + \Delta J$$

where $J^*$ is the solute flux at equilibrium. Zone spreading originates 
in the $\Delta J$ term. Using equation 3.42 it can be seen that,
\[ \Delta J = \frac{(1 - R).u^2}{k_d + k_a} \cdot \frac{\partial c^*}{\partial z} \]

\[ = \frac{(1 - R).u^2}{k_d + k_a} \cdot \frac{R \cdot \partial c}{\partial z} \quad 3.43 \]

since \( \frac{\partial c^*}{\partial z} = R \cdot \frac{\partial c}{\partial z} \).

The analogy to diffusion appears here. During diffusion along a concentration gradient, the flux of material is always \( -D \cdot \frac{\partial c}{\partial z} \)

where \( D \) is the diffusion coefficient. We can therefore write:

\[ \Delta J = -D \cdot \frac{\partial c}{\partial z} \quad 3.44 \]

where \( D \) is the apparent diffusion coefficient responsible for zone spreading. The value of \( D \) is seen to be:

\[ D = \frac{R(1 - R).u^2}{k_a + k_d} \quad 3.45 \]

By analogy with diffusion the plate height is given by:

\[ H = \frac{\chi^2}{L} = 2D \frac{t_R}{L} \quad 3.46 \]

where \( t_R \) is the time spent by the average solute molecule in the column.

\[ t_R = \frac{t_c}{R} = \frac{L}{u.A} \]

Substituting for \( t_R \) and \( D \) is equation 3.46 and cancelling we arrive at:

\[ H = \frac{2(1 - R).u}{k_d + k_a} \quad 3.47 \]

Since \( K' = \frac{k_a}{k_d}, k_a + k_d = k_d(1 + K') \)

\[ = \frac{(1 + K')}{t_d} \]

where \( t_d \) is the mean desorption time. Substituting for \( k_d + k_a \) in
equation 3.47 and replacing \((1 - R)\) by \(K'(1 + K')\) we arrive at:--

\[
H = \frac{2K'.u.t_d}{(1 + K')^2}
\]

This equation is identical to that derived from the random walk treatment.  (equation 3.16)

Generalising the arguments of this section for any non-equilibrium situation and effective diffusion coefficient \(D\) can be defined as:--

\[
D = \frac{-c_m^*\epsilon_m'u}{\partial c/\partial z}
\]

Using the fact that \(H = 2.D/R.u\), the plate height is given by:--

\[
H = \frac{2c_m^*\epsilon_m}{R.\partial c/\partial z} = \frac{-2.(R.c).\epsilon_m}{R.\partial c/\partial z}
\]

On cancelling the \(R\)'s and writing \(\partial c/c \partial z\) as \(\partial \ln c/\partial z\) we have:--

\[
H = \frac{-2.\epsilon_m}{\partial \ln c/\partial z}
\]

It should be noted that the equilibrium departure term \(\epsilon_m\) is always of such a nature as to introduce a velocity proportional term.

The plate height is now formulated in terms of the equilibrium departure term \(\epsilon_m\) which Giddings has evaluated for individual retention mechanisms and phase configurations.
3.5 Conclusion

An understanding of the mechanisms underlying chromatographic dispersion together with the development of chromatographic hardware has been responsible for the renaissance of liquid chromatography in the last decade. This understanding is largely due to the work of Giddings through his theories of "random walk" and "non-equilibrium".

An understanding of the roles of diffusivity, particle size and packing structure in the overall plate height of a chromatographic column has led to a rapid approach towards the optimum operating conditions in liquid chromatography. This is the subject of chapter 7.

The use of reduced parameters for plate height and eluent velocity has provided a useful diagnostic tool for the evaluation of chromatographic data.
The essential features of a liquid chromatograph are indicated in figure 4.1. The main components are:

1. The solvent reservoir.
2. The high pressure pump.
3. The analytical column.
4. The detector.
5. The electronics to amplify and record the detector signal.

In addition the following two features may be necessary:

6. When a single head reciprocating pump is used a device to damp out pressure pulsations is required.
7. If liquid/liquid partition chromatography is employed then a pre-column is necessary to ensure that the mobile phase is saturated with stationary phase.

4.1 Eluent pumping systems

The pumps used were:

(a) A single head reciprocating pump (Orlita Geissen Model DMP 1515).

Figure 4.2 is a schematic of the pump. The working piston acts directly on hydraulic oil which in turn acts on the solvent to be pumped via a flexible metal diaphragm. During the pressure stoke the diaphragm is forced outwards displacing a metered volume of eluent through the output valves. The volume of eluent introduced in the input part of the cycle is regulated by means of an adjustable stop on the regulating piston. By adjusting the stop it is possible to vary the extent to
which the regulating piston follows the working piston and hence the volume of eluent introduced into the pump.

The output from a pump of this type is a series of pressure/flow pulses. These pulses must be damped out if the detector is to work satisfactorily at high sensitivities. At low flow rates the pressure gauge provides sufficient damping but at higher flow rates a pressure dampening device must be fitted. We found that a 50 x 0.5 cm. tube filled with air and appendaged to the solvent delivery line was an adequate pressure damper for our requirements. The maximum flow rate produced by an Orlita pump is 10 mls. per minute and it can work against pressures up to 3000 p.s.i.

(b) A Haskel pressure intensifier pump. (Haskel Engineering and Supply Co.). Figure 4.3 is a schematic of this pump. The pump consists of a large surface area piston pressurised by compressed air which is connected to a small surface area piston acting on the liquid to be pumped. The pressure of the liquid is then the air pressure multiplied by the ratio of the surface areas of the pistons. Two such pumps were used in the slurry packing of columns, one with a maximum pressure of 3000 p.s.i. and the other with a maximum pressure of 4500 p.s.i. The volume of mobile phase delivered by one stroke of the hydraulic piston is 70 mls. Refill is automatically operated by air pressure when the stroke is completed and is sufficiently fast so as not to present any difficulties.

Other types of pump commercially available include the screw driven syringe pump (56), the dual head reciprocating pump (57) and simple
pneumatically operated pumps where the mobile phase, contained in a coil of tubing, is pressurised directly from a compressed air cylinder. A 'home made' pump of the later type constructed from nylon tubing was used for some plate height determinations where it was necessary to employ very low pressures, (less than 20 p.s.i.).

Pressures were measured by Bourdon gauges (Negretti and Zambra, London). Monitoring the pressure as a function of time was accomplished by recording the output from a pressure transducer on a dual pen recorder together with the detector signal.

Connections were made using nylon or stainless steel tubing and either Swagelok® or Wade® couplings.

4.2 Detectors

In all the experimental work reported here ultra-violet photometric detection was used. This is the most popular H.P.L.C. detector since it combines relatively high sensitivity with ease of operation and is capable (with variable wavelength) of detecting most samples likely to be encountered. The maximum sensitivity of a typical ultra violet detector is 0.01 absorbance units full scale deflection (with 1% noise). With this sensitivity it is possible to detect a concentration as low as 1 in $10^9$ mole fraction for a compound with an extinction coefficient of $10^4$.

The instruments used were:-

(a) DuPont Model 410

This is a monochromatic instrument employing a low pressure mercury lamp which emits radiation at 254 nm. This photometer has been described
by Kirkland. (61) In this instrument light from the mercury lamp is split by a semi-transparent mirror into an analyser beam and a reference beam. The reference beam intensity is approximately 10% of that incident on the beam splitter. The detector cell volume is 8 microlitres and it has a light path of 1 cm. The instrument was operated without a reference cell.

(b) Cecil Instruments Model CE 212 photometer.

This is a single beam variable wavelength instrument. It employs a deuterium lamp which emits radiation in the range 200 to 400 nm. The detector cell volume is 8 microlitres and it has a path length of 1 cm. The maximum sensitivity of the Cecil is equal to that of the DuPont 410 but the Cecil has the important advantage of variable wavelength which enables one to choose the wavelength of maximum absorbance of the samples to be detected and hence lowers the minimum detectable concentration.

The detector output was recorded on a Servoscribe® potentiometric chart recorder. These recorders have multiple chart speeds. Speeds ranging from 12 to 0.2 cm. per min. were useful for plate height studies.

Now that column efficiencies are approaching the theoretical optimum and high pressure pumping systems are readily available, the future development of H.P.L.C. is likely to be centred round improvements in detector design and the development of new detection methods. A brief description is given below of some of the other detectors (both universal and selective) which are either in use at present or show future potential.

Transport detectors

The transport detector is potentially a sensitive universal detector
but as yet it has not fulfilled this potential due to mechanical problems involved in transporting a large enough fraction of the column eluent to the detector. In its simplest form a moving wire is drawn through the eluent as it exits from the column and picks up a small fraction of the eluent. The mobile phase solvent is then evaporated as the wire is drawn through a low temperature oven leaving the solute to be detected adhering to the wire. The wire then travels through a pyrolysis oven within a nitrogen atmosphere. Finally the pyrolysis products are swept to a F.I.D. gas chromatograph detector to produce the output signal.

This detector is potentially sensitive since it employs a sensitive G.C. detector but at present the theoretical sensitivity cannot be achieved because of problems associated with the transport of the sample to the G.C. detector. The carrying capacity of a wire is severely limiting since it can only collect a small fraction of the eluting solvent and hence attempts have been made to replace the wire with an absorbent band or a rotating disc. There are also problems of high detector noise levels introduced by the mechanics involved in solute transfer.

**Refractive Index Monitor**

This is the second most popular detector in use at present and providing that a mobile phase can be chosen with a refractive index sufficiently different from that of the sample it is a "universal" detector. Unfortunately the sensitivity of the R.I. instruments is about a thousand times less than that of ultra violet detectors and this
severely limits their use in H.P.L.C. Nevertheless these detectors are widely used particularly in gel permeation chromatography where sample loadings are fairly large.

**Fluorescence detector**

The fluorescence detector monitors the fluorescent emission from a solute excited by ultra violet radiation. This is potentially an important detector since many biologically important compounds fluoresce. In the operation of a fluorimeter, monochromatic U.V. radiation is passed through the detector cell and excites the solute molecules to fluoresce (at longer wavelength). The fluorescent beam may be detected either parallel or perpendicular to the incident light after passing through a filter to remove radiation of the incident wavelength. (Even if the fluorescent light is detected perpendicular to the incident beam, the incident radiation is elastically scattered and has to be filtered out). The fluorescence detector is very sensitive but care must be taken in choosing the mobile phase since the fluorescence of a molecule is influenced by its environment.

**Polarographic Detection**

Polarography measures the current between a polarisable and a non-polarisable electrode as a function of the applied voltage. The non-polarisable electrode is conveniently a mercury pool while the polarisable electrode may be of the dropping mercury type. A disadvantage of the dropping mercury electrode is that the output signal must be smoothed before recording. Solid polarisable electrodes have been constructed from carbon, platinum and gold.
In a polarographic detector a constant voltage is applied between the electrodes which results in the oxidation or reduction (depending on the polarity of the electrodes) of the solute as it passes through the cell. The resulting current is measured as a function of time and recorded to give the usual detector trace. Nitro compounds, aromatic azo compounds, amino acids and inorganic ions can all be detected polarographically. Polarography is a highly selective detection method and has a sensitivity at least comparable with that of an ultra violet monitor. (62,63).

Other H.P.L.C. detectors have been suggested to monitor radioactive solutes (64) and changes in the dielectric constant due to the presence of the solute. (65) Detection by an electron capture G.C. detector involving complete evaporation and throughput of the solute plus eluent seems to show potential. (66) Finally the interfacing of H.P.L.C. with mass spectroscopy has been the subject of considerable interest.

4.3 Preparation and Packing of Columns

Columns were made from either regular glass tubing or stainless steel. The glass columns were sealed into the end pieces using Araldite epoxy resin while the stainless steel columns were hard soldered in. The column endpieces were constructed from brass or stainless steel. The column packing material was retained using a polyethylene or metal porous frit. A diagram of a typical column and injection head is shown in figure 4.4.

This system of on-column injection and minimum dead volume at the
column exit is vital in H.P.L.C. to eliminate tailing and extra-
column peak dispersion.

The names and characteristics of the packing materials used are
listed in table 4.1. Columns were dry and slurry packed using the
following methods:

(1) Dry packing using the "rotate tap bounce" method.

The column was put in a special rig which simultaneously rotated
and bounced the column. The speeds of both these operations could be
varied using variac transformers. The height of the bounce was about
1 cm. The packing was added continuously at a rate sufficient to
accumulate a bed at about 1 cm. per min. while the column was tapped
horizontally just above the level of the bed. This method is adapted
from that suggested by Sie and Van den Hoed. (58). The rates of
rotation, bouncing and tapping could be varied within quite wide limits
without noticeable deterioration in column performance. Typical values
used were: rotation - 120 r.p.m., bounce - 120 p.m. and tapping - 120 p.m.

(2) Dry packing using the "vibrator" method.

This method was suggested to us by Dr. D. Heinekey. (93) A glass
column was attached to a suitable motor rotating at a few revolutions
per second. One or two centimeters of packing were then added and the
column was vibrated using an engraving tool held against the glass just
above the level of the packing. The vibration was continued until no
further settling of the packing occurred. The next layer of packing was
then added and the procedure repeated. Crucial factors in this technique
are the amplitude of the vibration of the tool and the angle and manner
<table>
<thead>
<tr>
<th>Material</th>
<th>Description</th>
<th>Approx. Surface Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperisorb A.Y.</td>
<td>micro-particulate spherical porous alumina</td>
<td>120 m².g⁻¹</td>
</tr>
<tr>
<td>Sherisorb A.X.</td>
<td>&quot;</td>
<td>200 m².g⁻¹</td>
</tr>
<tr>
<td>Sherisorb S</td>
<td>micro-particulate spherical porous silica</td>
<td>200 m².g⁻¹</td>
</tr>
<tr>
<td>Partisil</td>
<td>micro-particulate irregular porous silica</td>
<td>400 m².g⁻¹</td>
</tr>
<tr>
<td>W.L.C.U. silica</td>
<td>micro-particulate spherical silica developed by the Wolfson Liquid Chromatography unit</td>
<td>200 m².g⁻¹</td>
</tr>
<tr>
<td>SAS silica</td>
<td>Chemically bonded hydrocarbon phase based on W.L.C.U. silica</td>
<td>200 m².g⁻¹</td>
</tr>
</tbody>
</table>
in which it is held against the glass. Some practice is necessary before reproducible columns can be obtained but once mastered efficiencies are comparable with columns packed using the "rotate tap bounce" method.

(3) Slurry packing.

Basically this technique consists of forming a uniformly dispersed slurry of the support in a suitable solvent. The slurry is then pumped rapidly into the analytical column which has been terminated by a fine porosity frit. The support is therefore filtered out and accumulates to form a uniform bed. Several slurrying solvents have been used. Kirkland (59) has used a dilute electrolyte which confers a surface charge on the support particles and therefore stabilises the suspension. Other workers have used "balanced density slurries" (60) where the slurrying medium is a mixture of dense halogenated solvents blended to give the same density as the support. A serious disadvantage of this method is the toxicity of these halogenated solvents.

At Edinburgh we have used methyl iodide (density 2.2) to slurry both silica with which it forms an approximately balanced slurry and also alumina with which it does not with equally good results. It is therefore evident that balanced density slurries are not necessary provided that settling of the support particles is sufficiently slow in relation to the time taken to pack the column. Later excellent results were achieved using an "unbalanced" slurry of silica in methanol.
The concentration of the slurry has also been varied over wide limits with good results produced from slurries composed of 1 - 29% w/v of support. The flow of slurry into the column should be as rapid as possible, i.e. the pressure should be as high as possible. Nevertheless excellent columns have been produced with relatively low packing pressures, (less than 2000 p.s.i.).

4.4 Experimental technique

The solvents, with the exception of hexane, were used without further purification. Hexane however contained a significant concentration of U.V. absorbing impurities. In order to remove these, the hexane was passed through a column containing activated silica. This process also served to dry the hexane. Water saturated hexane was produced by passing the 'purified' hexane through a column containing silica with a loading of 30% v/w of water. Hexane with a controlled water content was then easily produced by splicing the dry and saturated hexane in the required proportions, (usually 50 : 50).

Dissolved air must be removed from the eluent, particularly with polar solvents, before use. This was accomplished by refluxing the eluent or more quickly by rapidly stirring the eluent under a partial vacuum.

Generally, the passage of 25 to 50 column volumes of eluent was sufficient to stabilise detector response and solute retention times.

Samples were introduced by on-column injection of a dilute solution of the solutes in the mobile phase. When hexane was used as the eluent, it was necessary to inject "neat" pentane in order to
determine the unretained retention time. (Pentane has a different refractive index from hexane and in consequence the refractive index of the liquid in the detector cell changes as the pentane is eluted from the column. Since the light in its path through the cell is subject to a series of reflections, the change in refractive index of the mobile phase results in a perturbation of the detector signal. This perturbation has the form of a "differential peak".)

The volume injected ranged from 0.5 microlitres (in the plate height studies) to 10 microlitres. In order not to degrade the efficiency of a column it is vital that the dispersion of the solute on injection is small in relation to the subsequent dispersion within the column. The injection was therefore made as close to the top of the packing as possible. (The volume immediately above the packing was filled with glass beads).

Syringe injection was used against column head pressure in excess of 1000 p.s.i. but as the pressure was raised, blocking of the syringe needle became a problem and also septum life was diminished. Syringe injection has the additional problem that pieces of septa are torn off by the syringe needle and accumulate at the top of the column. This necessitates regular cleaning out of the top of the column if performance is to be maintained. A convenient way of avoiding disturbing the column packing during this cleaning process is to insert a stainless steel mesh disc between the column packing and the glass beads. The top of the column can then be easily washed out and replaced by fresh glass beads.
4.5 Calculation of results

Figure 4.5 is a typical chromatogram showing the measurements required to assess the column performance.

From chromatographic theory, fully resolved peaks should be eluted from a column with a Gaussian concentration profile. In H.P.L.C. this profile is readily obtainable if the column geometry is carefully selected. For a Gaussian peak the plate height is given by:

$$ H = \frac{L}{(4.\frac{t_R}{w})^2} $$

where $L$ is the column length and $t_R$ and $w$ are as defined in figure 4.5. For non-gaussian peaks (e.g. at the breakdown of infinite-diameter geometry), the plate heights as reported must only be treated as an empirical indication of deteriorating column performance.

The reduced plate height ($h$) is calculated from the equation:

$$ h = \frac{H}{dp} $$

where $dp.$ is the mean particle diameter. For large particles, fractionation is achieved by sieving so that the mean particle diameters are accurate. For small particles however (less than 30 microns), the mean particle diameter was assessed by measuring one hundred particles under a microscope and calculating the arithmetic mean. Due to experimental difficulties in resolving the smaller particles, the mean particle diameter from microscopic measurement may be in error by as much a 1 micron for particles less than 10 microns in diameter. An alternative method of assessing particle diameter is by measuring the permeability of a column packed with the material and calculating the "effective" particle
diameter from the equation:

\[ \phi' = \frac{P \cdot dp^2}{\eta \cdot u \cdot L} \]

where \( P \) is the pressure, \( \eta \) is the viscosity of the eluent, \( u \) is the solvent velocity and \( L \) is the column length. \( \phi' \) is the flow resistance parameter and has typical values of 500 for slurry packed columns and 1200 for dry packed columns. (These two values are only valid for materials which have been well fractionated.)

In order to characterise a column, both the mean particle diameter used and the \( \phi' \) value must be quoted. Since each is dependant on the value assumed for the other, one of them must be taken as the standard. ie. If the particle size is large and can be accurately assessed than that value of \( dp \) is used in order to calculate \( \phi' \). If on the other hand \( dp \) is too small to measure accurately then a value may be assumed for \( \phi' \) and the effective particle diameter calculated.

The reduced solvent velocity \((v)\) is given by the equation:

\[ v = u \cdot dp / D_m \]

where \( D_m \) is the diffusion coefficient of the solute in the mobile phase and \( u \) is the solvent velocity. \( u \) is simply calculated from the column length and the time taken to elute an un retarded solute. \( D_m \) was calculated from the Wilke-Chang equation:

\[ D_m = \frac{7.4 \times 10^{-8} (\nu^2 M_2)^{0.2}}{\eta V_1^{0.6}} \]

where \( M_2 \) is the molecular weight of the solvent, \( V_1 \) is the molar volume
of the solute and $\gamma_2$ is an association factor which takes the values, 1.0, 2.6, 1.9 and 1.5 for non-polar solvents, water, methanol and ethanol respectively. $T$ is the absolute temperature. $D_m$ is given in units of cm$^2$.sec$^{-1}$. For compounds with low molecular weights, the calculated value of $D_m$ in n-hexane is close to $3.3 \times 10^{-5}$ cm$^2$.sec$^{-1}$.

The capacity factors of the solutes were determined from the equation:

$$K' = \frac{(t_R - t_o)}{t_o}$$

where $t_R$ is the retention time of the solute and $t_o$ is the elution time of an unretained peak.
CHAPTER 5

The effect of "infinite diameter column geometry" on column performance

The concept of an "infinite diameter column" was first introduced by Knox and Parcher (22). They realised that in liquid chromatography, because of the relatively slow rate of radial dispersion, it was possible to construct a column such that solute molecules which were injected centrally onto the top of the column might fail to reach the walls by the time they emerged from the column.

From chemical engineering studies reviewed by Horne, Knox and McLaren (88), Knox and Parcher estimated a radial plate height of 0.15 particle diameters. Neglecting radial dispersion due to diffusion they deduced that for infinite diameter operation:-

\[
\frac{dc^2}{Ldp} \gg 2.4
\]

where dc is the internal diameter of the column, L is the column length and dp is the particle diameter.

Experimental confirmation of the "infinite diameter effect" was published by DeStefano and Beachell, (67,68). In two separate studies they found substantial increases in column performance when the column parameters were such as to permit infinite diameter operation as defined by equation 5.1. In the first study (67) they packed various size ranges of a controlled surface porosity support in 500 mm. X 8 mm. i.d. columns. Taking into account the theoretical effect of particle size on column efficiency, there was a substantial increase
in column performance for 29 and 41 micron particles for which equation 5.1 predicts infinite diameter operation over 58 and 68 micron particles for which condition 5.1 fails.

In the second study (68) DeStefano and Beachell packed 55 micron Porasil (a spherical totally porous silica) using 50 cm. columns of 2.1 and 10.9 mm. internal diameter. They found almost a two fold decrease in theoretical plate heights for the 10.9 mm. infinite diameter column over the 2.1 mm. column. In this paper the authors also note the increased gradient of the log H versus log u graph for the infinite diameter column resulting from the decreased contribution to the overall plate height arising from "eddy diffusion" and the consequently greater dependence on the mass transfer term.

Wolf (69) packed a series of 50 cm columns with diameters ranging from 2.1 to 23.6 mm. with Zipax Permaphase ODS (10 - 40 microns). He found that there was a steady rise in column efficiency with column diameter resulting in a four fold improvement in theoretical plate height for the 23.6 mm. column over the 2.1 mm. column. It is interesting to note that Wolf used an injection system designed to distribute the sample evenly over the top of the column. Although in a strict interpretation, this excludes infinite diameter operation, in practice if the central area of the column is sufficiently large in relation to the area of disturbed wall region then the observed efficiency will approach that expected from infinite diameter operation. This interpretation would suggest a gradual increase in column efficiency as the column diameter is increased (which was observed experimentally)
in contrast to the studies of Beachell and DeStefano where a clear cut distinction is made between infinite diameter and non-infinite diameter operation.

With the introduction of microparticulate packings with diameters equal to or less than 20 microns, the column dimensions required for "infinite diameter geometry" were reduced to more practical values. For example from equation 5.1, with $dp = 20$ microns and $L = 150$ mm., infinite diameter operation should result if $dc > 2.7$ mm. With the reduction in column lengths brought about by the use of smaller particles it was also important that the volume of the peaks eluted from a column did not become too small in relation to the volume of the detector system and the dead volume of connecting tubing. It therefore quickly became conventional to use 4 or 5 mm. internal diameter tubing for column construction. This gave the opportunity for infinite diameter operation while maintaining the peak volumes at reasonable values. (Greater than 30 microlitres).

Experimentally however some columns, particularly those greater than 150 mm. in length, had lower efficiencies than might be expected from infinite diameter operation and in addition peak assymetry was often observed. This assymetry took the form of "pre-tailing" or "fronting" of the peaks. Pre-tailing was dependant on the eluent velocity, increasing markedly at low eluent velocities. It was soon realised that this phenomena was due to the breakdown of infinite diameter geometry which resulted in a proportion of the solute molecules reaching a region of disturbed packing adjacent to the column wall.
splitting in a packed column. From chemical engineering studies a value of 0.15 had been estimated by Knox and Parcher (22) but more recent detailed work on glass beads by Knox and Raven (70) indicated a value of $A_r$ of about 0.06.

Knox and Raven performed a series of experiments on an infinite diameter column using a dual polarographic detector in which there was one central fixed platinum electrode and one platinum electrode which could be moved radially across the column exit frit. By moving the electrode they were able to determine the radial concentration distribution produced by a centrally injected solute at a series of eluent velocities. Graphically it was then possible to estimate radial plate heights and plot these against eluent velocity. Working with 64 micron glass beads packed in an 11.7 mm. bore column, they found that the best representation of the experimental results was given by:

$$h_r = \frac{1.4}{v} + 0.06$$

This value of $A_r = 0.06$ is very much smaller than the previously assumed value of 0.15 and highlights the fact that $A_r$ may not be a constant but be dependent on the quality of the column packing. A small value of $A_r$ would indicate a well packed uniform bed of particles (as one would expect with 64 micron glass beads) while the value of $A_r$ would be greater for a poorly packed column.

$A_r$ must therefore be regarded as a variable dependent on the packing material, particle size and on the column packing methods employed.

By studying the solute concentration produced at the column exit
from a solute solution spread evenly over the top of the column (using a stop flow method), Knox and Raven were also able to deduce something of the radial eluent velocity profile. They found that there was no evidence of a substantial increase in eluent velocity close to the walls even although the plate height of the eluted peaks within two millimeters of the walls increased more than two fold. (Eluent velocity within one or two particles of the walls could of course not be measured). The region of disturbed packing was found to extend 1 or 2 mm. from the wall ie. 15 to 30 particle diameters for 64 micron particles. Knox and Raven concluded that the original concept of infinite diameter geometry must be modified and refined to take into account both the extent of the region of disturbed packing and the variability of the value of $A_r$.

For a column to operate in the infinite diameter mode, the constraint is imposed that 95% of the solute molecules will not reach the walls of the column, ie.

$$dc \gg 4. \sigma'_r$$  \hspace{1cm} 5.7

Taking into account the area of disturbed packing extending $r$ particle diameters from the wall, the effective diameter of an infinite diameter column will be reduced by $2.r.dp.$, ie. for infinite diameter operation:

$$(dc - 2.r.dp.) \gg 4. \sigma'_r$$  \hspace{1cm} 5.8

but

$$\sigma'^2_r = \frac{H_r}{L}$$

$$= L (2. \psi_{.Dm.}/u + A_r .dp.)$$
Therefore for infinite diameter geometry:-

\[(dc - 2r.dp.)^2 \geq 16L(2.\frac{\gamma}{u} + A_r.dp.)\]

\[\geq 16L.dp.(2.\frac{\gamma}{v} + A_r)\]

ie.

\[I' \geq \frac{32}{v} + 16A_r\]  

\[\text{where } I' = (dc - 2r.dp.)^2/L.dp.\]  

It can be seen from this equation that for infinite diameter operation at high reduced velocities,

\[I' \geq 16A_r\]

while all columns will cease to behave as if of infinite diameter at sufficiently low reduced velocities because of radial molecular diffusion.

In the following discussion experimental data is compared with both the original simple theory of infinite diameter operation in which \(A_r\) is assumed to have a value of 0.15 and no account is taken of the extent of wall region and also the improved theory of infinite diameter operation where the effective diameter of the column is reduced by the disturbed wall region and \(A_r\) is smaller than had previously been supposed.

For the improved theory, in the absence of experimental data on porous particles the value of \(A_r\) and the extent of penetration of the disturbed wall region found by Knox and Raven will be assumed, ie. \(A_r = 0.06\) and \(r = 30\). From equations 5.9 and 5.10 with \(\gamma = 0.9\), this yields:-

\[(dc - 60.dp.)^2/L.dp. \geq \frac{28.8}{v} + 0.96\]
The original theory with $A_r = 0.15$ and $r = 0$, yields:

$$\frac{dc^2}{L dp} \geq \frac{28.8}{v} + 2.4$$

According to each of these two equations there is a critical reduced velocity below which radial molecular diffusion will cause the breakdown of infinite diameter geometry. For the "improved theory" we can define $v'_{\text{crit}}$ as:

$$v'_{\text{crit}} = \frac{28.8}{(dc - 60 dp)^2/L dp - 0.96}$$

While for the "original theory" $v_{\text{crit}}$ is given by:

$$v_{\text{crit}} = \frac{28.8}{dc^2/L dp - 2.4}$$

If the denominator of these equations is zero or negative then it is predicted that the column will not behave as if of infinite diameter.

5.2 Experimental

All columns were constructed from regular glass tubing of various diameters and were used with pressures up to 500 p.s.i. The columns were packed with Spherisorb Alumina AX having an average particle diameter of 21.5 microns. (Determined by microscopic measurement of 100 particles.)

Both the "rotate tap bounce" and the "vibrator" methods of column packing were used in these experiments. It was found experimentally that the "vibrator method" was more successful in packing active alumina while the "rotate tap bounce method" only produced good results when the alumina had been deactivated by the addition of two per cent w/w of water to the freshly activated support.
The water was equilibrated over the alumina surface by putting it in a sealed bottle and placing it on a rolling machine overnight.

Two sets of experiments were conducted to determine the effect of column geometry on efficiency.

(a) To study the effect of varying the column length with diameter constant, 5 mm. bore glass columns of lengths 82.5, 150, 220 and 280 mm. were packed by the "vibrator method". The efficiencies of these columns were determined using the solutes toluene, styrene, naphthalene and biphenyl. The eluent was 50% water saturated n-hexane.

(b) To study the effect of varying the column diameter with length constant 145 mm. columns with internal diameters of 2.3, 3.2, 4.2, 5.1 and 7 mm. were packed using the "rotate tap bounce method". Since the Alumina was deactivated before packing it was convenient to use a different test mixture. The solutes used were toluene, biphenyl and anthracene. The eluent was 50% water saturated n-hexane.

0.5 microlitres of the sample solution were injected centrally into a 2 mm. deep layer of 200 micron glass beads on the top of the packing.

The "unretained elution time" was found by injecting neat pentane and from this the capacity factor of toluene could be calculated. For each sample injection the eluent velocity \( u \) was then calculated from the elution time of toluene by the equation:

\[
u = \frac{L(1 + K'_{tol})}{t_{R,tol}}
\]

5.3 Results and Discussion

A graph of log \( h \) versus log \( v \) for an 82.5 mm. X 5 mm. column
packed using the vibrator method is shown in figure 5.1. The experimental data is given in table 5.2. For this column, the efficiencies were determined down to very low reduced velocities so that the effect of longitudinal diffusion (and the breakdown of infinite diameter geometry) could be observed.

By fitting the experimental data to the equation

\[ h = \frac{B}{\nu} + A \nu^{0.33} + C \nu \]

estimates of the values of the constants B, A and C can be made. The values found for a lightly retained solute (toluene) and a retained solute are shown in table 5.1.

**TABLE 5.1**

<table>
<thead>
<tr>
<th>Constant</th>
<th>B</th>
<th>A</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unretained solute</td>
<td>3.0</td>
<td>0.4</td>
<td>0.09</td>
</tr>
<tr>
<td>Retained solute</td>
<td>3.6</td>
<td>0.4</td>
<td>0.16</td>
</tr>
</tbody>
</table>

The very low value of A equals the best value previously obtained for 480 micron glass beads (22) and confirms the excellent packing characteristics of the Spherisorb Aluminas.

The high value of C (0.16 as against 0.05 for the silica support Porasil, (17)), indicates the relatively slow rate of mass transfer within the alumina support. However this does not present a serious
TABLE 5.2

Support: 21.5 micron Spherisorb AX. Column dimensions: 82.5 X 5 mm.
Packing method: "vibrator". Solutes: Toluene $K' = 0.5$, Styrene $K' = 1.0$, Naphthalene $K' = 2.2$, Biphenyl $K' = 3.4$.

<table>
<thead>
<tr>
<th>Pressure /p.s.i.</th>
<th>Eluent vol. /cm.sec.$^{-1}$</th>
<th>Toluene log $h$</th>
<th>Styrene log $h$</th>
<th>Naphth. log $h$</th>
<th>Biphenyl log $h$</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.097</td>
<td>0.80</td>
<td>0.24</td>
<td>0.25</td>
<td>0.30</td>
</tr>
<tr>
<td>11</td>
<td>0.074</td>
<td>0.68</td>
<td>0.25</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td>7</td>
<td>0.048</td>
<td>0.49</td>
<td>0.26</td>
<td>0.27</td>
<td>0.26</td>
</tr>
<tr>
<td>4</td>
<td>0.032</td>
<td>0.32</td>
<td>0.43</td>
<td>0.42</td>
<td>0.37</td>
</tr>
<tr>
<td>2.5</td>
<td>0.022</td>
<td>0.16</td>
<td>0.45</td>
<td>0.46</td>
<td>0.47</td>
</tr>
<tr>
<td>1.5</td>
<td>0.016</td>
<td>0.00</td>
<td>0.56</td>
<td>0.59</td>
<td>0.55</td>
</tr>
<tr>
<td>1.0</td>
<td>0.009</td>
<td>-0.25</td>
<td>0.82</td>
<td>0.85</td>
<td>0.82</td>
</tr>
<tr>
<td>1.5</td>
<td>0.014</td>
<td>-0.05</td>
<td>0.59</td>
<td>0.65</td>
<td>0.66</td>
</tr>
<tr>
<td>1.5</td>
<td>0.018</td>
<td>0.08</td>
<td>0.48</td>
<td>0.50</td>
<td>0.47</td>
</tr>
<tr>
<td>1.0</td>
<td>0.006</td>
<td>-0.40</td>
<td>0.84</td>
<td>0.86</td>
<td>0.81</td>
</tr>
<tr>
<td>1.0</td>
<td>0.008</td>
<td>-0.28</td>
<td>0.86</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.005</td>
<td>-0.53</td>
<td>0.97</td>
<td>0.96</td>
<td>0.99</td>
</tr>
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<td>1.0</td>
<td>0.011</td>
<td>-0.14</td>
<td>0.68</td>
<td>0.69</td>
<td>0.69</td>
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<tr>
<td>19</td>
<td>0.129</td>
<td>0.92</td>
<td>0.38</td>
<td>0.34</td>
<td>0.39</td>
</tr>
<tr>
<td>24</td>
<td>0.163</td>
<td>1.03</td>
<td>0.40</td>
<td>0.40</td>
<td>0.43</td>
</tr>
<tr>
<td>60</td>
<td>0.227</td>
<td>1.17</td>
<td>0.40</td>
<td>0.45</td>
<td>0.54</td>
</tr>
<tr>
<td>70</td>
<td>0.295</td>
<td>1.28</td>
<td>0.53</td>
<td>0.55</td>
<td>0.63</td>
</tr>
<tr>
<td>90</td>
<td>0.395</td>
<td>1.41</td>
<td>0.59</td>
<td>0.63</td>
<td>0.72</td>
</tr>
<tr>
<td>120</td>
<td>0.604</td>
<td>1.59</td>
<td>0.72</td>
<td>0.82</td>
<td>0.88</td>
</tr>
<tr>
<td>165</td>
<td>0.798</td>
<td>1.72</td>
<td>0.80</td>
<td>0.90</td>
<td>0.94</td>
</tr>
</tbody>
</table>
practical problem since the reduced velocities employed even with 20 micron material are fairly low. As will be shown in chapter 7, the dependence of column efficiency on the mass transfer term is reduced in importance still further as the particle size is decreased.

The value of $B$ of about 3.3 compares with the theoretical value of 2.8 for a porous packing of 1.8. It is not likely that longitudinal diffusion on the surface of the alumina would significantly increase the value of $B$ and in any case the dependence of $B$ on $K'$ is not sufficiently strong to suggest this. The most likely explanation of the large value of $B$ is the increased dispersion due to the loss of infinite diameter geometry at low reduced velocities.

The region of the plate height curve above a reduced velocity of about 3 shows the excellent efficiency obtainable from Spherisorb Alumina packed in an infinite diameter column. This section of the graph can be taken as a standard measure of efficiency against which columns of varying length can be compared.

(a) The effect of column length on efficiency with internal diameter constant

The experimental data for each of the four column lengths used in this study are shown in tables 5.3 to 5.6. Figures 5.2 to 5.5 show the $\log h$ versus $\log v$ plots obtained for each column. In each figure the standard curves for a lightly retained and a retained solute obtained from figure 5.1 are also shown.

It can be seen that the efficiency of all the columns at high reduced velocities (greater than $v = 30$) are identical within experimental error but as the eluent velocity is reduced, the
TABLE 5.3

Support: 21.5 micron Spherisorb AX. Column dimensions: 82.5 X 5 mm.
Packing method: "vibrator". Solutes: Toluene $K' = 0.5$, Styrene $K' = 1.0$, Naphthalene $K' = 2.3$, Biphenyl $K' = 3.6$.

<table>
<thead>
<tr>
<th>Pressure (p.s.i.)</th>
<th>Eluent vel. $v$ (cm/sec)</th>
<th>log $v$</th>
<th>Toluene $h$</th>
<th>log $h$</th>
<th>Styrene $h$</th>
<th>log $h$</th>
<th>Naphthalene $h$</th>
<th>log $h$</th>
<th>Biphenyl $h$</th>
<th>log $h$</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>0.521</td>
<td>1.53</td>
<td>0.70</td>
<td>0.71</td>
<td>0.74</td>
<td>0.75</td>
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<td>0.367</td>
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<td>0.61</td>
<td>0.54</td>
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<tr>
<td>60</td>
<td>0.237</td>
<td>1.19</td>
<td>0.43</td>
<td>0.40</td>
<td>0.48</td>
<td>0.50</td>
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<tr>
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<td>0.42</td>
<td>0.44</td>
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</tr>
<tr>
<td>50</td>
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<td>1.02</td>
<td>0.37</td>
<td>0.36</td>
<td>0.43</td>
<td>0.42</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>0.139</td>
<td>0.96</td>
<td>0.29</td>
<td>0.23</td>
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<td></td>
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<td>0.33</td>
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<td></td>
</tr>
<tr>
<td>37</td>
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<td></td>
</tr>
<tr>
<td>33</td>
<td>0.047</td>
<td>0.49</td>
<td>0.24</td>
<td>0.29</td>
<td>0.24</td>
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<tr>
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<td>0.27</td>
<td>0.36</td>
<td>0.35</td>
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<td>0.90</td>
<td>0.95</td>
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<tr>
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<td>0.717</td>
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<td>0.85</td>
<td>0.91</td>
<td>0.89</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 5.4

Support: 21.5 micron Spherisorb AX. Column dimensions: 150 X 5 mm.
Packing method: "vibrator". Solutes: Toluene K' = 0.5, Styrene K' = 1.1, Naphthalene K' = 2.7, Biphenyl K' = 4.4.

<table>
<thead>
<tr>
<th>Pressure /p.s.i.</th>
<th>Eluent vel. /cm.sec.</th>
<th>Toluene log v</th>
<th>Styrene log h</th>
<th>Naphthalene log h</th>
<th>Biphenyl log h</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>0.046</td>
<td>0.47</td>
<td>0.29</td>
<td>0.30</td>
<td>0.36</td>
</tr>
<tr>
<td>30</td>
<td>0.026</td>
<td>0.22</td>
<td>0.39</td>
<td>0.42</td>
<td>0.47</td>
</tr>
<tr>
<td>43</td>
<td>0.052</td>
<td>0.53</td>
<td>0.32</td>
<td>0.41</td>
<td>0.41</td>
</tr>
<tr>
<td>250</td>
<td>0.714</td>
<td>1.67</td>
<td>0.85</td>
<td>0.79</td>
<td>0.87</td>
</tr>
<tr>
<td>200</td>
<td>0.536</td>
<td>1.54</td>
<td>0.64</td>
<td>0.69</td>
<td>0.79</td>
</tr>
<tr>
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<td>0.60</td>
<td>0.64</td>
<td>0.66</td>
</tr>
<tr>
<td>120</td>
<td>0.353</td>
<td>1.36</td>
<td>0.59</td>
<td>0.55</td>
<td>0.65</td>
</tr>
<tr>
<td>90</td>
<td>0.234</td>
<td>1.18</td>
<td>0.48</td>
<td>0.45</td>
<td>----</td>
</tr>
<tr>
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<td>0.38</td>
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<td>45</td>
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<td>0.30</td>
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<td>0.043</td>
<td>0.45</td>
<td>0.32</td>
<td>0.32</td>
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</tr>
</tbody>
</table>
TABLE 5.5

Support: 21.5 micron Spherisorb AX. Column dimensions: 220 X 5 mm.
Packing method: "vibrator". Solutes: Toluene $K' = 0.5$, Styrene $K' = 1.1$, Naphthalene $K' = 2.7$, Biphenyl $K' = 4.4$.

<table>
<thead>
<tr>
<th>Pressure /p.s.i.</th>
<th>Eluent vgl. /cm.sec.</th>
<th>log v</th>
<th>Toluene log h</th>
<th>Styrene log h</th>
<th>Naphthalene log h</th>
<th>Biphenyl log h</th>
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<tr>
<td>80</td>
<td>0.141</td>
<td>0.96</td>
<td>0.58</td>
<td>0.51</td>
<td>0.51</td>
<td>0.51</td>
</tr>
<tr>
<td>110</td>
<td>0.207</td>
<td>1.13</td>
<td>0.48</td>
<td>0.71</td>
<td>0.72</td>
<td>0.69</td>
</tr>
<tr>
<td>155</td>
<td>0.308</td>
<td>1.30</td>
<td>0.60</td>
<td>0.71</td>
<td>0.76</td>
<td>0.78</td>
</tr>
<tr>
<td>200</td>
<td>0.425</td>
<td>1.44</td>
<td>0.64</td>
<td>0.71</td>
<td>0.76</td>
<td>0.78</td>
</tr>
<tr>
<td>260</td>
<td>0.550</td>
<td>1.55</td>
<td>0.65</td>
<td>0.71</td>
<td>0.76</td>
<td>0.78</td>
</tr>
<tr>
<td>50</td>
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<td>0.65</td>
<td>0.48</td>
<td>0.39</td>
<td>0.44</td>
<td>0.43</td>
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<td>0.101</td>
<td>0.82</td>
<td>0.40</td>
<td>0.43</td>
<td>0.45</td>
<td>0.55</td>
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<td>0.91</td>
<td>0.46</td>
<td>0.38</td>
<td>0.49</td>
<td>0.46</td>
</tr>
<tr>
<td>90</td>
<td>0.169</td>
<td>1.04</td>
<td>0.38</td>
<td>0.45</td>
<td>0.47</td>
<td>0.53</td>
</tr>
<tr>
<td>115</td>
<td>0.230</td>
<td>1.18</td>
<td>0.54</td>
<td>0.50</td>
<td>0.57</td>
<td>0.65</td>
</tr>
<tr>
<td>55</td>
<td>0.080</td>
<td>0.72</td>
<td>0.37</td>
<td>0.44</td>
<td>0.37</td>
<td>0.42</td>
</tr>
<tr>
<td>30</td>
<td>0.029</td>
<td>0.28</td>
<td>0.56</td>
<td>0.59</td>
<td>0.68</td>
<td>0.62</td>
</tr>
<tr>
<td>43</td>
<td>0.048</td>
<td>0.50</td>
<td>0.48</td>
<td>0.51</td>
<td>0.48</td>
<td>0.51</td>
</tr>
<tr>
<td>37</td>
<td>0.033</td>
<td>0.33</td>
<td>0.65</td>
<td>0.64</td>
<td>0.60</td>
<td>0.59</td>
</tr>
<tr>
<td>45</td>
<td>0.055</td>
<td>0.55</td>
<td>0.50</td>
<td>0.43</td>
<td>0.45</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 5.6

Support: 21.5 micron Spherisorb AX. Column dimensions: 280 X 5 mm.
Packing method: "vibrator". Solutes: Toluene K' = 0.5, Styrene K' = 1.0, Naphthalene K' = 2.1, Biphenyl K' = 3.1.

<table>
<thead>
<tr>
<th>Pressure (p.s.i.)</th>
<th>Eluent vel. (cm/sec)</th>
<th>Toluene log h</th>
<th>Styrene log h</th>
<th>Naphthalene log h</th>
<th>Biphenyl log h</th>
</tr>
</thead>
<tbody>
<tr>
<td>280</td>
<td>0.532</td>
<td>0.64</td>
<td>0.70</td>
<td>0.69</td>
<td>0.76</td>
</tr>
<tr>
<td>220</td>
<td>0.408</td>
<td>0.47</td>
<td>0.65</td>
<td>0.69</td>
<td>0.71</td>
</tr>
<tr>
<td>175</td>
<td>0.323</td>
<td>0.49</td>
<td>0.54</td>
<td>----</td>
<td>0.60</td>
</tr>
<tr>
<td>135</td>
<td>0.238</td>
<td>0.47</td>
<td>0.47</td>
<td>0.49</td>
<td>0.51</td>
</tr>
<tr>
<td>105</td>
<td>0.176</td>
<td>0.45</td>
<td>0.39</td>
<td>0.45</td>
<td>0.46</td>
</tr>
<tr>
<td>85</td>
<td>0.133</td>
<td>0.40</td>
<td>0.46</td>
<td>0.50</td>
<td>0.47</td>
</tr>
<tr>
<td>70</td>
<td>0.097</td>
<td>0.44</td>
<td>0.45</td>
<td>0.54</td>
<td>0.43</td>
</tr>
<tr>
<td>55</td>
<td>0.065</td>
<td>0.42</td>
<td>0.53</td>
<td>0.57</td>
<td>0.54</td>
</tr>
<tr>
<td>50</td>
<td>0.053</td>
<td>0.58</td>
<td>0.58</td>
<td>0.63</td>
<td>0.65</td>
</tr>
<tr>
<td>45</td>
<td>0.041</td>
<td>0.58</td>
<td>0.74</td>
<td>0.70</td>
<td>0.77</td>
</tr>
<tr>
<td>40</td>
<td>0.029</td>
<td>0.52</td>
<td>0.71</td>
<td>0.63</td>
<td>0.66</td>
</tr>
<tr>
<td>43</td>
<td>0.038</td>
<td>0.42</td>
<td>0.53</td>
<td>0.62</td>
<td>0.69</td>
</tr>
<tr>
<td>37</td>
<td>0.026</td>
<td>0.67</td>
<td>0.65</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>42</td>
<td>0.038</td>
<td>0.62</td>
<td>0.58</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>0.025</td>
<td>0.63</td>
<td>0.59</td>
<td>0.64</td>
<td></td>
</tr>
</tbody>
</table>
efficiencies of the columns break away from the "standard efficiency" curves. In accord with the theory of infinite diameter operation, the reduced velocity of the break away point increases with column length. The critical reduced velocity below which infinite diameter operation should cease according to equations 5.13 and 5.14 are listed in table 5.12. \( v_{\text{crit}} \) and \( v'_{\text{crit}} \) are shown in figures 5.2 to 5.5 by "dashed" and "solid" arrows respectively.

For each column length the experimental break away points are greater than \( v_{\text{crit}} \) and are in general agreement with \( v'_{\text{crit}} \), however the spread of experimental points precludes a definite choice between the two equations. It is interesting to note that in the region of reduced velocities of 4 to 10, the reduced plate heights for the 150 mm. column rise above the standard curve for the 82.5 mm. column but again fall on the curve below \( v = 4 \) as the 82.5 mm. column also ceases to be infinite diameter. For column lengths greater than 150 mm. the breakdown of infinite diameter geometry is more dramatic with pre-tailing of the peaks in evidence.

The data for the 82.5 mm. column plotted in figure 5.2 fall within the standard curves obtained from figure 5.1 and illustrate the excellent reproducibility of column packing which can be achieved with Spherisorb Alumina.

(b) The effect of column diameter on efficiency with length constant.

The experimental data for each of the five column diameters used in this study are shown in tables 5.7 to 5.11. Figures 5.6 to 5.10 show the log \( h \) versus log \( v \) plots obtained for each column.
TABLE 5.7

Support: 21.5 micron Spherisorb AX. Column dimensions: 145 X 7 mm.
Packing method: "rotate, tap, bounce". Solutes: Toluene $K' = 0.3$,
Biphenyl $K' = 0.9$, Anthracene $K' = 1.7$.

<table>
<thead>
<tr>
<th>Pressure /p.s.i.</th>
<th>Eluent vel. /cm.sec.</th>
<th>log v</th>
<th>Toluene log h</th>
<th>Biphenyl log h</th>
<th>Anthracene log h</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>0.546</td>
<td>1.55</td>
<td>0.49</td>
<td>0.69</td>
<td>0.69</td>
</tr>
<tr>
<td>125</td>
<td>0.463</td>
<td>1.48</td>
<td>0.49</td>
<td>0.63</td>
<td>0.70</td>
</tr>
<tr>
<td>100</td>
<td>0.389</td>
<td>1.40</td>
<td>0.45</td>
<td>0.59</td>
<td>0.62</td>
</tr>
<tr>
<td>90</td>
<td>0.356</td>
<td>1.36</td>
<td>0.42</td>
<td>0.55</td>
<td>0.62</td>
</tr>
<tr>
<td>80</td>
<td>0.295</td>
<td>1.28</td>
<td>0.32</td>
<td>0.47</td>
<td>0.52</td>
</tr>
<tr>
<td>60</td>
<td>0.220</td>
<td>1.16</td>
<td>0.25</td>
<td>0.38</td>
<td>0.48</td>
</tr>
<tr>
<td>45</td>
<td>0.171</td>
<td>1.05</td>
<td>0.17</td>
<td>0.33</td>
<td>0.41</td>
</tr>
<tr>
<td>35</td>
<td>0.130</td>
<td>0.93</td>
<td>0.21</td>
<td>0.29</td>
<td>0.36</td>
</tr>
<tr>
<td>25</td>
<td>0.095</td>
<td>0.79</td>
<td>0.20</td>
<td>0.23</td>
<td>0.29</td>
</tr>
<tr>
<td>17</td>
<td>0.068</td>
<td>0.65</td>
<td>0.24</td>
<td>0.18</td>
<td>0.29</td>
</tr>
<tr>
<td>12</td>
<td>0.047</td>
<td>0.48</td>
<td>0.25</td>
<td>0.24</td>
<td>0.33</td>
</tr>
<tr>
<td>9</td>
<td>0.040</td>
<td>0.41</td>
<td>0.39</td>
<td>0.35</td>
<td>0.36</td>
</tr>
<tr>
<td>7</td>
<td>0.030</td>
<td>0.29</td>
<td>0.49</td>
<td>0.39</td>
<td>0.47</td>
</tr>
</tbody>
</table>
TABLE 5.8

Support: 21.5 micron Spherisorb AX. Column dimensions: 145 X 5.1 mm.
Packing method: "rotate, tap, bounce". Solutes: Toluene $K' = 0.3$, Biphenyl $K' = 0.8$, Anthracene $K' = 1.3$.

<table>
<thead>
<tr>
<th>Pressure /p.s.i.</th>
<th>Eluent vel. /cm.sec.</th>
<th>log $V$</th>
<th>Toluene log $h$</th>
<th>Biphenyl log $h$</th>
<th>Anthracene log $h$</th>
</tr>
</thead>
<tbody>
<tr>
<td>205</td>
<td>0.908</td>
<td>1.77</td>
<td>0.73</td>
<td>0.80</td>
<td>0.97</td>
</tr>
<tr>
<td>150</td>
<td>0.692</td>
<td>1.65</td>
<td>0.63</td>
<td>0.78</td>
<td>0.87</td>
</tr>
<tr>
<td>115</td>
<td>0.527</td>
<td>1.54</td>
<td>0.47</td>
<td>0.66</td>
<td>0.74</td>
</tr>
<tr>
<td>90</td>
<td>0.401</td>
<td>1.42</td>
<td>0.44</td>
<td>0.56</td>
<td>0.63</td>
</tr>
<tr>
<td>70</td>
<td>0.342</td>
<td>1.35</td>
<td>0.36</td>
<td>0.45</td>
<td>0.57</td>
</tr>
<tr>
<td>50</td>
<td>0.240</td>
<td>1.19</td>
<td>0.32</td>
<td>0.42</td>
<td>0.55</td>
</tr>
<tr>
<td>36</td>
<td>0.178</td>
<td>1.06</td>
<td>0.26</td>
<td>0.34</td>
<td>0.50</td>
</tr>
<tr>
<td>24</td>
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<td>0.90</td>
<td>0.26</td>
<td>0.28</td>
<td>0.47</td>
</tr>
<tr>
<td>18</td>
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<td>0.79</td>
<td>0.31</td>
<td>0.28</td>
<td>0.45</td>
</tr>
<tr>
<td>13</td>
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<td>0.30</td>
<td>0.31</td>
<td>0.35</td>
</tr>
<tr>
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<td>0.049</td>
<td>0.51</td>
<td>0.41</td>
<td>0.39</td>
<td>0.49</td>
</tr>
<tr>
<td>6</td>
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<td>0.29</td>
<td>0.52</td>
<td>0.51</td>
<td>0.57</td>
</tr>
<tr>
<td>7</td>
<td>0.039</td>
<td>0.41</td>
<td>0.43</td>
<td>0.43</td>
<td>0.54</td>
</tr>
<tr>
<td>4</td>
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<td>0.22</td>
<td>0.52</td>
<td>0.52</td>
<td>0.54</td>
</tr>
</tbody>
</table>
**TABLE 5.9**

Support: 21.5 micron Spherisorb AX. Column dimensions: 145 X 4.2 mm.
Packing method: "rotate, tap, bounce". Solutes: Toluene $K' = 0.3$, Biphenyl $K' = 0.9$, Anthracene $K' = 1.7$.

<table>
<thead>
<tr>
<th>Pressure /p.s.i.</th>
<th>Eluent vel. /cm/sec.</th>
<th>log $v$</th>
<th>Toluene log $h$</th>
<th>Biphenyl log $h$</th>
<th>Anthracene log $h$</th>
</tr>
</thead>
<tbody>
<tr>
<td>170</td>
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<td>1.74</td>
<td>0.72</td>
<td>0.89</td>
<td>0.97</td>
</tr>
<tr>
<td>130</td>
<td>0.599</td>
<td>1.59</td>
<td>0.71</td>
<td>0.79</td>
<td>0.90</td>
</tr>
<tr>
<td>95</td>
<td>0.444</td>
<td>1.46</td>
<td>0.57</td>
<td>0.67</td>
<td>0.77</td>
</tr>
<tr>
<td>70</td>
<td>0.322</td>
<td>1.32</td>
<td>0.52</td>
<td>0.57</td>
<td>0.71</td>
</tr>
<tr>
<td>60</td>
<td>0.286</td>
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<td>0.35</td>
<td>0.57</td>
<td>0.64</td>
</tr>
<tr>
<td>45</td>
<td>0.224</td>
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<td>0.42</td>
<td>0.59</td>
<td>0.57</td>
</tr>
<tr>
<td>35</td>
<td>0.173</td>
<td>1.05</td>
<td>0.38</td>
<td>0.43</td>
<td>0.54</td>
</tr>
<tr>
<td>25</td>
<td>0.135</td>
<td>0.94</td>
<td>0.33</td>
<td>0.40</td>
<td>0.52</td>
</tr>
<tr>
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<td>0.83</td>
<td>0.32</td>
<td>0.37</td>
<td>0.47</td>
</tr>
<tr>
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<td>0.32</td>
<td>0.34</td>
<td>0.51</td>
</tr>
<tr>
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<tr>
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<td>0.50</td>
<td>0.41</td>
<td>0.37</td>
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</tr>
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<td>0.38</td>
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</tr>
<tr>
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<td>0.36</td>
<td>0.42</td>
<td>0.40</td>
<td>0.49</td>
</tr>
</tbody>
</table>
TABLE 5.10

Support: 21.5 micron Spherisorb AX. Column dimensions: 145 X 3.2 mm.
Packing method: "rotate, tap, bounce". Solutes: Toluene $K' = 0.3$, Biphenyl $K' = 0.8$, Anthracene $K' = 1.3$.

<table>
<thead>
<tr>
<th>Pressure /p.s.i.</th>
<th>Eluent vel. /cm.sec.</th>
<th>log v</th>
<th>Toluene log h</th>
<th>Biphenyl log h</th>
<th>Anthracene log h</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>0.213</td>
<td>1.14</td>
<td>0.73</td>
<td>0.72</td>
<td>0.82</td>
</tr>
<tr>
<td>160</td>
<td>0.248</td>
<td>1.21</td>
<td>0.72</td>
<td>0.75</td>
<td>0.79</td>
</tr>
<tr>
<td>200</td>
<td>0.297</td>
<td>1.29</td>
<td>0.68</td>
<td>0.72</td>
<td>0.83</td>
</tr>
<tr>
<td>260</td>
<td>0.365</td>
<td>1.38</td>
<td>0.85</td>
<td>0.83</td>
<td>0.97</td>
</tr>
<tr>
<td>320</td>
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<td>0.71</td>
<td>0.79</td>
<td>0.83</td>
</tr>
<tr>
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<td>0.165</td>
<td>1.03</td>
<td>0.67</td>
<td>0.66</td>
<td>0.79</td>
</tr>
<tr>
<td>70</td>
<td>0.139</td>
<td>0.96</td>
<td>0.64</td>
<td>0.68</td>
<td>0.77</td>
</tr>
<tr>
<td>53</td>
<td>0.114</td>
<td>0.87</td>
<td>0.70</td>
<td>0.74</td>
<td>0.83</td>
</tr>
<tr>
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<td>0.67</td>
<td>0.68</td>
<td>0.77</td>
</tr>
<tr>
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<td>0.67</td>
<td>0.66</td>
<td>0.76</td>
</tr>
<tr>
<td>23</td>
<td>0.058</td>
<td>0.58</td>
<td>0.59</td>
<td>0.68</td>
<td>0.77</td>
</tr>
<tr>
<td>16</td>
<td>0.045</td>
<td>0.46</td>
<td>0.63</td>
<td>0.66</td>
<td>0.74</td>
</tr>
<tr>
<td>11</td>
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<td>0.55</td>
<td>0.62</td>
<td>0.66</td>
</tr>
<tr>
<td>7</td>
<td>0.023</td>
<td>0.18</td>
<td>0.59</td>
<td>0.58</td>
<td>0.63</td>
</tr>
<tr>
<td>9</td>
<td>0.028</td>
<td>0.26</td>
<td>0.61</td>
<td>0.60</td>
<td>0.67</td>
</tr>
<tr>
<td>90</td>
<td>0.639</td>
<td>1.62</td>
<td>0.85</td>
<td>0.97</td>
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</tr>
<tr>
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<td>0.93</td>
<td>1.04</td>
<td></td>
</tr>
<tr>
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<td>0.967</td>
<td>1.80</td>
<td>0.94</td>
<td>1.12</td>
<td></td>
</tr>
</tbody>
</table>
Support: 21.5 micron Spherisorb AX. Column dimensions: 145 X 2.3 mm.
Packing method: "rotate, tap, bounce". Solutes: Toluene $K' = 0.3$,
Biphenyl $K' = 0.8$, Anthracene $K' = 1.3$.

<table>
<thead>
<tr>
<th>Pressure (p.s.i.)</th>
<th>Eluent vgl. (cm.sec.)</th>
<th>log $v$</th>
<th>log $h_{Toluene}$</th>
<th>log $h_{Biphenyl}$</th>
<th>log $h_{Anthracene}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>0.465</td>
<td>1.48</td>
<td>0.88</td>
<td>-----</td>
<td>1.08</td>
</tr>
<tr>
<td>175</td>
<td>0.365</td>
<td>1.38</td>
<td>0.76</td>
<td>0.84</td>
<td>0.98</td>
</tr>
<tr>
<td>125</td>
<td>0.288</td>
<td>1.27</td>
<td>0.79</td>
<td>0.84</td>
<td>0.97</td>
</tr>
<tr>
<td>90</td>
<td>0.211</td>
<td>1.14</td>
<td>0.88</td>
<td>0.93</td>
<td>1.06</td>
</tr>
<tr>
<td>70</td>
<td>0.160</td>
<td>1.07</td>
<td>0.78</td>
<td>0.84</td>
<td>0.98</td>
</tr>
<tr>
<td>53</td>
<td>0.139</td>
<td>0.96</td>
<td>0.69</td>
<td>0.78</td>
<td>0.92</td>
</tr>
<tr>
<td>41</td>
<td>0.114</td>
<td>0.87</td>
<td>0.70</td>
<td>0.77</td>
<td>0.92</td>
</tr>
<tr>
<td>30</td>
<td>0.086</td>
<td>0.75</td>
<td>0.69</td>
<td>0.75</td>
<td>0.88</td>
</tr>
<tr>
<td>21</td>
<td>0.065</td>
<td>0.63</td>
<td>0.60</td>
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</tr>
<tr>
<td>14</td>
<td>0.046</td>
<td>0.48</td>
<td>0.65</td>
<td>0.67</td>
<td>0.78</td>
</tr>
<tr>
<td>9</td>
<td>0.030</td>
<td>0.29</td>
<td>0.59</td>
<td>0.67</td>
<td>0.71</td>
</tr>
<tr>
<td>7</td>
<td>0.021</td>
<td>0.13</td>
<td>0.56</td>
<td>0.58</td>
<td>0.68</td>
</tr>
</tbody>
</table>
### TABLE 5.12
Reduced velocities below which columns cease to be infinite diameter.

\( dc = 5 \text{ mm.} \) columns packed using "vibrator method".

<table>
<thead>
<tr>
<th>( L / \text{mm.} )</th>
<th>( \nu_{\text{crit.}} )</th>
<th>( \log \nu_{\text{crit.}} )</th>
<th>( \nu'_{\text{crit.}} )</th>
<th>( \log \nu'_{\text{crit.}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>defined by eqn. 5.14</td>
<td>defined by eqn. 5.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>82.5</td>
<td>2.5</td>
<td>0.40</td>
<td>4.25</td>
<td>0.63</td>
</tr>
<tr>
<td>150</td>
<td>5.4</td>
<td>0.73</td>
<td>8.8</td>
<td>0.94</td>
</tr>
<tr>
<td>220</td>
<td>10.0</td>
<td>1.00</td>
<td>15.1</td>
<td>1.18</td>
</tr>
<tr>
<td>280</td>
<td>16.4</td>
<td>1.21</td>
<td>22.3</td>
<td>1.35</td>
</tr>
</tbody>
</table>

### TABLE 5.13
Reduced velocities below which columns cease to be infinite diameter.

\( L = 145 \text{ mm.} \) columns packed using the "rotate tap bounce method".

<table>
<thead>
<tr>
<th>( dc / \text{mm.} )</th>
<th>( \nu_{\text{crit.}} )</th>
<th>( \log \nu_{\text{crit.}} )</th>
<th>( \nu'_{\text{crit.}} )</th>
<th>( \log \nu'_{\text{crit.}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>defined by eqn. 5.14</td>
<td>defined by eqn. 5.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>2.2</td>
<td>0.34</td>
<td>3.0</td>
<td>0.48</td>
</tr>
<tr>
<td>5.1</td>
<td>4.8</td>
<td>0.68</td>
<td>7.9</td>
<td>0.90</td>
</tr>
<tr>
<td>4.2</td>
<td>8.8</td>
<td>0.94</td>
<td>16.8</td>
<td>1.23</td>
</tr>
<tr>
<td>3.2</td>
<td>33.0</td>
<td>1.52</td>
<td>169.0</td>
<td>2.23</td>
</tr>
<tr>
<td>2.3</td>
<td></td>
<td></td>
<td>&quot;non-infinite diameter at all reduced velocities&quot;</td>
<td></td>
</tr>
</tbody>
</table>
The plate height curves for the 7 mm. bore column (figure 5.6) illustrate the excellent efficiency which can be obtained using the "rotate tap bounce" packing method with relatively large (ie. 20 micron) Spherisorb Alumina. As with the 82.5 mm. vibrator packed columns, plate heights of about two particle diameters are obtained in the range of reduced velocities from 3 to 6.

Both equations 5.13 and 5.14 predict that the breakdown of infinite diameter geometry will only occur below a reduced velocity of 3 and therefore this column behaves as infinite diameter for all practical purposes.

The plate height curves obtained for a lightly retained and a retained solute for the 7 mm. diameter column are duplicated in figures 5.7 to 5.10 and are used as the standard measure of efficiency against which the remaining four column diameters are compared.

From figures 5.7 and 5.8 it can be seen that both the 5.1 and 4.2 mm. internal diameter columns operated at the efficiencies expected from infinite diameter operation at high reduced velocities but the reduced plate heights rise above the standard curves as the eluent velocity is lowered. \(v'_\text{crit}\) and \(v_{\text{crit}}\) are again indicated by "solid" and "dotted" arrows respectively. The break away point for each column can be seen to be greater than \(v_{\text{crit}}\) and to be in close agreement with \(v'_\text{crit}\).

From figure 5.9 it can be seen that the 3.2 mm. diameter column does not operate in the infinite diameter mode at the reduced velocities used in this study. \(v_{\text{crit}}\) predicted by equation 5.14 is equal to 33
and is clearly not applicable, however \( v'_{\text{crit}} \) is equal to 169 and is outside the experimental range of reduced velocities.

The 2.3 mm. diameter column does not operate as infinite diameter at any eluent velocity as is predicted by both equations. Plate heights for this column are about two or three times greater than for the 7 mm. column.

It seems clear from this study that the original "infinite diameter equation" is not applicable to this series of columns but that the modified theory fits in well with the experimental data. This is particularly evident for the 3.2 mm. column where equation 5.14 predicts infinite diameter operation within the range of experimental velocities but in practice its efficiency is similar to that of the non-infinite diameter 2.3 mm. column.

5.4 Conclusion

The concept of infinite diameter column operation is of paramount importance in the search for higher efficiencies in H.P.L.C. For optimum performance, column dimensions should be chosen taking into account both the particle diameter employed and also the lowest reduced velocity likely to be encountered. Thus a 145 X 5.1 mm. column of 20 micron particles may be run efficiently at reduced velocities above 10. Although a 7.0 mm. column would be theoretically more sound, the practical aspects of cost of packing material and volume of eluent used must also be taken into account. (Both the eluent flow rate and the weight of packing material used would be approximately doubled in going from a 5.1 to a 7.0 mm. column.)
The extent of the disturbed wall region suggested by Knox (70) and its effect in reducing the effective diameter of the column available to the solute as it migrates down the column has been supported by this study. It is not possible to obtain quantitative information on the "thickness of the wall layer", but a region of disturbed packing some 30 particle diameters deep is not inconsistent with the experimental data presented in this chapter.
CHAPTER 6
High Performance Liquid Chromatography of Aromatic Sulphonic Acids

The sulphonic acid group (-SO$_3$H) is widely used in the chemical industry, particularly in dye manufacture, in order to confer water solubility on otherwise hydrophobic compounds.

The chromatographic separation of sulphonic acids is a problem which has not yet been satisfactorily resolved. Their direct analysis by gas chromatography is precluded because of their low volatility and thermal instability. Therefore before G.C. can be used the sulphonic acid group must be derivatised, either to the acid chloride, (71) or to the sulphonate ester (72). The use of "pyrolysis gas chromatography" (73) has also been suggested.

Separations of sulphonic acids by "classical" liquid chromatography have been carried out using either cellulose or silica as the support. Results have been published from column chromatography (74), thin layer chromatography (75) and paper chromatography (76).

At the time of starting this work, the only H.P.L.C. separations of aromatic sulphonic acids had been by anion exchange chromatography on Zipax$^R$ SAX (77). Although the selectivity by anion exchange was more than adequate, the column efficiency of 40 micron Zipax SAX was unsatisfactory in comparison with the efficiencies current in other branches of H.P.L.C. Unfortunately at that time no high efficiency ion exchange material was yet commercially available. Such materials are now being produced and in the future ion exchange chromatography will
undoubtedly prove useful for the analysis of aromatic sulphonic acids.

The use of microparticulate silica with mixed aqueous/organic eluents was investigated but again the efficiencies were very low. For example with a Spherisorb silica adsorbent and a water : methylene chloride : methanol eluent, toluene was eluted with an efficiency of 1700 theoretical plates while sulphanilic acid was eluted with only 130 theoretical plates. This excessively poor efficiency was attributed to adsorption effects of the sulphonic acid group and therefore a chromatographic system was sought which relied on effects other than the adsorption of the sulphonic acid function. (In general it has been found that highly polar compounds cannot be satisfactorily chromatographed since they compete with water for the active sites on the silica surface.)

At this time considerable interest was being shown in partition chromatographic systems based on the ability to extract ionic species into organic solvents by forming an ion-pair with a suitable counter ion (78). Typical partitioning systems have an aqueous stationary phase supported on cellulose or silica and a mobile phase consisting of chloroform or methylene chloride containing a few per cent of a higher alcohol.

Systems of this type using tetra-alkylammonium counter ions to form ion-pairs with the sulphonic acids were tried but without success due to residual interactions of the sulphonic acid group with the support. However during these experiments it was discovered that excellent separations could be achieved using the hexadecyltrimethylammonium ion as a counter ion if a propanol/water eluent was used with a silica
support. The mechanism of solute retention in this system could not be rationalised in terms of a simple theory of ion-pair partition chromatography since there was no evidence for the development of a suitable stationary phase on the silica surface which could explain the retention behaviour.

Initial experiments were carried out using Partisil, a micro-particulate silica support, but later in order to elucidate the mechanism of retention, experiments were carried out using a chemically bonded stationary phase. Using data from these later experiments it has been possible to formulate a theory to explain the retention behaviour on the chemically bonded phase. The mechanism of retention on silica is more complex and is not yet fully understood.

6.1 Retention of Sulphonic acids on SAS

SAS is a chemically bonded phase material produced by the Wolfson Liquid Chromatography Unit and was manufactured by treating porous spherical silica with alkyl silanes. The basic mobile phase was a propanol/water mixture containing of the order of one per cent of hexadecyltrimethylammonium bromide. (Hexadecyltrimethylammonium bromide is commonly named cetrimide and will be referred to as such below.)

To obtain a reasonable degree of retention of sulphonic acids, the proportion of water to propanol in the eluent must be of the order of 2.5 to 1. The elution order of the acids is unaffected by the proportion of water to propanol so that variation of the concentration of propanol serves as a convenient method of obtaining the optimum separation time. An increase in the water content of the eluent
increases the retention of the acids while an increase in the propanol concentration decreases retention. This is the behaviour one would normally expect in reverse phase chromatography where a relatively non-polar molecule is partitioned into a lipophilic stationary phase.

The SAS stationary phase consists of a thin layer of hydrocarbon bonded to a silica surface, however with the passage of a water/propanol mobile phase the stationary phase will increase its polarity by selectively extracting propanol from the mobile phase. From bulk extraction experiments, organic solvents containing a substantial concentration of propanol are known to be very good solvents for ion-pairs. The "effective" stationary phase will therefore be a mixture of hydrocarbon and propanol and will also contain some cetrimide. In order to determine the amount of cetrimide adsorbed onto the stationary phase the following experiment was performed.

A column, packed with SAS, was equilibrated with a mobile-phase consisting of water and propanol in the ratio of 2.5 to 1. A given concentration of cetrimide in the same eluent was then pumped through the column. As the stationary phase extracted cetrimide from the mobile phase, a concentration front was formed. By measuring the volume of mobile phase required to elute this front, the amount of cetrimide adsorbed by the stationary phase could be calculated.

The volume of mobile phase necessary to elute the cetrimide front was measured by collecting 0.2ml. aliquots of eluent in a series of sample tubes containing approximately equal volumes of propanol, methylene chloride and a dilute aqueous solution of the dye sunset
yellow. When this mixture is shaken up and allowed to separate into two layers, the dye is partitioned strongly into the aqueous layer. However when the added aliquot of eluent contains cetrimide, the dye is extracted into the organic phase as the ion-pair. This therefore serves as a simple method of measuring the breakthrough volume of cetrimide.

Let the void volume of the column be \( V_0 \) and the volume of the mobile phase required to elute the cetrimide front be \( V_R \). The weight of adsorbed cetrimide is then found by multiplying the concentration of cetrimide in the mobile phase by the net retention volume \( (V_R - V_0) \).

The experimental results are shown in Table 6.1.

<table>
<thead>
<tr>
<th>Conc. of cetrimide in eluent. (gms./per 100 mls.)</th>
<th>( (V_R - V_0) ) (mls)</th>
<th>Wt. of adsorbed cetrimide per gm. of support. (mgs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>1.1</td>
<td>-12.9</td>
</tr>
<tr>
<td>1.0</td>
<td>1.4</td>
<td>8.2</td>
</tr>
<tr>
<td>0.25</td>
<td>1.8</td>
<td>2.7</td>
</tr>
</tbody>
</table>

The weight of SAS in the column was 1.7g., \( V_0 \) was 1.75 mls.

Figure 6.1 shows the plot of weight of cetrimide adsorbed per gm. of support against the percentage w/v of cetrimide in the eluent. The amount of cetrimide adsorbed per gm. of SAS with an eluent containing 2% cetrimide is about 13 mgs., ie. approximately \( 2 \times 10^{19} \) molecules. With a support surface area of 200 \( \text{m}^2 \) g.\(^{-1}\) this means that on average there will be one cetrimide molecule per 1000 \( \AA^2 \) of surface. The molecular volume of cetrimide is about 500 \( \AA^3 \). Assuming a "depth" for
the cetrimide molecule of the order of 5 Å then we can calculate that the degree of surface coverage will be between 5 and 15%. This relatively low surface coverage suggests that the molecule lies flat on the surface of the support.

6.2 Proposed theory of solute retention on SAS

From figure 6.1 it can be seen that the adsorption isotherm for cetrimide on SAS is of the simple Freundlich type and can be represented by the equation:

\[(c^+)_{ads} = \alpha \cdot (c^+)_{aq}^{0.8}\]  \[6.1\]

where the subscripts ads and aq refer to the cetrimide concentrations in the stationary and mobile phases respectively.

Partition of the sulphonic acid ions between the mobile and stationary phases can be represented by the equation:

\[(s^{n-})_{ads} = \beta \cdot (s^{n-})_{aq}\]  \[6.2\]

where \(n\) is the charge on the sulphonate ion.

The equilibrium constants for ion-pair formation in the mobile and stationary phases will be given by:

\[K_{aq} = \frac{(n \cdot c^+ s^{n-})_{aq}}{(c^+)_{aq}^n \cdot (s^{n-})_{aq}}\]  \[6.3\]

and

\[K_{ads} = \frac{(n \cdot c^+ s^{n-})_{ads}}{(c^+)_{ads}^n \cdot (s^{n-})_{ads}}\]  \[6.4\]

The distribution coefficient \(D\) between the stationary and mobile phase is given by:
D = \frac{\text{(total conc. of sulph. acid in stat. phase)}}{\text{(total conc. of sulph. acid in mobile phase)}} \\
= \frac{(S^{-})_{\text{ads}} + (n.C^{+}S^{-})_{\text{ads}}}{(S^{-})_{\text{aq}} + (n.C^{+}S^{-})_{\text{aq}}}

In the relatively lipophilic environment of the stationary phase there will be very little dissociation of the ion-pair, ie.

\[ (S^{-})_{\text{ads}} \ll (n.C^{+}S^{-})_{\text{ads}} \]

With this assumption and substituting using equation 6.4, equation 6.5 becomes:

\[ D = \frac{K_{\text{ads}} \cdot (C^{+})^{n}_{\text{ads}} \cdot (S^{-})_{\text{ads}}}{(S^{-})_{\text{aq}} + (n.C^{+}S^{-})_{\text{aq}}} \]

\[ = \frac{K_{\text{ads}} \cdot \alpha^{n} \cdot (C^{+})^{0.8n}_{\text{aq}} \cdot \beta \cdot (S^{-})_{\text{aq}}}{(S^{-})_{\text{aq}} + (n.C^{+}S^{-})_{\text{aq}}} \]

\[ = \frac{\alpha^{n} \cdot \beta \cdot K_{\text{ads}} \cdot (C^{+})^{0.8n}_{\text{aq}}}{1 + K_{\text{aq}} \cdot (C^{+})^{n}_{\text{aq}}} \]

This equation shows that the solute capacity factor, K', which is proportional to D will increase with (C^{+})_{aq} rapidly at low concentration and then more slowly as the magnitude of the denominator increases. At sufficiently high cetrimide concentrations D will become proportional to (C^{+})^{-0.2n}_{aq} and hence the solute capacity factor would start to decrease again.

At sufficiently high concentrations of cetrimide in solvents
containing a large proportion of water, it is likely that micelles will be formed. The presence of micelles is known to increase the solubility of sulphonic acids by association of the sulphonate ion with the positively charged surface of the micelle. When a sulphonated molecule also contains a significant hydrophobic portion then the solubility may be further enhanced by insertion of this portion into the interior of the micelle. (79) This process is termed solubilisation.

The critical micelle concentration of cetrimide in distilled water is $9.8 \times 10^{-4}$ moles per litre. (80) The addition of small amounts of propanol decreases the critical micelle concentration (CMC) due to a lowering of the free energy of the micelle resulting from the incorporation of the alcohol into the micelle structure. By X-ray diffraction studies it has been established that the propanol molecules are orientated within the micelle such that the hydrocarbon "tail" penetrates into the interior of the micelle with the hydroxyl group remaining on the surface. (81) Quantitative data on the effect of added propanol on the CMC of cetrimide has not been published but its effect on the CMC of decyltrimethylammonium bromide has been reported by Harkin et al.. (82) They found that the addition of 15% of propanol lowers the CMC of decyltrimethylammonium bromide by about a factor of three. At higher concentrations of alcohols the CMC rises again due to a lowering of the dielectric constant of the solvent medium but again no quantitative data has been published. It should also be noted that the CMC is less easily defined in a solution containing a substantial concentration of propanol. (81).
In the context of these experiments it is sufficient to say that it is likely that as the cetrimide concentration is increased cetrimide ions will tend to form clusters such that a number of cetrimide ions will be associated with each sulphonate ion. ie. 

\[ \text{m} \ C^+_{\text{aq}} + \text{n} \ S^-_{\text{aq}} \leftrightarrow (\text{m} \ C^+ \text{n} S^-)_{\text{aq}} \]

Incorporating this into equation 6.6 gives:

\[ D = \frac{\alpha^n \beta \cdot K_{\text{ads}} \cdot (C^+)_{\text{aq}}^{0.2n}}{1 + K^*_{\text{aq}} \cdot (C^+)_{\text{aq}}^{m}} \]

where \( K^*_{\text{aq}} \) replaces the ion-pair dissociation constant and \( (C^+)_{\text{aq}} \) appears in the denominator with an exponent of \( m \).

When no micelle formation takes place \( m = n \) and equations 6.6 and 6.7 are identical, however as clusters of cetrimide ions start to form at higher concentrations (ie. \( m > n \)), the denominator will increase more rapidly than the numerator and solute retention will start to decrease. Whereas equation 6.6 predicts a maximum negative gradient of a graph of \( \log K' \) against \( \log (C^+)_{\text{aq}} \) of 0.2\( n \), equation 6.7 shows that association of cetrimide molecules to form clusters will result in an increase in the magnitude of the negative gradient.

6.3 Experimental

The chromatographic supports used in this work were Partisil\textsuperscript{R}, an irregular microparticulate silica and SAS, a short chain hydrocarbon chemically bonded phase. The particle size of the Partisil used in the bulk of the work was 10 micron, however practical applications were performed using 6 micron Partisil in order to achieve higher efficiencies.
The SAS chemically bonded phase which was prepared by the Wolfson Liquid Chromatography Unit had a surface area of about 200 m$^2$ g$^{-1}$ and an average particle size of 7 microns.

Columns were constructed from 5mm. i.d., 1/4 inch o.d. stainless steel tubing and were either 100 or 120 mm. in length. Both supports were slurry packed, methyl iodide was used as the slurrying solvent for SAS and methanol was used for Partisil.

A Pye gas chromatography oven was used to run columns at elevated temperatures. A coil of stainless steel tubing positioned before the analytical column ensured temperature equilibration of the eluent.

Diffusion coefficients were calculated using the Wilke Chang equation (9). The value found for anisole and nitrobenzene in n-hexane was $3.3 \times 10^{-5}$ cm$^2$ s$^{-1}$ while the values for sulphanilic acid, R-acid and Ponceau MX in 3:1, propanol:water were 1.1, 0.74 and $0.68 \times 10^{-6}$ cm$^2$ s$^{-1}$ respectively.

The samples of sulphonic acids used in this study were kindly supplied by I.C.I. Organics Division (Manchester) and are listed in table 6.2. Cetrimide was purchased from BDH (Poole, Dorset).

1 to 5 microlitres of sample solution (about 1 mg.ml$^{-1}$) were injected on-column using a micro-syringe and septum injector.

6.4 The effect of Cetrimide concentration on solute retention on SAS

With a ratio of water to propanol in the eluent of 2.5 to 1, the effect of varying the cetrimide concentration on the retention of six sulphonlic acids was investigated. Cetrimide concentrations from 1/32 to 2% w/v i.e. $8.6 \times 10^{-4}$ to $5.5 \times 10^{-2}$ M. were used. Table 6.3
TABLE 6.2

Systematic names of the sulphonic acids used in chapter 6

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Systematic Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schaffers acid</td>
<td>2-naphthol-6-sulphonic acid</td>
</tr>
<tr>
<td>Neville and Winthers acid</td>
<td>1-naphthol-4-sulphonic acid</td>
</tr>
<tr>
<td>Naphthionic acid</td>
<td>1-naphthylamine-4-sulphonic acid</td>
</tr>
<tr>
<td>Sulphanilic acid</td>
<td>aniline-4-sulphonic acid</td>
</tr>
<tr>
<td>R-acid</td>
<td>2-naphthol-3,6-disulphonic acid</td>
</tr>
<tr>
<td>G-acid</td>
<td>2-naphthol-6,8-disulphonic acid</td>
</tr>
<tr>
<td>J-acid</td>
<td>6-amino-1-naphthol-3-sulphonic acid</td>
</tr>
<tr>
<td>Gamma acid</td>
<td>7-amino-1-naphthol-3-sulphonic acid</td>
</tr>
<tr>
<td>Di-oxy-J-acid</td>
<td>2,5-dihydroxynaphthalene-7-sulphonic acid</td>
</tr>
<tr>
<td></td>
<td>5-sulpho-isatin</td>
</tr>
<tr>
<td></td>
<td>1(4'-sulphophenyl)-3-carboxy-5-pyrazalone</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Azo Dye</th>
<th>Diazotized Component</th>
<th>Coupled Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunset yellow</td>
<td>sulphanilic acid</td>
<td>schaffers acid</td>
</tr>
<tr>
<td>Tartrazine</td>
<td>sulphanilic acid</td>
<td>1(4'-sulphophenyl)-3-carboxy-5-pyrazalone</td>
</tr>
<tr>
<td>Ponceau MX</td>
<td>2,4-dimethyl-aniline</td>
<td>R-acid</td>
</tr>
<tr>
<td>Ponceau 4R</td>
<td>naphthionic acid</td>
<td>G-acid</td>
</tr>
<tr>
<td>Carmoisine</td>
<td>naphthionic acid</td>
<td>neville &amp; winthers acid</td>
</tr>
<tr>
<td>Amaranth</td>
<td>naphthionic acid</td>
<td>R-acid</td>
</tr>
</tbody>
</table>
shows the experimental results obtained. A plot of the logarithms of the capacity factors of the six sulphonic acids against the concentration of cetrimide in the mobile phase is shown in figure 6.2.

It can be seen that the capacity factors increase with the number of sulphonic acid groups in the solute molecule. Sulphanilic and shaffers acids have one sulphonic group, sunset yellow, tartrazine and ponceau MX have two while ponceau 4R has three. Ponceau 4R was very strongly retained at intermediate cetrimide concentrations and its capacity factor could not be measured.

With no cetrimide present in the mobile phase, all the sulphonic acids were unretained. With the addition of cetrimide, the retention of the solutes initially increased in accordance with equation 6.6, however when the concentration of cetrimide was increased beyond 0.25% w/v the capacity factors began to decrease again. The negative gradients were about 0.5 for the mono-acids, 1.0 for the di-acids and 1.3 for the tri-acid. These values compare with the maximum negative gradients allowed by equation 6.6 of 0.2, 0.4 and 0.6 respectively. It would appear therefore that the solutes are complexing with more than their stoichiometric equivalent of cetrimide ions and therefore behaving according to equation 6.7.

As discussed, the CMC of cetrimide in this eluent is not known but it is likely to be within an order of magnitude of the CMC in distilled water. In fact the cetrimide concentration at maximum retention (0.25%) is equivalent to $6.9 \times 10^{-3}$ M compared to $9.8 \times 10^{-4}$ M for the CMC of cetrimide in distilled water. The experimental value for the concentration
TABLE 6.3

The effect of Cetrimide concentration on the capacity factors of six sulphonic acids on SAS.

Eluent was 1 : 2.5 propanol : water with x% w/v Cetrimide

<table>
<thead>
<tr>
<th>Solute</th>
<th>1/32% Cet.</th>
<th>1/16% Cet.</th>
<th>1/8% Cet.</th>
<th>1/4% Cet.</th>
<th>1/2% Cet.</th>
<th>1% Cet.</th>
<th>2% Cet.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K’</td>
<td>log K’</td>
<td>K’</td>
<td>log K’</td>
<td>K’</td>
<td>log K’</td>
<td>K’</td>
</tr>
<tr>
<td>Schaffer’s acid</td>
<td>0.37</td>
<td>-0.43</td>
<td>0.70</td>
<td>-0.15</td>
<td>1.06</td>
<td>0.03</td>
<td>1.26</td>
</tr>
<tr>
<td>Sulphanilic acid</td>
<td>0.12</td>
<td>-0.92</td>
<td>0.34</td>
<td>-0.47</td>
<td>0.56</td>
<td>-0.24</td>
<td>0.69</td>
</tr>
<tr>
<td>Sunset yellow</td>
<td>1.05</td>
<td>0.02</td>
<td>2.12</td>
<td>0.33</td>
<td>3.90</td>
<td>0.59</td>
<td>4.26</td>
</tr>
<tr>
<td>Tartrazine</td>
<td>1.86</td>
<td>0.27</td>
<td>5.17</td>
<td>0.71</td>
<td>11.99</td>
<td>1.08</td>
<td>13.43</td>
</tr>
<tr>
<td>Ponceau MX</td>
<td>4.22</td>
<td>0.63</td>
<td>7.61</td>
<td>0.88</td>
<td>13.26</td>
<td>1.12</td>
<td>12.97</td>
</tr>
<tr>
<td>Ponceau 4R</td>
<td>11.40</td>
<td>1.06</td>
<td>---------</td>
<td>strongly retained</td>
<td>27.50</td>
<td>1.44</td>
<td>12.97</td>
</tr>
</tbody>
</table>
of cetrimide at maximum retention is therefore within the range of values expected for micelle formation and thus the decrease in solute retention as the cetrimide concentration is increased can be ascribed to the association of the sulphonate ion with positively charged clusters of cetrimide ions. From figure 6.2 it can be seen that the concentration of cetrimide corresponding to maximum retention is identical within experimental error for all the solutes. This mitigates strongly in favour of an explanation for the decrease in solute retention which is dependent on the cetrimide concentration and is essentially independent of solute parameters.

6.5 Applications of SAS in the separation of sulphonic acids

Two separations of sulphonic acids are shown in figures 6.9 and 6.10. Figure 6.9 shows the separation of four 1-naphthylamine-sulphonic acid isomers which are useful intermediates in the dye industry. Baseline separation was achieved in ten minutes with a column head pressure of only 700 p.s.i.

Figure 6.10 shows the separation of J-acid from three major impurities which are present in industrial samples. The separation of the closely related isomers, J and Gamma acids (6-amino-1-naphthol-3-sulphonic acid and 7-amino-1-naphthol-3-sulphonic acid respectively) illustrates the excellent selectivity of the system.

The high efficiency of the 7 micron SAS used in these two separations combined with the excellent selectivity make these systems potentially very useful in the analysis of complex reaction mixtures.
6.6 Chromatography of sulphonic acids on Partisil\textsuperscript{R} silica

Partisil\textsuperscript{R} is an irregular silica which has proved to be a very efficient support for H.P.L.C. (20) In order to obtain a reasonable degree of retention of sulphonated compounds in the presence of cetrimide the proportion of water to propanol in the eluent should be of the order of one to three. An increase in the proportion of water in the eluent decreases retention which is the behaviour one would expect in a "normal phase" system, i.e. with a polar stationary phase. As with SAS, the retention order is not effected by the proportion of water to propanol in the eluent so that variation of the water content serves as a convenient method of optimising solute retention.

As one would expect, lowering the pH of the eluent decreases the retention of the carboxylated compounds relative to the purely sulphonated compounds by discouraging the dissociation of the carboxylic group and hence its ability to complex with a cetrimide ion. Data showing the effect of sulphuric acid concentration on the retention of five sulphonic acids is given in table 6.4. A plot of log $K'$ against log (H\textsubscript{2}SO\textsubscript{4}) for the five acids is shown in figure 6.3. This figure illustrates that pH can be a useful variable in optimising a separation if one of the components of the mixture has a carboxylic acid function, however pH has little effect on the selectivity among purely sulphonated compounds. The capacity factors of the four purely sulphonated compounds are reduced by a factor of about four over the range of sulphuric acid concentrations used due probably to the competing effect of the sulphate ions for the cetrimide. The molarity of the two ions in the eluent are
comparable, the highest molarity of sulphuric acid used was $2.6 \times 10^{-2}$ M compared to the molarity of a 1% solution of cetrimide of $2.7 \times 10^{-2}$ M.

All the sulphonic acids tested were retained less strongly as the temperature was raised. This is shown in figure 6.4 where the logarithms of the solute capacity factors of three sulphonic acids are plotted against the reciprocal of the absolute temperature for the **basic eluent composition:** propanol:water 3:1, with 1% cetrimide. The data is tabulated in table 6.5.

The linearity of the plot allows the heats of adsorption to be calculated. The values found for Ponceau 4R, Ponceau MX and R-acid are 32, 21 and 17 kJ.mole$^{-1}$ respectively.

Elevated temperatures have been used to increase the speed and/or resolution in analysis by H.P.L.C. but the advantages gained are usually outweighed by the added complication of thermostating the column. Consequently all the practical separations in this chapter were carried out at ambient temperatures.

6.7 The effect of Cetrimide concentration on solute retention on Partisil

The effect of the concentration of cetrimide on the retention of sixteen sulphonic acid dyes and dye intermediates is shown in figure 6.5 for the **basic eluent:** propanol:water with 1% cetrimide. The experimental data are listed in table 6.6.

The following general comments can be made on the retention behaviour of the sulphonic acids:

(a) The capacity factors of all the sulphonic acids increase with the concentration of cetrimide in the eluent.
TABLE 6.4

The effect of adding sulphuric acid to the eluent on the solute capacity factors on Partisil®. Eluent was 3:1, propanol:water, containing 1% cetrimide and varying concentrations of sulphuric acid.

<table>
<thead>
<tr>
<th>Solute</th>
<th>2.6 X 10^{-2} M</th>
<th>8.5 X 10^{-3} M</th>
<th>2.8 X 10^{-3} M</th>
<th>0.0 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>K' log K' K' log K' K' log K' K' log K' K' log K'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schaffer's acid</td>
<td>0.09 -1.05</td>
<td>0.19 -0.72</td>
<td>0.28 -0.55</td>
<td>0.34 -0.47</td>
</tr>
<tr>
<td>Sunset yellow</td>
<td>0.32 -0.49</td>
<td>0.70 -0.15</td>
<td>0.98 -0.01</td>
<td>1.28 0.11</td>
</tr>
<tr>
<td>Tartrazine</td>
<td>0.28 -0.55</td>
<td>0.72 -0.14</td>
<td>1.46 0.16</td>
<td>3.50 0.54</td>
</tr>
<tr>
<td>Ponceau MX</td>
<td>0.53 -0.28</td>
<td>1.06 0.03</td>
<td>1.50 0.18</td>
<td>1.91 0.28</td>
</tr>
<tr>
<td>Ponceau 4R</td>
<td>1.44 0.16</td>
<td>3.33 0.52</td>
<td>5.04 0.70</td>
<td>---------</td>
</tr>
</tbody>
</table>

TABLE 6.5

The effect of temperature on solute retention on Partisil®. The eluent was 3:1, propanol:water, with 1% cetrimide.

<table>
<thead>
<tr>
<th>Solute</th>
<th>18.5°C</th>
<th>34°C</th>
<th>48°C</th>
<th>63°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>K' log K' K' log K' K' log K' K' log K'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-acid</td>
<td>1.09 0.04</td>
<td>0.82 -0.09</td>
<td>0.53 -0.28</td>
<td>0.44 -0.36</td>
</tr>
<tr>
<td>Ponceau MX</td>
<td>2.15 0.33</td>
<td>1.37 0.14</td>
<td>0.94 -0.03</td>
<td>0.74 -0.13</td>
</tr>
<tr>
<td>Ponceau 4R</td>
<td>9.64 0.98</td>
<td>4.80 0.68</td>
<td>2.87 0.46</td>
<td>1.99 0.30</td>
</tr>
</tbody>
</table>
(b) The selectivity is virtually independent of the concentration of cetrimide in the eluent.
(c) The degree of retention increases with the number of sulphonic groups present in the molecule and to a lesser extent with molecular size.
(d) The presence of a carboxylic acid function in anthranilic acid, sulpho-phenylcarboxypyrazalone and the dye tartrazine substantially increases their retention.

The variation of solute capacity factors over a wider range of cetrimide concentrations is shown in figure 6.6 for the same six sulphonic acids used for the study on SAS. The experimental data is given in table 6.7. (These capacity factors differ in magnitude from those in table 6.6 due to a difference in the surface area of the Partisil used. However the selectivity, i.e. the capacity factor of each acid relative to all the others, is unchanged.)

From figure 6.6 it can be seen that the elution order of the six sulphonic acids on Partisil is very similar to that found when SAS was used as the support. In particular a retention mechanism involving a reversal of the phases from the SAS system cannot be reconciled with the similarity of retention order.

Solute retention is therefore probably due to adsorption of the sulphonate/cetrimide ion-pair onto the silica support. Below 0.125% cetrimide in the eluent, the proportion of ion-pairs is insufficient to provide reasonable retention while at high concentrations (greater than 2%), most of the solute molecules are present as ion-pairs and
TABLE 6.6

The effect of cetrimide concentration on the capacity factors of sixteen sulphonic acids on Partisil R. The eluent was 3:1, propanol: water, with x% w/v cetrimide.

<table>
<thead>
<tr>
<th>Solute</th>
<th>1/2 % Cet.</th>
<th>1 % Cet.</th>
<th>2 % Cet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K'</td>
<td>log K'</td>
<td>K'</td>
</tr>
<tr>
<td>Sulphanilic acid</td>
<td>0.23</td>
<td>-0.64</td>
<td>0.33</td>
</tr>
<tr>
<td>Schaffer's acid</td>
<td>-----------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Naphthionic acid</td>
<td>0.19</td>
<td>-0.72</td>
<td>0.22</td>
</tr>
<tr>
<td>Neville and Winthers acid</td>
<td>-----------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>R-acid</td>
<td>0.37</td>
<td>-0.43</td>
<td>0.60</td>
</tr>
<tr>
<td>5-sulphoanthranilic acid</td>
<td>0.87</td>
<td>-0.06</td>
<td>1.34</td>
</tr>
<tr>
<td>G-acid</td>
<td>0.36</td>
<td>-0.44</td>
<td>0.57</td>
</tr>
<tr>
<td>5-sulphoisatin</td>
<td>-----------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Sulphophenylcarboxy pyrazalone</td>
<td>1.34</td>
<td>0.13</td>
<td>2.12</td>
</tr>
<tr>
<td>Sunset yellow</td>
<td>0.38</td>
<td>-0.42</td>
<td>0.63</td>
</tr>
<tr>
<td>Tartrazine</td>
<td>0.82</td>
<td>-0.09</td>
<td>1.44</td>
</tr>
<tr>
<td>Amaranth</td>
<td>0.61</td>
<td>-0.21</td>
<td>1.01</td>
</tr>
<tr>
<td>Ponceau MX</td>
<td>0.67</td>
<td>-0.17</td>
<td>1.09</td>
</tr>
<tr>
<td>Carmoisine</td>
<td>0.64</td>
<td>-0.19</td>
<td>0.99</td>
</tr>
<tr>
<td>Ponceau 4R</td>
<td>2.29</td>
<td>0.36</td>
<td>4.04</td>
</tr>
<tr>
<td>Blue X</td>
<td>0.29</td>
<td>-0.54</td>
<td>0.47</td>
</tr>
</tbody>
</table>
hence further increases in the concentration of cetrimide have only a small effect on the capacity factors. There is no indication of a decrease in retention of the sulphonamic acids at high cetrimide concentrations as was found with the chromatography on SAS. This is as one would expect since no micelle formation will take place in an eluent containing 75% propanol.

In contrast to the SAS system, there is no evidence for the formation of a hydrophobic stationary phase which could solvate the ion-pair. More work must be done to establish the mechanism of retention but at present the best explanation is that of adsorption of the sulphonate/cetrimide ion-pair onto the silica surface.

6.8 The effect on solute retention of adding "solvent modifiers"

Nine different solvents were screened to ascertain their effect on solute retention by measuring the capacity factors of seven sulphonamic acids in eluents with the general composition: propanol:water: solvent, 15:5:4, with 1% cetrimide. The results are given in table 6.8 and shown graphically in figure 6.7.

It can be seen that the addition of polar organic solvents to the basic 3:1, propanol:water eluent increases retention while the addition of water decreases retention. The addition of tetrahydrofuran and hexane have little effect while methylene chloride and acetonitrile decrease retention. This behaviour is qualitatively consistent with a retention mechanism based on the adsorption of ion-pairs since polar organic molecules such as ethyl acetate and the higher alcohols are very effective in solvating ion-pairs and hence their addition to the
TABLE 6.7

The effect of cetrimide concentration on the capacity factors of six sulphonic acids on Partisil\textsuperscript{R}.

The eluent was 3:1, propanol:water, with x% w/v of cetrimide.

<table>
<thead>
<tr>
<th>Solute</th>
<th>1/8 % Cet.</th>
<th>1/4 % Cet.</th>
<th>1/2 % Cet.</th>
<th>1 % Cet.</th>
<th>2 % Cet.</th>
<th>4 % Cet.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K' log K'</td>
<td>K' log K'</td>
<td>K' log K'</td>
<td>K' log K'</td>
<td>K' log K'</td>
<td>K' log K'</td>
</tr>
<tr>
<td>Schaffer's acid</td>
<td>-----------</td>
<td>0.04 -1.40</td>
<td>0.14 -0.85</td>
<td>0.25 -0.60</td>
<td>0.27 -0.57</td>
<td>0.30 -0.52</td>
</tr>
<tr>
<td>Sulphanilic acid</td>
<td>-----------</td>
<td>0.21 -0.68</td>
<td>0.32 -0.49</td>
<td>0.51 -0.29</td>
<td>0.51 -0.29</td>
<td>0.52 -0.28</td>
</tr>
<tr>
<td>Sunset yellow</td>
<td>-----------</td>
<td>0.35 -0.46</td>
<td>0.59 -0.23</td>
<td>0.99 0.00</td>
<td>1.23 0.09</td>
<td>1.35 0.13</td>
</tr>
<tr>
<td>Ponceau MX</td>
<td>0.23 -0.64</td>
<td>0.68 -0.17</td>
<td>1.04 0.02</td>
<td>1.70 0.23</td>
<td>2.28 0.36</td>
<td>2.68 0.43</td>
</tr>
<tr>
<td>Tartrazine</td>
<td>0.23 -0.64</td>
<td>0.89 -0.05</td>
<td>1.58 0.20</td>
<td>2.84 0.45</td>
<td>3.45 0.54</td>
<td>3.82 0.58</td>
</tr>
<tr>
<td>Ponceau 4R</td>
<td>0.93 -0.03</td>
<td>2.75 0.44</td>
<td>4.05 0.61</td>
<td>6.75 0.83</td>
<td>10.44 1.02</td>
<td>13.50 1.13</td>
</tr>
</tbody>
</table>
eluent will increase the concentration of ion-pairs in the eluent and thus increase the probability of adsorption. Conversely increasing the water content of the eluent decreases retention by discouraging ion-pair formation. The addition of organic solvents which are not effective in solvating cetrimide ion-pairs either leave retention virtually unchanged or in the case of methylene chloride and acetonitrile decrease retention, presumably by inhibiting ion-pair formation.

The overall effect of adding a particular solvent to the eluent is however very complex. Not only will the equilibrium of the ion-pair/"free anion" be perturbed by the addition of a solvent but also the net adsorption energy of the ion-pair will depend on the competing solvent molecules and on the degree of solvation of the ion-pair.

From a practical point of view, it can be seen from figure 6.7 that the addition of auxiliary solvents can markedly change the selectivity of the system. This has been particularly useful in the separation of J-acid and gamma-acid which are not resolvable in the basic propanol/water eluent but can be separated by the addition of methylene chloride to the eluent. Figure 6.11 shows a separation of dioxy-J-acid, J-acid, gamma-acid and di-J-acid on Partisil with a propanol/methylene chloride/water eluent containing 2% w/v cetrimide.

6.9 Column performance of Partisil for the chromatography of sulphonic acids

With the development of a new chromatographic system it is important to ascertain whether a column is operating as efficiently as possible. As discussed in chapter three, the efficiency of a chromatographic column is best characterised by plotting the logarithm of the reduced plate
The effect of solvent 'modifiers' on solute capacity factors on Partisil®.

Eluents have the composition 15 : 5 : 4, propanol : water : solvent, with 1% cetrimide.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Ethyl acetate</th>
<th>n-Butanol</th>
<th>n-Propanol</th>
<th>Ethanol</th>
<th>Tetrahydrofurfan</th>
<th>n-hexane</th>
<th>Methylene chloride</th>
<th>Acetonitrile</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schaffer's acid</td>
<td>0.38</td>
<td>0.35</td>
<td>0.32</td>
<td>0.34</td>
<td>0.30</td>
<td>0.22</td>
<td>0.14</td>
<td>0.11</td>
<td>0.08</td>
</tr>
<tr>
<td>Sulphanilic acid</td>
<td>0.72</td>
<td>0.77</td>
<td>0.61</td>
<td>0.61</td>
<td>0.53</td>
<td>0.53</td>
<td>0.50</td>
<td>0.26</td>
<td>0.16</td>
</tr>
<tr>
<td>R-acid</td>
<td>1.72</td>
<td>1.44</td>
<td>1.43</td>
<td>1.33</td>
<td>1.17</td>
<td>0.77</td>
<td>0.49</td>
<td>0.39</td>
<td>0.33</td>
</tr>
<tr>
<td>Sunset yellow</td>
<td>1.74</td>
<td>1.46</td>
<td>1.48</td>
<td>1.49</td>
<td>1.25</td>
<td>0.70</td>
<td>0.39</td>
<td>0.50</td>
<td>0.34</td>
</tr>
<tr>
<td>Ponceau MX</td>
<td>3.20</td>
<td>2.49</td>
<td>2.80</td>
<td>2.55</td>
<td>2.02</td>
<td>1.29</td>
<td>0.70</td>
<td>0.97</td>
<td>0.77</td>
</tr>
<tr>
<td>Tartrazine</td>
<td>6.90</td>
<td>5.15</td>
<td>5.20</td>
<td>5.18</td>
<td>4.22</td>
<td>1.55</td>
<td>1.14</td>
<td>1.49</td>
<td>0.70</td>
</tr>
<tr>
<td>Ponceau 4R</td>
<td>17.63</td>
<td>10.98</td>
<td>14.95</td>
<td>12.65</td>
<td>9.98</td>
<td>5.16</td>
<td>1.94</td>
<td>3.88</td>
<td>3.01</td>
</tr>
</tbody>
</table>
height against the logarithm of the reduced velocity. From chromatographic theory (17), log h versus log v plots should be obtained for a given column which are essentially independent of the solute and mobile phases employed, provided that only kinetic factors are contributing to peak dispersion.

The efficiency of column operation when using aqueous eluents is however often low in comparison with that obtained with non-polar mobile phases. This can usually be assigned to added contributions to the overall peak dispersion by thermodynamic factors. Such factors may arise from non-linearity of partition coefficients or to inadequate "buffering" in one of the phases resulting in non-linearity of dissociation equilibria.

In order to establish that only kinetic factors are contributing to the overall peak dispersion, the use of reduced parameters enables one to compare the column efficiency in the system of interest with the efficiency when it is operated in a simple adsorption mode where it is known that only kinetic factors are contributing to peak dispersion.

The efficiency of a 9.5 cm. column of 10 micron Paritsil was therefore first measured for the compounds anisole and nitrobenzene with an eluent of one per cent acetonitrile in n-hexane. The eluent was then changed to one per cent cetrimide in 3:1, propanol:water and the efficiency measured for the compounds, sulphanilic acid, R-acid and Ponceau MX. The experimental data for the two systems are given in tables 6.9 and 6.10 respectively. (The diffusion coefficients of sulphanilic acid, R-acid and Ponceau MX were calculated on the
assumption that they are present in the eluent in association with cetrimide ions. The very low values of the diffusion coefficients are mainly due to the high viscosity of the propanol/water eluent.)

Figure 6.8 shows the plot of log h versus log v obtained from both sets of data. It can be seen that the experimental results from the column operated with both eluent systems fall on the same curve. This clearly demonstrates that the performance of the column when it is used for the separation of sulphonic acids is dependent only on the quality of the column and is not influenced by the polar nature of the solutes. The full performance of microparticulate silica columns can therefore be utilised for the separation of sulphonic acids. Figure 6.12 shows a separation achieved on 6 micron Partisil where the high efficiency (of the order of 5000 theoretical plates) enables sulphanilic acid to be resolved from another impurity in Sunset yellow.

6.10 Application of Partisil in the separation of sulphonic acids

A series of important food dyes are manufactured by coupling a diazotised aromatic component (often a sulphonic acid) with an aromatic sulphonic acid. Chromatograms showing the separations of two of these dyes from their intermediates are given in figures 6.12 and 6.13.

Figure 6.12 shows the separation of sulphanilic acid (the component which is diazotised), schaffers acid (the coupled component) and the final dye Sunset yellow. The position of triazine, a known impurity is Sunset yellow is also shown. Other peaks in the chromatogram are probably isomeric subsidiary dyes produced in the coupling reaction.
# Table 6.9

Data used to assess the efficiency of a 9.5 cm. column of 10 micron Partisil with a "non-polar" eluent. (1% v/v acetonitrile in n-hexane).

Solutes: Toluene (unretained), Anisole $K' = 0.8$, Nitrobenzene $K' = 2.0$.

<table>
<thead>
<tr>
<th>Pressure /p.s.i.</th>
<th>Eluent vel. /cm/sec$^{-1}$</th>
<th>log $v$</th>
<th>Anisole log $h$</th>
<th>Nitrobenzene log $h$</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>0.50</td>
<td>1.22</td>
<td>0.52</td>
<td>0.42</td>
</tr>
<tr>
<td>500</td>
<td>0.50</td>
<td>1.22</td>
<td>0.51</td>
<td>0.42</td>
</tr>
<tr>
<td>380</td>
<td>0.44</td>
<td>1.13</td>
<td>0.39</td>
<td>0.35</td>
</tr>
<tr>
<td>380</td>
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<td>0.84</td>
<td>0.26</td>
<td>0.20</td>
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</table>
**TABLE 6.10**

Data used to assess the efficiency of a 9.5 micron Partisil® column.

Eluent: 3 : 1, propanol : water with 1% cetrimide.

<table>
<thead>
<tr>
<th>Pressure /p.s.i.</th>
<th>u. /cm.s⁻¹</th>
<th>Sulphanilic acid Dm. = 1.1 X 10⁻⁶</th>
<th>R-acid Dm. = 0.74 X 10⁻⁶</th>
<th>Ponceau MX Dm. = 0.68 X 10⁻⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log v</td>
<td>Log h</td>
<td>Log v</td>
<td>Log h</td>
</tr>
<tr>
<td>760</td>
<td>0.167</td>
<td>2.17 1.35</td>
<td>2.35</td>
<td>2.40 1.57</td>
</tr>
<tr>
<td>760</td>
<td>0.158</td>
<td>2.15 1.43</td>
<td>2.33</td>
<td>2.38 1.55</td>
</tr>
<tr>
<td>580</td>
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<td>1.97 1.05</td>
<td>2.15 1.23</td>
<td>2.20 1.40</td>
</tr>
<tr>
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<td>2.22 1.45</td>
</tr>
<tr>
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<td>1.85 0.97</td>
<td>2.03 1.04</td>
<td>2.08 1.20</td>
</tr>
<tr>
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<td>1.85 0.94</td>
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</tr>
<tr>
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<td>0.064</td>
<td>1.76 0.58</td>
<td>1.94 0.92</td>
<td>1.98 1.02</td>
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<tr>
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<td>1.75 0.86</td>
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<td>1.75 0.87</td>
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<tr>
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<td>1.55 0.69</td>
<td>1.59 0.72</td>
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<tr>
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<td>1.37 0.49</td>
<td>1.55 0.63</td>
<td>1.59 0.76</td>
</tr>
<tr>
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<td>0.016</td>
<td>1.13 0.40</td>
<td>1.30 0.54</td>
<td>1.35 0.59</td>
</tr>
<tr>
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<td>1.30 0.61</td>
<td>1.35 0.63</td>
</tr>
<tr>
<td>95</td>
<td>0.009</td>
<td>0.93 0.34</td>
<td>1.11 0.50</td>
<td>1.15 0.53</td>
</tr>
<tr>
<td>95</td>
<td>0.009</td>
<td>0.92 0.34</td>
<td>1.09 0.45</td>
<td>1.14 0.55</td>
</tr>
</tbody>
</table>
Figure 6.13 shows a separation of a synthetic mixture of naphthionic acid (the component which is diazotised), G-acid (the coupled component) and the final dye Ponceau 4R.

6.11 Conclusion

Two chromatographic systems have been developed for the separation of aromatic sulphonic acids which make use of the cetrimide ion.

The usefulness of silica as a support for the analysis of residual intermediates in commercial food dyes has been demonstrated. The mechanism of solute retention on silica appears to be by adsorption of the ion-pair onto the silica surface.

Since this work was done on SÅS, the use of reversed phase chromatography in the presence of ion-pairing agents has become a popular technique in H.P.L.C. and it is now clear that the use of cetrimide is a 'special case' (due to its long hydrocarbon chain) of what is now commonly referred to as "reverse phase ion-pair chromatography".

The technique usually makes use of relatively short hydrocarbon chain counter ions such as tetra-n-butylammonium (83) and hexane or heptane sulphonates. (84,85). Interest in more hydrophobic ions has however been illustrated by two recent papers. Horvath et. al. (86) studied the increased retention made possible by incrementing the chain length of alkyl sulphonates while Gloo and Johnson (87) experimented with a range of ion-pairing agents both basic and acidic.

Reversed phase ion-pair chromatography is now rapidly becoming an established chromatographic technique.
CHAPTER 7

The effect of particle size and packing technique on column performance

The renaissance in liquid chromatography was based on the use of moderately small particles, typically 40 to 50 microns, which could be easily and repeatable dry packed. (17,89) Although it was known that the use of very small particles was theoretically beneficial, it is only in the last few years that the technology for packing small particles has been developed. (90,91,92)

Dry packing of columns has now been generally succeeded by slurry packing methods which have been found to be more effective for small particles. Nevertheless, excellent results have been achieved by dry packing small spherical particles, particularly the Spherisorb \textsuperscript{R} aluminas which, because of their relatively high density, uniformity of size and spherical shape, can be dry packed efficiently.

7.1 Experimental

20 and 10 microns Spherisorb A.Y. were dry packed in 12 cm. X 5 mm. glass columns using the "rotate, tap, bounce" method. 10, 7.5 and 6 micron Spherisorb A.Y. were slurry packed in a stainless steel columns using iodo-methano as the slurry medium. The packing pressure of 4500 p.s.i. was supplied by a Haskel \textsuperscript{R} pressure intensifier pump. Spherisorb A.Y. is a spherical totally porous alumina with a surface area of 120 m\textsuperscript{2}g\textsuperscript{-1}.

Before slurry packing the alumina was activated for four hours at 400 degrees Centigrade to remove all the adsorbed water. Failure to remove this water results in poor dispersion of the particles in
the slurry and consequently poor column performance.

Before dry packing, 1.5% w/w of water was added to the freshly activated adsorbent. The water was then allowed to equilibrate over the adsorbent surface by putting the alumina in a sealed bottle on a rolling machine overnight.

The dry packed columns were run without any conditioning being necessary, but with the slurry packed columns, 150 mls of ether containing 0.75% v/v water was passed through the column before use.

The eluent used throughout this study was 50% water saturated n-hexane. (Made by mixing equal volumes of dry and saturated hexane).

The solutes employed were toluene, biphenyl, anisole and nitrobenzene. They were injected in solution in pentane which was used as a measure of the unretained elution time. The diffusion coefficients of all the solutes were taken as 3.3 x 10^{-5} cm^2 sec^{-1}.

7.2 Results and Discussion

The experimental results for 20 and 10 micron particles (dry packed) and 10, 7.5 and 6 micron particles (slurry packed) are listed in tables 7.1 to 7.5 respectively. Results are given for duplicate columns of each type.

Figure 7.1 shows the log h versus log v plot obtained for the first packing of 20 micron particles and illustrates the spread of experimental points among the four solutes. The range of plate heights is about 30% which is equivalent to a range of about 15% in resolution, \( R_s \propto N^2 \).

Because the data for the four solutes is so extensive, interpretation of the effects of particle size and packing technique on column
TABLE 7.1

Support: 20 micron Spherisorb AY. Column dimensions: 120 X 5 mm.
Packing method: "rotate, tap, bounce". Solutes: Toluene $K' = 0.3$,
Biphenyl $K' = 1.0$, Anisole $K' = 1.9$, Nitrobenzene $K' = 6.3$.

<table>
<thead>
<tr>
<th>Pressure (p.s.i.)</th>
<th>Eluent vel. /cm.sec.</th>
<th>Toluene log v</th>
<th>Biphenyl log h</th>
<th>Anisole log h</th>
<th>Nitrobenzene log h</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>0.25</td>
<td>1.18</td>
<td>0.33</td>
<td>0.39</td>
<td>0.45</td>
</tr>
<tr>
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<td>0.15</td>
<td>0.95</td>
<td>0.37</td>
<td>0.30</td>
<td>0.40</td>
</tr>
<tr>
<td>75</td>
<td>0.09</td>
<td>0.72</td>
<td>0.38</td>
<td>0.31</td>
<td>0.35</td>
</tr>
<tr>
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<td>0.35</td>
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<td>0.50</td>
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<td>0.59</td>
<td>0.66</td>
</tr>
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<td>0.36</td>
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</table>

2nd packing:

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<tr>
<th>Pressure (p.s.i.)</th>
<th>Eluent vel. /cm.sec.</th>
<th>Toluene log v</th>
<th>Biphenyl log h</th>
<th>Anisole log h</th>
<th>Nitrobenzene log h</th>
</tr>
</thead>
<tbody>
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<td>1.07</td>
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<td>0.71</td>
<td>0.76</td>
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</table>
Support: 10 micron Spherisorb AY. Column dimensions: 120 X 5 mm.

Packing method: "rotate, tap, bounce". Solutes: Toluene $K' = 0.3$, Biphenyl $K' = 1.0$, Anisole $K' = 2.0$, Nitrobenzene $K' = 6.5$.

<table>
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<th>Pressure</th>
<th>Eluent vel.</th>
<th>Toluene $K'$</th>
<th>Biphenyl $K'$</th>
<th>Anisole $K'$</th>
<th>Nitrobenzene $K'$</th>
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</thead>
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<td>log h</td>
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<td>0.71</td>
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<tr>
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<tr>
<td>1100</td>
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<td>1.05</td>
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</table>

2nd packing

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<th>Biphenyl $K'$</th>
<th>Anisole $K'$</th>
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</tr>
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<tr>
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<tr>
<td>130</td>
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</tr>
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</table>
TABLE 7.3

Support: 10 micron Spherisorb AY. Column dimensions: 120 X 5 mm.
Packing method: "slurry in CH₂I". Solute: Toluene $K' = 0.2$,
Biphenyl $K' = 0.7$, Anisole $K' = 1.3$, Nitrobenzene $K' = 4.0$.

<table>
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<tr>
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<th>Eluent vel. /cm.sec⁻¹</th>
<th>log $v$</th>
<th>Toluene log $h$</th>
<th>Biphenyl log $h$</th>
<th>Anisole log $h$</th>
<th>N-benz. log $h$</th>
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</thead>
<tbody>
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<td>0.58</td>
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</tr>
<tr>
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<tr>
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2nd packing

<table>
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<tr>
<th>Pressure /p.s.i.</th>
<th>Eluent vel. /cm.sec⁻¹</th>
<th>log $v$</th>
<th>Toluene log $h$</th>
<th>Biphenyl log $h$</th>
<th>Anisole log $h$</th>
<th>N-benz. log $h$</th>
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<tbody>
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</tr>
<tr>
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<td>0.45</td>
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</table>
TABLE 7.4

Support: 7.5 micron Spherisorb AY. Column dimensions: 120 x 5 mm.

Packing method: "slurry in CH₂I". Solutes: Toluene $K' = 0.25$, Biphenyl $K' = 0.8$, Anisole $K' = 1.5$, Nitrobenzene $K' = 5.0$.

<table>
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<tr>
<th>Pressure /p.s.i.</th>
<th>Eluent vel. /cm.sec.⁻¹</th>
<th>log v</th>
<th>Toluene log h</th>
<th>Biphenyl log h</th>
<th>Anisole log h</th>
<th>N-benz. log h</th>
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</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>180</td>
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<td>0.38</td>
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</tr>
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<tr>
<td>80</td>
<td>0.07</td>
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<td>0.44</td>
<td>0.33</td>
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</tr>
<tr>
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<td>1.27</td>
<td>0.77</td>
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2nd packing

<table>
<thead>
<tr>
<th>Pressure /p.s.i.</th>
<th>Eluent vel. /cm.sec.⁻¹</th>
<th>log v</th>
<th>Toluene log h</th>
<th>Biphenyl log h</th>
<th>Anisole log h</th>
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<td>0.38</td>
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<tr>
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<td>0.56</td>
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<td>0.76</td>
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<td>0.48</td>
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<td>0.56</td>
</tr>
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</table>
**TABLE 7.5**

Support: 6 micron Spherisorb AY. Column dimensions: 120 X 5 mm.

Packing method: "slurry in CH$_2$I". Solutes: Toluene $K' = 0.2$, Biphenyl $K' = 0.6$, Anisole $K' = 1.2$, Nitrobenzene $K' = 4.0$.

<table>
<thead>
<tr>
<th>Pressure (p.s.i)</th>
<th>Eluent vel. (cm/sec$^{-1}$)</th>
<th>Toluene log $v$</th>
<th>Toluene log $h$</th>
<th>Biphenyl log $h$</th>
<th>Anisole log $h$</th>
<th>Nitrobenz. log $h$</th>
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</thead>
<tbody>
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<td>0.16</td>
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<td>0.62</td>
<td>0.64</td>
<td>0.54</td>
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<tr>
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<td>0.62</td>
<td>0.62</td>
<td>0.46</td>
</tr>
<tr>
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<td>0.73</td>
<td>0.58</td>
</tr>
<tr>
<td>540</td>
<td>0.66</td>
<td>1.08</td>
<td>0.92</td>
<td>0.78</td>
<td>0.72</td>
<td>0.57</td>
</tr>
<tr>
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<td>1.00</td>
<td>0.93</td>
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<td>0.99</td>
<td>0.88</td>
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<td>0.85</td>
<td>0.60</td>
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<td>0.54</td>
</tr>
<tr>
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<td>0.64</td>
<td>0.58</td>
<td>0.69</td>
<td>0.54</td>
</tr>
<tr>
<td>625</td>
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<td>1.20</td>
<td>0.68</td>
<td>0.64</td>
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<tr>
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2nd packing

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<th>Pressure (p.s.i)</th>
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<th>Toluene log $v$</th>
<th>Toluene log $h$</th>
<th>Biphenyl log $h$</th>
<th>Anisole log $h$</th>
<th>Nitrobenz. log $h$</th>
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<tr>
<td>330</td>
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<td>0.80</td>
<td>0.56</td>
<td>0.54</td>
<td>0.54</td>
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<tr>
<td>550</td>
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<td>0.77</td>
<td>0.66</td>
<td>0.66</td>
<td>0.48</td>
</tr>
<tr>
<td>570</td>
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<td>1.08</td>
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<td>0.80</td>
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<td>0.55</td>
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<tr>
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<td>1.15</td>
<td>0.88</td>
<td>0.80</td>
<td>0.84</td>
<td>0.55</td>
</tr>
</tbody>
</table>
efficiency is simplified if only the experimental points for nitrobenzene are plotted. Figures 7.2 and 7.3 show the aggregate data for nitrobenzene for duplicate columns of each of the five support/packing method combinations. Figure 7.2 shows the efficiencies of dry packed columns of 10 and 20 micron particles while figure 7.3 compares the efficiencies of 10, 7.5 and 6 micron particles using slurry packing.

From figure 7.2, it can be seen that the reduced plate heights found for 10 micron particles are about 35 per cent higher than those for the 20 micron material in the range of reduced velocities 7 to 40. However, although the 10 micron material is less well packed than the 20 micron, the inherent advantages of employing small particles makes the use of the 10 micron material preferable for most applications. This aspect is dealt with in detail in section 7.5.

Attempts to dry pack particles with diameters less than 10 microns by the "rotate, tap, bounce" method and also the "vibrator" method have proved unsatisfactory in that the theoretical advantages of employing smaller particles were outweighed by the decrease in packing efficiency.

Figure 7.3 shows the reduced parameter plots for 10, 7.5 and 6 micron particles packed using the slurry method. At reduced velocities above the minimum reduced plate height, the plot for the 10 micron particles is to all intents and purposes identical to the plot for the dry packed 10 micron columns. Below the minimum reduced plate height however, it is interesting to note the more gradual increase in the plate heights for the slurry packed columns.
As discussed in chapter 5, the relatively large negative gradient in the plate height curves of dry packed "infinite diameter" columns is due to longitudinal diffusion superimposed on which is the added dispersion (and asymmetry) caused by the breakdown of infinite diameter geometry. The nature of the radial velocity profile and also the radial variation in plate height in dry packed columns has been investigated by Knox and Raven. (70) However no investigation of this effect in slurry packed columns has been published. It is clear nevertheless, from the indirect evidence of the reduced plate height curves, that the contribution to peak dispersion caused by the inclusion of the wall region in slurry packed columns is less than in the corresponding dry packed columns. This suggests that the wall region is less extensive in slurry packed columns than in dry packed columns where trans-column fractionation of the packing may greatly enhance the effect of the wall region. The further reduction of the effect of the wall region in slurry packed columns by choosing the best "finish" on the column (polished or unpolished) has probably only a small effect on column efficiency. Although the columns used in this study were internally polished, excellent results have been obtained by other workers using unpolished columns. (93)

As can be seen from figure 7.3, the efficiency of slurry packed columns also decreases as the particle size is reduced. The values of reduced plate heights for the 6 micron particles used in this study were of the order of 30% higher than for the 10 micron particles with 7.5 micron particles intermediate between the two. The reasons for
this are probably analogues to the problems associated with dry packing small particles, i.e.

(a) The tendency to form aggregates is much greater for small particles. This causes "bridging" to occur in dry packed columns and there is probably a similar effect in slurry packed columns due to a failure to produce a mono-disperse slurry. Some later experiments have indicated that slight improvements in the efficiency of slurry packed columns of particles less than 10 microns in diameter may result from ultrasonic dispersion of the slurry before packing.

(b) Since the flow rate for a given packing pressure is proportional to the square of the particle diameter, the impact of "new" particles on the sedimenting bed will be less effective in fabricating a uniform structure. The effect of flow rate on the efficiency of slurry packed columns has also been blamed for the fall off in efficiency with increasing column length (95), but this is a more complex problem since considerations of infinite diameter geometry are also involved.

7.3 Comparison of dry and slurry packing techniques

(a) Reproducibility The reproducibility of a particular packing method is largely dependent of the degree of skill required. Since both of the packing methods used in this study were largely "automated", the reproducibility of column efficiencies was excellent. Figures 7.2 and 7.3 illustrate this. The divergence in terms of the plate heights of nitrobenzene for duplicate columns is of the order of ten per cent which is equivalent to less than a five per cent variation in resolution.
In terms of the time taken to pack a particular column, there is also little to choose between the two packing techniques, both of which take of the order of thirty minutes to produce. The choice between dry and slurry packing a particular support should therefore be based solely on the efficiency and permeability of the columns produced.

(b) Column permeability Column permeability is most conveniently measured in terms of the flow resistance parameter $\phi'$, defined as,

$$\phi' = \frac{P \cdot dp^2}{L \cdot u \cdot \eta}$$

where $P$ is the pressure, $L$ is the column length, $dp.$ is the particle diameter, $u$ is the eluent velocity and $\eta$ is the viscosity of the eluent. (A low value of $\phi'$ indicates a high permeability and visa versa).

From theory, $\phi'$ should be independent of the particle diameter for columns equally "densely" packed with a given support. This is experimentally verified by this study as is shown in table 7.6. The dry packed columns of 10 and 20 micron particles had $\phi'$ values of about 1200 while the slurry packed columns of 10, 7.5 and 6 micron particles had $\phi'$ values of around 500. The consequences of this increase in column permeability for slurry packed columns relative to dry packed columns on column performance are discussed later in this chapter. The higher permeability of the slurry packed columns is in agreement with the findings of Kirkland (59) for the packing of Zorbax$^R$, a spherical silica material.

The particularly low values of $\phi'$ for slurry packed columns of
spherical particles has been attributed to their spherical nature, but it now seems that the reason for the low values is largely due to the narrowness of the particle size distribution of commercially available spherical packing materials. In contrast, the particle size distribution in commercial irregular materials is often large. By careful sedimentation of these irregular packings, narrow ranges of particle sizes can be produced which give similar permeabilities to columns of spherical particles.

(c) Efficiency As discussed above, the dry packing of 20 micron particles produced the "best packed" columns as assessed by plotting log h against log v. 10 micron particles could be packed equally well using either the dry or slurry packing methods but could not be packed as well as the 20 micron material.

With slurry packed columns, there is a gradual fall off in packing efficiency with particle diameter.

The effect of the "quality of packing" and column permeability on the overall performance of the different particle sizes is the subject of the rest of this chapter.

7.4 Derivation of an equation for the optimisation of column performance

Previous optimisation equations (94) have been derived from Snyder's empirical plate height equation,

$$H = D.u^n$$

where D and n are constants and u is the eluent velocity.

While this is satisfactory at high eluent velocities and moderately sized particles (greater than 10 micron), with the use of smaller
particles and consequently lower reduced velocities, axial molecular diffusion makes a significant contribution to the observed plate height. An equation of the form,

$$ h = \frac{B}{v} + D.v^n $$  \hspace{1cm} \text{(7.2)}

must therefore be used where low reduced velocities are involved. B, D and n in equation 7.2 are purely empirical constants and should not be related to the constants in the "Knox equation":

$$ h = \frac{2h}{v} + A.v^{0.33} + C.v $$

The performance of a particular column in liquid chromatography is a complex function of both its packing efficiency (as represented by the log h against log v plot), and its flow resistance characteristics. The inherent advantages of using small particles in order to reduce the height equivalent to a theoretical plate must be balanced against the slight decrease in packing efficiency as the particle size is reduced and more importantly the increased pressure required.

As a solution to this problem of assessing overall column performance, we have developed an equation which relates the time required for a given separation in terms of the available pressure, number of theoretical plates required, the particle size of the support and the efficiency and permeability of the packed material. The derivation of this equation is as follows:

The elution time of an unretained peak is given by,

$$ t_o = \frac{L}{u} $$

Substituting for u from equation 7.1 we have,
The number of theoretical plates, \( N \), is given by,

\[
N = \frac{L}{h_0} = \lambda / h
\]

Using this equation to substitute for \( \lambda \) in equation 7.3 gives,

\[
\tau_0 = \frac{\eta \cdot \varphi'}{P} \cdot \frac{L^2}{dp^2} = \frac{\eta \cdot \varphi'}{P} \cdot \lambda^2
\]

In practice however, the reduced plate height is dependent on the eluent velocity. The reduced eluent velocity is given by,

\[
v = \frac{u \cdot dp}{Dm} = \frac{\frac{dp}{Dm}}{\frac{L}{\eta' \varphi'} \cdot \frac{P}{dp^2}} = \frac{\frac{P}{dp^2}}{Dm \cdot \eta' \varphi'} \cdot \frac{1}{\lambda}
\]

Substituting for \( \lambda \) from equation 7.4 leads to,

\[
v = \frac{\frac{P}{dp^2}}{Dm \cdot \eta' \varphi' \cdot N} \cdot \frac{1}{h} = \frac{F}{h}
\]

where \( F = \frac{P dp^2}{(Dm \cdot \eta' \varphi' \cdot N)} \)

Substituting for \( v \) in equation 7.2 yields,
\[ h = \frac{B}{h} + D \frac{(P/h)^n}{F} \]

Solving this equation for \( h \) leads to,

\[ h = \frac{P}{(D/(F - B))^1/(1 + n)} \]

\( h \) can then be substituted in equation 7.5 and the resulting expression simplifies to,

\[ t_o = \frac{P \cdot dp^2}{\eta \cdot \phi_{im}^2} \cdot \left\{ \frac{D}{(P \cdot dp^2/(Dm \cdot \eta \cdot \phi_{im}^2)) - B} \right\} \frac{2}{1 + n} \]

When \( B \) is small in relation to \( P \cdot dp^2/(Dm \cdot \eta \cdot \phi_{im}^2) \), i.e. at high reduced velocities, \( t_o \) is given by,

\[ t_o = \left( \frac{\eta \cdot \phi'}{P} \right)^{\frac{1 - n}{1 + n}} \cdot Dm \cdot \left( \frac{1 + n}{N \cdot D} \right)^{\frac{2}{1 + n}} \cdot dp^2 \cdot \frac{A_n}{1 + n} \]

This equation is equivalent to the one developed by Majors (94) in terms of "unreduced parameters".

Equation 7.10 is preferred however since it takes account of peak dispersion produced by axial diffusion. In fact whereas equation 7.11 implies that a reduction in particle size will always decrease the analysis time, equation 7.10 shows that there is a theoretical optimum particle size to minimise the analysis time for a given set of experimental conditions.

The column length required for the separation can be calculated from \( t_o \) by the equation,

\[ L = (P \cdot dp^2 \cdot t_o / (\eta \cdot \phi'))^{0.5} \]

The total analysis time is approximately equal to \( t_R \), the retention time of the last eluting peak, i.e.

\[ t_R = (1 + K') \cdot t_o \]
7.5 Use of the "optimisation equation".

The experimental plots of log $h$ versus log $v$ shown in figures 7.2 and 7.3 can be represented by the equation,

$$h = \frac{B}{v} + Dv^n$$

The values found for $B$, $D$ and $n$ and also the flow resistance parameter $\phi'$, for 20 and 10 micron Spherisorb AY dry packed and 10, 7.5 and 6 micron slurry packed are given in table 7.6. The plate height data for nitrobenzene was used since as the most retained solute it was least likely to be affected by extra-column peak broadening.

Equations 7.10 and 7.12 were then used to predict values of $t_o$ and $L$ as a function of the applied pressure for each of the five column types in order to obtain 4000 theoretical plates. The plots of $t_o$ against $P$ and $L$ against $P$ are shown in figures 7.4 and 7.5 respectively.

These figures can be used directly if a separation requires 4000 theoretical plates. For example, if 500 p.s.i. is available, then it can be seen that either 7.5 or 6 micron particles would give the shortest analysis time whereas if only 100 p.s.i. was available either 7.5 of 10 micron particles slurry packed would be preferred.

It can be seen that dry packed columns are inferior to slurry packed columns at all realistic pressures and therefore columns should only be dry packed when the equipment for slurry packing is not available.

Figures 7.4 and 7.5 can also be used to predict analysis times if a different number of theoretical plates is required. For example,
suppose that the required value of N is 10,000 and the pressure available is 1000 p.s.i.. The value of the reduced velocity remains unchanged if N, P, L and t₀ are all increased (or decreased) in proportion. Therefore from figures 7.4 and 7.5, we can see that we could generate 4000 plates using 400 p.s.i. across a column packed with 6 micron particles if the column length was 9 cms. and t₀ was 13 seconds. By multiplying by 2.5 we deduce that a column length of 22.5 cms. should generate 10,000 theoretical plates with t₀ = 32.5 seconds and P = 1000 p.s.i..

**TABLE 7.6**

<table>
<thead>
<tr>
<th>Particle size /packing method</th>
<th>B</th>
<th>D</th>
<th>n</th>
<th>φ'</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mic. dry packed</td>
<td>8.3</td>
<td>0.20</td>
<td>0.82</td>
<td>1230</td>
</tr>
<tr>
<td>10 mic. dry packed</td>
<td>7.7</td>
<td>0.35</td>
<td>0.77</td>
<td>1190</td>
</tr>
<tr>
<td>10 mic. slurry packed</td>
<td>4.5</td>
<td>0.37</td>
<td>0.77</td>
<td>510</td>
</tr>
<tr>
<td>7.5 mic. slurry packed</td>
<td>5.5</td>
<td>0.33</td>
<td>0.84</td>
<td>470</td>
</tr>
<tr>
<td>6 mic. slurry packed</td>
<td>5.5</td>
<td>0.58</td>
<td>0.68</td>
<td>500</td>
</tr>
</tbody>
</table>
Effect of pressure on analysis time

The effect of pressure on \( t_o \) is illustrated in figure 7.4. It can be seen that at low pressures where the operating reduced plate height is near the minimum and \( h \) is nearly independent of \( v \), \( t_o \) is inversely proportional to \( P \) as we would expect from equation 7.5. At higher pressures however, \( h \) increases rapidly with \( v \) and we can see from equation 7.11 that,

\[
t_o \propto \frac{n-1}{P^{n+1}}
\]

For 6 micron particles, \( n \) is equal to 0.68, i.e.

\[
t_o \propto P^{-0.19}
\]

This means that the pressure must be increased by a factor of 1.75 in order to decrease the analysis time by 10 per cent. The use of increased pressure without changes in other parameters is therefore not an "economic" way of decreasing the analysis time.

Effect of \( \phi' \) on analysis time.

The \( \phi' \) values for the slurry packed columns of Spherisorb Alumina are about a factor of 2.3 lower than those found for the dry packed columns. This results in the obvious advantage that less than half the pressure is required to accomplish a given separation. On the other hand, if the extra pressure capability made available through a low value of \( \phi' \) is used in making the column longer, then the same limitations apply as in the previous section.

This effect can be seen in table 7.8 for the 10 micron particles which were packed by both dry and slurry techniques. The \( \phi' \) value
for the slurry packed column is 2.3 times less than that of the dry packed column but the analysis time for the same separation is only shortened by a factor of 1.4.

**Effect of solvent viscosity on analysis time**

Since the diffusion coefficient is inversely proportional to the solvent viscosity (Wilke Change equation), the term $D_m \eta$ is independent of the solvent viscosity. From equation 7.10 therefore we can see that the analysis time is directly proportional to the eluent viscosity. Eluents with low viscosities should therefore be used in liquid chromatography whenever possible.

**Effect of the solute diffusion coefficient on analysis time**

A decrease in the diffusion coefficient as a result of changes other than in the viscosity of the eluent, increases the analysis time if the particle diameter is invariant. However if the particle diameter is scaled to the square root of the diffusion coefficient, then we can see from equation 7.10 that the analysis time is unchanged. This has important consequences since it implies that the separation of large molecules should not necessarily be less efficient or slower than separations of relatively small molecules.

**Effect of particle diameter on analysis time**

The decrease in particle diameter from the 80 to 150 micron range used in classical column chromatography is largely responsible for the great improvements made in the efficiency of liquid chromatography in the last decade. At present the lower size limit for commercially available packings is 5 micron, but this is still much larger than the
theoretical optimum particle size.

If the packing parameters $B$, $D$, $n$ and $\phi'$ found for 7.5 micron Spherisorb AY are taken as representative of what we might eventually achieve for particles down to one micron, then from equation 7.10 we can plot $t_0$ as a function of the mean particle size used. Four plots corresponding to four different values of $P$ with $N = 4000$ are shown in figure 7.6. The corresponding column lengths are shown in figure 7.7. The large savings in analysis time obtained by decreasing the particle diameter can be contrasted with the small decreases brought about by increased pressures.

If equation 7.10 is differentiated with respect to the particle diameter and $d(t_0)/d(dp)$ equated to zero, we find that the particle diameter required to produce the minimum analysis time is given by the equation,

$$dp_{\text{min}} = \left( (1+n/n) \cdot B \cdot D \cdot \eta \cdot \phi' \cdot N/P \right)^{0.5}$$

It can be seen that the particle diameter required to minimise the analysis time is a function of two sets of variables. Firstly $n$, $B$, $Dm$, $\eta$, and $\phi'$ which characterise the chromatographic system used and secondly the operating parameters $N$ and $P$ which can be varied independently. If the ratio of $N/P$ remains constant then the optimum particle diameter is set by the chromatographic system used.

The optimum particle diameter is listed as a function of $N/P$ in table 7.7 using the packing parameters found for the 7.5 micron Spherisorb AY columns. It can be seen that if high resolution
is required with a limited pressure available, then relatively large particles are preferred (about 5 microns). If on the other hand, only three or four thousand plates are required and high pressures are available, then the optimum particle diameter decreases to about 1 micron.

TABLE 7.7
Optimum particle diameters required to minimise analysis time as a function of the ratio N/P.

<table>
<thead>
<tr>
<th>N/P (p.s.i.⁻¹)</th>
<th>dp. (mic.)</th>
<th>P for N = 4000 (p.s.i.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>5.9</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>4.2</td>
<td>200</td>
</tr>
<tr>
<td>10</td>
<td>2.9</td>
<td>400</td>
</tr>
<tr>
<td>5</td>
<td>2.1</td>
<td>800</td>
</tr>
<tr>
<td>2</td>
<td>1.3</td>
<td>2000</td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
<td>4000</td>
</tr>
</tbody>
</table>

7.6 Experimental verification of the "optimisation equation".

Using the values of B, D and n given in table 7.6, equations 7.10 and 7.11 were used to predict values of \( t_o \) and \( L \) for each of the five particle size/packing method combinations which would be required in order to generate 4400 theoretical plates with an available pressure of 330 p.s.i..

The column length predicted for 20 micron particles, dry packed, was 370 mm. This column would therefore be operating on the borderline
of infinite diameter geometry and hence its efficiency would probably be impaired. With column dimensions as large as 370 X 5 mm., the amount of packing material required also becomes prohibitive and therefore no attempt was made to pack this column. Instead a chromatogram produced from a 500 X 2 mm. column of 20 micron particles is shown in figure 7.8.

Columns of the predicted lengths for 10 micron particles, dry packed and 10, 7.5 and 6 micron particles, slurry packed, were then produced. Table 7.8 lists the predicted and experimental values of $t_o$, $L$ and $N$ for each of the four column types. The chromatograms obtained for the test mixture of toluene, biphenyl, anisole and nitrobenzene are shown in figures 7.8 and 7.9. $N$ was measured for the nitrobenzene peak.

**TABLE 7.8**

Comparison of experimental and predicted values of the parameters, $N$, $L$ and $t_o$ with $P = 330$ p.s.i..

<table>
<thead>
<tr>
<th>Particle size /packing method</th>
<th>Predicted values</th>
<th>Experimental values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N$</td>
<td>$L$ (cm.)</td>
</tr>
<tr>
<td>20 mic. dry packed</td>
<td>4400</td>
<td>37.0</td>
</tr>
<tr>
<td>10 mic. dry packed</td>
<td>4400</td>
<td>13.8</td>
</tr>
<tr>
<td>10 mic. slurry packed</td>
<td>4400</td>
<td>18.8</td>
</tr>
<tr>
<td>7.5 mic. slurry packed</td>
<td>4400</td>
<td>12.2</td>
</tr>
<tr>
<td>6 mic. slurry packed</td>
<td>4400</td>
<td>8.9</td>
</tr>
</tbody>
</table>
It can be seen from table 7.8 that the agreement between the experimental and predicted values of $t_o$ and $N$ are excellent for all four columns. Figure 7.8 illustrates the savings in analysis time which can be obtained by using 6 micron particles. $t_o$ for a non-infinite diameter column of 20 micron particles was 104 seconds. For virtually the same separation (in fact more efficient), $t_o$ was reduced by a factor of three by decreasing the particle size to 10 microns and taking advantage on "infinite diameter column operation". Finally a further reduction in analysis time by a factor of two was possible by slurry packing 6 micron particles.

Figure 7.9 shows the chromatograms obtained from the slurry packed columns of 10, 7.5 and 6 micron particles. It is interesting to note that the number of theoretical plates found for the 6 micron column exceeded the predicted value, the 7.5 micron column produced almost exactly the predicted value but the efficiency of the 10 micron column fell short of that predicted. This is in line with the general finding that the longer the column, the smaller is the probability of packing it successfully. (Even although the column would be operating well within the limits of "infinite diameter geometry".)

7.7 Conclusion

Although columns dry packed with 10 and 20 micron Spherisorb AX give very low reduced plate heights (indicating how well they are packed), slurry packing has two major advantages. Firstly dry packing becomes inefficient if particles below 10 microns are used whereas slurry packing can be successfully used with particle sizes down to five microns and probably below. The theoretical advantages
of using very small particles can therefore be realised. Secondly slurry packed columns are more than twice as permeable as the corresponding dry packed columns. Slurry packing is therefore now established as the standard method of column preparation in H.P.L.C.

The future improvement of chromatographic performance lies with a further reduction in the particle size used for column packing, probably to two microns. This will undoubtedly create problems for both sample injection and detection systems since the column lengths required will decrease in proportion to the particle diameter used.
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FIGURE 2.1

Types of hydroxyl group present on a Silica surface

Free hydroxyl

Bound hydroxyl

Geminal hydroxyl

Reactive hydroxyl
FIGURE 2.2

CALIBRATION CURVE

Molecular weight

\[ \begin{align*}
A & \quad 10^6 \\
B & \quad 10^5 \\
C & \quad 10^4 \\
D & \quad 10^3 \\
E & \\
F & \quad \text{region of selective permeation}
\end{align*} \]

Elution volume

\[ V_M \rightarrow V_T \]

CHROMATOGRAM

Detector response

\[ \begin{align*}
A+B & \\
C & \\
D & \\
E+F & \quad \text{Elution volume}
\end{align*} \]
FIGURE 2.3

The effect of pH on the retention of a weak acid in reverse phase chromatography

\[ AH \rightarrow A^- + H^+ \]
Contrast between coupling and classical expressions for plate height as a function of velocity. The coupling $H$ is always less than $H_f$ and $H_D$, while the classical $H$ is always greater than either of these.

Diagram showing the displacements of the solute concentration profiles in the mobile and stationary phases with respect to the equilibrium concentration profile.
FIGURE 4.1

Essential features of a Liquid Chromatograph.

Eluent Reservoir

Pressure gauge

Pump

Injector

Analytical Column

Recorder

Detecto

Solvent waste

Precolumn Preamplifier
FIGURE 4.2
Schematic representation of an 'Orlita' pump.

FIGURE 4.3
Schematic representation of a 'Haskel' pump.
FIGURE 4.4

H.P.L.C. column and injection head.

FIGURE 4.5

Measurements required to calculate column efficiency.
FIGURE 5.1
Log h versus log v curves for a column of Spherisorb AX packed using the "vibrator" method.
L = 82.5 mm. dc. = 5 mm.
FIGURE 5.2

Log h versus log v curves for a column of Spherisorb AX packed using the "vibrator" method.
L = 82.5 mm, dc. = 5 mm.
FIGURE 5.3
Log h versus log v curves for a column of Spherisorb AX packed using the "vibrator" method.
L = 150 mm. dc. = 5 mm.
Log $h$ versus log $v$ curves for a column of Spherisorb AX packed using the "vibrator" method.

$L = 220$ mm, $d_c = 5$ mm.
FIGURE 5.5

Log h versus log v curves for a column of Spherisorb AX packed using the "vibrator" method.
L = 280 mm, d.c. = 5 mm.
FIGURE 5.6

Log h versus log v curves for a column of Spherisorb AX packed using the "rotate, tap, bounce" method.
L = 145 mm, dc. = 7.0 mm.
FIGURE 5.7

Log h versus log v curves for a column of Spherisorb AX packed using the "rotate, tap, bounce" method.
L = 145 mm. dc. = 5.1 mm.
FIGURE 5.8
Log h versus log v curves for a column of Spherisorb AX packed using the "rotate, tap, bounce" method.
L = 145 mm, dc. = 4.2 mm.

- Anthracene
- Biphenyl
- Toluene
FIGURE 5.9
Log h versus log v curves for a column of Spherisorb AX packed using the "rotate, tap, bounce" method.
L = 145 mm. dc. = 3.2 mm.
Log h versus log v curves for a column of Spherisorb AX packed using the "rotate, tap, bounce" method.

L = 145 mm, d.c. = 2.3 mm.
FIGURE 6.1

Plot of weight of cetrimide adsorbed per gm. of SAS against the percentage cetrimide in the eluent.
The effect of cetrimide concentration on the retention of sulphonylic acids on SAS. Eluent - 2.5:1, water: propanol with % cetrimide.
FIGURE 6.3

The effect of added sulphuric acid on the retention of sulphonic acids on Partisil. Eluent - 3:1, propanol:water with 1% cetrimide.

[Diagram showing the effect of added sulphuric acid on the retention of sulphonic acids on Partisil. Eluent - 3:1, propanol:water with 1% cetrimide. The graph plots Log K' against Log (H₂SO₄) for different compounds: Ponceau 4R, Tartrazine, Ponceau MX, Sunset Yellow, and Schaffer's acid.]
FIGURE 6.4

The effect of temperature on the retention of sulphonic acids on Partisil. Eluent – 3:1, propanol:water with 1% cetrimide

Log $K'$

$\frac{1}{T} \times 10^3$ ($^\circ K^{-1}$)
The effect of cetrimide concentration on the retention of sixteen sulphonic acids on Partisil.

Eluent - 3:1, propanol:water with x% cetrimide.

```
FR - Ponceau 4R
SP - Sulphophenylcarboxypyrazalone
TZ - Tartrazine
SA - Sulphoanthranilic acid
PM - Ponceau MX
CM - Carmoisine
AM - Amaranth
RA - R - acid
SY - Sunset Yellow
GA - G - acid
BX - Blue X
SU - Sulphanilic acid
NA - Naphthionic acid
NW - Neville & Winthers acid
SH - Schaffers acid
SI - 5-sulphoisatin
```
FIGURE 6.6

The effect of cetrimide concentration on the retention of six sulphonic acids on Partisil. Eluent 3:1, propanol : water with x% cet.

[Graph showing the relationship between Log K' and % w/v Cetrimide for Ponceau 4R, Tartrazine, Ponceau MX, Sunset yellow, Sulphanilic acid, and Schaffers acid.]
The effect of added solvent "modifiers" on the retention of sulphonic acids on Partisil.

Eluent - 15:5:4, propanol:water:solvent, with 1% cetrimide. 

- Log $K'$ vs. solvent type
- Chart includes markers for Ponceau 4R, Tartrazine, Ponceau MX, Sunset yellow, R-acid, Sulphanilic acid, Schaffers acid, with various solvent types indicated:
  - BuOH
  - EtOH
  - THF
  - CH$_2$Cl$_2$
  - CH$_3$CN
  - H$_2$O

No added solvent
FIGURE 6.8
Plot of log h versus log v for a column of 9.5 micron Partisil operated with (a) The solutes anisole and nitrobenzene with an eluent of 1% acetonitrile in hexane. (b) The solutes sulphanilic acid, R-acid and Ponceau MX with an eluent of 3:1, propanol:water with 1% cetrimide.
FIGURE 6.9

Separation of 1-naphthylamine sulphonie acid isomers.

<table>
<thead>
<tr>
<th>Peak identity</th>
<th>Eluent</th>
<th>Support</th>
<th>Pressure</th>
<th>Column dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) 1-naphthylamine-5-sulpho</td>
<td>2.5:1, water:propanol, with 1% cetrimide</td>
<td>7 micron SAS</td>
<td>700 p.s.i.</td>
<td>120 X 5 mm.</td>
</tr>
<tr>
<td>(2) 1-naphthylamine-6-sulpho</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3) 1-naphthylamine-7-sulpho</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(4) 1-naphthylamine-8-sulpho</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FIGURE 6.10

Separation of J-acid from three major impurities.

Peak identity: (1) gamma acid, (2) J-acid, (3) Dioxy-J-acid, (4) Di-J-acid.
Eluent: 2.5:1, water:propanol, with 1% cetrimide
Support: 7 micron SAS
Pressure: 700 p.s.i.
Column dimensions: 120 X 5 mm.
Separation of sulphonic acids on Partisil using methylene chloride as a "solvent modifier".

Peak identity: (1) dioxy-J-acid, (2) J-acid, (3) gamma-acid, (4) di-J-acid.
Eluent: 70:40:12, PrOH:CH₂Cl₂:H₂O, with 2% cetrimide
Support: 5 micron Partisil
Pressure: 700 p.s.i.
Column dimensions: 100 X 5 mm.
Separation of Sunset Yellow from its intermediates and impurities.

Peak identity: (1) Schaffer's acid, (2) unknown, (3) sulphanilic acid, (4) Triazine, (5) Sunset Yellow, (6) unknown.

Eluent: 3:1, propanol:water, with 1% cetrimide.

Support: 6 micron Partisol.

Pressure: 1200 p.s.i.

Column dimensions: 120 X 5 mm.
FIGURE 6.13

Separation of Ponceau 4R from its intermediates.

Peak identity: (1) naphthionic acid, (2) G-acid, (3) Ponceau 4R
Eluent: 10:3, propanol:water with 2% cetrimide
Support: 10 micron Partisil
Pressure: 300 p.s.i.
Column dimensions: 120 X 5 mm.
FIGURE 7.1

Plot of log h versus log v for a column of 20 micron Spherisorb AY packed using the "rotate, tap, bounce" method.
Plot of log h versus log v for 10 and 20 micron Spherisorb AY packed using the "rotate, tap, bounce" method. Aggregate data for nitrobenzene from duplicate columns of each particle size is shown.
FIGURE 7.3

Plot of log h versus log v for 10, 7.5 and 6 micron Spherisorb AY packed using the "slurry" method. Aggregate data for nitrobenzene from duplicate columns of each particle size is shown.
$t_o$ for a separation requiring 4000 theoretical plates plotted as a function of the applied pressure for each of the five particle size/packing method combinations.

(Plotted using equation 7.10.)
FIGURE 7.5

L for a separation requiring 4000 theoretical plates plotted as a function of the applied pressure for each of the five particle size/packing method combinations. (plotted using equation 7.12.)
FIGURE 7.6:

t₀ for a separation requiring 4000 theoretical plates plotted as a function of dp.

for four applied pressures. (Plotted using equation 7.10.)

---

φ' = 470
B = 5.5
D = 0.33
n = 0.84
FIGURE 7.7
L for a separation requiring 4000 theoretical plates plotted as a function of dp. for four applied pressures. (plotted using equation 7.12.)

\[ \phi' = 470 \]
\[ B = 5.5 \]
\[ D = 0.33 \]
\[ n = 0.84 \]
Comparison of the chromatograms of a test mixture obtained with:
(a) 20 micron particles dry packed in a 500 X 2 mm. column.
(b) 10 micron particles dry packed in a 135 X 5 mm. column.
(c) 6 micron particles slurry packed in a 90 X 5 mm. column.
FIGURE 7.9

Comparison of the chromatograms of a test mixture obtained with:
(a) 10 micron particles slurry packed in a 185 X 5 mm. column.
(b) 7.5 micron particles slurry packed in a 120 X 5 mm. column.
(c) 6 micron particles slurry packed in a 90 X 5 mm. column.

(a) 150 secs. 120 90 60 30 0
N = 3800
NITROBENZENE
ANISOLE TANNOHATE TOLUENE
\( t_0 = 23 \text{ secs.} \)

(b) 120 secs. 90 60 30 0
N = 4350
\( t_0 = 17.5 \text{ secs.} \)

(c) 90 secs. 60 30 0
N = 5000
\( t_0 = 16 \text{ secs.} \)
INTERACTION OF RADIAL AND AXIAL DISPERSION IN LIQUID CHROMATOGRAPHY IN RELATION TO THE "INFINITE DIAMETER EFFECT"

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SUMMARY

The dynamics of radial dispersion of small solute samples injected centrally into a chromatographic column have been examined using a dual-electrode polargraphic detector having one electrode fixed and the other movable radially across the face of the column exit frit. It is shown that the radial dispersion can be characterised by a reduced radial plate height, \( h_r \), whose dependence on reduced velocity, \( v \), obeys the equation

\[
h_r = 1.4/v + 0.06
\]

Experiments with trans-column injection using the same equipment show that there is little variation of peak-maximum velocity but that the mean axial plate height is increased up to threefold near the wall. The region of high dispersion extends about 30 particle diameters inwards from the walls.

Experiments with conventional high-performance liquid chromatographic columns and equipment, carried out with aromatic hydrocarbon solutes and columns of various lengths and bores packed with 21.5 \( \mu \)m spherical alumina, show that serious loss of efficiency occurs when solute can reach the wall regions. The data are consistent with the view that for efficient chromatography using microparticles the solute should not spread closer to the walls than about 30 particle diameters.

INTRODUCTION

In previous papers\(^1-3\) it has been shown that column performance may be most effectively assessed by plotting the logarithm of the reduced plate height \( h \) (= \( H/d_p \), where \( H \) = plate height and \( d_p \) = particle diameter) against the logarithm of the reduced velocity \( v \) (= \( u d_p/D_m \), where \( u \) = mean linear eluent velocity and \( D_m \) = diffusion coefficient of solute in eluent). This method of representation permits easy comparison of data obtained using different columns, packing materials, solutes and
eluent standards and sets readily remembered standards of good performance. The dependence of $h$ upon $v$ can be interpreted in terms of Giddings' theories of peak dispersion using the semi-empirical equation

$$h = B|v + A v^{0.33} + C v$$

(1)

The $(h,v)$ curves represented by eqn. 1 have minima which in practice occur at values of $h$ between 2 and 5 and of $v$ between 2 and 10. It has been shown both in practice and in theory that optimum performance in chromatography using a given pressure drop is obtainable by adjusting the column geometry and particle size so that $v$ is held around this minimum.

In eqn. 1 the first term arises from axial molecular diffusion, the second from velocity variations within the streaming part of the mobile phase, and the third from resistance to mass transfer between the streaming and static parts of the column (the latter comprising mobile and stationary phase held with the particles of the packing).

In 1969 Knox and Parcher pointed out that if the column diameter was sufficiently large in relation to the column length and particle diameter, solute molecules injected centrally onto the top of the column would fail to reach the walls in traversing the length of the column. If therefore additional peak dispersion arises due to wall effects, central injection into a sufficiently wide column should result in improved chromatographic performance. The general validity of this idea in true chromatographic systems has been fully vindicated by several studies. However, no detailed examination of lateral dispersion and its effects on chromatographic performance have been reported.

As shown by Horne et al., radial dispersion in a packed bed can be characterised by a radial plate height, $H_r$, defined as the rate of increase of the radial variance of the concentration profile with distance migrated:

$$H_r = d\sigma_r^2/dz$$

(2)

where $\sigma_r$ is the standard deviation of the concentration profile and $z$ is the distance measured along the axis of the column. For a point injection in a column of length $L$ this is equivalent to

$$H_r = \sigma_r^2/L$$

(3)

The reduced radial plate height is then

$$h_r = H_r/d_p = \sigma_r^2/Ld_p$$

(4)

It has been established by chemical engineering studies reviewed by Horne et al. that the velocity dependence of $H_r$ may be expressed as

$$H_r = B D_m/u + A_r d_p$$

(5)

or in reduced terms

$$h_r = B|v + A_r$$

(6)
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where $B$ has the same significance as in eqn. 1 for axial dispersion and arises from molecular diffusion, and $A_r$ represents the contribution to radial dispersion from stream splitting as the flow divides around particles of packing. From the chemical engineering studies and theoretical calculation it appeared that $A_r$ lay between 0.1 and 0.2, and a value of 0.15 has generally been used in previous chromatographic work.\textsuperscript{7-9}

To ensure that a given column behaves as if it were of infinite diameter, it has been proposed\textsuperscript{7} that the column diameter $d_c$ should exceed about four standard deviations of the radial dispersion, that is

$$d_c \geq 4 \sigma_r$$

Combining eqns. 4, 6 and 7 yields the condition for a column to be effectively of infinite diameter, namely

$$d_c^2/Ld_p \geq 16 B/v + 16 A_r$$

(Eqn. 8) shows that some columns will behave as if of infinite diameter at values of $v$ above a certain critical reduced velocity given by

$$v_{crit} = 16B/(I - 16A_r)$$

where $I$ is the dimensionless parameter defined by

$$I = d_c^2/Ld_p$$

Taking $A_r = 0.15$ and $B = 1.8$ (a typical value for a porous packing)\textsuperscript{11} eqn. 9 then gives

$$v_{crit} = 28.8/(I - 2.4)$$

For a typical column with $d_c = 5$ mm, $L = 0.25$ m and $d_p = 20$ $\mu$m, $I = 5$, and $v_{crit} = 11$. For the standard short column described later for which $L = 0.0825$ m, $d_c = 5.0$ mm, and $d_p = 21.5$ $\mu$m, $I = 14$ and $v_{crit} = 2.5$; this value of $v_{crit}$ is sufficiently low that this column should behave as if of infinite diameter at all velocities which are of practical interest.

It must, however, be recognised that the onset of wall effects due to solute penetration into the wall regions will be gradual, and that any condition such as given by eqns. 7, 8, 9 or 11 must be quite diffuse. Furthermore, factors other than the rate of radial dispersion will determine the overall effect of walls on chromatographic performance. The shape of the injected sample will be clearly critical, and very different results may be expected from a centrally injected “point sample” and a uniform injection over the entire cross-section of the bed. Most practical injections will be of some intermediate and probably indeterminate type. Equally important will be the configuration of the packing close to the wall of the column, the “extent of wall region” (as a fraction of the total column cross-section), the linear velocity variations within this region, and their deviation from the mean over the column as a whole. We believe that the wall region can probably be thought of as extending a definite
number of particle diameters, say $r$ particle diameters, from the column wall and that the "extent of wall region" can therefore be expressed as

$$\text{"extent of wall region"} = \frac{\text{area of wall region}}{\text{area of column}} = \frac{4r}{\rho}$$

where $\rho = d_c/d_p$, $d_c$ being the column diameter. This view was first advanced by Knox and Parcher\(^7\) in order to explain why the $(h,v)$ curves for glass bead columns were independent of $\rho$ over the range $8 < \rho < 20$. They proposed that the wall region was about 3 particle diameters thick.

At present we know little about the "extent of wall region" in chromatographic columns with very large values of $\rho$ (say $50 < \rho < 1000$) such as are used with microparticles in liquid chromatography (LC), and we have very meagre data on whether peak migration rates differ between the wall regions and the core, or whether axial dispersion of solute migrating in the wall regions is significantly different from that for solute migrating in the core region. Finally, we have little information on whether, in a particular column, it makes any difference if solute is allowed to penetrate to the wall regions or not. Conceivably, the effects reported in support of the view that wide columns give better performance than narrow columns could be caused more by differences in the quality of packing achieved than by differences in the importance of wall effects, as is suggested by Wolf\(^10\).

It is the purpose of this paper to clarify some of these points.

The work falls into two more or less independent parts. In Part A, by using a dual polarographic detector with one electrode fixed and one movable radially across the face of the frit at the column exit we have determined the radial dispersion of centrally injected samples as a function of $\nu$ in order to validate eqns. 5 and 6. Then, by making injections across the entire column cross-section we have measured the mean band velocity and the mean axial plate height as functions of distance from the column axis. The data give direct information about the thickness of the wall region.

In Part B standard chromatographic columns packed with 21.5 $\mu$m Spherosorb alumina were used. Dimensions were chosen so that $v_{mf}$ fell within the experimental velocity range. The object of this part of the work was to determine the extent to which column performance deteriorated when solute was permitted to penetrate to the wall regions by reducing $\nu$. We examined two series of columns. In Series I, the column bore and "extent of wall region" remained constant while $\nu$ was varied by altering the column length. In Series 2, the length was kept constant while the column bore was altered, thereby changing both $\nu$ and the "extent of wall region".

PART A — EXPERIMENTS WITH A DUAL POLAROGRAPHIC DETECTOR

**Experimental**

A high-pressure reciprocating pump (Orlia Giessen, Type DMP 1515) was used to pump the eluent, consisting of 0.1 M KCl kept free of oxygen by continual reflux under nitrogen. Pulsations due to the pump cycle were damped out by a 300-mm length of 5-mm bore stainless-steel tubing containing nitrogen which was teed into the flow-line between the pump and the injector head. The column, mounted vertically, was a glass tube 0.775 m in length and 11.7 mm in bore. A PTFE frit was
inserted into the outlet end and a stainless-steel ferrule was fixed to the inlet end with Araldite. This ferrule mated with an injector head fitted with a central guide which ensured that injections could be made strictly centrally into the top of the column packing. The general design of these fittings has been described elsewhere\textsuperscript{4,13}. The column was packed with clean dry glass beads by the rotate, bounce, and tap method (RTB-method)\textsuperscript{4,15}. The beads had a mean diameter of 64\,\mu m with a standard deviation of 3\,\mu m as found by microscopic measurement. The solute used was p-nitrophenol, which was dissolved in oxygen-free eluent. The value of $D_m$, used in the calculation of $r$, was obtained by the Wilke–Chang equation\textsuperscript{5,16} as $0.97 \times 10^{-2}\,\text{mm}^2\text{sec}^{-1}$.

The dual-electrode polarographic detector is outlined in Fig. 1. It consisted of two spherical platinum electrodes, 0.3 mm in diameter, melted on the ends of platinum wires. One electrode was fixed 0.5 mm from the column axis and the other could be moved radially by a micrometer across the face of the PTFE frit which retained the column packing. The two electrodes were positioned 0.1 mm below the lower surface of the frit. The platinum electrodes were sealed into glass sleeves and led out of a Perspex housing through 0.25-mm.-I.D. nylon tubing. They were held in PTFE plugs fitted into glass tubes which passed through PTFE plugs screwed into the sides of the housing. The platinum electrodes were maintained at a potential of $-1.0\,V$ relative to a mercury counter electrode which was an annular pool a few centimeters downstream of the Pt electrodes. The electrode currents were simultaneously recorded by a dual-pen recorder (Servoscribe Model RE 520.20) which measured the voltages developed across two resistors (1–5 k$\Omega$ according to the working current) connected in series with the electrodes.

Radial concentration profiles were determined by injecting successive 5-\mu l portions of p-nitrophenol solution about 2 mm below the top of the bed of glass beads. This produced a near spherical injection about 2 mm in diameter. For the first injection the movable electrode was placed centrally, and for subsequent injections it was moved in successive steps of 0.5 mm towards the column wall. For each injection two concentration–time profiles were obtained, one from the reference electrode and
one from the movable electrode. The area under each peak gave the amount of solute reduced at the electrode. The peak area obtained with the reference electrode was used to correct the areas obtained with the movable electrode for small variations in the quantity injected. The radial concentration profile was then obtained by plotting the corrected peak areas obtained with the movable electrode against the distance from the column axis after dividing each area by the corrected area for the axial position. Profiles were recorded in this way for several eluent velocities. Peak shapes were always Gaussian if the electrode was not less than 1.5 mm from the column wall, but became increasingly skewed and sometimes double when the electrode approached the wall.

Trans-column velocity and plate height variations were obtained by introducing solute samples across the entire column section. The eluent flow was first arrested and about 30 µl of a solution of p-nitrophenol in 1.0 M KCl was slowly and carefully injected just above the top of the bed of glass beads. Because of the higher density of the sample solution than eluent, the 30 µl sample quickly spread out as a thin layer about 0.3 mm deep over the top of the bed. When this was seen to have occurred the flow of eluent was restarted. Concentration–time profiles were again recorded at both electrodes for successive injections. By using the dual recorder it was possible to obtain accurate peak migration rates for different positions across the column, and to determine the plate height as a function of the radial position.

Results and discussion

Fig. 2 shows three plots of the radial distribution for 5-µl samples injected

Fig. 2 Radial dispersion of a 5-µl sample of p-nitrophenol injected centrally into a 775 mm long × 11.7 mm I.D. column packed with 64-µm glass beads. Distributions are shown for three reduced velocities. Gaussian curves are drawn to fit each of the three sets of data points, the upper two sets of which are displaced vertically. The lowest curve shows three Gaussian curves with $\sigma_x = 1.85$, 1.90 and 1.95 mm, respectively.
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centrally at different flow velocities. At the highest velocity \( v = 247 \) the solute barely reached the walls. The trans-column concentration distribution is Gaussian, as expected, and within experimental error follows the equation

\[
c/c_{\text{axis}} = \exp \left(-x^2/2\sigma^2\right)
\]

where \( c_{\text{axis}} \) is the concentration at the column axis, and \( c \) is the concentration at a distance \( x \) from the column axis. Three curves drawn through the lowest set of data points show that \( \sigma = 1.90 \pm 0.05 \text{ mm} \).

According to the theory of second moments, the final variance should be given by

\[
\sigma_r^2(\text{tot}) = \sigma_r^2(\text{inj}) + \sigma_r^2(\text{col})
\]

where \( \sigma_r^2(\text{inj}) \) and \( \sigma_r^2(\text{col}) \) are the variances due to the injector and column processes, respectively. It is, of course, \( \sigma_r(\text{col}) \) with which eqns. 2 and 4 are concerned, but it is \( \sigma_r(\text{tot}) \) with which we are really concerned in eqn. 7. If the injected sample is regarded as a 2-mm-diameter sphere, then \( \sigma_r(\text{inj}) \) is equal to the radius of gyration of this sphere, that is 0.63 mm, and \( \sigma_r^2(\text{inj}) = 0.4 \text{ mm}^2 \). We therefore obtain for the variance and standard deviation due to the column processes

\[
\sigma_r^2(\text{col}) = 1.90^2 - 0.4 = 3.21 \text{ mm}^2
\]

whence

\[
\begin{align*}
\sigma_r(\text{col}) &= 1.79 \text{ mm} \\
H_r &= 3.21/775 = 0.0041 \text{ mm} \\
h_r &= 4.1/64 = 0.065
\end{align*}
\]

In the second curve of Fig. 2, for which \( v = 48 \), there is now some penetration of sample to the wall regions and reflection from the wall. By fitting the portion of the distribution out to 4.0 mm from the axis, we obtain \( \sigma_r(\text{tot}) = 2.18 \text{ mm} \) and \( \sigma_r(\text{col}) = 2.09 \text{ mm} \). When \( v = 15 \) (uppermost curve), there is considerable penetration to the wall and \( \sigma_r(\text{col}) = 2.68 \text{ mm} \). The dependence of the reduced radial plate height upon reduced velocity is shown in Fig. 3. The best fit of eqn. 6 to the data is

\[
h_r = 1.4/v + 0.060
\]

The value of \( B = 1.4 \) is, within experimental error, in agreement with the previous value of 1.2 obtained for axial dispersion in a bed of packed spheres by Knox and McLaren\(^1\). The surprising result is the very low value of \( A, = 0.060 \) obtained in this study, which is about half that derived from chemical engineering work\(^9\). It is possible that the lower value now obtained is the result of our use of impermeable spheres rather than angular porous particles and further work using different packings would be required to clarify this point. The present value is also somewhat lower than that predicted theoretically on the basis of a simple stream-splitting mechanism\(^1\).

Fig. 3 also shows the dependence of the axial plate height upon reduced velocity.
In agreement with Knox and Parcher, $h$ rises very gradually with $v$ according to a low exponent of about 0.2 compared to the exponent of about 0.33 obtained with narrow columns. Fig. 3 also demonstrates the dramatic difference in the rate of axial and radial band spreading in chromatographic columns. Under conditions widely used in high-performance liquid chromatography (HPLC) with $v$ around 20, $h_{\text{axial}}$ is about ten times as large as $h_{\text{radial}}$. However, when $v = 1$, typically used in thin-layer chromatography (TLC), $h_{\text{axial}}$ and $h_{\text{radial}}$ are comparable. Thus, spots in TLC tend to be circular, while bands arising from point injections in HPLC are considerably elongated, the more so the higher the velocity.

Fig. 4 obtained using trans-column injections, shows concentration-time profiles obtained centrally and 1 mm from the column wall alongside the profiles obtained with the fixed electrode. In the first case the chromatograms (taken with the electrodes 0.5 mm apart) are essentially superimposable after allowing for the pen-displacement of the dual recorder. The traces in the second case differ markedly. The peak arising from the wall region emerges about 9 sec earlier than that from the reference position in a total elution time of 440 sec. It is also lower, wider and significantly asymmetric, showing considerable "fronting".

Fig. 5 shows how the velocity obtained from the peak maximum depends upon radial position. Surprisingly there is little evidence of a sudden upsurge in velocity near the wall. Indeed the main velocity variation occurs between 1 and 3 mm from the axis. This almost certainly results from the packing procedure, which either causes some fractionation of particles across the column or causes slight compaction towards the centre relative to the outer regions. The same result was obtained for different radial directions. There is therefore no evidence here of large velocity variations near the wall. However, the development of peak asymmetry as the wall is approached does
Fig. 4. Dual recorder traces for elution of a trans-column injection with the movable electrode placed centrally (left-hand trace) and 1 mm from the column wall (right-hand trace). The reference traces are taken by the fixed central electrode. For column details, see Fig. 2.

Fig. 5. Dependence of axial reduced plate height (a) and peak maximum velocity (b) upon radial position. O, Trans-column injection, v = 115; Q, central injection, v = 15. For column details, see Fig. 2.

suggest that the flow velocity very close to the wall is somewhat higher when averaged around any annulus than it is in the centre. In interpreting the data on both velocity variations and plate heights across the column, it is important to remember that the peak maximum velocity is not the same as the mean eluent velocity at any distance
from the axis because of radial mixing which will smooth out gross variation. Probably the most that can be said on the basis of the present data is that there may well be substantially increased velocities very near to the wall, as supposed by Knox and Parcher\(^1\), but that they extend only a very short distance into the packing proper, so that peak maximum velocities are little affected, although substantial peak skewing inevitably occurs.

The variation in plate height as a function of radial position also shown in Fig. 5 is much more dramatic; \(h\) rises from about 1.7 in the core region to about 4.7 at 1 mm from the wall. When the movable electrode was placed closer to the wall than 1 mm, severe peak distortion was noted with both peak shape and base width becoming irreproducible. Similar results were obtained from 5-\(\mu\)l injections made centrally at flow velocities sufficiently low that a significant part of the sample reached the walls before emergence from the column. It is thus clear from Fig. 5 that serious band broadening and peak distortion begins to occur for solute emerging up to 2 mm from the wall, where there is virtually no disturbance of the mean peak velocity. Again lateral mixing will to some extent smooth out the variation in axial dispersion with distance from the column axis, but we believe that the effect will not be substantial and that the results shown in Fig. 5 imply that the region of disturbed packing extends much farther from the wall than we had previously imagined. We believe that the wall region in this column extends at least 1 mm into the column (\(\approx 15\) particle diameters) and more probably about 2 mm (\(\approx 30\) particle diameters). This is much farther than suggested by Knox and Parcher\(^3\), but their widest column had \(\phi = 20\). It would now seem that there may be a thin layer of about 3 \(d_p\) in which wide velocity variations occur and also a much deeper region in which a highly dispersive packing arrangement is present, but without any gross variation in the mean velocity.

Further experiments are clearly required to obtain more information on the relationship between axial and radial dispersion near column walls. The most fruitful modification of the present experiment would be to use much shorter columns in which the extent of radial dispersion would be less so that the measured axial plate height at any distance from the column axis would be a better measure of the axial dispersive power of the column at that particular distance. It is also clear that experiments should now be carried out with porous and sorbing column packings so that the results are more directly related to real chromatographic situations.

**PART II -- EFFECT OF COLUMN GEOMETRY ON \((h,v)\) CURVES**

*Experimental*

The HPLC equipment was again home assembled. For moderate and high flow-rates an Orlita pump was used with a pulse damper as before; but for very low flow-rates the eluent was directly pressurised by nitrogen in a coil of nylon tubing. Detection was by a fixed-wavelength (254 nm) UV photometer (DuPont Model 410). All columns were made from normal heavy-walled glass tubing and were able to withstand at least 30 atm. Column end pieces and injectors were of brass and fixed to the glass by Araldite. They enabled injections of 0.5 \(\mu\)l of sample to be made centrally into a 2-mm deep layer of 200-\(\mu\)-m-diameter glass beads on the top of the column packing proper. The eluent was hexane from which aromatics had been removed by passage through silica gel, and which had been degassed by reflux. Sample solutions were
made up from a selection of the following aromatic hydrocarbons dissolved in hexane: toluene, styrene, naphthalene, biphenyl, and anthracene. Their diffusion coefficients in hexane were taken as $3.3 \times 10^{-3} \text{ mm}^2 \text{ sec}^{-1}$.

Columns were packed with Spherisorb alumina, Type X, kindly gifted by the Material Preparations Unit, A.E.R.E., Harwell, Great Britain. Alumina Type X had a surface area of $290 \text{ m}^2 \text{ g}^{-1}$. The mean particle diameter of the spherical alumina particles was $21.5 \mu\text{m}$ with a standard deviation of $1.9 \mu\text{m}$, as found by microscopic measurement of about 100 particles.

Two different dry-packing methods were used, the “RTB” method previously described and a “vibrator” method, which was carried out as follows: a 10-20 mm depth of packing was slowly added to the column which was rotated at about 1 revolution per second. The tip of an engraving tool, vibrating at 50 Hz, was held against the glass column until no further settling of the packing occurred. Further layers of packing were added and the procedure repeated until the column was filled. This method has the attraction of simplicity and cheapness, but the technique of the operator is critical and considerable practice is required before reproducible results can be obtained. Crucial factors are the amplitude of the vibration of the tool, and the angle and manner by which it is held against the column. Once mastered, the technique produces columns which have the same efficiency as those produced by the RTB method. The main advantage of the RTB method is that it can be used successfully and reproducibly by operators with widely different experience in chromatography.

When using the RTB method to ensure efficient packing, it was necessary to standardise the activity of the alumina before packing the column. The alumina was first heated for 4 h at 400° to desorb water, $2\%$ (w/w) of water was then added, and the alumina + water allowed to equilibrate overnight in a sealed bottle on a rolling machine. When using the vibrator method the alumina could be used without any pretreatment.

Results and discussion

The validity of eqn. 1 for fine-particle alumina was checked over an extended range of reduced velocity using a standard short column 82 mm long and 5.0 mm bore. Fig. 6 shows the data fitted by eqn. 1 with the following values of the constants $A$, $B$ and $C$

Upper line: $B = 3.6$, $A = 0.4$, $C = 0.16$
Lower line: $B = 3.0$, $A = 0.4$, $C = 0.09$

It is noticeable in this and later figures that at high values of $r$, $h$ increases as $k'$ increases, while at low values of $r$, $h$ is roughly independent of $k'$. This implies similar $A$ and $B$ values for all solutes but $C$ values increasing with $k'$. This is in broad agreement with general experience in gas and liquid chromatography (see ref. 1, for example).

The minimum reduced plate height is around $h = 2$ at a reduced velocity $r = 5$. The curve clearly shows the upward trend in $h$ due to molecular diffusion at low velocities, which is familiar to gas chromatographers.

For a porous solid the value of $B$ is expected to lie between the value of 1.2 for random-packed impervious spheres and 2.0 for diffusion in a pure liquid. The value of about 3.3 is therefore unexpectedly high. There are three possible explanations,
Fig. 6. Reduced plate height–velocity plots for 21.5-μm Spherisorb Alumina X over a wide velocity range. Column, 82 × 5.0 mm I.D. Solute samples: O, toluene (k' = 0.5); Q, styrene (1.0); ⊥, naphthalene (2.3); ⊕, diphenyl (3.5). For upper and lower lines, see text.

viz. (a) The assumed solute diffusion coefficient is low by a factor of 1.6–2.0. We believe this to be unlikely. (b) Some diffusion occurs on the adsorbent surface. This would undoubtedly raise B (see ref. 4, for example), but, contrary to our data, one would then expect different values of B for the different solutes, whose values of k' ranged from 0.5–3.5. (c) At reduced velocities below about 2.5 (the value of \( v_{cr} \)) solute can reach the walls and therefore performance is likely to be impaired. An increase in h due to wall effects might therefore be expected at low values of v and in fitting eqn. 1 to the data this would show up as a larger B term. In view of the data which follow, this seems to be the most likely explanation.

The value of A is very low and equals the best value previously obtained, namely 0.4 for 480-μm glass beads\(^7\). The very low value confirms the excellent packing characteristics of Spherisorb aluminas. The values of C on the other hand are somewhat higher than those previously found for porous materials, which were closer to 0.05\(^4\). High C values suggest that mass transfer is relatively slow in Alumina X. However, the disadvantage of the higher C values is not a serious limitation if conditions are chosen so that one works at reduced velocities close to the minimum. This is best achieved by working with particles in the 5- or 10-μm range rather than with 20-μm particles\(^3\).\(^4\).

For the further experiments on the effect of column geometry on (h, v) curves, the band shown in Fig. 6 is taken as the standard for a column in which there is negligible penetration of centrally injected solute to the walls at eluent velocities of practical significance (i.e., at values of v equal to or greater than that for minimum h).

In Series 1, columns with \( d_e = 5.0 \) mm and \( d_p = 21.5 \) μm were used with lengths varying from 82 to 280 mm. The columns were packed by the vibrator method and the test mixture contained toluene (k' = 0.5), styrene (1.0), naphthalene (2.3), and biphenyl (3.5).

In Series 2, columns with \( L = 145 \) mm were used, having bores from 2.3–7.0 mm. These columns were packed with deactivated alumina using the RTB method.
TABLE I

COLUMN PARAMETERS

| Series 1: $d_e = 5.00$ mm; $d_p = 21.5$ μm; vibrator packing method |
|-------------------------|-------------------------|-------------------------|-------------------------|
| $L$ (mm)               | 82                      | 150                     | 220                     | 280                     |
| $l$                    | 14.1                    | 7.75                    | 5.3                     | 4.15                    |
| $e$                    | 235                     | 235                     | 235                     | 235                     |
| $v_{crit}$ (eqn. 11)   | 2.4                     | 5.3                     | 9.9                     | 16.5                    |
| $l'$                   | 7.75                    | 4.27                    | 2.91                    | 2.29                    |
| $v'_{crit}$ (eqn. 16)  | 4.25                    | 8.8                     | 15.1                    | 22.3                    |

| Series 2: $L = 145$ mm; $d_p = 21.5$ μm; RBT packing |
|-------------------------|-------------------------|-------------------------|-------------------------|
| $d_e$ (mm)              | 7.0                     | 5.1                     | 4.2                     | 3.2                     |
| $l$                     | 15.7                    | 8.34                    | 6.66                    | 3.28                    |
| $e$                     | 325                     | 225                     | 195                     | 150                     |
| $v_{crit}$ (eqn. 11)    | 2.2                     | 4.8                     | 6.8                     | 33                      |
| $l'$                    | 10.4                    | 4.63                    | 2.70                    | 1.15                    |
| $v'_{crit}$ (eqn. 16)   | 3.1                     | 7.9                     | 16.9                    | 192                     |

* Asymptotic values of length and diameter at which $v_{crit}$ becomes infinite.

For this less active alumina the test mixture was toluene ($k' = 0.3$), biphenyl (0.8), and anthracene (1.5).

The geometrical dimensions and characteristics of the nine columns used are given in Table I. For each column a value of $v_{crit}$ can be calculated according to eqn. 11. These values are shown in Fig. 7. For columns which are sufficiently long (Series 1) or sufficiently narrow (Series 2) solute will always penetrate to the walls and limiting lengths and diameters therefore appear as asymptotes in Fig. 7.

![Fig. 7. Dependence of $v_{crit}$ upon column length for 5-mm-bore columns (Series 1), and upon column diameter for 145-mm-long columns (Series 2), packed with 21.5-μm particles.](image-url)
Fig. 8 shows the \((h, v)\) plots for the columns of Series I. The plots are displaced vertically for convenience and in each case the band containing the standard data points of Fig. 8 is shown along with the values of \(v_{ctr}\), calculated by eqn. 11. It is evident that the data obtained from the longer columns coincide within experimental error with those for the standard short column at high values of \(v\), but deviate upwards at lower values of \(v\). The break-away generally occurs at velocities somewhat above \(v_{ctr}\) as calculated by eqn. 11.

These curves provide clear evidence that penetration of solute to the wall regions leads to higher plate heights and poorer performance. The observation that the plate height deteriorates for a given packing as the column is lengthened has been observed before and has generally been put down to the difficulty of packing long columns\(^7\). This explanation is unlikely to apply in our experiments since the columns were all packed dry and the bed should be packed in the same way at all depths.

Fig. 9 shows the equivalent data for the columns of Series 2. While the data show more spread between high and low values of \(k'\), similar trends are apparent to those found in Series 1. For the 7-mm-bore column the \((h, v)\) curve is in good agreement with that for the shorter 5-mm-bore standard column, which has a similar value of \(I\). For the 5.0- and 4.2-mm columns of Series 2 the \((h, v)\) curves again break away from the standard curve at velocities just above \(v_{ctr}\), calculated from eqn. 11. For the 3.2- and 2.3-mm-bore columns very different \((h, v)\) curves are obtained. The data
Fig. 9. Reduced plate height—velocity plots for columns of Series 2 (see Table I). Column diameters (in mm) are given beside each set of data points. Solute identification: ○, toluene ($k' = 0.3$); ●, biphenyl (0.8); □, anthracene (1.5). For significance of bands and arrows, see Fig. 8.

for the 3.2-mm-bore column are particularly striking, since this column has roughly the same $I$ value as the 280 x 5 mm-bore column of Series 1, yet the $(h,v)$ curve is almost the same as that for the 2.3-mm-diameter column, which, according to eqn. 11, can never behave as if of infinite diameter.

Two observations suggest that the use of eqn. 11 underestimates the importance of wall effects. Firstly, the $(h,v)$ curves break away from the standard curve at velocities considerably above $v_{crit}$. Secondly, the 145 x 3.2 mm column behaves as if it could never show infinite diameter behaviour in spite of $v_{crit}$ according to eqn. 11 being about 30. There are two ways in which such discrepancies could arise, viz. (a) The assumed value $B = 1.8$ could be too low (this would make all the values of $v_{crit}$ too low), but as already argued, it is difficult to justify a value of $B$ above 2. (b) The wall region may have a substantial thickness, as suggested by the results of Part A. The appropriate diameter to be used to calculate $v_{crit}$ should then be not the column diameter $d$, but the diameter of the homogeneously packed central core of the column. The results of Part A suggest that this core does not reach beyond 30$d_c$ from the wall. For 21.5-μm alumina this would produce a wall region about 0.65 mm thick. Thus the core diameter which should be used in calculating $I$ for $v_{crit}$ should be taken as 1.3 mm less than $d_c$. 
Taking full account of the results of Part A using the new value of 0.062 for $A_r$ and taking $B = 1.8$ for porous particles, the condition that a column exhibits no wall effects becomes

$$I' \equiv \frac{(d_e - 60 d_p)^2}{Ld_p} \geq 16 \left( \frac{1.8}{v + 0.062} \right)$$

(15)

We then obtain for $v_{crit}$

$$v_{crit} = \frac{28.8}{\frac{(d_e - 60 d_p)^2}{Ld_p} - 1.00}$$

(16)

Table 1 compares the value of $v_{crit}$ obtained from eqns. 11 and 16. Those from eqn. 16 are 50-100\% higher for all columns, with one notable exception. For the $145 \times 3.2$ mm column $v_{crit}$ is increased from 33 to the very high value of 192. For practical purposes this column can never be free of wall effects by eqn. 16.

The modification of the basic condition given in eqn. 7 to allow for a wall region of substantial thickness as proposed in Part A removes the two anomalies and so generally substantiates this proposal.

**CONCLUSIONS**

The major conclusion from this work is that the wall region in an otherwise well packed LC column extends about 30 particle diameters into the column. Within this region there is only a small increase in mean peak velocity but a drastic increase in dispersive power as the wall is approached. Thus penetration of solute into this region greatly affects column performance and in the worst cases can increase the apparent plate height threefold.

It therefore seems important to study methods for improving the packing characteristics in the wall region.

However, all effects can be avoided if a column is sufficiently wide. The condition for wall effects to be avoided which we now recommend is

$$d_e - 2r_d \geq 4\sigma_t$$

which leads to

$$I' \equiv \frac{(d_e - 2r_d)^2}{Ld_p} \geq 16 \left( \frac{B}{v} + A_r \right)$$

The recommended values of the constants are: $r \approx 30$, $B = 1.4$ (im pervious particles), 1.8 (porous particles), and $A_r = 0.060$.

**REFERENCES**


INTERACTION OF RADIAL AND AXIAL DISPERSION IN LC

SOAP CHROMATOGRAPHY — A NEW HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC TECHNIQUE FOR SEPARATION OF IONIZABLE MATERIALS

DYESTUFF INTERMEDIATES

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Department of Chemistry, University of Edinburgh, Edinburgh EH9 3JJ (Great Britain)

SUMMARY

A new form of high-performance ion-pair chromatography is described in which a detergent (cetyltrimethylammonium bromide) is added at around the 1% level to a propanol-water eluent. The column packing may either be a reversed-phase material (e.g., SAS silica) or a silica gel (e.g., Partisil). The method allows high-resolution separations of a wide range of sulphonic acids and derived dyestuffs containing one to three \(-\text{SO}_3\text{H}\) groups. The degree of retention can be varied by changing cetrimide concentration, acidity of eluent, concentrations, and nature of additives.

In the reversed-phase system it is probable that cetrimide-sulphonate ion pairs are extracted from the water-rich eluent into an adsorbed layer rich in propanol and cetrimide. In the silica gel system ion pairs are probably present in the eluent phase and are adsorbed onto the silica gel surface with propanol acting as a protective agent for both ion pairs and surface. Column performance in terms of plate height is equivalent to that obtainable in normal adsorption chromatography. Specific separations are shown of naphthylaminesulphonic acid isomers, including J-acid and \(\gamma\)-acid, and of sunset yellow from impurities.

INTRODUCTION

The chromatography of ionizable substances such as amines and organic acids often presents problems in respect of retention, plate efficiency, and peak symmetry. In order to separate such compounds by adsorption chromatography it is necessary to use polar eluents, which reveal the inhomogeneity of the adsorbent surface and produce tailed peaks. This is particularly liable to occur if the substance contains several readily ionizable or highly polar functional groups. It is also a general experience that buffering of an ionizable material either with acid or base is necessary since changes in the degree of ionization throughout any chromatographic band due to change in concentration over the band will cause peak asymmetry. In such cases it
is often better to employ ion-exchange chromatography. Buffering is again required to maintain the optimum balance between ionized and neutral forms but most ion exchangers give relatively poor performance compared to adsorbents.

Recently ion-pair chromatography\(^2\) has been shown to be valuable for high-performance liquid chromatography (HPLC) of ionizable compounds and to possess great versatility. In “normal-phase” ion-pair chromatography of say amines, \(\text{RNH}_3^+\), an aqueous acid, such as 0.1 \(M\) \(\text{HClO}_4\), might be used as stationary phase, the eluent being, say, a halocarbon containing a few per cent of a higher alcohol. The amine is then present in the stationary phase as a cation and is partitioned into the eluent as an ion pair according to the following equation:

\[
\text{RNH}_3^+ + \text{ClO}_4^- \rightleftharpoons (\text{RNH}_3^+, \text{ClO}_4^-)
\]

Schill, Persson and co-workers\(^2\) have pioneered this technique and it has recently been applied successfully by Karger and co-workers\(^3\) to the separation of biogenic amines, sulfonamides and other pharmacologically active material. Knox and Jurand\(^4\) have applied the technique to tricyclic tranquilizers.

“Reversed-phase” ion-pair chromatography can also be carried out where the stationary phase is organic and the eluent is an aqueous acid or base. Some separations on bonded supports may be of this type.

While ion-pair partition systems have great flexibility\(^5\), it is always difficult to maintain the stability of two-phase partition systems over long periods and, of course, gradient elution is not possible except with reversed-phase bonded supports. In some cases the complexities of ion-pair equilibria may again result in tailed peaks, but this can usually be avoided.

In the present study of the separation of various naphthalenesulphonic acid derivatives, we were unable to solve the problem of tailing either by conventional adsorption or ion-pair partition methods using tetraalkylammonium counter ions in an aqueous stationary phase and we ascribed this to the very strong polarity of the \(-\text{SO}_3^-\) group, which is probably able to displace water from silanol sites on the silica surface even when a strongly aqueous mobile or stationary phase is present. It occurred to us that this problem might be solved by employing an ion-pairing substance which might be expected to form highly stable ion pairs which would not dissociate in the presence of strongly hydrogen-bonding surface groups. We therefore examined the effect of adding the quaternary ammonium detergent cetrimide, “cetrimide”, to the aqueous alcoholic eluent for the chromatography of sulphonic acids. Initial experiments were unexpectedly successful and led to the more detailed study reported below. For conciseness we have termed this new technique “soap chromatography”.

It was found in the initial experiments that while the addition of about 1% of “cetrimide” greatly improved the adsorption chromatography of a range of mono-, di- and trisulphonic acids on silica gel, the dependence of retention upon cetrimide concentration and other operating parameters was difficult to interpret. Accordingly, we turned to a reversed-phase support, Wolfson SAS silica, in which the surface of silica is covered by short alkyldimethyl groups bonded to the surface by Si–C bonds.
With this material the detergent was expected to be directly adsorbed by the support and we anticipated that the dependence of retention upon cetrimide concentration would be more readily interpretable.

Subsequently to this work it was pointed out to us that the effect of detergents on the paper chromatography of dyestuff intermediates *inter alia* had previously been studied by Farulla *et al.* They found that while addition of up to 1% cetrimide to the eluent increased $R_F$ values (and so reduced $k'$ values), pretreatment of the paper with cetrimide, or its addition to the solute sample had undesirable effects producing tailing and double spots in many cases. These results, for a rather different chromatographic system, contrast sharply with our own where cetrimide addition decreased $R_F$ values (increased $k'$) and brought about remarkable improvements in resolution, retention, peak symmetry and peak sharpness.

In regard to the chromatography of sulphanilic acids, previous separations have been carried out by gas chromatography after derivatization\(^8,9,10\), and by various classical liquid chromatographic methods such as column\(^12\), thin-layer\(^13\), and paper chromatography\(^9,14\), but results have generally been unsatisfactory. This inadequacy of conventional methods was confirmed by our initial experiments using Spherisorb silica as adsorbent and water-methylene chloride-methanol as eluent. These showed that while toluene could be eluted with a column efficiency of 1700 theoretical plates, the peak for sulphanilic acid corresponded to only 130 plates.

Results previously obtained for HPLC of sulphanic acids were equally disappointing. Schnit and Henry\(^15\) in 1970 separated some naphthalenesulphonic acids on Zipax\(^16\) SAX but the column efficiency was again poor with plate numbers around 100 on a column which for ideal solutes would have been expected to give 1000-2000 plates.

**EXPERIMENTAL**

The chromatographic equipment comprised an Orliat DMP 1515 reciprocating pump, an air-filled pulse damper (a 300-mm \times 5-mm-bore stainless-steel tube capped at one end), a pressure gauge, an injector and column as described elsewhere\(^16\), and a DuPont Type 410 single-wavelength (254 nm) UV detector.

Two column packing materials were used: For normal-phase chromatography Partisil 10 (Whatman Biochemicals, Springfield Mill, Great Britain) was used. Some experiments were carried out using a pre-production batch of characteristics slightly different from the later commercial material. For the high-performance chromatograms and plate height measurements we used a 6.5-$\mu$m fraction obtained from Partisil 10 by fractionation in water. The surface area of Partisil is $\sim 400 \text{ m}^2\text{ g}^{-1}$. For the reversed-phase experiments we used 7-$\mu$m SAS silica with a surface area of $\sim 200 \text{ m}^2\text{ g}^{-1}$ prepared by the Wolfson Liquid Chromatography Unit (Department of Chemistry, University of Edinburgh). This material is a spherical silica gel having short-chain hydrocarbon groups bonded to the surface. The material has virtually no adsorptive activity attributable to residual silanol groups.

Columns were of 5-mm-bore polished stainless steel and 100 or 120 mm in length. They were packed by pumping a slurry of the packing material (roughly 2 g for a column in 25 ml of supporting solvent) into the column with a constant pressure pump set at 2500 p.s.i. Methanol was used as the supporting liquid for Partisil and methyl iodide for SAS silica.
Column efficiencies were assessed by determining the reduced plate height, \( h \), at various reduced velocities, \( v \) (for further discussion of the method see ref. 1) and comparing with previous data obtained with normal- and reversed-phase materials. The solutes used in the study were kindly gifted by I.C.I. Dyestuffs Division (Macclesfield, Great Britain) and are listed under their common names and formulae in Table I.

Sample solutions in water generally contained \( \sim 0.1\% \) of each solute and injections were normally in the range of 1–5 \( \mu l \).

Cetrimide was obtained from BDH (Poole, Great Britain).

### Table I

**SULPHONIC ACIDS AND DYESTUFFS**

In the formulae the \(-SO_2H\) group is represented by \( $ \). Bracketted numbers give the elution order on Partisil for the six components also studied by the reversed-phase method.

<table>
<thead>
<tr>
<th>Name</th>
<th>Formula</th>
<th>Elution order</th>
<th>Reversed phase: Silica gel</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Sulphonic acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monosulphonic acids</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>5-Sulphoisatin</td>
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<td>1</td>
<td></td>
</tr>
<tr>
<td>Sulphanilic acid</td>
<td><img src="image" alt="Image" /></td>
<td>1</td>
<td>5 (2)</td>
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<tr>
<td>Naphthionic acid</td>
<td><img src="image" alt="Image" /></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Schäffer's acid</td>
<td><img src="image" alt="Image" /></td>
<td>2</td>
<td>1 = (1)</td>
</tr>
<tr>
<td>Nevile and Winthers acid (NW-acid)</td>
<td><img src="image" alt="Image" /></td>
<td>3</td>
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<tr>
<td>5-Sulphoanthraniolic acid</td>
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<th>Reversed phase</th>
<th>Silica gel</th>
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<td>1-(4'-Sulphophenyl)-3-carboxy-5-pyrazaloue</td>
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<td>γ-Acid</td>
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<tr>
<td>Disulphonic acids</td>
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<td>Blue-X</td>
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<td>G-acid</td>
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<td>Di-J-acid</td>
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</table>

(Continued on p. 22)
### TABLE I (continued)

<table>
<thead>
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<th>Name</th>
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<th>Elution order</th>
<th>Reversed phase</th>
<th>Silica gel</th>
</tr>
</thead>
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<tr>
<td><strong>B. Azo dyes</strong></td>
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<td>Disulphonic acids</td>
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<td>Carmosine</td>
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<td>Tartrazine</td>
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<td></td>
</tr>
<tr>
<td>Trisulphonic acids</td>
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<td>Ponceau 4R</td>
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</tr>
</tbody>
</table>

### RESULTS AND DISCUSSION

**A. Separations on SAS silica (Wolfsen Unit reversed-phase material)**

A 5:2 mixture by volume of water and propanol was used as eluent with the addition of between 0.031 and 2 g of cetrimide per 100 ml of eluent (denoted below by the % concentration of cetrimide) giving cetrimide concentrations from $8.6 \times 10^{-4}$ to $5.5 \times 10^{-2} M$. The dependence of $\log k'$ upon cetrimide concentration is shown in Fig. 1. With no cetrimide present all solutes were unretained. The elution order is scarcely affected by the concentration of cetrimide. The curves for a variety of sulphonlic acids all show an increase of $k'$ up to a concentration of about 0.25% ($7 \times 10^{-3} M$) cetrimide followed by a gradual decline as the concentration rises to 2%.
Fig. 1. Dependence of log $k'$ upon w/v cetrimide concentration (g/100 ml eluent) for six dyestuffs and intermediates. Eluent, water–propanol (5:2); column packing, 7 μm SAS silica (Wellson Unit).

The ratio of water to propanol has no effect on elution order but $k'$ increases with increase in water content as might be expected in a reversed-phase system. Finally $k'$ in general increases as the number of sulphonate acid groups in the solute molecules increases.

The results are most simply explained if it is supposed that with the dilute solutions of cetrimide the detergent and sulphonate acid exist in the ionised form in the eluent phase, while cetrimide–sulphonate ion pairs are sorbed into the hydrophobic stationary phase, which is probably associated with a tightly held layer of propanol, for it is well known that organic solvents containing propanol are good solvents for cetrimide ion pairs. It may also be anticipated that the surface of the reversed-phase packing will adsorb cetrimide cations which will form a molecular layer at the interface between the stationary and mobile phases. The overall situation may be represented by the interlinked equilibria shown in scheme A, where the subscripts aq and ads refer to the eluent and interface phases, respectively, and where underlined species are those thought to be present in high relative concentrations at low cetrimide concentrations.

$$
\begin{align*}
  nC^*_\text{aq} + S^{n-}_\text{aq} &\rightleftharpoons K_{\text{aq}}^{-n} (nC^+S^{n-})_\text{aq} \\
  nC^*_\text{ads} + S^{n-}_\text{ads} &\rightleftharpoons K_{\text{ads}}^{-n} (nC^+S^{n-})_\text{ads}
\end{align*}
$$
Fig. 2 shows that cetrimide is indeed adsorbed by the support in the concentration range of interest. The adsorption was measured in the following way: a column, packed with SAS silica, was equilibrated by passing water–propanol (5:2). The eluent was then switched to a mixture containing a known concentration \( C \) of cetrimide. A cetrimide concentration front thus moved down the column. If cetrimide was adsorbed by the packing material the break-through volume of the front \( V' \) would exceed the void volume of the column \( V_0 \). The amount of cetrimide adsorbed would then be \( (V' - V_0)C \). The break-through volume, \( V' \), was determined by collecting 0.2-ml aliquots of eluent in sample tubes containing a dilute solution of sunset yellow in a two-phase mixture made from equal volumes of water, methylene chloride and propanol. Initially the dye was partitioned strongly into the aqueous phase but where cetrimide was present in any aliquot the dye was extracted as the \((C^+S^-)\) ion pair into the organic layer.

\[
\%
\text{ w/v Cetrimide} \\
\text{wt. adsorbed/mg g}^{-1}
\]

\[
\%
\text{ w/v Cetrimide} \\
\% \text{ w/v Cetrimide}
\]

Fig. 2. Adsorption of cetrimide by SAS silica (Wolfson Unit) as a function of cetrimide concentration in eluent.

The adsorption isotherm shown in Fig. 2 is significantly curved and within experimental error obeys the simple Freundlich-type equation

\[
[C^+_{\text{ads}}] = \alpha [C^+_{\text{aq}}]^{0.8} \tag{1}
\]

For a 2\% cetrimide concentration, 13.0 mg of cetrimide is adsorbed per gram of support. With a support surface area of about 200 m\(^2\)g\(^{-1}\) this implies an area per molecule of about 1000 Å\(^2\). Since the molecular volume of the cetrimide cation is about 500 Å\(^3\), the degree of surface coverage by cetrimide is between 5 and 15\%, depending upon the “depth” assigned to the cetrimide chain on the surface. This relatively low surface coverage suggests that the chain lies flat on the surface and that a considerable part of the surface must be covered by adsorbed propanol, the less polar component of the eluent phase.
SOAP CHROMATOGRAPHY OF DYE STUFF INTERMEDIATES

If we now assume that the sorption of the sulphonate acid ions, $S^{n-}$, onto the surface is linear with respect to concentration in the eluent phase (which is reasonable since the $S^{n-}$ concentration is very low), and that the equilibria in the eluent and surface phase obey the normal laws, we can write the following equilibrium conditions in addition to eqn. 1

$$[S^{n-}_{ads}] = \beta [S^{n-}_{aq}]$$

(2)

$$[(nC^+ S^{n-})_{aq}] = K_{eq} [(C^+_{aq})^n (S^{n-}_{aq})]$$

(3)

$$[(nC^+ S^{n-})_{ads}] = K_{ads} [(C^+_{ads})^n (S^{n-}_{ads})]$$

(4)

Assuming that the concentration of $S^{n-}_{ads}$ is negligible compared to that of the sulphonate ion pair, we obtain for the distribution coefficient $D$ between the surface and eluent phase

$$D = \frac{n^n \beta K_{ads} [C^+_{ads}]^{0.8n}}{1 + K_{eq} [C^+_{aq}]^{0.8n}}$$

(5)

This expression shows that at low $C^+$ concentrations, $k'$, which is proportional to $D$, will rise with $[C^+_{aq}]$. The exponent of $[C^+_{aq}]$, being 0.8 $n$, should increase with the number of sulphonate acid groups which are paired with cetrimide cations. In Fig. 1 the gradients at the lowest cetrimide concentrations are between 0.9 and 1.5, and therefore in the correct region. That for sulphamic acid is high, but this could be due to experimental error since measurement of the lowest value of $k'$, viz. 0.12, is sensitive to the elution time of the solute assumed to be unretained. The gradient for Schäffer’s acid is about 0.9, in fair agreement with the predicted value of 0.8 for a monosulphonic acid. The values for the three disulphonic acids are generally somewhat higher than unity but the correlation with the number of $-SO_3H$ groups is poor.

A rather better correlation exists between gradient and number of sulphonate acid groups for the decline of $k'$ with increasing concentration of cetrimide in the 0.5–2% concentration range. The negative gradients for the mono-acids are about 0.5, for the di-acids 1.0 to 1.1, and 1 for the tri-acid about 1.3. It is clear from eqn. 4 that if $K_{eq}$ or $[C^+]$ are very large so that virtually all $S^{n-}$ in the eluent phase are in the form of ion pairs at the highest cetrimide concentration, the largest negative gradient which can be allowed is 0.2 $n$, whereas the experimental data show negative gradients closer to 0.5 $n$. The likely explanation is the formation of micelles or at least of cetrimide ion clusters which can solubilize the sulphonate ions in the eluent phase. Formally this can be accommodated by writing the equilibrium in the eluent phase as

$$mC^+_{aq} + S^{n-}_{aq} \rightarrow (mC^+ S^{n-})_{aq}$$

(B)

Eqn. 5 then becomes

$$D = \frac{n^n \beta K_{ads} [C^+_{ads}]^{0.8n}}{1 + K'_{eq} [C^+_{aq}]^{0.8n}}$$

(5')

where $K'_{eq}$ is the equilibrium constant for reaction B. Evidently, when $m > n$, the negative gradient can be large. The data are explained if we set $m \sim 1.3$ with $K'_{aq}$
relatively large but not so large that the second term in the denominator dominates at low concentrations of cetrimide.

In pure water the critical micelle concentration (CMC) of cetrimide is $9.8 \times 10^{-4} \text{M}$, corresponding to 0.036\% cetrimide. The addition of propanol is known to lower the CMCs of long-chain quaternary ammonium salts but larger quantities may raise them again; for example Emerson and Holtzer found that in 2.0 M propanol (water-propanol (approx. 11:2)) the CMC of dodecyltrimethylammonium bromide was reduced from about 0.017 M to about 0.01 M. But they also found that by this concentration it was becoming extremely difficult to detect the onset of micelle formation by the conductivity method employed. It is therefore probable that at the concentration used in the present work, equivalent to about 3.7 M, there is no precise concentration of cetrimide at which micelle formation could be said to occur. It is probably more realistic to suppose that with increase in detergent concentration there is an increasing tendency for cetrimide cations to associate into micelle-like clusters of a range of sizes, the mean size increasing with concentration. Within this range of concentration increasing solubilization of the sulphonic acid groups in the eluent phase would be expected as the cetrimide concentration increased. From the present data it appears that each $-\text{SO}_3$ group is on the average associated with about 1.3 cetrimide cations when the cetrimide concentration is between 1 and 2\%. Further experiments at higher cetrimide concentrations would clearly be of interest.

The excellent chromatography which can be achieved in the reversed-phases system is illustrated by two representative chromatograms shown in Figs. 3 and 4. In the former the high selectivity of soap chromatography for isomeric naphthylamine-sulphonic acids is demonstrated, while in the latter the difficult separation of $\gamma$- and
J-acids is shown. The plate height for the final peak in Fig. 3 is 32\,\mu m, giving a reduced plate height of \( h = 4.5 \) at a reduced velocity of about 25 [based upon the Wilke-Chang equation\(^\text{20}\)] with an eluent viscosity of 2.2\,cP]. This result implies a very high efficiency for solutes of exceedingly high polarity.

\[ B. \text{ Separations on silica gel (Partisil)} \]

Whereas with cetrimide present a 5:2 water–propanol mixture was required for reasonable retention of solutes by SAS silica, a much less hydrophilic 1:3 mixture was necessary for adequate retention on silica gel.

Fig. 5 shows the dependence of the capacity ratio, \( k' \), on cetrimide concentration over a relatively small concentration range for sixteen sulphonic acids using 1:3 water–propanol mixtures. The data were obtained with the pre-production batch of Partisil. In the absence of cetrimide all solutes were unretained. Fig. 6 shows the dependence of \( k' \) over a wider range of cetrimide concentrations for the more restricted group of acids examined under reversed-phase conditions. These data were obtained on a commercial sample of Partisil; the absolute values of \( k' \) are somewhat different with these two samples although the elution order is unchanged. Under the propanol-rich conditions used the formation of micelles is unlikely and the cetrimide is probably present as ion pairs solubilised by propanol\(^\text{22}\). The surface of the silica gel is likely to be covered by a layer containing water and propanol in a ratio considerably higher than 1:3 due to the strong adsorption of water by silanol groups.

The broad trends are clear. The degree of retention increases continually as the cetrimide concentration increases, and there is no indication of the maximum found with the reversed-phase system, although there is a flattening out of the plots.

\[ \text{Fig. 5. Dependence of } k' \text{ upon cetrimide concentration for sixteen dyestuffs and intermediates.} \]

*Eluent, water-propanol (1:3); packing, Partisil 10. The \(-\text{SO}_3\text{H}\) group is represented by $.$*
Fig. 6. As Fig. 5 but for six compounds over a wider range of cetrimide concentrations.

at high concentrations of cetrimide. The order of elution is essentially unaffected by cetrimide concentrations and is much the same as that observed with the reversed-phase packing. Although retention generally increases with the number of sulphonic acid groups in the molecules, there are notable exceptions in that 5-sulphoantraniilic acid and 1-(4'-sulphophenyl)-3-carboxy-5-pyrazalone, both monosulphonic acids containing carboxyl groups, are eluted towards the end of the disulphonic acid group. The carboxyl group in these and in tartrazine apparently increases retention substantially.

Fig. 7 shows that $k'$ is reduced by increasing the acidity of the eluent and that the change from neutral to 0.025 $M$ sulphuric acid produces roughly a fourfold decrease in $k'$. The gradients of the log $k'$ vs. pH plots at low pH approach unity for all solutes irrespective of the number of sulphonic acid groups in the molecules. Tartrazine, a disulphonic acid containing a carboxyl group, shows a rather steeper gradient at higher pH than the other acids due probably to ionization of the carboxyl group but approximately the same gradient at low pH when the carboxyl group will be fully protonated.

Fig. 8 shows the effects on $k'$ of diluting the standard 1:3 water-propanol mixture containing 1% cetrimide by adding 20% v/v of a number of additives. The addition of water reduced $k'$, while the addition of propanol and other alcohols increased $k'$. The addition of relatively large polar molecules produced effects similar to those produced by the addition of alcohols, while the addition of a non-polar
Fig. 7. Dependence of \( k' \) upon acid concentration for elution of five dyestuffs and intermediates. Standard eluent, water–propanol (1:3), containing 1% w/v cetrimide; packing, Partisil 10.

Fig. 8. Effects upon \( k' \) of addition of 20% v/v of a number of additives to the standard eluent for seven dyestuffs and intermediates. For other details, see Fig. 7.
Increasing the concentration of $\text{H}_2\text{SO}_4$ may have two general effects. The first is to reverse the equilibrium $\text{D}$ by increase in $(\text{H}^+)$

$$\text{SO}_3\text{H} \rightleftharpoons \text{SO}_4^- + \text{H}^+ \quad (\text{D})$$

The second is to decrease the availability of cetrimide for formation of ion pairs by reaction $\text{E}$

$$2\text{C}^+ + \text{SO}_4^{2-} \rightleftharpoons (2\text{C}^+ \text{SO}_4^{2-}) \quad (\text{E})$$

If the effect of $\text{H}_2\text{SO}_4$ is largely due to the former, then it would be necessary for $n$ to equal unity in the general scheme $\text{C}$ or that the ionization of only one of the $-\text{SO}_3^-$ groups in di- and trisulphonic acids could be suppressed. Since neither of these seem probable, it is most likely that the addition of $\text{H}_2\text{SO}_4$ reduces retention mainly by competing with the sulphoninic acids in the formation of ion pairs and effectually reducing the cetrimide concentration available for this purpose. In this connection it is notable that the cettrimide concentration was around $2.7 \times 10^{-5}$ M, while that of the $\text{H}_2\text{SO}_4$ when $k'$ is significantly reduced was between $10^{-2}$ and $3 \times 10^{-2}$ M.

The effect of water in reducing retention and the reverse effect of alcohols are readily interpreted in terms of their effect on the composition of the layer in contact with the adsorbent surface. Retention of the ion pairs which themselves will tend to adsorb propanol rather than water molecules will be greater the higher the proportion of the solvating molecules which the adsorbent surface can accommodate. Replacement of water on the surface by propanol will make the surface layer more lipophilic, so will tend to increase retention, whereas the reverse will be true if propanol is replaced by water. Ethyl acetate and tetrahydrofuran apparently behave in much the same way as alcohols. Acetonitrile, on the other hand, will be rather strongly adsorbed by a silanol surface yet will not solvate ion pairs so well as propanol. It will therefore reduce retention. Hexane will be excluded from the surface layer and should therefore have little effect on retention as is observed. Methylene chloride has an intermediate effect, sometimes behaving like acetonitrile and sometimes like hexane.

We conclude that the most satisfactory model for the retention on silica gel is one wherein cettrimide sulphonate ion pairs are present in the eluent and are solvated predominantly by propanol molecules. These solvated ion pairs are then adsorbed most readily onto a surface which can itself be heavily solvated by propanol: the propanol molecules thus act as a binder to hold the lipophilic ion pairs onto the hydrophilic silanol surface. Any additive which interferes with this binding function, say, by displacing propanol from the surface of either the adsorbent or the ion pair or any additive which prevents the formation of ion pairs in the eluent (e.g., a strong acid), will reduce retention since neither cetrimide nor the sulphonic acids are individually retained to a significant extent with water–propanol $(1:3)$ as eluent. By contrast to the situation with a reversed-phase packing, where a predominantly aqueous eluent was used, micelles or detergent clusters are not involved.

The variation of retention with temperature is in the direction expected, that is, increase of temperature decreases $k'$. The usual Van 't Hoff type of plot indicates heats of adsorption in the range of 20–30 kJ mol$^{-1}$.

Column performance was assessed by determining the reduced plate height.
vs. reduced velocity curves for two standard solutes in normal adsorption chromatography (anisole and nitrobenzene) and for three sulphonie acids in soap chromatography. The results are shown as reduced plate height vs. reduced velocity plots in Fig. 10. Diffusion coefficients required for the calculation of the reduced velocity were calculated by the Wilke-Chang equation, which gave $D_m$ (anisole) = 3.3 x $10^{-5}$ cm$^2$/sec$^{-1}$ and $D_m$ (acetrimide-sulphanilic acid ion pair) = 1.5 x $10^{-6}$ cm$^2$/sec$^{-1}$. The second value was based upon a directly determined viscosity of 2.8 cP for water-propanol (1:3) containing 1% acetrimide. The presence of acetrimide increased the viscosity of water-propanol (1:3) by about 5%. It is clear that there is little if any difference in performance between the two systems and that a minimum plate height of around two particle diameters is attainable in both. The full performance of HPLC which we are accustomed to expect in adsorption chromatography can therefore be obtained in soap chromatography. In practice, eluents for soap chromatography are likely to be several times more viscous than for normal adsorption chromatography and the absolute mass transfer rate correspondingly lower. This means that analyses of equivalent plate performance can be performed on columns of the same dimensions operated at the same pressure drops, but that the time taken will be significantly longer. This disadvantage can be removed by working at a higher temperature where the viscosity of the eluent is reduced, and mass transfer rates proportionately increased, or by using higher pressures.

![Logarithmic plot of reduced plate height, $h$, against reduced velocity, $v$, for the elution of standards and dyestuff intermediates. Packing, 6.5-μm Porasil. Eluents: hexane for adsorption chromatography of anisole and nitrobenzene; standard eluent (see Fig. 7) for soap chromatography of sulphanilic acid, R-acid, and Ponceau MX.](image)

Fig. 10. Logarithmic plot of reduced plate height, $h$, against reduced velocity, $v$, for the elution of standards and dyestuff intermediates. Packing, 6.5-μm Porasil. Eluents: hexane for adsorption chromatography of anisole and nitrobenzene; standard eluent (see Fig. 7) for soap chromatography of sulphanilic acid, R-acid, and Ponceau MX.

Two separations carried out using the ion pair adsorption system are shown in Figs. 9 and 11. Fig. 9 again illustrates the excellent resolution of γ- and J-acids which can now be achieved, but in the reverse order to that obtained using SAS silica. Fig. 11 shows the analysis of the dye sunset yellow for impurities including residual uncoupled sulphanilic and Schäffer’s acids. A small proportion of triazine, a known impurity in sunset yellow, has been added to the mixture.
CONCLUSIONS

The technique of "soap chromatography" has proved to be remarkably effective for the resolution of sulphonic acids of interest in the dyestuff industry. The technique employs a long-chain cationic detergent, in the present work cetyltrimethylammonium bromide, which forms ion pairs with the sulphonate groups.

Separations can be carried out either on bonded reversed-phase materials or on adsorbents. The chromatographic efficiency is similar in both cases and equivalent in terms of plate height to that obtainable in high-performance adsorption chromatography.

The technique can equally well be applied to amines if an anionic detergent is used.

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New Columns for Old in Liquid Chromatography

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The purpose of this paper is to focus attention on the tremendous improvement in column performance that has occurred in liquid chromatography over the last 5 years, and to outline some of the recent work on high-performance liquid chromatographic columns and column packing materials carried out in Edinburgh.

Fig. 1 shows a typical classical chromatogram taken from the literature of 1969 with an analysis time of 30 h. It can be compared with Kirkland's famous chromatogram of the same year (Fig. 2), showing a 6-min separation of herbicides with comparable resolution, a speed increase of 300 times. Fig. 3 shows a recent chromatogram obtained in Edinburgh using 6-μm Spherisorb alumina in which six aromatic compounds are separated in 1 min with even better resolution. Such separations are now becoming common and represent a further 10-fold improvement in performance over that originally obtained by Kirkland in 1969. Further refinement may yield another 10-fold increase in speed or resolution but we shall then be close to the theoretical limit for a pressure capability of about 5000 p.s.i.

These improvements in speed have arisen from a better understanding of the kinetic features of chromatography and have been associated with the use of high pressures and small particles. In the search for higher performance, i.e., higher speed and resolution, the key question to be answered is "how can we reduce the time taken for an analysis requiring a given number of theoretical plates, N, when a specified pressure drop, ΔP, is used?" We emphasise specified pressure drop because one can nearly always increase speed by increasing ΔP and column length.

In a series of papers, it has been shown that performance in the sense of speed of analysis for a specified ΔP can best be treated in terms of the reduced or dimensionless parameters defined in equation (1):

\[ h = H/d_p \]
\[ v = u d_p/D_m \]

where \( H \) = height equivalent to a theoretical plate, \( d_p \) = particle diameter, \( u \) = linear velocity of mobile phase and \( D_m \) = solute diffusion coefficient in the mobile phase.
The reduced plate height, \( h \), is a function of the reduced velocity, \( v \), as shown in equation (2),

\[
h = \frac{B}{v} + A v^{0.33} + C v
\]

and can be regarded as made up of three contributions which arise from axial diffusions, flow and mass transfer, respectively, as originally proposed by Van Deemter in connection with liquid chromatography.

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**Fig. 1.** Classical liquid chromatogram of food additives by Nagasawa et al. Column, 430 x 17 mm; packing, Wotelm polyamide; eluent, water-acetic acid (11 : 8 + 1). Peaks: A, dehydroacetic acid; B, sorbic acid; C, benzoic acid; D, \( p \)-hydroxybenzoic acid; and E, salicylic acid. Reproduced from Journal of Chromatography by permission of Elsevier Publishing Co., Amsterdam.

**Fig. 2.** High-speed liquid chromatogram of urea herbicides by Kirkland. Column, 1000 x 2.1 mm; packing, 37–44-\( \mu \)m Zipax coated with 1 per cent. \( \mu \)m \( \beta \beta^\prime \)-oxydipropionitrile; eluent, dibutyl ether. Reproduced by permission of Preston Technical Abstracts Co., Illinois, U.S.A.

**Fig. 3.** High-speed liquid chromatogram of aromatic hydrocarbons. Column, 125 x 5 mm; packing, 6-\( \mu \)m Sphersorb alumina type AY; eluent, hexane. Peaks: A, \( \beta \)-terphenyl; B, pyrene; C, anthracene; D, \( \alpha \)-terphenyl; E, biphenyl; F, toluene; and G, injection.

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with gas chromatography. A log-log plot enables one to see at a glance which term dominates in any practical case. Thus if \( A \) is high the log \( h \) versus log \( v \) curve is flat around the minimum and somewhat high, which means poor packing. If \( C \) is large, the right wing is steep. Because dimensionless variables are used, we expect \( h \) versus \( v \) curves to be similar for all packings, irrespective of particle size. For a good packing, \( h \) should be 2-3 at the minimum and about 10 at \( v = 100 \). The \( h \) versus \( v \) curves are thus the ideal method of comparing different packings.

High-speed liquid chromatography is still relatively new and chromatographers are often at a loss to know which of the many packing materials to select from the wide range available. We have therefore compared several of the commercially available 10-14-\( \mu \)m silica gels using 5 mm bore, 125 mm long columns that were slurry packed. The results are shown in Fig. 4; similar results were obtained with 5-7-\( \mu \)m materials. All materials show a relatively flat minimum at \( h \) values of about 3 or 4. The value of \( h_{\text{min}} \) shows that we have achieved moderately good but not extremely good packing. Merckosorb shows a rather steep curve at \( v > 10 \), which implies a high \( C \) term and probably indicates that mass transfer is somewhat slower than with the other materials. Our results for Merckosorb are in close agreement with those of Majora. Partisil (marketed by Whatman) performs better at high \( v \) but comparably at lower \( v \). Spheronex (marketed by Perkin-Elmer Ltd.) and Sil-X (marketed by Perkin-Elmer Ltd.) are marginally better, but surprisingly Corning Controlled Pore Glass chips gave the best results of all materials examined; this is in agreement with the earlier work of Kennedy and Knox. The results obtained by Kirkland for Zorbax (Du Pont) shows that this material is comparable to the other silica gels studied by us in terms of its \( h \) versus \( v \) curve. From this comparison, we conclude, as did Kennedy and Knox, that little if any advantage arises from the use of spherical particles rather than broken chips.

A question often asked is what packing method should be used, dry or slurry packing. As noted above, columns equally well packed with the same material but with different particle sizes should give identical \( h \) versus \( v \) curves, but in practice smaller particles usually give somewhat higher curves, especially at velocities around the minimum \( h \) where packing is most important. Fig. 5 shows results for samples of 10- and 20-\( \mu \)m Spheronex alumina that were packed dry. The curves nearly coincide but that for the 10-\( \mu \)m material is a little higher around the minimum, as expected if the smaller material is more difficult to pack. The dimensionless column resistance, parameter \( \phi \) (defined in equation (4) below), is about as expected for porous spheres, around 1200. When the same materials are slurry packed, slightly lower values of \( h \) are obtained, as shown in Fig. 6, and the resistance para-
Fig. 5. Comparative \((h, \nu)\) curves for 10- and 20-\(\mu m\) Spherisorb alumina type AY. Column, 125 \(\times\) 5 mm; dry packing technique; eluent, hexane; solute, nitrobenzene. \(\phi\) and \(k'\) below are the column resistance parameter [see equation (4)] and the column capacity ratio, respectively:

<table>
<thead>
<tr>
<th>Spherisorb AY</th>
<th>10 (\mu m)</th>
<th>20 (\mu m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\phi)</td>
<td>1150</td>
<td>1200</td>
</tr>
<tr>
<td>(k')</td>
<td>6-6</td>
<td>6-6</td>
</tr>
</tbody>
</table>

It is also the only successful packing method for particles smaller than 10 \(\mu m\), in our experience.

While \(h\) versus \(\nu\) curves may indicate to the expert which packing performs best on an absolute basis, they do not immediately indicate to the user which packing will give him the fastest analysis under specified conditions: \(h\) versus \(\nu\) curves do in fact provide the answer, but only after calculation.

We have carried out a theoretical analysis in order to obtain elution times for a stated \(\Delta \phi\) and \(N\) using experimental \(h\) versus \(\nu\) curves and tested these in practice for Spherisorb aluminas with \(d_p\) values of 6, 7-5, 10 and 20 \(\mu m\) and using different packing methods.

Fig. 6. Comparative \((h, \nu)\) curves for 6-, 7-5- and 10-\(\mu m\) Spherisorb alumina type AY. Conditions as for Fig. 5 except columns were packed by the slurry technique.

<table>
<thead>
<tr>
<th>Spherisorb AY</th>
<th>6 (\mu m) (O)</th>
<th>7-5 (\mu m) (X)</th>
<th>10 (\mu m) (Q)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\phi)</td>
<td>600</td>
<td>470</td>
<td>610</td>
</tr>
<tr>
<td>(k')</td>
<td>3-9</td>
<td>6-5</td>
<td>6-6</td>
</tr>
</tbody>
</table>
The mathematical analysis requires manipulation of equations (3)–(6) together with equation (1):

Retention time for an unretained solute
\[ t_0 = \frac{L}{H} \quad \ldots \ldots \]  

Pressure drop
\[ \Delta p = \frac{\phi \eta L t}{d_p^2} \quad \ldots \ldots \]  

Plate number
\[ N = \frac{L}{H} = \frac{H d_p}{b} \quad \ldots \ldots \]  

Simplified plate height equation
\[ h = B \gamma + D \phi^n \quad \ldots \ldots \]

The parameters \( d_p, \phi, B, D \) and \( n \) are determined by the method of column packing and the plate height curve; \( D_m \) and \( \eta \), the viscosity of the eluting agent, are determined by the solute and eluent; \( \Delta p \) and \( N \) are set by the desired operating conditions. From these basic equations, we can readily derive expressions for the analysis time, \( t_0 \), and the necessary column length, \( L \). These are given by equations (7) and (8):

\[ t_0 = \frac{d_p^2}{D_m} \cdot X \left\{ \frac{D}{(X/N) - B} \right\}^{2(1 + n)} \quad \ldots \ldots \quad (7) \]

\[ L = \frac{d_p^2}{D_m} \cdot X \left\{ \frac{D}{(X/N) - B} \right\}^{1(1 + n)} \quad \ldots \ldots \quad (8) \]

where \( X = (\Delta p d_p^2)/(\phi \eta D_m) \).

Using the values of relevant parameters for each packing, we can now calculate \( t_0 \) for each packing as a function of \( \Delta p \). The results are shown in Fig. 7. It can be seen that except at very low pressures, the analysis time required to obtain 4000 theoretical plates should be lowest for 6-\( \mu \)m Spherosorb slurry packed and highest for 20-\( \mu \)m Spherosorb dry packed. For 10-\( \mu \)m material, the analysis should be faster if the column is slurry packed than if dry packed, largely as a result of the better permeability. The appropriate column lengths for each packing are obtained from equation (8) for each pressure.

![Fig. 7](image_url)

**Fig. 7.** Dependence of \( t_0 \) on pressure drop for columns packed with Spherosorb AY of various \( d_p \) by different techniques and calculated to give 4000 theoretical plates [equation (7)]. \( D_m = 3.3 \times 10^{-8} \text{ cm}^2 \text{s}^{-1} \), \( \eta = 3.2 \text{ cP} \). A. 6 \( \mu \)m slurry packed; B. 7.5 \( \mu \)m slurry packed; C. 10 \( \mu \)m slurry packed; D. 10 \( \mu \)m dry packed; and E. 20 \( \mu \)m dry packed.

In order to check the reliability of the above treatment, we constructed columns of the appropriate length to obtain 4000 theoretical plates with a moderate pressure drop of 330 p.s.i.

Fig. 8 shows three chromatograms on columns of the calculated length that were slurry packed with 6-, 7.5- and 10-\( \mu \)m Spherosorb A. The time scales are the same and the fastest analysis is indeed obtained with the 6-\( \mu \)m material. The number of plates is close to the
target figure of 4000, although the shorter columns (with smaller particles) actually gave more than 4000 plates.

Fig. 9 compares columns containing particles covering a wider range of size and packed by different methods. As the time scales double from the bottom of the figure, the topmost analysis is about 15 times slower than the lowest. Evidently, there is a marked difference between the 6-μm slurry packed and 10-μm dry packed material in 5 mm bore columns, and a further worsening when 20-μm material is used in a 2 mm bore column. This last configuration was used as we did not have enough 20-μm material to fill a long 5 mm bore column. There seems to be no doubt that the small particle material packed in a short wide column gives the highest performance. Again, we note that the target of 4000 plates is readily met by columns shorter than the standard length of 125 mm but that we fail to reach it for longer columns. We believe this can be explained by wall effects.

As shown by Horne, Knox and McLaren, a sample injected centrally into a column spreads slowly towards the walls. The spreading is due partly to flow and partly to transverse molecular diffusion. As the flow is seriously disturbed in the neighbourhood of the walls, highest efficiencies are obtained only if the sample can be eluted before a significant part of it reaches the walls. For a given length of column, the extent to which a sample reaches the walls depends upon velocity, as this affects the time for diffusion (the radial dispersion by flow in unaffected by velocity). At high velocities, wall effects can be avoided by choosing a sufficiently wide column, but at low enough velocities they must eventually become important. Thus there is a critical velocity below which wall effects will become increasingly important. The condition connecting column length, L, column diameter, d, particle diameter, dp, and velocity when 5 per cent. or less of solute reaches the wall was shown by Horne et al. to be given by equation (1):

\[ I = d^2/2Ld_p > 2.4 + 20/v \]
Evidence supporting the existence of a critical velocity is given in Fig. 10, where the experimental plate height has been measured for 20-μm Spherosorb alumina dry packed into 5 mm bore columns of different lengths. The arrows indicate the critical velocity given by equation (9). At velocities below those marked, \( h \) is higher for the longer than for the shorter columns, which behave as if of infinite diameter over the entire velocity range. At velocities above those marked, \( h \) is similar for long and short columns. The results are taken as clear evidence that poorer performance is obtained wherever molecules can reach the wall regions.

The reason why columns of more than the standard length of 125 mm gave less than the target figure of 4000 plates is now clearer. In the longer columns, wall effects are more important and performance suffers. In shorter columns with smaller particles, \( J \) is larger and performance is slightly better than expected in comparison with the standard column.

The above experiments emphasise the importance of achieving "infinite diameter" geometry if one is to obtain highest performance using modern microparticles. Such geometry is most readily achieved by using small particles, say of 10 μm diameter or less, and relatively wide bore columns, of say 5 mm. Column lengths can then be increased to 250 mm without loss of performance in terms of \( h \).

**EXPERIMENTAL—**

Experimental plate height measurements were carried out using both home-made chromatographs and a Du Pont Model 830 instrument.

The home-made instruments consisted of an Orilta DMP 1515 reciprocating pump (Orilta KG, Giessen, E. Germany) or a Haskel pressure intensifier pump with a 22:1 intensification ratio (Haskel Engineering and Supply Co., Burbank, Calif., U.S.A.). Pressures were measured by all-stainless-steel Bourdon gauges (American Chain and Cable Co., Bridgeport, Conn., U.S.A.). Columns were made to designs developed in this Department

![Fig. 10](image-url)  
*(8, v) curves for 5-mm bore columns of different lengths dry packed with 21-μm Spherosorb alumina type AX. Curves for columns longer than 83 mm are successively displaced vertically by 0-5 log unit. The curve for the 83-mm column is repeated with appropriate displacement for comparison purposes. For significance of arrows, see text.*
were terminated at the outlet end by 6 μm porosity stainless-steel frits (B.S.A. Sintered Components Ltd., Birmingham). Detection was carried out by ultraviolet spectrophotometry using either a Cecil Model 212 variable-wavelength ultraviolet spectrophotometer, or a Du Pont Model 410 fixed-wavelength (254 nm) ultraviolet spectrophotometer. Flow cells were generally 8 μl in volume, but for work with 6-, 7.5- and 10-μm packings a specially built 2-μl flow cell was used with the Du Pont 410 instrument. Chromatograms were recorded on a single-pan Servoscribe recorder (Smiths Instruments Ltd., Croydon).

Columns were dry packed using a specially constructed packing machine that enabled columns to be simultaneously rotated at about 60 r.p.m. and banced at a similar but different frequency. The bounce height was 2-5 mm. Packing was added in a slow continuous stream so as to build up the bed at the rate of about 10 mm min⁻¹. While the column was being packed, it was tapped with a light object at about 100 times per minute at the level of the top of the bed.

For slurry packing, the packing was dried at 120 °C and then suspended in about twice its volume of a dense solvent; dry methyl iodide proved the most satisfactory. The slurry was transferred into a 500 x 5 mm guard column connected to the analytical column, which had previously been filled with the dense solvent. The guard column was then topped up with dense solvent and the pump (a pressure intensifier) filled with a suitable driver liquid (carbon tetrachloride or hexane). The columns were connected to the pump through an on-off valve and with the valve closed the pump pressure was set at 4500 p.s.i. The valve was then opened quickly to drive the slurry at high velocity into the column. The isolation valve was then closed and opened about five times so as to "hammer" the packing home and give the densest possible packing.

Experimental plate heights were obtained from the chart records by use of the equation

\[ H = \frac{L}{16} \left( \frac{\sigma}{t_R} \right)^2 \]  

(10)

where \( \sigma \) is the base width of the peak obtained by drawing tangents at the points of inflection of the peaks and extending these to the base line, and \( t_R \) is the retention distance between the injection point and the peak maximum.

Materials

We are much indebted to the following manufacturers and suppliers for gifts of packing materials: A.E.R.E. Harwell (Materials Preparation Unit) and Phase Separations Ltd. for samples of Spherical alumina and silicas; Reeve Angel and Scientific Ltd. for samples of Partisol; Perkin Elmer Ltd. for a sample of Sil-X; and E. Merck Ltd. for samples of Merckosorb S1100.

The sample of 10-14-μm Corning Controlled Pore Glass CPG240 was obtained by grinding 200-400-mesh material (from Electro-Nucleonics, Fairfield, N.J., U.S.A.) followed by gravity fractionation in water.

References