HIGH SPEED LIQUID CHROMATOGRAPHY

by

Paul Raven, B.Sc.

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In Part I, the history of chromatography is briefly described from the time of its inception to the present day, with particular emphasis being given to the development of liquid chromatography. The thermodynamic theory of chromatography is developed and the factors determining retention in liquid-liquid and liquid-solid chromatography are considered. The kinetics of band broadening are discussed in terms of the random walk and nonequilibrium models and the pertinent equations that describe the mass transfer phenomena are quoted.

The second part of the thesis reviews the support materials that are commercially available for high speed liquid chromatography and considers the general criteria for ideal materials. Novel methods for the preparation of pellicular and totally porous silica gel supports are reported and the performance of these materials in partition or adsorption chromatography is examined in terms of the empirical relation

$$h = \frac{2Y}{V} + Av^{0.33} + Cv$$

The coefficients A and C reflect the importance of band dispersion within the flowing part of the mobile phase and dispersion due to nonequilibrium between the flowing and stationary regions of the column, whether it is stationary phase proper or stagnant mobile phase. The materials produced are compared with commercially available materials (Zipax, Corasil, Porasil, Spherisorb S) for which high quality performance data are readily available.

In Part III, polarographic detection systems for high speed
liquid chromatography are reviewed. The factors governing the
sensitivity of such detectors are delineated and the performance
characteristics of several electrodes are reported. Results are
presented to demonstrate that the low dead volume of a suitably
constructed polarographic electrode is particularly suitable for the
measurement of solute concentration at points across the column cross
section. The radial dispersion of an unretained solute in a wide
bore column is examined over a range of mobile phase velocities and
the 'infinite diameter column' equation

\[ \frac{d_c^2}{L} = 2.4 \frac{d_p}{L} \]

is examined in the light of the results obtained.

Finally, the technique of packing columns for high speed
liquid chromatography is considered in Part IV. Several methods of
varying sophistication are evaluated and the optimum method, used
throughout the work reported here, is described.
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PART I

INTRODUCTION
HIGH SPEED LIQUID CHROMATOGRAPHY has emerged over the past six or seven years as a fast quantitative technique to complement gas chromatography. The latter technique is unable to handle analyses of thermally unstable or involatile compounds without prior chemical modification of the sample. Liquid chromatography, however, is not subject to this limitation and is ideally suited to the separation of the high molecular weight, thermally labile and ionic species which make up a major part of all known organic compounds. Before the advent of High Speed Liquid Chromatography (HSLC) the separation of such compounds was carried out by the traditional form of liquid chromatography which would invariably mean analysis times of hours or days, comparing adversely with characteristic gas chromatography analysis times of minutes.

It is interesting that liquid chromatography is the oldest of chromatographic processes and yet appears to have been the least developed until recently. It seems certain that, in the past, observers had noted separations of substances by filtration through columns of finely divided adsorbents, but it is evident that in 1903, the Russian botanist, Tswett (1), was the first to be aware of the great possibilities of chromatography. He described in detail the separation of pigments and colourless substances by filtration through columns, followed by development with pure solvents. His discovery, however, received little notice and more than twenty-five years passed before Kuhn, Lederer and Winterstein (2) achieved a breakthrough in 1931.
by resolving, among other pigments, carotenes and xanthophylls on a preparative scale column of alumina and calcium carbonate. This heralded an explosive growth in the practical application of adsorption chromatography to diverse separations. A notable development of this time was the discovery of TLC in 1938 by Ismailov and Schraiber (3). There was, nevertheless, little understanding of the basic principles behind the separations until 1941 when Martin and Synge (4) published the results of their experiments in liquid-liquid partition chromatography. In this paper the authors establish the foundations of plate theory and predict that "the smallest height equivalent to a theoretical plate should be obtainable by using very small particles and a high pressure difference across the length of the column". Although their brilliant theoretical analogy could have paved the way to improved performance, their example was largely ignored. Similarly, their suggestion that the liquid mobile phase could be replaced by a gas was not followed up for more than ten years. In the interim, some advances in chromatographic technique were made by Gordon and Martin (5) in 1941, with the invention of paper chromatography and by Kirchner et al. (6) in 1950, with improvements in TLC technique. The first practical implementation of gas chromatography in 1952 by Martin and James (7) stimulated a great deal of interest in this field and its rapid development followed. Although the broad applicability of gas chromatography accounted for its initial impact, its subsequent advancement was largely as a result of greater theoretical insight into the chromatographic mechanism. De Vault (8) and Weiss (9) had modified the original plate theory and further contributions by Thomas (10) Lapidus and Amundsen (11) and Glueckhauf (12) cleared
the ground for the introduction of the van Deemter equation in 1956 (13). By the end of the 1960's, developments by Golay (14), Khan (15) and especially Giddings (16), had placed gas chromatography on a firm theoretical footing. In contrast to this, liquid chromatography had progressed little since the days of Martin and Synge. This apparent neglect undoubtedly resulted from the lack of a theoretical framework on which to base any developments. Analysts, wishing to separate compounds unsuited to gas chromatography, had to resort to the slow and tedious practice of classical liquid chromatography.

In 1963 Giddings (17), by elucidating the inter-relation of pressure drop, particle diameter, mobile phase velocity and column efficiency, showed that the theory developed for gas chromatography was equally applicable to liquid chromatography. A few years before, Hamilton (18) had applied gas chromatography principles in an attempt to improve amino acid separations. He reported that as particle diameter was reduced the peaks became sharper and narrower, but his findings were ignored, possibly because ion exchange had developed as a separate field. In a similar way, Snyder had shown great understanding of the mechanism of adsorption chromatography. Giddings' comprehensive theory had the important effect of unifying the different branches of chromatography and several workers now began the search to achieve the objectives that the theory had revealed. It was clear that improved performance could be realised by attention to a number of key factors. Firstly, because liquid viscosities are typically one hundred times larger than gas viscosities, it would be necessary to use pressurised flow of liquid to the column of up to three or four hundred atmospheres in order to achieve elution times comparable to those found in gas chromatography.
Secondly, because of the lower solute diffusion coefficients, support materials would have to be much smaller in order to give rapid mass transfer. Thirdly, special attention would have to be paid to the design of the injection port and detector in order to avoid unswept volumes that would seriously affect performance. Finally, sensitive post column detectors analogous to those used in gas chromatography would need to be developed.

The credit for the breakthrough of high speed liquid chromatography in the years 1967 - 69 falls to a number of workers: Huber (19, 20), Horvath and Lipsky (21, 22), Kirkland (23) and Waters et al. (24). The tremendous potential of the technique was now quite evident and work continued towards full realisation of the goals predicted by theory. The significant improvement in performance that Kirkland achieved was due to his use, and subsequent development of, a pellicular material as stationary phase support. The pellicular material consisted of an impervious siliceous particle coated with a porous layer of controlled thickness and pore size. This material had several advantages over the totally porous G.C. support materials then being tried in liquid chromatography, and established the criterion for subsequent liquid chromatography packing materials.

Since 1969 the practice of high speed liquid chromatography has undergone some advances both in instrument design and in the technique necessary to achieve higher performance. Improvements in instrument design have mainly been motivated by the demand for an easily operable, semi-automatic, versatile system. A range of such instruments is now commercially available, differing little in basic component structure from Kirkland's apparatus (23) but incorporating facilities for programmed gradient elution, fraction collection, column
temperature control, choice of detector etc. (25, 26, 27). The main development in the area of instrumentation has been in the increased range of detection systems. The introduction of variable or dual wavelength U.V. photometers has enhanced the applicability of this popular detector. Other detectors in general use are the refractive index monitor and wire transport system. Selective detectors have also been reported employing polarography, heat of adsorption, fluorescence etc. The more important systems have been reviewed recently in the literature (28, 29, 30). Increased efficiency has been achieved by advances on two fronts, namely, the development of suitable support materials and the perfection of specialised techniques to facilitate their packing into columns. At present 10 - 15 μm particles can be reasonably well packed without much difficulty. Supports as small as 5 μm have been used but they require a more complex packing technique. The introduction of chemically bonded stationary phases has opened up new horizons for the practice of partition chromatography. The materials initially produced by Halasz (31) were of limited stability but later materials, Du Pont Permaphases for example, can be used in a wide range of solvents without degradation. The absence of column "bleed" obviates the need for solvent pre-saturation and eluted samples are not contaminated with stationary phase. Further details of column packing materials are given in Part 2 and in recent reviews (32, 33, 34). The work of DeStefano, Beachell (35, 36, 37) and others (38, 39, 40) has brought the prospect of scaling up present technique to preparative level into perspective and it is plain that the problems this entails can be solved within the next few years. As the gap between experimental performance and theoretically
predicted performance closes, the interest in the parameters that determine minimum analysis time is liable to intensify (41, 42). This was first done by Knox (43) for gas chromatography but the principles are, of course, equally applicable in liquid chromatography. Some attempts have been made to rationalise the complex factors governing retention in adsorption and partition chromatography but the rules for selection of mobile and stationary phases are still somewhat rudimentary. In the future, this is liable to be an area that will come under close scrutiny.

The versatility of high speed liquid chromatography is readily shown by the separations reported in current literature. The wide range of sample types covered demonstrates the applicability of the technique, not only in chemistry and the chemical industry, but in biochemistry and medicine (44 - 47).

This thesis deals mainly with significant problems encountered in high speed liquid chromatography. Theory and experiment have shown that the pore structure and shape of packing materials is crucial to performance in liquid chromatography. The production and subsequent evaluation of suitable low cost materials is described in Part 2. Sensitive, quantitative detectors have played a key role in the advancement of HS LC. The polarograph holds promise as a sensitive, selective detector and its readily optimised geometry makes it ideally suitable for studies of column performance. These aspects are dealt with in Part 3. In Part 4, various methods of packing materials into columns are experimentally evaluated and the optimum method, used throughout the work reported here, is described. The initial part of the thesis is, however, devoted to the fundamental theory of chromatography with particular emphasis being given to the kinetics and thermodynamics of liquid chromatography.
CHAPTER 2

THE THEORY OF CHROMATOGRAPHY

The separation of compounds on a chromatographic column arises from the different solute migration rates along the column. The degree of separation of adjacent solute zones is quantitatively described by the resolution, Rs, defined by equation 2.1,

$$Rs = \frac{\Delta z}{w}$$  \hspace{1cm} (2.1)

where $\Delta z$ is the distance between adjacent peak maxima and $w$ is the width of the peak at its base. For a peak whose concentration profile is Gaussian, the base width is equal to four standard deviations. In the case of adjacent peaks the standard deviations, and hence the base widths, will differ slightly and an average value is normally taken. Resolution is, therefore, given by

$$Rs = \frac{t_2 - t_1}{\frac{1}{2}(w_1 + w_2)}$$  \hspace{1cm} (2.2)

where $t_1$, $t_2$ are the elution distances of peaks of base width $w_1$ and $w_2$.

The extent of peak separation, $\Delta z$, is governed by the thermodynamic properties of the system and is proportional to the distance migrated along the column. The peak width, on the other hand, is determined almost entirely by hydrodynamic and kinetic considerations and is then proportional to the square root of the distance migrated. Consequently, resolution will improve as the bands move down the column. As the thermodynamic and kinetic aspects of chromatography are essentially independent it is possible to produce satisfactory resolution by their separate optimisation.
When a band of solute is introduced onto a chromatographic column a dynamic equilibrium is established between solute in the mobile and stationary phases. This equilibrium is described by the distribution coefficient, $K$, where

$$K = \frac{\text{solute concentration in stationary phase}}{\text{solute concentration in mobile phase}} \quad (2.3)$$

The value of $K$ is essentially the same as the equilibrium value $K^e$ when averaged over a solute band although small departures from equilibrium, within the band, are inevitable when the mobile phase velocity is other than zero. These small departures from equilibrium are extremely important as shown in a later section.

The observed velocity of migration of a band down the column can vary from zero, corresponding to complete retention, to $u$, the mobile phase linear velocity corresponding to complete exclusion. This zone velocity, when expressed as a fraction of the mobile phase velocity, is given by $R$,

$$R = \frac{\text{rate of movement of band}}{\text{rate of movement of mobile phase}} = \frac{u_b}{u} \quad (2.4)$$

Any given molecule will move at a speed $u$ while in the mobile phase but will not move at all while in the stationary phase. If a large number of molecules are being considered the velocity of the solute band relative to the mobile phase is equal to the mean fractional residence time of the molecules in the mobile phase. This, in turn, is equal to the fraction of all molecules of solute which are instantaneously in the mobile phase. That is:
\[ R = \frac{u_a}{u} = \frac{t_m'}{t_m' + t_s'} = \frac{n_m}{n_m + n_s} \quad (2.5) \]

\( t_m', t_s' \), being the mean residence times of the solute molecules in the mobile and stationary phases and \( n_m, n_s \), being the number of solute molecules in the mobile and stationary phases respectively.

The fraction of solute molecules in the mobile phase at equilibrium, \( R \), is related to the distribution coefficient \( K \) by equations 2.6 and 2.7.

\[ R = \frac{n_m}{n_m + n_s} = \frac{c_m V_m}{c_m V_m + c_s V_s} \quad (2.6) \]

\[ R = \frac{V_m}{V_m + KV_S} \quad (2.7) \]

where \( c_m, c_s \), are the equilibrium concentrations in the two phases and \( V_m, V_s \), are the volumes of the mobile phase and stationary phase. This last expression is equivalent to the expression developed by Martin and Synge (4) using the plate theory.

The ratio of the quantity of solute in the stationary phase to that in the mobile phase is called the column capacity ratio, \( k' \). That is

\[ k' = \frac{c_s V_s}{c_m V_m} = K \frac{V_s}{V_m} \quad (2.8) \]

\( k' \) is readily found from the elution time of the band, \( t_r \), and that of an unretained solute, \( t_o \).
The retention ratio, $R$, is simply related to $k'$ by 2.9

$$R = \frac{u_b}{u} = \frac{t_0}{t_r} = \frac{1}{1 + k'} \quad (2.9)$$

and $k' = \frac{t_r - t_o}{t_o} \quad (2.10)$

The retention volume of a solute $V_R$ is defined as that volume of mobile phase passing through any section of the column from the moment of injection until the moment when half of the symmetrical solute band has been eluted. i.e.

$$V_R = t_r \cdot (\text{volume flow rate through the column}) \quad (2.11)$$

Equation 2.10 can therefore be rewritten as

$$k' = \frac{V_R - V_m}{V_m} \quad (2.12)$$

where $V_m$ is the interstitial volume of the column occupied by the mobile phase, the elution volume of an unretained substance. Combining this with equation 2.8 gives

$$V_R = V_m + KV_s \quad (2.13)$$

the fundamental relationship between retention volume and $K$.

The net retention volume $V_n$ is

$$V_n = V_R - V_m = KV_s \quad (2.14)$$

and finally the specific retention volume, $V_g$, is defined
It is clear that for the Gaussian concentration profile to be maintained as the solute is eluted along the column, \( K \) must be independent of concentration. This is not always the case and many of the practical problems of liquid chromatography arise from a dependence of \( K \) on concentration, which leads to peak distortion. However, for simplification of the theoretical treatments the distribution isotherm between the mobile and stationary phases will be taken to be linear in what follows. Under these circumstances the distribution coefficient can readily be related to the thermodynamic functions describing the equilibrium situation.

The selection of mobile and stationary phases to give the optimum separation of particular compounds should have some theoretical basis. Martire and Locke (48, 49) have considered the case of solute retention in partition chromatography and Snyder (50) has dealt comprehensively with the adsorption mode. The situation is, however, of some complexity and the accuracy in predicting \( k' \) values depends to a large extent on the simplicity of the system.

\section{Liquid-Liquid Partition Chromatography}

Martire and Locke (48, 49) consider the liquid-liquid partition system in terms of a binary solution of two compounds in equilibrium, namely the solvent and the solute. From this model they show that the solute activity coefficient \( g_2(T,P) \) in either phase of the system is related to the solute mole fraction and chemical potential in that phase at a standard pressure \( P^* \) by

$$
\frac{V_g}{w_s} = \frac{V_n}{w_s} = \frac{K}{\zeta_s}
$$

\( w_s \) is the weight of stationary phase in the column and \( \zeta_s \) its density.
\[ RT \ln x^i_2 (T,P) = \mu^i_2 (T,P) - \mu^*_{2i} (T,P*) - (P-P*) v^o_2 - RT \ln x^i_2 \] (2.15)

\[ \mu^i_2 (T,P) \] is the solute chemical potential in phase \( i \) at the system temperature \( T \) and pressure \( P \). \[ \mu^*_{2i} (T,P*) \] is this chemical potential at standard pressure \( P* \). \( v^o_2 \) is the molar volume of the pure solute, assumed to be independent of pressure, and \( x^i_2 \) is the mole fraction of the solute in phase \( i \).

Applying the condition that at equilibrium the chemical potentials in the two phases are equal, and defining the solute standard state as the pure liquid state, it can be shown that

\[ \ln \frac{x^s_2}{x^m_2} = \ln \frac{\gamma^m_2 (T,P*)}{\gamma^s_2 (T,P*)} + \frac{(P-P*) (\bar{v}^m_2 - \bar{v}^s_2)}{RT} \] (2.16)

The superscripts \( s \) and \( m \) denote the stationary and mobile phases, respectively. \( \bar{v}^m_2 \), \( \bar{v}^s_2 \) are the partial molar volumes of the solute in the appropriate phase.

The distribution coefficient, \( K \), is defined as the ratio of solute concentration in the stationary phase to that in the mobile phase.

In very dilute solutions this can be expressed as

\[ K = \frac{x^s_2 v^o_m}{x^m_2 v^o_s} \] (2.17)

\( v^o_m \), \( v^o_s \) being the molar volumes of the pure mobile and stationary phases.

In view of equations 2.16 and 2.17 it can be stated that

\[ \ln K = \ln \frac{\gamma^m_{2,\infty} (T,P*) v^o_m}{\gamma^s_{2,\infty} (T,P*) v^o_s} + \frac{(P-P*) (\bar{v}^m_{2,\infty} - \bar{v}^s_{2,\infty})}{RT} \] (2.18)
The additional superscript $\infty$ indicates that the condition of infinite dilution prevails.

Equation 2.18 can be written in terms of the specific retention volume. By convention, the standard pressure $P^\text{\#}$ is 1 atmosphere.

\[
\ln \frac{V}{g} = \ln \frac{\gamma_2 m^{\infty} (T, 1) M_m}{\gamma_2 s^{\infty} (T, 1) M_s \rho_m(T)} + \frac{(P-1) (V_2 m^{\infty} - V_2 s^{\infty})}{RT} \quad (2.19)
\]

$M_m$ and $M_s$ are the molecular weights of the mobile and stationary phases and $\rho_m(T)$ the eluent density at the system temperature.

At the pressures used in liquid chromatography, the second term on the right hand side of the last equation is negligible and to a good approximation.

\[
\frac{V}{g} = \frac{\gamma_2 m^{\infty} M_m}{\gamma_2 s^{\infty} M_s \rho_m} \quad (2.20)
\]

The equation enables a priori prediction of retention from the physical properties of the sample, stationary phase and mobile phase when the conditions imposed are met. In practice, most experimental systems will fulfill these conditions. Small sample sizes ensure that the system operates in the linear portion of the sorption isotherm and that the solutions are effectively infinitely dilute. From practical considerations, the use of phases that are virtually immiscible preserves the integrity of the thermodynamic parameters. Partition equilibrium is established rapidly enough that departure from equilibrium, summed over the whole column, is negligible. The expression is, however, only valid for bulk liquid-liquid partitioning.
If other separation mechanisms are operating, such as solute adsorption at the liquid/liquid or liquid/solid interface, a correction must be made.

2.2 Liquid-Solid Adsorption Chromatography

The role played by the adsorbent, solvent and sample in determining solute retention in adsorption chromatography has been considered in depth by Snyder [50]. At the outset the adsorption system is described in terms of the Langmuir isotherm. The adsorbent surface is considered to consist of a number of distinct and identical adsorption sites for which solvent and sample molecules are in competition. It is assumed that adjacent molecules in the adsorbed monolayer do not interact, i.e. sample concentrations are low. The thermodynamic equilibrium constant for adsorption of a sample molecule X is

\[
K_{th} = \frac{N_{xa}}{N_{xm}}
\]  

(2.21)

where \( N_{xa} \) is the mole fraction of X in the adsorbed phase and \( N_{xm} \) is the mole fraction of X in the nonadsorbed phase. At low sample concentrations the total moles of sample in the adsorbed (\( n_{xa} \)) and non-adsorbed (\( n_{xm} \)) phases is small compared to the total moles of solvent in the adsorbed (\( n_{sa} \)) and nonadsorbed (\( n_{sm} \)) phases. Mole fractions of X in the two phases are then approximated by

\[
N_{xa} = \frac{n_{xa}}{n_{sa}} \quad \text{and} \quad N_{xm} = \frac{n_{xm}}{n_{sm}}
\]  

(2.22)
The distribution coefficient given in equation 2.3 is, in this case,

\[ K = \frac{(n_{xa}/W)}{(n_{m}/V_m)} \]  \hspace{1cm} (2.23)

where \( W \) is the weight of the adsorbent in grams.

Combination of the last three equations yields

\[ K = \frac{K_{th} n_{sa} V_m}{n_{sn} W} \]  \hspace{1cm} (2.24)

If \( V_a \) is defined as the volume of an adsorbed solvent monolayer per unit weight of adsorbent

\[ V_a = \frac{n_{sa} V_m}{n_{sn} W} \]  \hspace{1cm} (2.25)

then

\[ K = K_{th} V_a \]  \hspace{1cm} (2.26)

\( K_{th} \) is related to the standard free energy of adsorption by

\[ \log K_{th} = -\frac{\Delta G^o_a}{2.3RT} \]  \hspace{1cm} (2.27)

The quantity \(-\frac{\Delta G^o_a}{2.3RT}\) can be regarded as a dimensionless net adsorption free energy \( \Delta E \). Equation 2.26 can now be expressed as

\[ \log K = \log V_a + \Delta E \]  \hspace{1cm} (2.28)

The origin of the net adsorption energy term can be more fully understood by considering the adsorption of a sample molecule \( X \) from the liquid phase.

\[ X_l + mS_a \rightleftharpoons X_a + mS_l \]  \hspace{1cm} (2.29)
As the surface is considered to be initially covered with a monolayer of adsorbed solvent molecules, $S$, the sample molecule in the liquid phase must displace $m$ of these to give an adsorbed species and $m$ solvent molecules in the liquid phase. The net energy of adsorption is thus given by

$$\Delta E = E_{xa} + mE_{sl} - E_{xl} - mE_{sa} \quad (2.30)$$

where $x$, $s$ refer to the solute and solvent and $a$, $l$ the adsorbed and liquid phases. There is justification on both theoretical and empirical grounds for neglecting the liquid phase energy terms, so that we can approximate 2.30 and write

$$\Delta E = E_{xa} - mE_{sa} \quad (2.31)$$

If these remaining adsorption energies are considered in terms of a standard activity adsorbent, $E_{xa}$ may be replaced by $S^0_x$, the sample adsorption energy on the standard activity surface. Similarly, if $c^0$ is the adsorption energy of the solvent per unit area of the standard activity surface $E_{sa}$ may be replaced by $c^0A_s$, where $A_s$ is the area required by the adsorbed solvent molecule. $m$ is simply the ratio of adsorbed sample area, $A_x$, to that of the adsorbed solvent molecule, i.e.

$$m = \frac{A_x}{A_s}$$

Hence, allowing for different surface activities by introducing an activity factor, $\alpha$, (where $\alpha = 1$ for the standard activity adsorbent) we obtain

$$\Delta E = \alpha(S^0_x - A_x c^0) \quad (2.32)$$
Substitution of this into equation 2.28 yields Snyder's basic equation

$$\log K = \log V_a + \alpha (S_x^o - A_x e^o)$$  \hspace{1cm} (2.33)

Sample K values can therefore be predicted from the fundamental properties of the adsorbent \((V_a, \alpha)\), sample \((S_x^o, A_x)\) and solvent \((e^o)\). This equation has been found to be reasonably accurate for solvents of weak to moderate strength. For strong solvents the liquid phase energy terms of equation 2.30 cannot be totally ignored. A correction term must then be added to the right hand side of equation 2.32 to account for these secondary solvent effects.

\(V_a\) values are simply calculated from the specific surface area of the adsorbent, corrected for added water, and the thickness of the adsorbed monolayer.

The solvent parameter \(e^o\) is a measure of solvent strength and is evaluated or estimated relative to pentane, for which \(e^o\) is defined as zero. Snyder has listed the \(e^o\) values of many pure solvents on silica and has developed empirical relationships to enable their calculation for other adsorbents and also for solvent mixtures.

The calculation of the area covered by an adsorbed sample molecule is generally straightforward for aromatic compounds where the adsorbed configuration can usually be assumed to be flat. Organic molecules can be considered as consisting of various combinations of a limited number of separate chemical groups. The area of the adsorbed solute can then be calculated on a group contribution basis:

$$A_s = \sum_{i} a_i$$  \hspace{1cm} (2.34)
Tabulated values of the group areas, $a_i$, are available. The configuration and $A_g$ value of an adsorbed aliphatic molecule is more difficult to quantify. The flexibility of the aliphatic chain or alkyl group attached to an aromatic nucleus allows a variety of configurations, in some of which only a part of the molecule is adsorbed.

The sample adsorption energies, $S_x^0$, are primarily determined by the types and total numbers of groups present in the molecule and to a lesser extent by the arrangement of these groups within the sample molecule. The total energy of interaction between a sample molecule and the adsorbent surface can be regarded as the sum of the energies of interaction of each atom or group $i$ in the molecule with the surface. To a first approximation

$$S_x^0 = \sum Q_i^0$$

(2.35)

where $Q_i^0$ is the dimensionless free energy of adsorption of the group $i$ on the standard activity surface. Unfortunately the direct application of this simple relation gives realistic results only for the simplest of systems. In a sample molecule containing a number of groups $i,j,k$ etc., the strongest adsorbing group will be localised on a strong adsorbent site. The geometry of the molecule is then likely to prevent optimum adsorption of the remaining groups and in some cases they may be virtually unadsorbed. A correction for the loss in adsorption energy should then be made, but its magnitude is difficult to predict. The secondary effect of sample structure, the influence of different arrangements of groups in the molecule, can make an important contribution to $S_x^0$ through interactions
such as steric hindrance and intramolecular hydrogen bonding. These, and other interactions can be corrected for by inclusion of additional terms in equation 2.35. The final form is now

\[
S_i^0 = \sum Q_i^0 - f(Q_k^0) \sum Q_i^0 - \sum \sum i \cdot j
\]  

(2.36)

The second term on the right hand side is the correction for group k's localisation. The final term represents the contribution to i's adsorption energy due to interaction with j.

While Snyder has greatly clarified many of the factors that determine the extent of sample adsorption, the accuracy of predicted k' values is limited in some respects by the lack of published values of the parameters described above. As a consequence, the theory is best described as semi-quantitative, although in well defined systems the agreement between experimental and predicted data is impressive.

2.3 **Kinetics of Chromatography**

It has already been pointed out that band broadening in the chromatographic column is mainly kinetic in origin. The development of a theory dealing with the phenomenon has been a major challenge since the early days of chromatography and remarkable advances have been made. Martin and Synge (4) were the first to attempt a simple explanation of band spreading under conditions of a linear isotherm. They considered the column to consist of a number of discrete layers or plates and defined the height equivalent to a theoretical plate (HETP or H) as the thickness of a layer such that the solution issuing from it is in equilibrium with the mean concentration of solute in the non-mobile phase throughout the layer.
Although their theory has been criticised for its inability to describe the essential kinetic processes, their treatment showed that an initially sharp solute pulse would gradually acquire a Gaussian profile as it progressed through the bed. In addition, Martin and Synge, using intuitive arguments, deduced that \( H \) depended upon diffusion rates, particle size and flow velocity, indicating that there was an optimum rate of flow for minimum plate height.

The concept of the theoretical plate has survived in present day usage largely because of its convenience in describing quantitatively the extent of zone dispersion. \( H \) is now defined as the rate of increase of variance of the Gaussian profile per unit length of column, i.e.

\[
H = \frac{\partial \sigma_z^2}{\partial z} \quad (2.37)
\]

For a uniform column of length \( L \), this becomes

\[
H = \frac{\sigma_z^2}{L} \quad (2.38)
\]

\( N \), the number of theoretical plates is then

\[
N = \frac{L}{H} \quad (2.39)
\]

Martin and Synge assumed that the effects of longitudinal diffusion were negligible and that equilibrium between the two phases was maintained throughout the plate. They were, however, aware that this was an approximation and discussed the effects of non-equilibrium.

The assumption of plate wide equilibrium persisted through subsequent improvements of plate theory until Thomas (10) derived complex mathematical expressions to describe the nonequilibrium situation. To obtain a tractable solution, Thomas imposed the
condition that the departure from equilibrium was only slight, an approximation that was used to advantage in later theories. Lapidus and Amundsen (11) argued that nonequilibrium could only be considered as slight at low flow velocities, where the effect of longitudinal molecular diffusion would be pronounced. In 1952, therefore they deduced a general equation which accounted for band broadening from a finite rate of mass transfer and axial diffusion. Gluskauf (12) and his associates were the first to show how such factors as particle size, intraparticle diffusion and diffusion in the film around the particle could be related to HETP. A more general approach to this combination of plate and rate theories was inspired by van Deemter, Zuidereg and Klinkenberg (13) whose work enjoyed great popularity following its experimental application to gas-liquid chromatography by Keulemans and Kwantes (51). The van Deemter equation

\[ H = 2d_p \frac{2Y_D}{u} + 23D \frac{(k')^2}{(1+k')^2} \frac{u}{D} + \frac{d_s^2}{u} \]  

is normally expressed as

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

The terms on the right hand side of the equation represent the contributions to the overall plate height from particular column processes: The A term is the contribution resulting from variations in the carrier flow velocity at different points within the bed. This is purely a flow pattern effect determined by the packing material geometry. The B term arises from axial molecular diffusion and effective diffusion rate in the mobile phase. The C term arises
from the finite rate of equilibrium of solute between the moving and stationary phase. Van Deemter et al. originally neglected the contribution of mass transfer in the mobile phase on the grounds that for gas chromatography it was several orders of magnitude smaller than the liquid diffusion term. In practice this is not always so in gas chromatography and in liquid chromatography it is certainly not so. The C term for liquid chromatography must be modified to include both a mobile phase and stationary phase mass transfer contribution.

The use of statistical concepts to describe the chromatographic phenomena had been introduced by Giddings and Eyring (52). From these concepts evolved a simple model that described the role of the underlying kinetic and diffusion processes (53, 17). The Random Walk Model, as it is known, provides simple expressions for the dependence of plate height on flow velocity, particle diameter, diffusion coefficients, rate constants and stationary phase dimensions which are essentially correct except for the values of geometrical constants. According to the random walk model, zone spreading will arise whenever high and low velocities exist side by side with molecules exchanging between them. Through this transfer the molecules undergo a random motion back and forth with respect to the zone centre. Velocity inequalities occur between the mobile phase and stationary phase, and within the mobile phase itself, both parallel and perpendicular to the column axis. When considering a large number of molecules undergoing a random process, the variance of the resulting Gaussian concentration profile after a period of time is given by

\[ \sigma^2 = 1^2 n \]  

(2.42)
where \( l \), the step length, is the average length of the displacement relative to the zone centre and \( n \) is the number of steps taken. The variance of the Gaussian profile resulting from a number of independent and simultaneous random processes is given by the sum of the variances of the component mechanisms:

\[
\sigma^2 = \sigma_1^2 + \sigma_2^2 + \sigma_3^2 + \ldots
\]  

(2.43)

From this it follows that

\[
H_{\text{total}} = H_1 + H_2 + H_3 + \ldots
\]  

(2.44)

The problem of relating plate height to the inherent dispersive processes is now simply one of expressing each of the processes in terms of equation 2.42. Following this scheme, Giddings has estimated the plate height contributions for the various dispersive processes occurring in the column. The spreading due to axial molecular diffusion is quite independent of the others and results in a plate height contribution given by 2.45

\[
H = \frac{2 \delta D_m}{u} + \frac{2 \delta' D_s}{u}
\]  

(2.45)

\( D_m \) is the solute diffusion coefficient in the mobile phase. The obstructive factor \( \delta \) is included because the particles in the bed force the molecules to take a roundabout path. In liquid chromatography a second term, \( \delta' \), is required to allow for diffusion in the stationary phase. The value of \( \delta' \) is uncertain and may be very small.

Two fundamentally different mechanisms control solute sorption and desorption. The kinetics can be determined by either of the mechanisms or a combination of both. The adsorption of solute onto a surface site and its subsequent desorption are abrupt molecular processes. On the other hand, solute absorption and desorption within
a liquid stationary phase is by gradual diffusion. In liquid-solid chromatography, therefore, the component processes are solute diffusion in the mobile phase to and from the adsorbent surface, accompanied by adsorption/desorption steps determined by energetic factors. In contrast, partition chromatography kinetics are determined solely by diffusion processes in both phases. From the random walk theory, the contribution to the plate height of adsorption–desorption kinetics for a simple first order process is given by

\[ H = \frac{2}{(1+k')^2} \cdot t_d \cdot u \]  (2.46)

where \( t_d \) is the solute mean desorption time. A similar expression is derived for diffusion in a liquid stationary phase where the desorption time is governed by the diffusivity of the solute in the phase, \( D_s \), and the average thickness of the film, \( d_s \).

\[ H = \frac{k'}{(1+k')^2} \cdot \frac{d_s^2}{D_m} \cdot u \]  (2.47)

The effects of solute diffusion in the mobile phase are complicated by the non-uniformity of velocity across the column. Imperfections in the packed bed structure mean that the dimensions of channels and void spaces between particles are varied. As flow is more rapid in open unrestricted flow paths the solvent velocity will fluctuate from channel to channel. In addition, because of viscous drag, the velocity at the centre of each interstitial flow channel is greater than that near the walls. Finally, a velocity inequality exists between the streaming part of the mobile phase and the stagnant solvent entrained in the space within a porous support.
Solute molecules caught up in the fast streampaths will be displaced further downstream than those caught up in the slower streampaths. The complex flow pattern within the column ensures that the molecules are carried to subsequent parts of the channel network where other velocities prevail. The solute molecules, therefore, experience a random succession of velocities as they pass along the bed; a regime that will in itself result in band dispersion. If the flow is laminar, as is generally the case, this effect is purely geometrical and no velocity dependence is expected. In agreement with this, the random walk treatment yields

$$H_f = \sum_i 2\lambda_i d_p$$

(2.48)

$H_f$ being the contribution due to flow alone. $\lambda_i$ is the eddy diffusion coefficient for the $i$th velocity inequality identified within the column and is determined purely by the bed structure. This flow mechanism of band spreading is termed "classical eddy diffusion". The superposition of molecular diffusion on the flow pattern has important consequences. Lateral diffusion of the solute molecules into adjacent streampaths also results in a random exchange of velocities. The plate height contribution, $H_d$, due to this diffusive mechanism alone is

$$H_d = \sum_i \frac{\omega_i d_p^2}{D_m} u$$

(2.49)

$\omega_i$ is the mobile phase contribution coefficient for the $i$th velocity inequality. Giddings has argued that although the flow and lateral diffusion mechanisms of molecular exchange between velocities are occurring simultaneously, their effect is co-operative so that the step in a flow dominated random walk may be prematurely terminated by lateral
diffusion, while the opposite may happen in a diffusion dominated random walk. As a result, the plate height contribution of the two mechanisms, $H_f + d$, is not a simple summation as the classical approach would predict. Giddings has proposed that the expression

$$H_{f+d} = \frac{1}{H_f} + \frac{1}{H_d}$$

reflects more accurately the effect of the "coupling" of flow and diffusive mechanisms.

The coupling equation

$$H_{f+d} = \frac{1}{M} \left[ \frac{1}{2 \Lambda_p d_p} + \frac{d_m}{\omega_d d_p^2 u} \right]^{-1} \quad (2.50)$$

contrasts significantly with the classical expression. The value of $H_{f+d}$ is less than either $H_f$ or $H_d$. At high velocities $H_{f+d}$ approaches the constant term $2 \Lambda_p d_p$, reflecting the dominance of the flow mechanism. At low velocities $H_{f+d}$ will be approximately proportional to velocity as the diffusive term $\omega_d d_p^2 u/d_m$ is dominant.

In summary, the random walk has shown that longitudinal molecular diffusion gives a plate height inversely proportional to the mobile phase linear velocity. This can be abbreviated as $B/u$. The contribution due to a finite rate of mass transfer in the stationary phase can arise from adsorption-desorption kinetics or originate in a diffusion controlled process in the stationary phase. This contribution is proportional to velocity and can be written as $Cu$. Finally, diffusion in the mobile phase gives a velocity dependent contribution, $C_m u$, that is coupled with a velocity independent contribution, $A$, arising from the bed geometry. Over a broad range of velocities the full expression can then be written as

$$H = \frac{B}{u} + Cu + \left[ \frac{1}{A} + \frac{1}{C_m u} \right]^{-1} \quad (2.51)$$
The random walk approach to describing the origin of zone dispersion is acceptable in view of its simplicity and absence of mathematical complexities, especially in dealing with the complication of the coupling mechanism. Its validity is, nevertheless, limited.

Molecular motion and velocity transitions within the column are continuous and not discrete changes. The values of $\lambda_1$ and $\omega_1$ are inexact because of the arbitrary assignment of a step length to a particular process, nor is it clear how many terms are required in the summation.

The relative importance of the flow and diffusive mechanisms is given by the ratio of $H_d$ to $H_f$

$$\frac{H_d}{H_f} = \frac{\omega_1}{2\lambda_1} \frac{ud_p}{D_m}$$

The dimensionless quantity $ud_p/D_m$ scales the mobile phase velocity to the rate of diffusion over a particle diameter. This quantity is defined as the reduced velocity, $v$.

$$v = \frac{ud_p}{D_m}$$

At the same reduced velocity the relative role of flow and diffusive mechanisms is the same in all chromatographic systems. This implies that support materials in different systems can be compared under standardised mobile phase conditions.

The plate height can be similarly scaled to the particle diameter by defining a reduced plate height, $h$

$$h = \frac{H_d}{D_p}$$
Knox (54) has shown that the simple form of the coupling expression suggested by Giddings, presented here in reduced terms

$$ h = \frac{1}{2\lambda} + \frac{1}{\omega v} $$

does not fit the experimental data and can be replaced by a more accurate integral form. The intricacies that this form would introduce are avoided by a simpler empirical equation proposed by Huber (55)

$$ h = \left[ \frac{1}{2\lambda} + \frac{1}{CV} \right]^{-1} $$

The deficiencies of the random walk model in describing sorption-desorption kinetics can largely be overcome by Giddings' nonequilibrium theory (17). The errors that arise from the random walk approach can be traced to errors in the model and in the development of the theory. In the nonequilibrium approach all significant errors reside in the model itself. Failure of the model and its theory to predict chromatographic events merely requires that the model be modified to represent the basic dynamic processes more realistically. The random walk theory dealt with the column events on a micro-statistical level, observing the individual random motion of the molecules. The nonequilibrium theory deals with precisely the same random processes but on a macro-statistical level, studying the overall effect of the mechanisms. The nature of nonequilibrium can best be understood by reference to Figure 1. The solute in the mobile phase is in complete equilibrium with the solute in the stationary phase while the mobile phase is at rest. When the mobile phase is moving with velocity u, the concentration profile is carried forward and a moving concentration gradient is established between
the two phases. At the leading edge of the profile this gradient is negative, and, in an attempt to maintain equilibrium, molecules will tend to be sorbed into the stationary phase. At the trailing edge the gradient is positive and molecules will tend to be desorbed. Because the sorption-desorption processes require a finite amount of time to occur, the mobile phase solute concentration is displaced ahead of its equilibrium concentration, while the actual concentration in the stationary phase lags behind its equilibrium value. This view of nonequilibrium also demonstrates the origin of zone spreading. Previously, it has been noted that the band velocity along the column is proportional to the fraction of solute molecules, \( R \), in the mobile phase at a given point. The picture of nonequilibrium just described has shown that the fraction of molecules in the leading edge of the zone is greater than \( R \), while at the trailing edge the fraction is less than the equilibrium value. Thus the velocity of the molecules in the leading edge is greater than that of the zone centre. This in its turn is greater than the velocity of the molecules in the trailing edge. Hence, as it moves down the column, the band is observed to broaden. The rate of broadening is directly proportional to the mobile phase velocity and inversely proportional to the rate of sorption-desorption. Because of the similarity of this kinetic band spreading to molecular diffusion it can be described by an effective diffusion coefficient, \( D \), which is related to the plate height by

\[
H = \frac{2D}{u} (1 + k') = \frac{2D}{Ru} \quad (2.57)
\]
In order to demonstrate the nonequilibrium approach to describing
mass transfer processes, a typical development is outlined below.
For this purpose a simplified model is chosen, in which case the need
for detailed mathematics is unnecessary. It is understood that the
idealised situation presented below shows little resemblance to the
real chromatographic circumstances but the primary advantage of
the nonequilibrium theory is that it is equally applicable to
complex models by extension to include more complicated mass transfer
phenomena.

The simple model for adsorption chromatography is that of a
uniform surface consisting of equivalent sites. The solute concen-
tration per unit volume of column packing $c_0$ may be split into
two parts $c_m$ and $c_s$, corresponding to the amounts in the mobile and
stationary phase respectively. $c_m^*$ and $c_s^*$ denote these concentrations
when equilibrium is attained. The degree of nonequilibrium can then
be described for each phase by the fractional departure from
equilibrium $\varepsilon_m, \varepsilon_s$, where

$$\varepsilon_m = \frac{c_m - c_m^*}{c_m^*} \quad \text{and} \quad \varepsilon_s = \frac{c_s - c_s^*}{c_s^*} \quad (2.58)$$

The departure from equilibrium in one phase is plainly matched by
the departure in the other so that

$$\varepsilon_m c_m^* + \varepsilon_s c_s^* = 0 \quad (2.59)$$

The objective of the ensuing development is to relate the extent
of nonequilibrium to the zone dispersion by considering both in terms
of the rate of mass transfer. In a system where the apparent first
order rate constant for solute adsorption is $k_a$, and that for
desorption $k_d$, the rate of mass transfer is the net rate of increase of $c_m$

$$\left( \frac{dc_m}{dt} \right)_{mt} = k_d c_s - k_a c_m \quad (2.60)$$

substituting for $c_m$ and $c_s$ (equation 2.58) this can be written

$$\left( \frac{dc_m}{dt} \right)_{mt} = k_d c_s - k_a c_m + k_d c_s c_m - k_a c_m c_m \quad (2.61)$$

At equilibrium the rate of mass transfer is zero, therefore

$$k_d c_s - k_a c_m = 0 \quad (2.62)$$

In view of this and equation 2.59 the rate of mass transfer under nonequilibrium is

$$\left( \frac{dc_m}{dt} \right)_{mt} = -c_m (k_a + k_d) c_m \quad (2.63)$$

We can now turn our attention to considering the effect of additional solute material being carried into a particular region by flow. This must be redistributed between the phases. General principles of mass conservation show that the net change in the amount of material in the region is related to the gains and losses due to mass transfer and flow

$$\frac{\partial c_m}{\partial t} = \left( \frac{dc_m}{dt} \right)_{mt} - u \frac{\partial c_m}{\partial z} \quad (2.64)$$

The second term on the right hand side is the contribution due to flow, $\partial c_m/\partial z$ being the concentration gradient within the region.
One of the fundamental assumptions of nonequilibrium theory is that departure from equilibrium is not large. In a real chromatographic process the departure at a particular location may indeed be great but over the whole system the near-equilibrium assumption is generally valid. This greatly simplifies the solution to the last equation for \( c_m^* \) and \( c_m \) can now be equated. Equation 2.64 can be rewritten and rearranged to give

\[
\left( \frac{dc_m}{dt} \right)_{mt} = \frac{\partial c_m^*}{\partial t} + u \frac{\partial c_m^*}{\partial z} \tag{2.65}
\]

Because \( R \) is equal to \( c_m^*/c \) the first term on the right hand side is

\[
\frac{\partial c_m^*}{\partial t} = R \frac{\partial c}{\partial t} \tag{2.66}
\]

In physical terms \( \partial c/\partial t \) is the rate at which the overall concentration of solute increases within the region. This can only be affected through flow as mass transfer only redistributes solute within the region. Hence an equation of mass conservation shows

\[
R \frac{\partial c}{\partial t} = -uR \frac{\partial c_m}{\partial z} \tag{2.67}
\]

If the degree of nonequilibrium is small this becomes

\[
R \frac{\partial c}{\partial t} = -uR \frac{\partial c_m^*}{\partial z} \tag{2.68}
\]

a form that is suitable for inserting back into equation 2.65 which now reads

\[
\left( \frac{dc_m}{dt} \right)_{mt} = (1 - R) u \frac{\partial c_m^*}{\partial z} \tag{2.69}
\]
The two expressions derived for \( \frac{dc_m}{dt} \) (equations 2.63 and 2.69) can now be equated and solved for \( \epsilon_m \):

\[
\epsilon_m = - \frac{(1 - R)u}{k_a + k_d} \epsilon_m + \frac{1}{\epsilon_m} \frac{\partial \epsilon_m}{\partial z} \quad (2.70)
\]

The final stage of this development serves to relate \( \epsilon_m \) to the plate height in the following way. Zone dispersion can be seen as an excess (above the equilibrium amount) of solute being carried forward at the leading edge and a deficiency at the trailing edge. The amount of solute, \( J \), carried through unit cross sectional area of the column by the mobile phase in unit time is

\[
J = c_m u \quad (2.71)
\]

If equilibrium were established this amount is \( J^* \) and

\[
J^* = c_m^* u \quad (2.72)
\]

The amount of solute associated with band spreading is the excess amount \( J - J^* \). From the last two expressions this is equal to \( u(c_m - c_m^*) \). From the definition of \( \epsilon_m \) (equation 2.58) this can be written

\[
J - J^* = uc_m^* \epsilon_m \quad (2.73)
\]

Substitution for \( \epsilon_m \) from equation 2.70 yields

\[
J - J^* = - \frac{(1 - R)u^2}{k_a + k_d} \frac{\partial \epsilon_m}{\partial z}
\]

\[
= - \frac{R(1 - R)u^2}{k_a + k_d} \frac{\partial c}{\partial z} \quad (2.74)
\]
The link between $J - J^*$ and the plate height is forged by Fick's law of diffusion which in this case is stated as

$$J - J^* = -D \left( \frac{\partial c}{\partial z} \right)$$  \hspace{1cm} (2.75)

$D$ has been defined previously as the effective diffusion coefficient, related to plate height by equation 2.57. Therefore we can now write

$$H = \frac{2(1 - R)}{k_a + k_d} u$$  \hspace{1cm} (2.76)

Comparison of this with the form obtained from the random walk theory is facilitated by substituting for $(k_a + k_d)$.

$$k_a = k_d \frac{c_s^*}{c_m^*} \text{ (from equation 2.62)}$$

Therefore,

$$k_a + k_d = k_d \left( \frac{c_s^*}{c_m^*} + 1 \right)$$

$$= k_d \left( \frac{c^*}{c_m^*} + 1 \right)$$

$$= k_d \frac{c}{c_m}$$

$$= k_d / R$$

Equation 2.76 now reads

$$H = \frac{2R(1 - R)}{k_d} u = 2R (1 - R) t_d u$$  \hspace{1cm} (2.77)

This is identical to that derived from the random walk theory. A parallel approach is used in the partition model to derive the contribution due to diffusional mass transport in a uniform layer of stationary phase, although the procedure is somewhat more complicated because of concentration gradients within the stationary phase layer.

The idealised models described above serve little purpose apart
from delineating the general approach. Giddings has extended the models to more realistic systems that are characterised by non-uniform adsorption sites, non-uniform layers of partitioning material and mixed mechanisms of retention. From the generalised non-equilibrium theory the contribution to the plate height due to diffusion in the stationary phase is given as

\[ H = q_s \frac{k'}{(1+k')^2} \cdot \frac{d^2_s}{D_s} u \]  \hspace{1cm} (2.78)

The form of the equation is identical to that developed from the random walk theory, differing only in the numerical constant. The configurational factor, \( q_s \), depends upon the shape of each unit of stationary phase. Diffusion within a porous support particle containing mobile phase contributes

\[ H = q_m \left( 1 + \frac{\alpha k'}{1+k'} \right)^2 \frac{d_{1}^2}{D_m} u \]  \hspace{1cm} (2.79)

\( q_m \) and \( \alpha \) depend upon the geometry of the mobile phase within the intraparticle space. \( d_1 \) is the thickness of the porous layer and is taken as \( d_p \) for a totally porous support.
PART II

SUPPORT MATERIALS FOR HIGH SPEED LIQUID CHROMATOGRAPHY
3.1 General Criteria for HSCLC Supports

The design and manufacture of chromatographic support materials specifically for use in HSCLC has been of prime importance in the achievement of high efficiency separations. The classical form of liquid chromatography employs large diameter porous particles (>200μm) and liquid flow is produced by gravity feed. Efficiencies of this technique are of the order $10^{-2} - 10^{-3}$ theoretical plates per second. It is true that the use of pressurised flow alone would lead to some increased speed of separation but other factors, crucial to improved resolution, must be considered. The theory outlined in the previous chapter shows that the slow diffusion of solutes in liquids imposes two criteria upon the design of suitable porous supports. Firstly, the material, when packed in a column, must form a homogenous bed in order that the flow of liquid through the bed is uniform and no transcolumn velocity differences occur. As the flow velocity in an interparticle channel in the bed depends upon the shape and diameter of that channel, it is clear that careful control of particle shape and size will produce channels of similar dimensions throughout a regular packed bed. Classical column packings, which are roughly graded materials characterised by a wide size distribution and irregular particle shape, are clearly unsuitable for homogeneous packing. The second criterion concerns the porous nature of the materials. Slow solute diffusion into and out of deep pores containing stagnant liquid, either stationary phase or stagnant mobile phase, results in a major contribution to band spreading. The efficiency of this process is
improved by shortening the distance the solute molecules must diffuse i.e. by decreasing the diameter of the particle. Obviously, the use of particle diameters as large as 200\(\mu\)m associated with classical techniques is not conducive to fast mass transfer. In addition, because of the high pressures used to achieve reasonable eluent flow velocities through the packed bed, the particles are required to be inflexible and resistant to fragmentation. Fragile particles tend to produce "fines" that interfere with homogeneous packing and column permeability.

By the mid 1960's it was obvious to chromatographers that LC performance could be significantly improved by turning attention to these points. However, it was found that as particle size was reduced it became increasingly difficult to pack the particles homogeneously. Hence the benefit gained through faster mass transfer was more than offset by the increased band dispersion due to transcolumn velocity differences. For this reason the predicted improvement in performance failed to materialise and particle sizes below 30 - 40\(\mu\)m were seldom used. In 1967, Horvath, Preiss and Lipsky (21) demonstrated that this drawback could be circumvented. In the context of gas chromatography Golay (56), emphasising the significance of rapid mass transfer between the two phases and within the stationary phase, had suggested that the rate of mass transfer could be increased by the use of materials that were impervious except for a thin porous outer layer. Later, Knox (54) pointed out that fast liquid chromatography should be possible using glass beads bearing a thin surface layer of partitioning material easily accessible to the mobile phase. This idea was exploited by Lipsky et al. who used glass beads coated with a pellicle of ion exchange resin for rapid nucleotide separations. Kirkland (57) had previously developed controlled surface porosity (CSP) materials for gas chromatography and showed, in 1969, that these could successfully be employed for HS LC (23). Since then a number of pellicular materials
have been introduced and it has been firmly established that the performance of good pellicular materials is superior to that of porous materials in the 30 - 50μm range (23, 58, 59). The success of porous layer beads did not preclude the use of totally porous supports, for the small surface area of the former (1 - 15m²g⁻¹) compares unfavourably with that of the latter (>200m²g⁻¹). As a consequence, the linear capacity of the pellicular support is considerably reduced and losses in performance occur with large samples. In addition, the use of low sensitivity detectors such as the refractive index monitor is impracticable except for major component analysis. Until recently, therefore, porous supports of 40 - 50μm were in common use - the overall efficiency being sacrificed for increased capacity. Advances in packing techniques have now enabled porous particles as small as 5μm to be packed and on performance grounds there is no longer a need for pellicular materials. It is probable, however, that their use will persist because they are easily dry packed for use in either liquid-liquid or liquid-solid chromatography.

The range of packing materials currently available presents a wide choice. Packings for adsorption and partition chromatography are listed in Table 3.1. Materials for gel permeation and ion exchange chromatography are reviewed elsewhere (32) and permanently bonded stationary phase materials have been comprehensively covered by Pryde (34). Liquid-solid chromatography can be performed with all the supports in Table 3.1, except Zipax which has an intentionally low surface area. All the supports are suitable for liquid-liquid chromatography, the loading of stationary phase depending on the support used. Large loadings are desirable from the point of view of sample capacity but performance may be lost because of a reduced mass transfer rate.
<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>PARTICLE DIAMETER (MICRONS) AND SHAPE</th>
<th>SURFACE AREA (m²g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pellicular Silica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zipax (Du Pont)</td>
<td>37 - 44 S</td>
<td>~1</td>
</tr>
<tr>
<td>Corasil I (Waters)</td>
<td>37 - 50 S</td>
<td>7</td>
</tr>
<tr>
<td>Corasil II (Waters)</td>
<td>37 - 50 S</td>
<td>14</td>
</tr>
<tr>
<td>Pellosil HS (Reeve Angel)</td>
<td>37 - 44 S</td>
<td>4</td>
</tr>
<tr>
<td>Pellosil HC (Reeve Angel)</td>
<td>37 - 44 S</td>
<td>8</td>
</tr>
<tr>
<td>Perisorb (Merck)</td>
<td>30 - 40 S</td>
<td>10</td>
</tr>
<tr>
<td>Vydac (Anachem)</td>
<td>30 - 40 S</td>
<td>12</td>
</tr>
<tr>
<td>Pellicular Alumina</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pellumina HS (Reeve Angel)</td>
<td>37 - 44 S</td>
<td>4</td>
</tr>
<tr>
<td>Pellumina HC (Reeve Angel)</td>
<td>37 - 44 S</td>
<td>8</td>
</tr>
<tr>
<td>Pellicular Polyamide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pellidon (Reeve Angel)</td>
<td>55 - 65 S</td>
<td>~1</td>
</tr>
<tr>
<td>Pellicular Charcoal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pellicarb (Reeve Angel)</td>
<td>- S</td>
<td>-</td>
</tr>
<tr>
<td>Porous Silica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porasil A (Waters)</td>
<td>37 - 75 S</td>
<td>350 - 500</td>
</tr>
<tr>
<td>Porasil B (Waters)</td>
<td>37 - 75 S</td>
<td>125 - 250</td>
</tr>
<tr>
<td>Porasil C (Waters)</td>
<td>37 - 75 S</td>
<td>50 - 100</td>
</tr>
<tr>
<td>Porasil D (Waters)</td>
<td>37 - 75 S</td>
<td>25 - 45</td>
</tr>
<tr>
<td>Porasil E (Waters)</td>
<td>37 - 75 S</td>
<td>10 - 20</td>
</tr>
<tr>
<td>Porasil F (Waters)</td>
<td>37 - 75 S</td>
<td>2 - 6</td>
</tr>
<tr>
<td>Porasil T (Waters)</td>
<td>15 - 25 I</td>
<td>300</td>
</tr>
<tr>
<td>υPorasil (Waters)</td>
<td>10 S</td>
<td>400</td>
</tr>
<tr>
<td>Lichrosorb (Merck)</td>
<td>10-20-30-40 I</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Partisil (Reeve Angel)</td>
<td>5-10-20 I</td>
<td>-</td>
</tr>
<tr>
<td>MATERIAL</td>
<td>PARTICLE DIAMETER (MICRONS) AND SHAPE</td>
<td>SURFACE AREA (m²g⁻¹)</td>
</tr>
<tr>
<td>--------------------------------</td>
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<td>----------------------</td>
</tr>
<tr>
<td>Spherisorb S (Phase Sep)</td>
<td>5-10-20 S</td>
<td>190</td>
</tr>
<tr>
<td>Zorbax Sil (Du Pont)</td>
<td>S</td>
<td>300</td>
</tr>
<tr>
<td><strong>Porous Alumina</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Woelm Alumina (Woelm)</td>
<td>10 - 30 I</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Bio Rad Ag (Bio Rad Labs)</td>
<td>&lt; 74 I</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Spherisorb (Phase Sep)</td>
<td>5-10-20 S</td>
<td>-</td>
</tr>
<tr>
<td>Lichrosorb Alox-T (Merck)</td>
<td>5, 10 I</td>
<td>400</td>
</tr>
</tbody>
</table>

* S: Spherical
I: Irregular
Higher liquid loadings also result in the supports becoming sticky and difficult to dry pack. Pellicular materials generally bear about 1% by weight of stationary phase, whereas porous supports may be coated with up to 20% for dry packing. Columns packed by slurry methods can be coated in situ by a dynamic coating method (60) although the amount of stationary phase deposited is difficult to control and equilibration of the column may take some time.

It is generally accepted that spherical particles pack more homogeneously than irregular ones. For this reason the majority of materials are manufactured in a spherical form. The surface structure may also be important in determining how well a support will pack. The superior packing of Zipax compared to glass beads of the same size is attributed to the micro-rough texture of the porous pellicle. The problem of packing particles homogeneously has complicated the evaluation of materials for HSLC and care must be taken to differentiate between the poor performance due to bad packing and poor performance resulting from unfavourable kinetic and thermodynamic properties.

3.2 Specific criteria for silica gels

Nearly all the materials in Table 3.1 are composed of silica or alumina. Of these two, silica is the more widely used chromatographic support and a vast amount of literature exists on its structure and the mechanism of adsorption onto its surface (61-63). Chromatographic silica gels are porous solids consisting of a three dimensional network of silica units. These units or primary particles may vary from polysilicic acid units each containing a few silicon atoms to colloidal silica particles containing thousands of silicon atoms. The variation in size of these units that are linked together forming the gel makes
a wide range of specific surface areas and mean pore diameters possible. The basic building block of the gel structure is monosilicic acid, Si(OH)₄. However, this is rarely found except in dilute solutions because it readily polymerises. Thus, a supersaturated solution of monosilicic acid will eventually form a solid phase of amorphous silica which will appear in the form of either colloidal particles, a precipitate or a gel. The polymerisation involves the condensation of silanol (Si–OH) groups to form siloxane (Si–O–Si) bonds.

In the absence of soluble salts the rate of polymerisation of silicic acid to form a gel depends to a large extent upon the pH and concentration of the solution. Experimental observations show that in the presence of small amounts of alkali, silicic acid may
polymerise to form a stable dispersion of polymeric silica of colloidal dimensions (10 - 100 Å), while in acid solution silica gel is formed. The effect of pH on the stability of a silicic acid solution is shown in Figure 2. Below pH 2 the rate of polymerisation is proportional to the concentration of H⁺ and to the concentration of fluoride impurities which have a catalytic effect. Above pH 2 the rate is catalysed by OH⁻ ion and the stability falls as pH increases. In very dilute acid solution, polymeric silica particles will slowly grow in size and may reach colloidal dimensions but when the silica concentration exceeds about 1% the primary particles are able to condense to give a weak gel or precipitate. In concentrated solution an immense number of nuclei are formed close to one another and rapid gel formation follows. In this case the polysilicic acid units are unable to reach as large a size as with more dilute reagents and the resulting gel has a high density, relatively small pore diameter and a primary particle size not more than one or two nanometres. At about pH 4 - 5 the polysilicic acid molecules begin to adsorb OH⁻ ions and become negatively charged. This surface charge increases rapidly with the concentration of OH⁻. The mutual repulsion of these charged molecules means that fewer effective collisions occur and gel formation is slowed or prevented. Thus, in alkaline solution, polymeric silica of colloidal dimensions can be tolerated in quite high concentrations before gelling occurs. As the repulsive potential energy of a charged particle is proportional to its radius, the precise value of the maximum concentration depends upon the size of the polymeric particles. It follows from this that the rate of gelation of an alkali stabilised silica sol is dramatically increased by any mechanism that removes or diminishes the surface charge. For example, gelation can be brought about by the addition of water
miscible organic solvents that lower the dielectric constant of the medium and effectively reduce the surface charge. The presence of soluble salts has a similar effect. The silica particles adsorb cations from solution and the negative charge is neutralised.

The mode of preparation of a gel influences its physical properties and performance. Silica gels are easily produced either by reacting solutions of alkali metal silicates with acid or by aggregation of colloidal silica particles. In the former case the pH at which the reaction is carried out determines the final surface area of the gel. For the reasons discussed above, the primary particle size that can be reached before gelation occurs increases with the pH of the solution and the specific surface area falls accordingly. Gelation tends to occur before the primary particles reach any appreciable size because the reaction also produces soluble salts. Hence silica gels formed in this way have a higher surface area than those formed by aggregation of colloidal silica. The concentration of the reactants determines the strength of the gel. Low concentrations produce weak gels or precipitates, whereas high concentrations produce strong firm gels.

The behaviour of the gel on drying is largely dependent upon the pore diameter of the gel and the strength of the bond between primary particles. As the liquid phase is evaporated from the pores, menisci are formed at the surface. The surface tension exerts a strong compression on the gel mass which increases with smaller pore diameters. Gels of primary particle size in the region of 1 - 2 nanometres have a theoretical surface area of about $1000 \text{ m}^2 \text{ g}^{-1}$ when initially formed. After drying, these gels have a specific surface area typically in the
range 200–800 m² g⁻¹. Evidently surface area is lost by coalescence of the very small micelles and by spontaneous coarsening of the structure. The gel shrinkage is essentially irreversible and will proceed until the mechanical strength of the gel can withstand the pressure exerted upon the structure by the surface tension of the liquid around the boundary of the gel. The process has the overall effect of increasing the packing density of the primary particles and diminishing the pore diameter. Slow drying of the gel is essential to prevent the gel lumps from shattering because of greater shrinkage of the exterior portions. It is extremely difficult to predict precisely the surface area of a gel prepared by the reaction of sodium silicate with acid. Even when the pH during the reaction and the rate of drying are carefully controlled the resulting gel may still be unsuitable for chromatography because of the geometrical and chemical inhomogeneity of the surface.

Instead of gelling polysilicic acid and forming a fine gel structure which is then coarsened during processing, it is possible to produce gels of predetermined surface areas from sols of known particle size. The packing density of the silica particles in such gels can be controlled by varying the concentration of silica in the sol before gelling. Sols containing 20 to 30% silica produce gels having packing densities of 0.2 to 0.3, and these shrink to only a minor extent when dried slowly. The pore size distribution of such gels is clearly dependent upon the particle size distribution of the particular sol. Gelling of the sol can be brought about in a number of ways; for example, dehydration, adjustment of pH, addition of soluble salts or a water miscible organic solvent. Several methods for preparing stable, concentrated silica sols of narrow size range have been patented. A few, relevant to this thesis are described briefly below. The first, which was developed by Bird (64), enables
sols of up to 20% silica to be prepared. A dilute solution of sodium silicate containing up to 3.5% silica is passed through a bed of cation exchange resin in the hydrogen form to give a silicic acid effluent containing up to 3% silica. Although this solution is virtually free of soluble salts, it is acidic and will gel within a few hours unless the growing polymeric silica particles are stabilised by the addition of alkali. Enough alkali is added so that after the solution is evaporated to 20% silica it will contain the equivalent of 0.4% Na₂O. More concentrated solutions of colloidal silica of up to 40% can be stabilised against gelling by careful control of the particle size and the amount of alkali added to maintain a negative charge on the particles. This is the basis of the Bechtold and Snyder process (65). A portion of the dilute, alkali stabilised effluent from the ion exchanger is heated to over 60°C to form larger particles or nuclei (the "heel"). The remainder of the effluent (the "feed") is slowly added so that the additional silica polymerises on these nuclei. The heating of the small silica particles of the "feed" sol in the presence of the larger silica particles in the "heel" effects a build up of the smaller particles upon the larger. This build up produces a family of larger silica particles which are substantially spherical in shape, uniform in size and consisting of dense amorphous silica. A third method developed by Stoever, Fink and Bohn (66) can produce colloidal silica spheres as large as 1μm by the reaction of silicates in alcoholic solution with water in the presence of ammonia as morphological catalyst. This reaction leads to the formation of uniform silica spheres within a few hours and the sol can be concentrated by evaporation after addition of alkali. The size of the spheres can be controlled by adjusting the proportions of ester, water and ammonia. The type of silica particle found in sols may vary, depending upon the method of preparation used. A particle of silica in a sol prepared by the
Bechtold and Snyder method has a surface area which is about equal to the surface area of a sphere of equivalent diameter, i.e., the particle has no internal surface area and is dense. Other methods may produce sols whose particles are of colloidal size but consist of aggregates of smaller, heavily hydrated, primary particles which retain, at least to some degree, their individual structure. The surface area of the sol particle is, therefore, much greater than that of a sphere of the same diameter. The presence of micropores in the gels made from these sols is not desirable from the point of view of efficient chromatography.

The porosity and surface area of dried silica gels can be altered by thermal treatment and hot steam treatment. The mechanism by which this occurs is readily understood if examined in terms of the chemical structure of the silica surface. Physically adsorbed water is removed from the surface of the gel by drying at 110 - 150°C, leaving the surface covered with a monolayer of hydroxyl groups. These surface hydroxyls can be roughly divided into two types: free hydroxyls and reactive hydroxyls. The distinction is that reactive hydroxyls are in close enough proximity for hydrogen bonding to occur, whereas free hydroxyls are not. At temperatures between 200°C and 400°C the reactive hydroxyls condense with loss of water to give surface siloxane groups:

At higher temperatures free hydroxyls begin to migrate about the surface forming transient reactive groups and eventually undergo condensation. At temperatures greater than 600°C silanol groups on adjacent adsorbent
planes begin to react to give particle to particle fusion. In this sintering process the small capillaries close first with permanent loss of surface. The total pore volume and surface area are thus reduced while the remaining pores change little in size. Steam treatment (67), on the other hand, results in a comparatively larger surface area reduction by increasing the pore size. Steam treatment promotes the migration of the smaller silica primary particles about the surface. The larger primary particles grow at the expense of the smaller ones, becoming more uniform and smoother. Consequently, the overall effect is to make the pore size more uniform and the gel stronger. Improvements in pore size distribution have also been reported by dehydrating gels upon which certain cations are adsorbed (68). This method has the advantage that it can be carried out at atmospheric pressure and comparatively low temperatures.

Silica gels are generally characterised in terms of their specific surface area, total pore volume and mean pore diameter. The specific surface area can be calculated from \( d_s \), the mean diameter of the primary particles measured from electron micrographs, on the assumption that the particles are dense amorphous silica with a specific gravity of 2.2. Thus

\[
S_c = \frac{2727}{d_s}
\]

(3.1)

where \( S_c \) is in \( m^2g^{-1} \) and \( d_s \) is in \( nm \).

This method is subject to error for it assumes that the primary particles in the gel are barely touching and that no area is lost at the point of contact. In addition \( S_c \) does not allow for the presence of pores invisible in the electron microscope. A more reliable measure of the actual surface area is that obtained by nitrogen adsorption, \( S_n \). The mean pore diameter and specific pore volume can be calculated from comprehensive data derived from the
nitrogen adsorption and desorption isotherms. Alternatively, mercury intrusion can be used. In the case of impervious primary particles the ratio of $S_c / S_n$ is a measure of the degree to which the ultimate particles are cemented together at the points of contact. Experiment has shown that if very little contact exists, $S_c / S_n$ will vary from 0.8 to 1.0. Where the particles have coalesced to form a strong union, $S_c / S_n$ is expected to be greater than unity. The packing density, $S$, of the primary particles is given by equation 3.2

$$S = \frac{\text{Volume occupied by solid spheres}}{\text{total volume of the gel}}$$

$$= \frac{(1/2,2)}{V_p + (1/2,2)}$$

$$= \frac{0.455}{V_p + 0.455}$$

where $V_p = \text{specific volume of the gel in cm}^3 \text{ g}^{-1}$.

Values of $S$ give a rough idea of how many other spheres each individual sphere is touching; e.g., values of $S$ between 0.12 and 0.34 indicate a co-ordination number of four. Values in the region of 0.52 indicate a co-ordination of six.

3.3. Evaluation of Chromatographic Performance

An evaluation of the chromatographic performance of a column packing material is conveniently carried out by plotting the reduced plate height against reduced velocity for both retained and unretained solutes. As the theory of chromatography (17) has shown, plotting the data in reduced terms enables the direct comparison of systems differing in particle size, mobile phase and support type. From the slope and position of the plot much can be learned about the relative
importance of the multiple band spreading processes occurring in the column.

As indicated in the previous chapter, band spreading arises from axial molecular diffusion, flow nonequilibrium between different parts of the mobile phase and nonequilibrium between the mobile and stationary phases. In reduced terms, axial molecular diffusion in the mobile and stationary phases gives the plate height contribution.

\[ h = 2 \left( \frac{\gamma}{v} + k'D' \frac{D_s}{D_m} \right) \]  

(3.3)

The obstructive factor, \( \gamma \), is generally assumed to have a value 0.6 for non porous materials and 0.9 for porous supports. \( \gamma' \) is thought to be approximately unity. Equation 3.3 can be written in the form

\[ h = \frac{B}{v} \]  

(3.4)

According to Giddings (17), nonequilibrium between mobile and stationary phases will give rise to a plate height term

\[ h = q_s \frac{k'}{(1+k')^2} \frac{\partial^2 D_s}{\partial z^2} \frac{D_m}{D_s} v \]  

(3.5)

where the mechanism of equilibration is diffusion within the stationary phase or

\[ h = \frac{2k'}{k_d(1+k')^2} \frac{D_m}{D_p} v \]  

(3.6)
where nonequilibrium is maintained because of a finite rate of adsorption-desorption. This can be written

\[ h = C v \quad (3.7) \]

where \( C \) is the coefficient for mass transfer in the stationary phase. The \( h \) term originating in nonequilibrium within the mobile phase can be split into two components: The first arising from nonequilibrium between the flowing part of the mobile phase in the interparticle space and the stagnant part held within the pores of the particles, and the second arising from velocity variations within the streaming part of the mobile phase itself. The first component is given by equation \( 3.8 \)

\[ h = C_m \left[ 1 + \frac{\alpha k'}{1+k'} \right]^2 v \quad (3.8) \]

which is simplified by writing it as

\[ h = C_m v \quad (3.9) \]

where \( C_m \) is the coefficient for mass transfer in the stagnant mobile phase. As pointed out in the previous chapter the second contribution is best expressed by the empirical relation

\[ h = \left[ \frac{1}{2\lambda} + \frac{1}{C'v^{n''}} \right]^{-1} \quad (3.10) \]

with \( n'' \leq 1 \).

Over a range of \( v \) covering less than two orders of magnitude this can be approximated by

\[ h = Av^{n'} \quad (3.11) \]

with \( n' \leq 1 \).
Compiling the independent contributions, the overall plate height is given by

\[ h = \frac{B}{v} + A v^n + C v \]  

(3.12)

where \( C = C_s + C_m \) and \( n \) lies between 0.25 and 0.35 for unsorbed solutes (58) and is normally taken as 0.33.

Kennedy and Knox (59) have shown that equation 3.12 provides a reasonably accurate and useful description of the dependence of plate height upon velocity for a number of supports used in LC.

At high reduced velocities the third term of equation 3.12 will be of major importance. As \( v \) is decreased the contribution of the second term will be more in evidence. At reduced velocities below 10 axial molecular diffusion ceases to be negligible and the plate height, which up to this point has been decreasing with \( v \) under the influence of the second and third terms, begins to rise. The curve of reduced plate height against reduced velocity, therefore, shows a minimum in the region \( v = 3 - 6 \). The value of \( B \) can be found from \( k' \), diffusion coefficients and estimates of the obstructive factors. Values of \( A \) and \( C \) are best found by curve fitting. The parameter \( A \), determined by the extent of nonequilibrium within the flowing part of the mobile phase, exhibits a weak dependence on \( k' \) but will, in the main, reflect how well a column has been packed. The criterion of efficient packing has been established by Knox and Parcher (69) using 480\( \mu \)m glass spheres for which a value of \( A = 0.37 \) was recorded. However, in view of the difficulties in packing spheres of 40\( \mu \)m or smaller a value of \( A = 1 \) is considered to be reasonable. The parameter \( C \) depends strongly on \( k' \) and is a quantitative measure of the extent of nonequilibrium between the phases. The variation of \( C \) with \( k' \) depends
on the behaviour of its components $c_m$ and $c_s$. According to equations 3.6 and 3.8, $c_m$ will increase to a maximum as $k'$ increases from zero to infinity, whereas $c_s$ will reach a maximum at $k' = 1$. Therefore, any tendency for $c$ to decline as $k'$ rises above unity indicates the importance of slow mass transfer within the stationary phase.

Over a limited range of reduced velocity, typically ten to a hundredfold, in the region where axial diffusion is not important, equation 3.12 may be approximated by

$$h = dv^n$$

with $1 > n > n'$.

This is the reduced form of an approximate empirical equation proposed by Snyder (70):

$$H = Du^n$$

(3.13)

where $D$ and $n$ are constants for a given column and set of experimental conditions. Snyder took the exponent to be 0.4 but values between 0.3 and 0.6 have been reported (24, 71). Because reduced velocities of interest cover a wide range, experimental results are usually displayed on logarithmic plots. In this case $n$ will be the gradient of the log $h$ - log $v$ curve at any particular value of $v$ and will reflect the importance of equilibration by diffusion in the stationary phase or in the stagnant parts of the mobile phase. Hence for non porous materials $n$ will be about 0.33 while for porous particles $n$ will be larger and should rise with $v$. The proportion of $h$ contributed by either mechanism can be determined from $n$ in this region and hence serves as a simple method for finding $A$ and $C$ (72).
Although values of $A$ and $C$ offer a convenient method for the comparison of supports, the overall performance cannot be assessed fully without considering the speeds of analysis achieved with the materials under comparable conditions. When the maximum pressure drop is set by the equipment, the resistance of the column to eluent flow plays a critical part in determining the time of analysis. Knox and Saleem (73) have shown that for a particular separation requiring $N$ theoretical plates the time of elution, $t$, is given by equation 3.14

$$t = \frac{N^2 \eta k'}{\Delta P} \quad (3.14)$$

where $\Delta P$ is the pressure drop developed across a column of length $L$ with a mobile phase of viscosity $\eta$. $\phi'$, the flow resistance parameter is derived from the Kozeny-Carman equation (74) which gives the mean interstitial eluent velocity in a bed of non-porous spheres as

$$u = \frac{\Delta P d_p^2}{\phi' \eta L} = \frac{K^o \Delta P}{\eta L} \quad (3.15)$$

$K^o$ is the specific permeability of the column and is proportional to $d_p^2$. $\phi'$ is dimensionless and is more useful as a measure of the flow resistance, being very sensitive to porosity. Equation 3.14 shows that for a given separation and set of experimental conditions the shortest analysis time is arrived at by minimising $h^2 \phi'$. The particle diameter providing this shortest analysis time found from

$$hv = \frac{\Delta P d_p^2}{\phi' \eta^2 \bar{m}} \quad (3.16)$$
Under the prevailing experimental conditions each particle diameter will provide a value of $h v \phi'$. By measurement of pressure drop and linear flow rate, $\phi'$ can be found through equation 3.15 and the value of $h v$ calculated. The individual values of $h$ and $v$ for each particle size are determined from the $h - v$ plot for each particle diameter. Insertion of the values of $h$ and $\phi'$ so derived for each $d_p$ into equation 3.14 then allows the analysis times for different particle sizes to be calculated. It is then a simple procedure to obtain the particle diameter giving the minimum analysis time.
EXPERIMENTAL

4.1 Equipment and Procedure for High Speed Liquid Chromatography

(A) Equipment: Figure 3 shows the equipment used for the evaluation of packing material performance. The main features are described below: The solvent reservoir and degassing system consisted of a two litre "Quickfit" flask fitted with reflux condenser and solvent delivery tube. The flask was mounted in a heating mantle controlled by variable transformer. To prevent foreign matter being carried to the pump, a 5 - 10μm porosity stainless steel filter was included in the tubing from the reservoir to the pump.

Two types of pump were used in the work described here:

(i) Orlita DMP 1515 (Orlita, Giessen). This pump (Figure 4) operates on the counter-plunger system. A motor driven plunger reciprocates in and out of the oil filled cavity, thus creating a volume variation that is translated via the metal diaphragm to the valve chamber containing the pumping liquid. The volume of liquid pumped during each cycle depends upon the swept volume of the plunger which is controlled by a regulating plunger positioned opposite the working plunger. The regulating plunger is spring-loaded and its position is determined by an adjustable stop. As the working plunger moves out of the oil cavity the regulating plunger follows, remaining in contact with it until it is retained by the stop. During the rest of the stroke the working plunger sucks up the quantity of pumping liquid through the inlet valve. On the return pressure stroke this volume is forced through the outlet valve until the working plunger again meets the regulating plunger pushing it back against the spring
and completing the cycle. This pump is capable of delivering solvent at continuously variable flow rates from 0 - 10ml per minute against back pressures of up to 3,000 psi. In general this pump performed satisfactorily, although at back pressures less than about 30 psi the long term flow rate tended to vary. A further disadvantage, common to all pumps of this kind, is that the mode of pumping gives rise to pressure pulsing which may not be compatible with the detection system. At the lower flow rates associated with 2mm bore analytical columns, the pulsing was adequately damped by the Bourdon type pressure gauge (Negretti and Zambra, London). However, the pulsing produced at the higher flow rates associated with 5mm bore columns required an additional damping system.

(ii) Haskel Pressure Intensifier (Haskel Engineering and Supply Co.). (Figure 5). A hydraulic piston bearing on the liquid to be pumped is coupled directly to a pneumatic piston which is driven by gas pressure from an air cylinder. The area of the air driven piston relative to that of the hydraulic piston is designed to give a pressure amplification factor of 20. At the end of the delivery stroke the hydraulic piston is automatically returned to its starting position, thereby refilling the liquid chamber from the external reservoir via a non-return valve. The volume of the chamber is about 60ml. This method of pumping is pulse free but suffers from the minor disadvantage that the detector base line signal is interrupted during pump refilling (about 1 sec). Flow rate variations occurred at driving pressures less than about 50 psi, probably due to frictional resistance to the piston movement. Constant low flow rates were generated by connecting a 30cm pre-column containing small glass beads between the pump and analytical
column. The flow resistance created in this way meant that the pump experienced a high back pressure while the pressure drop across the column remained low.

Introduction of the solute onto the top of the analytical column was implemented by syringe injection through a rubber septum. Two types of on-column injection port were used, differing only slightly in design. These are illustrated in Figure 6. Both were constructed to ensure that no volume remained unswept by the eluent flow. The stainless steel injection port could be used with all solvents in general use but the brass port was limited to non-corrosive mobile phases.

The analytical columns were constructed from precision bore stainless steel (Tube Sales Ltd.) or glass tubing. End fittings of brass or stainless steel were sealed onto glass columns by "Araldite" epoxy resin, and onto stainless steel columns by solder. Stainless steel columns were polished internally to a mirror finish by hand with fine grade carborundum paper. Particular care was taken to ensure that the tubing ends were ground flush with the surface of the end fitting flange. This ensured that no unswept volume occurred between the point of injection and the detector. Stainless steel columns so constructed could withstand at least 3,000 psi. The maximum working pressure of the glass columns depended upon the wall thickness, which was generally chosen to enable the columns to be used up to 1,000 psi. To retain the column packing material the column outlet was plugged with a porous frit of P.T.F.E. or stainless steel.

Two detectors were used in the work reported here. Both are ultra-violet absorption photometers designed for use in HSLC.

(i) Du Pont Model 410 (Du Pont Co.). This instrument (Figure 7) operates at a fixed wavelength of 254nm. Light from the source is collimated by a lens before being split into a reference beam
and a sample beam by a semi-transparent mirror. The sample beam passes through a sample cell before impinging on a photocell. The reference beam focuses directly on another photocell. The output from the photocells is fed to a differential log circuit which amplifies the electrical signal from each of the photocells logarithmically and then subtracts them. The resultant output is fed to a recorder. The manufacturer's specification gives the short term noise at the highest full scale sensitivity (0.01 AU) as $\pm 0.0002$ AU. Long term drift over 12 hours is 0.001 AU maximum, without any ambient temperature control. Upper limit of linear operation is 1.2 - 2.0 AU.

(ii) Cecil 212 photometer (Cecil Instruments Ltd).

This single beam instrument (Figure 8) may be operated at wavelengths between 220 and 400nm. The beam generated at the deuterium lamp source is attenuated before monochromation by a series of mirrors, collimator and grating. The beam then passes through the sample cell and focuses on the photocell. Wavelength accuracy is specified as better than 2nm and reproducibility less than 0.5nm. Highest full scale sensitivity is 0.01 AU and zero stability better than $\pm 0.1\%$ short term.

Sample cells for both instruments have a 1cm optical path length and cell volume of 8 - 10μl.

"Swagelok" stainless steel unions and 1/16" or 1/8" stainless steel or nylon tubing were used to connect the component parts of the apparatus.

Elution chromatograms were recorded by a "Servoscribe" potentiometric recorder.

(B) **Column Preparation:**

(i) Dry packing: Columns were dry packed using the mechanical device illustrated in Figure 9. Free running packing material
was introduced into the column in a fine stream from a feed bottle. During the filling process the column was rotated about its longitudinal axis at 100 - 120 r.p.m. and bounced lengthways at about 180 r.p.m., the height of bounce being about 1cm. The column was tapped laterally at the level of the packing with a light, hard object to help consolidate the accumulating bed. Supports for adsorption chromatography were activated before packing by heating for a few hours at 120°C in vacuo. Supports for partition chromatography were coated with \(\beta, \beta^\prime\)-oxy-dipropionitrile (BOP) by weighing out an appropriate amount of support in a small beaker and adding the calculated weight of BOP dissolved in dry acetone. The particles were slurried in the solution and the beaker gently warmed until the acetone evaporated.

(ii) Slurry packing: In this method, the packing material was shaken vigorously with a dense organic solvent (e.g. methyl iodide) to give a homogeneous balance density slurry. This was then rapidly pumped with a less dense, immiscible liquid (e.g. n-hexane) into a stainless steel column blank. The equipment used (Figure 10) was similar to that used by several authors (75). The column blank, fitted with a porous stainless steel frit, was filled with methyl iodide and the pre-column, which acts as a slurry reservoir, was bolted on. The packing material, thoroughly dispersed in methyl iodide, was poured into the pre-column and any remaining volume filled up with the dense solvent. The injection head was bolted on, and, with the valve closed, the pump pressure raised. At 2000 - 3000psi the release valve was opened. The flow was then undisturbed until pure eluent issued from the column. After compacting the bed by alternately restoring and releasing the pressure on the
column several times by manipulation of the valve, the column was detached and flushed with methylene chloride or ether to remove the remaining traces of methyl iodide. Columns packed by this method were activated for adsorption chromatography by flushing the packed column with anhydrous ether. Kirkland recommends a dynamic method for BOP coating slurry packed columns for partition chromatography (60).

Specific amounts of water were added to an activated support directly, prior to packing, or by conditioning the packed column by flushing it with a solvent partially saturated with water until equilibrium was reached. Partially water saturated solvents were made up by mixing the appropriate proportions of anhydrous and water saturated solvents. Passing the solvent through a column of activated silica gel was sufficient to render it anhydrous. Trace quantities of aromatic impurities that would interfere with the u.v. detection system were also removed by this process. Water saturation was accomplished by passing the solvent through a column of silica gel to which 30% w/w of water had been added.

In liquid-liquid partition chromatography the solubility of the polar stationary phase in the non-polar mobile phase is small. The continued passage of the mobile phase, however, eventually strips the stationary phase from the column. To prevent this the mobile phase was pre-saturated with the stationary phase by passing it through a pre-column containing "Chromosorb" bearing 30% w/w of stationary phase. This pre-column, incorporated in the eluent flow between the pump and the column, consisted of a 30cm length of 1/4" stainless steel tubing packed by slowly pouring in the coated "Chromosorb" while gently
(C) **Experimental Procedure and Calculation of Results:**

Columns, packed as described above, were flushed with mobile phase until all air had been removed and the $k'$ values of the test solutes remained constant. The pump was set to the required pressure or flow rate and the eluent flow velocity given time to stabilise.

0.5 - 1.0% solutions of the solutes were made up in the mobile phase and these were injected directly, through a rubber septum, into the top of the column packing by glass microsyringe. Sample size was generally 1 - 5 μl. Plate heights were calculated for an unretained solute and at least one retained solute for each of the column packings examined. The mobile phase linear velocity was determined by injecting a sample that was not retained by the stationary phase in the column. The solute, therefore, eluted at the mean velocity of the moving phase. Unretained solutes are generally compounds that are chemically similar to the mobile phase. Solutes that did not absorb in the u.v., e.g. pentane, could be detected by the perturbation in the base line resulting from the refractive index change as the solute passed through the photometer cell.

The performance of the packing materials was compared on the basis of their respective plots of log reduced plate height against log reduced velocity. The plate height, $H$, is defined as

$$H = \frac{L}{16} \left( \frac{w}{t} \right)^2$$  \hspace{1cm} (4.1)

where $w$ is the baseline width of the gaussian peak, $t_r$ is the elution distance of the peak maximum and $L$ is the column length.

The reduced plate height, $h$, is given by

$$h = \frac{H}{d_p}$$  \hspace{1cm} (4.2)
where $d_p$ is the mean particle diameter.

The reduced velocity, $v$, is defined as

$$v = \frac{ud_p}{D_m}$$  \hspace{1cm} (4.3)

where $u$ is the mobile phase linear velocity and is

$$u = \frac{L}{t_o}$$  \hspace{1cm} (4.4)

to is the elution time of an unretained solute i.e. the elution distance measured from the chart, divided by the chart speed.

Diffusion coefficients, $D_m$, were found from the Wilke-Chang equation (76) quoted below:

$$D_m = \frac{7.4 \times 10^{-3} L \psi_2 M_2^{0.5} T}{\eta V_1^{0.5}}$$  \hspace{1cm} (4.5)

$\eta$ is the viscosity of the solvent in centipoise, $M_2$ the molecular weight of the solvent, $V_1$, the molar volume of the solute in cm$^3$ mol$^{-1}$ and $\psi_2$ an association factor which takes the values 1.0 for non-polar solvents, 2.6 for water, 1.9 for methanol and 1.5 for ethanol.

$T$ is the absolute temperature. $D_m$ is in units of cm$^2$ sec$^{-1}$.

Figure (11) shows how the necessary data is derived from the elution record. Tangents are drawn at the points of inflexion on the Gaussian profile to intersect with the base line. The distance between these intersection points is $w$. $t_r$ is measured from the point of injection to the peak maximum.

$\phi'$ values for the packed columns were calculated by plotting column pressure drop $\Delta P$ against linear velocity $u$. The gradient of this plot was used to calculate $\phi'$ from equation 4.6

$$\phi' = \frac{\Delta P d_p^2 6.89 \times 10^{-2}}{u L \eta}$$  \hspace{1cm} (4.6)

where $\Delta P$ is in units of psi, $u$ in cm sec$^{-1}$, $d_p$ in $\mu$m, $\eta$ in...
4.2 Preparation of Supports for High Speed Liquid Chromatography

(A) Preparation of Pellicular Supports: Glass beads (English Glass Company) were used as the impervious support for the silica gel coating. They were cleaned by washing with hot water and detergent, followed by rinsing with warm distilled water. The beads were filtered off and washed with acetone before drying in an oven. The free flowing beads were sieved and the 37 - 44µm (400 - 325 mesh) fraction retained. The procedure described below was used for coating batches of beads with silica gel or sodium silicate. Aqueous silica sols were between 20 and 30% by weight SiO₂. Aqueous sodium silicate solution was 25% v/v commercial water glass.

(i) 3 - 6g clean dry glass beads (37 - 44µm) were slurried with the coating liquid (silica sol or sodium silicate solution) and the excess decanted, leaving the beads just covered.

(ii) The slurry was stirred into 150mls of viscous liquid paraffin containing 1% v/v Span 80 surfactant and the beads dispersed by vigorous agitation.

(iii) The temperature of the suspension was raised to 95°C and a volume of hot agar solution (0.3ml of 4% agar solution per gram of beads) added. Agitation was continued to disperse the agar over the beads' surface.

(iv) After allowing the suspension to cool to room temperature the beads were filtered, washed with light petroleum (b.p. 100 - 120°C), acetone, and then dried.

Silica sol coated beads were fired at 500°C for a few hours to burn out the agar binding agent and to bond the silica gel coating to
the bead surface. Sodium silicate coated beads were acid treated to precipitate the gel under the following conditions: 2M HCl was added dropwise to the coated beads until the solution became permanently acid to methyl orange indicator. This acid was decanted after one hour and the beads washed with distilled water before immersing in 0.2M HCl for eight to ten hours. Finally, the beads were washed with distilled water to remove all traces of acid and transferred to a drying oven. The gel coating was dried slowly by raising the temperature to 110°C over a period of 3 - 4 hours. Firing of the beads at 500°C ensured that the pellicular gel was firmly bound to the surface.

A simplified procedure utilising the gelling properties of a de-stabilised silica sol is described below. 30 - 40% silica sols of the Bechtold and Snyder type were used:

(i) 3 - 6g clean, dry glass beads (37 - 44μm) were slurried with silica sol that had been acidified to pH 5 - 7 and the excess sol decanted.

(ii) The slurry was transferred into 150ml liquid paraffin containing 2% v/v Span 80 and the beads dispersed by rapid stirring.

(iii) The beads were maintained in suspension for 2 - 3 hours until gelation occurred.

(iv) The silica gel coated beads were filtered off, washed with petroleum ether (b.p. 100 - 120°C), acetone, and then dried. Firing at 500°C for a few hours made the coating more resistant to abrasion.

(B) Preparation of Porous Supports: 30% and 40% silica sols of the Bechtold and Snyder type were used to prepare porous silica spheres, ranging in size from 10 to 40μm by the method outlined below:

(i) 50mls silica sol were acidified to pH 7, giving a gel time of about two hours at room temperature.
(ii) The sol was dispersed by rapid stirring in 100ml petroleum ether (b.p. 100 - 120°C) containing 4% v/v Span 80 surfactant.

(iii) Gentle shaking for 2 - 3 hours kept the globules in suspension until gelation had occurred.

(iv) The spheres were then allowed to settle out and the supernatant suspension medium decanted. The beads were washed with petroleum ether and acetone before transferring to water for fractionation.

(C) Particle Fractionation and Determination of Particle Size:

Ungraded batches of spherical porous silica gel were sieved to remove particles greater than 37um in diameter (Endecott Test Sieves Ltd, London). Narrow fractions of particles smaller than this were obtained by a sedimentation method. The terminal velocity, \(v_t\), of a sphere of radius \(r\) and density \(d\), falling under the influence of gravity in a fluid of density \(d'\) and viscosity \(\eta\) is given by

\[
v_t = \frac{2}{9} \frac{r^2(d - d')}{\eta} g = \frac{d^2}{18} \frac{(d - d')}{\eta} g \quad (4.7)
\]

where \(g\) is the gravitational constant.

In a particular system, therefore, the time a sphere takes to fall a given distance is inversely proportional to the square of its diameter and a pair of sedimentation times, corresponding to the limits of each desired fraction, can be calculated for the given distance. In practice the particles were shaken up with water in a suitable container and then allowed to settle out. When the greater of the two calculated sedimentation times had lapsed, the particles still in suspension, i.e. those that had not traversed the given distance, were drawn off by siphon. The water in the container was restored to its original level and the cycle continued until no further particles were being removed.
The process was then repeated at the shorter of the two sedimentation times and the intermediate fraction retained for subsequent drying and measurement of particle size. The mean particle diameter of a sieved fraction of particles was taken as the mean diameter of the sieve openings that defined the fraction. For particles fractionated by sedimentation, at least fifty individual particles were sized by a measuring microscope calibrated against a graticule. The mean particle diameter and the standard deviation were then calculated.

(D) Hydrothermal Treatment of Silica Gels: Hydrothermal treatment (67) of silica gels was accomplished by sealing the gel material and the required amount of water into a stainless steel tube fitted with "Swagelok" end caps. The tube was placed in a furnace at the necessary temperature until the treatment period was complete. The tube was removed, cooled to room temperature and the contents washed out with distilled water.

4.3 Preparation of Silica Sols

Silica sols were prepared by two methods:

(i) Ammoniacal hydrolysis of tetraethyl silicate in ethanolic solution.

(ii) Controlled polymerisation of silicic acid in aqueous solution.

Method (i) is that of Stober, Fink and Bohn (66). The reagents were simply stirred for several hours at room temperature and the resulting sol concentrated by distillation of the ethanol. 1% sodium hydroxide based upon the weight of silica in the sol was added along with enough water so that on removal of the ethanol a 15 to 20% aqueous sol remained.

Typical quantities used were:

- 58g tetra-ethyl silicate
- 880ml absolute ethanol
- 54ml concentrated aqueous ammonia (14M)
The sol particle size could be varied from about 40 to 800 nm by altering the ammonia concentration. The monodisperse nature of sols prepared in the laboratory by this method was confirmed by the observation of second order Tyndall effects with dilute solutions.

Method (ii) is that of Bechtold and Snyder (65). A silica sol was made by passing a dilute solution of sodium silicate (10% v/v commercial "water glass") through a bed of ion exchange resin in the hydrogen form. The aqueous sol contained about 3% SiO₂ by weight and was stabilised by addition of sodium hydroxide to pH 9. 100mls of this was refluxed for 1 hour. This "heel", as it is termed, was concentrated by adding fresh "feed" sol while simultaneously distilling off the water. 700mls of fresh "feed" sol were added in this way over a period of about eight hours, keeping the volume in the evaporator constant. The product contained about 25% silica by weight and was stable to gelling.

Commercial "water glass" is a viscous solution characterised by a Na₂O:SiO₂ ratio of 1:3.3. The material contains about 30% silica in the form of mono and polyanions.
RESULTS AND DISCUSSION

5.1 Pellicular Materials

The most satisfactory commercially available pellicular material is Zipax (Du Pont Co.). The performance of this material has been examined in depth (59, 77, 78) and it has been shown that its excellent chromatographic properties are attributable to rapid mass transfer between phases and favourable packing characteristics. The plot of log h against log v for this material is shown in Figure 12 and is taken from the comprehensive data of Done and Knox (78). The full lines are drawn according to the equation

\[ h = \frac{2\gamma}{v} + A v^{0.33} + C v \]

The values of \( \gamma \), A and C are shown in the figure.

The manufacture of Zipax is based on a patent by Iler (79) for coating surfaces with layers of silica microspheres. Glass beads are alternately washed with a positively charged organic polymer and a negatively charged silica sol, the principle being that layers of silica microspheres, separated by a binding layer of polymer, are thus built up to the required thickness on the bead. The organic layer is later burned out by firing at high temperature. Electron micrographs (EM 1, 2, 3 and ref. 81) show that the microspheres are of a narrow size range, but their packing within the porous layer is not as regular as the method would suggest and it is difficult to argue that the structure of Zipax is that claimed by the patent (80). Thus, building up the pellicle in thin regular layers does not appear to be an essential requirement for a surface of controlled pore size. The silica microspheres are about 0.2 \( \mu m \) in diameter, which approaches the upper limit of the colloidal size range. Consequently, the porous surface has a relatively large
pore size and low surface area. The thickness of the pellicle is estimated to be in the region of 2 to 3μm. Corasil is another pellicular material for which comprehensive data are available (77). Electron micrographs (EM 4, 5, 6) show that physically, Corasil bears little resemblance to Zipax. Although the method of manufacture is not available it is known that a Corasil particle consists of an impervious glass bead coated with one or two layers of silica gel. The material's performance (Figure 13) is considerably poorer than that of Zipax but is still of such a standard that it is accepted as a satisfactory pellicular material.

The collection of high quality data on pellicular and other materials is hampered by the failure of many authors to achieve a satisfactory standard of column packing. The interpretation of chromatographic data derived from a badly packed column is rarely easy, for the poor performance may be due to the bad packing, or poor mass transfer, or both. The data for Zipax, Corasil and other materials quoted in this chapter are derived from columns in which a satisfactory standard of packing has been achieved. The (h, v) curve for unretained solutes on 480 μm glass spheres obtained by Knox and Parcher (69) is drawn in both Figures 12 and 13. Their data gave a value of A = 0.4 which is the lowest ever achieved for the random packing of spheres. As yet, it has not been possible to attain similar values with small particles and a value of A = 1.0 is considered acceptable.

(A) Pellicular silica gel from sodium silicate: The method devised for the preparation of pellicular materials that is described here, and is briefly described above, endeavours to establish the porous pellicle in one single process by coating each glass bead with a layer of sodium silicate which
is subsequently treated with acid to yield a coating of silica gel. In general, two conditions must be met in the batch preparation of pellicular beads. Firstly, each bead should have the same amount of silica gel associated with it, and secondly, aggregation of the beads must be prevented before the gel layer has been hardened. The former requirement was met in practice by slurrying the beads with sodium silicate solution, then dispersing them in a hydrophobic liquid. Each individual bead was then enveloped by hydrophilic silicate solution. Aggregation of the beads while in suspension can be discouraged in a number of ways. In this case, viscous liquid paraffin, to which a surfactant had been added, was chosen as the dispersion medium. The effect was twofold: The high viscosity of the paraffin retarded the settling out of the dispersed beads, while the surfactant discouraged the coalescence of the hydrophilic envelopes of beads in close proximity. In addition the suspension was heated and a small quantity of molten agar added. This was distributed over the surface of each bead so that, on cooling, a hard agar gel protected the beads from contact with each other. At this stage the beads could be filtered, washed free of the dispersion medium and treated with acid to precipitate the silica gel. The final stages involved drying the silica gel and firing the beads to burn out the agar and bond the porous pellicle to the support bead.

The optimum reagent proportions for the process were found from experiment. The surfactant concentration in the dispersing medium was critical in determining the thickness of the silicate solution envelope around each bead. Dispersion of the slurried beads within the medium was favoured by high concentrations of surfactant. This had the disadvantageous effect, however, of enhancing the tendency for silicate solution in the envelope to detach from the bead and form an independent globule in suspension. The eventual gel thickness was thus reduced
considerably. To prevent this, just enough surfactant was added to aid the preliminary dispersion of agglomerated beads held together by the surface tension of the silicate solution. The strength and rigidity of the silica gel coating was promoted by high silicate concentrations in the envelope. Unfortunately the high viscosity of such solutions prevented the dispersion of the slurried beads in the paraffin, even with vigorous agitation. A 25% v/v solution of water glass proved a satisfactory compromise. Agar solutions of 1% w/v were used in all experiments. At this concentration the molten solution was satisfactorily mobile and, on cooling, formed a hard gel. The volume of solution added to each suspension was calculated from the weight of beads so as to give a thin coating over each bead. The amount was not critical, however, and excess could be added without affecting its function. Optimum reagent proportions and method of coating are given in the experimental section. The method resulted in a very low proportion of aggregated beads and these were easily removed by sieving.

A superficial examination of the beads was conducted by microscope before the gel precipitation stage. The silicate coating appeared to cover each bead completely but was not uniformly distributed over the whole surface. As a result the beads were only approximately spherical. At this point two courses were possible: The agar binding agent could be burned out before, or after the gel precipitation stage. In the former case, the thermal treatment made the silicate coating more resistant to abrasion but left the texture and uniformity of the surface unchanged. Similarly, after treatment with acid the gel pellicle remained rough and uneven. Not surprisingly, this irregular exterior was detrimental to the packing characteristics of the beads. Figure 14 shows the variation of reduced plate height with reduced velocity for retained and unretained solutes on a column of material bearing BOP stationary phase. The value of A describing the curve k' = 0 is 1.4, underlining
the relatively poor packing. Mass transfer within the layer was encouragingly low and peak symmetry excellent, as can be seen in the phenols separation in Figure 15. The maximum loading of stationary phase that the beads could bear without becoming sticky was 0.5 - 0.6% indicating a rather low surface area. The alternative procedure of heat treating the beads after gel formation evolved a material of even lower surface, bearing a maximum loading of 0.3 - 0.4% BOP. Agar is slowly hydrolysed by acid so that the freshly precipitated silica gel was unprotected from abrasion during the washing and drying stages. Consequently, a proportion of the layer was inevitably lost. The general appearance of these beads differed significantly from those fired before acid treatment. The gel coating viewed by optical and electron microscope (EM 7, 8) was smoother and more regular. Presumably the parts of the coating most susceptible to abrasion were the more prominent irregularities in the gel layer. The effect of this on packing is clear from Figure 16 which presents the (h, v) curves for various solutes in partition chromatography. The value of A describing the unretained solute curve is 0.8, which compares favourably with that of 0.87 quoted for Zipax (Figure 12). However, the behaviour of h with k' for this particular column differed markedly. For k' values less than unity, h rose much more sharply than for Zipax, emphasising the difference in pore structure of the two materials. The symmetry of eluted peaks (Figure 17) was excellent. The reproducibility of the process was not particularly good. Figure 18 shows the data for a column of material prepared under similar precipitation conditions to that of the material in Figure 16. Again, good packing has been attained but the former column shows a slower rise of h with k', closely resembling the performance of Zipax. These performance differences highlight the critical nature of the gel formation
and drying conditions. The surface area of this material could be increased by repeating the silicate coating and gel precipitation procedures. For example, the maximum BOP loading compatible with good packing characteristics could be increased from 0.3% to 0.6 - 0.7%. The \((h, v)\) data of Figure 19 and the chromatogram, Figure 20, demonstrate that the overall performance of beads bearing two coats of silica gel differed little from those bearing a single coating.

(B) Pellicular silica gel from silica sols: The method described above for coating glass beads with silicate was equally adaptable to coating with silica sols. The advantage of silica sols is that they only require to be dehydrated to form the gel, thereby simplifying the experimental procedure. Samples of pellicular beads prepared by coating with Stober, Fink and Bohn sols had very low surface areas. Two coatings were needed to create a large enough surface to bear 0.5% BOP. The performance of the material, shown in Figure 21, was disappointing. Although the unretained solute plate height was satisfactorily low, the plate heights of retained solutes were high, even at the lowest velocities examined. The values of \(A\) and \(C\), 1.9 and 4.6 respectively, describing the \(k' > 0\) curve, indicated that the pore structure of the gel was far from ideal. Irregularities in pore structure could spring from a variety of sources: A broad sol particle size distribution, the failure of the sol particles to close pack together during gelation or from the presence of micropores within the sol particles themselves. Modification of the gel structure by thermal treatment was impracticable, for the sintering of silica gel occurs at a temperature well above the melting point of the glass bead support. Moderate hydrothermal treatment of the material caused severe erosion of the glass bead rendering the material useless. A sample of beads
coated with sol that had previously been hydrothermallytised for two hours at 250°C showed a marked deterioration in efficiency (Figure 21).

The preparation of silica sols by the method of Bechtold and Snyder does not differ fundamentally in mechanism from that of Stober, Fink and Bohn. Both are hydrolysis reactions, one in acid, the other in ammoniacal solution. The hydrolysis of tetraethyl silicate, however, may produce a less dense polymeric silica particle than that generated in the Bechtold and Snyder process. In addition, in this latter process the concentration of silica is slowly increased throughout the process, whereas the method of Stober et al. entails polymerisation in a solution that remains dilute until the final evaporation stage. Although the solution is dilute enough to prevent total gelation, there is little to discourage the aggregation of small particles to form larger sol particles that will, as a result, be sponge-like, containing micropores that mar chromatographic efficiency.

In contrast to the pellicular material derived from Stober et al. sol, the beads coated with Bechtold and Snyder sol required only a single coating to create a reasonable surface area. The curves in Figure 22 show the (h, v) relationship for solutes retained and unretained on 1% BOP. The figure also shows the effect of diluting the coating sol from 20% to 10%. The surface area was diminished accordingly and was able to bear only 0.3% stationary phase. The shape and position of the curves for corresponding k' values were unaffected. The notable similarity in performance between these materials and that prepared from Stober et al. sol made it doubtful that the poor efficiency arose from a structural difference in the sol particles prepared by the two methods. Examination of the pellicular coating by electron microscope revealed a structure of compact, roughly spherical particles of silica (EM 9, 10). Although it was not clear whether these were the ultimate sol particles or clusters of sol particles, the broad variation in size of these visible spheres would not conform with
a uniform pore structure. A sol prepared by the Bechtold and Snyder process is more likely to be monodisperse if the rate of addition of "feed" sol to the "heel" is slow and constant. If "feed" is added at a faster rate than the growing nuclei in the "heel" can accommodate, the excess active silica will commence formation of new nuclei. The overall uniformity of particle size is thus destroyed and the efficiency of the pellicular bead suffers. In one particular preparation the rate of addition of "feed" sol was increased from about 1.5 ml per minute to 3 ml per minute. The loss in efficiency of the pellicular support prepared from this sol was immediately apparent. Comparison of Figure 24, the (h, v) plot for this material, with that of Figure 22 shows that the increased resistance to mass transfer was particularly pronounced at low k' values: The value of C for k' = 0.4 increased from $1.8 \times 10^{-2}$ to $5.4 \times 10^{-2}$. Chromatograms for both materials are shown in Figures 23 and 25. The similarity of these materials to Corasil, both in appearance and performance, is notable.

In view of the possible dispersion in particle size that a sol preparation on the laboratory scale could produce, comparison with a sol of assured uniformity would be advantageous. Silica sols prepared by the Bechtold and Snyder process are available commercially. The controlled conditions under which they are manufactured ensure that the particle size of the final product falls within narrow, well defined limits. Figure 26 gives the data for the pellicular support prepared using such a commercial sol in the coating process. Technical data supplied with the sol, specified as having a particle diameter between 13 and 14 mm, is reproduced in Table 5.1. As the figure shows, the material coated with the commercial sol exhibited no obvious improvement in performance over that coated with the laboratory prepared sol. The similarity in performance
TABLE 5.1

Colloidal Silica Solutions:

<table>
<thead>
<tr>
<th></th>
<th>13 - 14 nm</th>
<th>7 - 8 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific surface area (m² g⁻¹)</td>
<td>210 - 230</td>
<td>375 - 420</td>
</tr>
<tr>
<td>Silica as SiO₂ weight per cent</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>pH (25°C)</td>
<td>9.7</td>
<td>9.9</td>
</tr>
</tbody>
</table>

TABLE 5.2

Silica gels:

<table>
<thead>
<tr>
<th></th>
<th>13 - 14 nm sol</th>
<th>7 - 8 nm sol</th>
<th>Bechtold &amp; Snyder sol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific surface area (m² g⁻¹)</td>
<td>187 (177)</td>
<td>285 (288)</td>
<td>62 (75)</td>
</tr>
<tr>
<td>S = S₀/Sₙ</td>
<td>1.2</td>
<td>1.4</td>
<td>-</td>
</tr>
<tr>
<td>Specific pore volume (cc g⁻¹)</td>
<td>0.50</td>
<td>0.66</td>
<td>0.42</td>
</tr>
<tr>
<td>Mean pore diameter (nm)</td>
<td>8</td>
<td>5</td>
<td>15</td>
</tr>
</tbody>
</table>

Results returned by Coulter Limited. Figures in parentheses refer to data obtained by the author. Both figures were found by the low temperature nitrogen adsorption (BET) method.
between pellicular materials coated with Stober et al. sol, and Bechtold and Snyder sols (commercial and laboratory preparations) indicated that the principal source of slow mass transfer could not be due to physical differences in sol particle structure, nor to broad distributions in particle size.

When a silica sol is dehydrated, the surface tension of the remaining liquid draws the sol particles close enough together for interparticle bonding to occur. When gelation is complete the particles are packed closely together and each sphere is in contact with several neighbouring spheres. Providing their particle sizes are similar, the pores throughout the gel will be of uniform diameter. The presence of agar, which is itself colloidal, within the silica sol may alter this regime. Extensive penetration of the agar solution into the sol layer must occur before the agar gel forms. It can be visualised that, upon dehydration, the agar matrix physically obstructs the process by which the silica spheres are normally brought into close contact with each other. When the agar is finally displaced by firing at high temperature, voids and breaks in the silica gel remain. The intended function of agar was to protect the sol envelope around each bead until a hard silica gel could be formed. Fortunately, the properties of silica sols are such that an alternative method of accomplishing this is readily available: When an alkali stabilised silica sol is acidified, the rate of polymerisation to form a gel is increased by a factor depending on the amount of acid added. The general relationship between gel time, pH and sol concentration is presented graphically in Figure 27. In the case of the 40% commercially prepared sol, addition of acid to pH 7 caused an increase in viscosity until the hard gel formed in just over two hours. The preparation of pellicular beads utilising this phenomenon, described in the experimental section, has several advantages over that using agar. Firstly, as the gel
formation takes place at room temperature there is no need to heat the suspension. Thus, the viscosity of the dispersing medium remains high throughout the process and re-aggregation of beads is virtually eliminated. Secondly, in the agar process the glass bead supports are in contact with highly alkaline sols and silicate solution. This must result in some erosion of the glass surface that may significantly affect results. In this process the coating sol is neutral. Finally, in the absence of agar the gelling mechanism can proceed unobstructed. Figure 28 shows the excellent results obtained with this material. Values of A and C for retained and unretained solutes are comparable with those of Zipax. The material only required one coating of sol to create a surface area that could bear up to 1% stationary phase without showing signs of overloading. As the sol particle size is much smaller than that used in Zipax manufacture, 13nm compared with 200nm, the gel coating need not be very thick to generate an appreciable surface area. The smooth gel coating of the material, seen in electron micrographs (EM 11, 12, 13) contrasts sharply with that of material using agar as binding agent (EM 9, 10). The limited resolution of the microscope prevents the discernment of the gel fine structure.

5.2 Totally Porous Materials
The principle of controlled polymerisation of silica sols to form gels is immediately applicable to the preparation of totally porous silica gel spheres for adsorbents in HSIC. Hydrophilic silica sol, acidified to pH 7, is simply dispersed in a hydrophobic medium to give a suspension of polymerising silica spheres. This suspension is maintained until gelation is complete and the hard gel particles are recovered.

Preliminary experiments were carried out using sols prepared in the laboratory by the method of Bechtold and Snyder. The performance data of
21μm and 16μm fractions of materials prepared from these sols are given in Figures 29 and 30 respectively. Both curves show a minimum value of h as v falls to about 4 due to the increasing importance of longitudinal diffusion. The positions of the minima show that the columns are well packed. The minimum for the 16μm material lies at a slightly higher value of h than that for the 21μm support, indicating a slight deterioration in the goodness of packing. The materials performed well in adsorption chromatography, the eluted peaks showing no signs of asymmetry (Figure 31). One advantage of using silica sols is that the final surface area of the material can be predetermined by choosing a sol of suitable particle size. This is well illustrated by the materials prepared from two commercially available sols whose particle sizes were specified as 13 - 14nm and 7 - 8nm. The data supplied with the sols are reproduced in Table 5.1. The specific surface areas of the product gels were measured by low temperature nitrogen adsorption (BET method) and are given in Table 5.2 together with specific pore volume data determined by mercury intrusion. Figures 32, 33, 34 show the (h, v) curves for 40.5μm, 25.5μm and 12.5μm fractions of material prepared from 13 - 14nm sol. Again, the 40.5μm and 25.5μm materials packed better than the 12.5μm support. h for retained solutes is slightly higher than that for unretained solutes and, in reduced terms, the overall performance is similar to data obtained by Kennedy and Knox (59) on 48.5μm Porasil. Their data are reproduced in Figure 35. Further comparison may be made with Figure 36 which shows the (h, v) curve for 12μm Spherisorb S. Chromatograms for the 40.5 and 12.5μm supports in Figure 37 underline the increased speed of analysis that the use of small particles can bring about. Figures 38 and 39 show the curves obtained on 20μm and 12μm fractions of material prepared from 7 - 8nm sol. The curve for the retained solute
on the 20µm material lies unexpectedly high above that for the unretained solute. This behaviour was apparently anomalous, arising perhaps from the choice of retained solute, for the performance of the smaller fraction showed no similar inconsistency.

The mean pore diameter and specific pore volume data, Figures 40, 41, 42 and Table 5.2, supply additional information on the physical character of the gels. The curves each show two distinct parts: The volume of mercury penetrating the sample at pressures less than 40 - 50 psi penetrated the void spaces between the particles, while the volume above this pressure penetrated the pores within the spheres. The steepness of rise in this second part is representative of the distribution in pore size in the material which, in this case, appeared to be narrow in all three supports. In contrast to the gel prepared from 13 - 14nm sol the curves for the other two materials showed significant hysteresis as the pressure of mercury diminished. This is evidence that a proportion of the pores were "ink-well" shaped. In the case of the sols of known particle size the ratio S_c/S_n gives some indication of the coalescence at the points of contact of primary particles during gelation. The degree of coalescence depends on the surface tension of the evaporating liquid within the pores. This, in turn, increases with decreasing pore diameter. Hence, as expected, the value of S_c/S_n for the sol of smaller particle size is marginally greater than that for the larger particle sol. Values of S, calculated from equation 3.2, indicated that the primary particles in the gel had a co-ordination number approaching six. As would be expected, the gel formed from the 30% sol showed a lower value than that for the 40% sol.

Column resistance parameters, ϕ', were recorded for the packed columns. Values for dry packed columns ranged from about 1000 to 1700 whereas
the more permeable bed structure of slurry packed columns gave values between 800 and 900.

Electron micrographs of the porous materials (EM 14 - 21) showed them to be reasonably spherical and generally free from surface flaws. Further resolution of the fine structure of the gel was not possible with the particular instrument used.
PART III

THE APPLICATION OF POLAROGRAPHY IN HIGH SPEED LIQUID CHROMATOGRAPHY
6.1 Introduction

It is generally accepted that one of the major contributing factors to the rapid evolution of HSLC was the development of suitably stable and sensitive post column detectors. The characteristics of an ideal detector for the continuous monitoring of the effluent from an HSLC column include versatility, high sensitivity, large linear dynamic range, low internal volume and independence of column parameters such as mobile phase composition and flow rate. Many detection systems have been examined but only three are in widespread use and are considered to be satisfactory. They are the refractive index (RI) monitor, UV photometer and wire or chain transport system. These exemplify the three basic types of detector: those which monitor a general property of the eluate, those which monitor a particular property selectively possessed by the solute and those which physically separate the solute from the eluent before assay of the solute.

The RI monitor \((19, 28, 29, 82, 83)\) compares the refractive indices of pure eluent and eluate and in common with other bulk property detectors it is relatively insensitive. Commercially available models will detect differences in RI between the sample and reference stream of as little as \(10^{-7}\) RI units, but as the total change between pure eluent and pure solute is unlikely to be larger than 0.1 RI units the overall detection limit is no better than 1 part of solute in \(10^6\) of eluent. To achieve this level of sensitivity, temperature stabilisation to at least \(10^{-3}\) \(^\circ\)C is required. Many of the column packing materials
used in HSLC have a limited capacity and such a level of detection means that the analysis of trace components in mixtures is virtually impossible. The detector, however, is popular in gel chromatography where, invariably, only major components are of interest.

As many compounds absorb UV radiation, photometric detectors are the most widely used in HSLC (28, 29, 84, 85). The simplest monitors are generally dual beam systems operating on low pressure mercury lamps which emit the major proportion of their radiation at 254 nm. The applicability of the photometric detector has been extended by the introduction of single and dual beam variable wavelength models. The UV photometer is a selective detector and as such is more sensitive than bulk property detectors. As the noise level is typically in the region of $10^{-4}$ absorbance units the detection level is about 1 part of solute in $10^9$ of eluent, assuming the solute has a molar extinction coefficient of about $10^4$ mole$^{-1}$ cm$^{-1}$. With normal flow and temperature fluctuations the photometer does not require any thermostating but the choice of eluents is, of course, limited to those which have no UV absorbance.

In a transport detection system (86 - 91) the column eluate is collected on a moving wire or band which carries it first into a low temperature evaporation oven where the solvent, which must be volatile, is removed. Involatile solutes remain on the wire and are carried to an assaying monitor. In one form of the detector (88) the solutes are pyrolysed to carbon dioxide and water. The CO$_2$ is catalytically reduced to methane which is passed to a flame-ionisation detector. Although the general principle of detectors of this type is ingenious, models currently available are noisy and their performance is only slightly better than the RI monitor. This lack of sensitivity stems
from the small proportion (0.1 - 1.0%) of the total eluate that the transport mechanism picks up. Further development work on this problem may eventually produce a satisfactory solute detection level. The transport detector is useful in the analysis of lipids, polymers, carbohydrates and other substances which do not absorb in the UV and is potentially useful in that it can be made specific for certain elements.

The micro-adsorption detector (29, 92-96) which measures the heat of adsorption of solutes as the eluate is passed through a bed of adsorbent such as silica, has been examined for use in HSLC. It is no more sensitive than the RI monitor and has a particular disadvantage in that it produces a differential peak shape due to the adsorption and desorption of the solute.

Because of the drawbacks associated with each of these detectors, other more specific detectors based on fluorimetry (97), radiometry (98), dielectric constant (99, 100) and polarography (101 - 105) have been reported but are not widely used. Of these the polarograph holds particular promise as a selective detector capable of giving a sensitivity comparable with the UV monitor. Polarography is an electro-chemical analysis method in which a solution is electrolysed by using a small polarisable working electrode and a large non-polarisable electrode. If the solution contains compounds whose oxidation or reduction potentials fall within the potential range of the working electrode material, the current increases as a function of the voltage applied at the electrode. Under the appropriate conditions the maximum current is controlled by the diffusion of the electrochemically active compound to the polarisable electrode and is proportional to its concentration. The potential at which a current step appears is termed the half wave potential. The half wave potential for a given compound
in a specified system is characteristic for that compound. In the classical form of polarography the precise measurements of components having similar half-wave potentials is difficult. Similarly, the determination of a trace component is often made impossible by the masking effect of the polarographic wave due to a main component. The combination of liquid chromatography and polarography fully exploits the polarographic technique, enabling the components of the mixture to be separated and the individual fractions to be analysed. When used purely as a specific means of detection a constant potential is applied across the electrodes and the limiting current produced by the eluting electrochemically active species is measured as a function of time. The first reported use of a platinum electrode in a flowing stream for electroanalysis was in 1947 (106) and later in 1952, Kemula used a dropping mercury electrode (DME) (107). Since then the technique of 'chromatopolarography' has been used in a range of applications including the analysis of inorganic anions (108), metal ions (109), nitro compounds (110), amino acids (111), alkaloids (112), and pesticides (101).

Previous work has nearly all been carried out with a dropping mercury electrode designed to continuously monitor the column eluent stream. For HSLC the cell volume must be kept to a minimum and microdetectors employing the DME can successfully by constructed for use with high efficiency columns (101, 113). Such a detector based on the design of Huber (101) was found to function satisfactorily in the laboratory. Extra-column volume and effective cell volume were kept to a minimum by arranging that the effluent from the chromatographic column was passed through a short length of 0.020" stainless steel tubing and sprayed directly onto the developing mercury drop. The sensitivity of the laboratory
constructed detector compared well with that obtained by Huber (typically $2 \times 10^{-2}$ Amp litre mole$^{-1}$) but the noise level was significantly higher ($2 \times 10^{-8}$ Amp). Polarography using a DME has important advantages, namely a constantly renewed and reproducible electrode surface and an extensive cathodic working range owing to the large overpotential for the evolution of hydrogen. Also, there is a very extensive bibliography of organic polarography at the DME (114, 115). Unfortunately its limited anodic potential range precludes the use of the electrode in the electro-oxidation mode. As a detector for continuous analysis the DME has several other disadvantages: The current oscillation over the lifetime of the drop with constant applied potential requires some form of damping which inevitably influences the minimum detectable peak width. In addition, the drop rate and sensitivity are both functions of the eluent flow rate.

Detectors employing solid electrodes have been constructed and show considerable promise. A low volume detector constructed by Joynes and Maggs (116) used a carbon impregnated silicone rubber membrane electrode with an anodic and cathodic working potential, low noise level and low standing current. This type of electrode material has been studied by Pungor (117). Two designs of low volume solid electrode cell have been evaluated by MacDonald and Duke (118) using a pulse polarographic technique to minimise electrode surface contamination. These detectors utilise only a very small portion of the available electroactive species for their response i.e. the electrolytic efficiency is low. For a detector operating with an electrolytic efficiency of 100% all electroactive species passing through the cell undergo electrode reaction.

Such a detector is called a coulometric detector since the time integral of the current through the electrode is related by Faraday's law of
electrolysis to the number of equivalents of electroactive species
flowing in the stream. Efforts to develop a detector that functions with
100% electrolytic efficiency even at high flow rates have centered on the
need to increase the surface area of the electrode while decreasing the
volume of mobile phase entrained by the cell and the thickness of the
diffusion layer at the electrode-solution interface. Several types of
coulometric detector for flow analysis have been described using a
variety of electrode materials (102-104, 119).

Polarographic electrodes act as concentration detectors and the
general relationship between detector response and the concentration of
electroactive species in the bulk solution, \( c \) can be expressed by
equation 6.1

\[
I = I_o + Sc
\]

(6.1)

\( I \) is the total detector current and \( S \) is the detector sensitivity. \( I_o \)
is the residual or standing current arising mainly from traces of
electroactive species adsorbed onto the electrode or present in the
mobile phase.

Mass transfer to the electrode occurs by three modes: migration,
convection and diffusion. In the presence of a large excess of supporting
electrolyte the first of these can be neglected. The limiting current
arising from the diffusion mode is proportional to concentration while
that arising from convection is a function of flow rate. For this reason
the detector sensitivity is dependent not only on the electrode
characteristics but also on the mobile phase flow rate.

The sensitivity at any particular flow rate is the increase in signal
intensity per unit increase of solute concentration. Therefore within the
linear dynamic range of the detector the sensitivity can be determined from
where $I_e$ is the limiting current of the electroactive solute. Levich (120) has studied many electrolytic systems under various hydrodynamic restraints and has shown that the general relationship between the limiting electrical current, the solute concentration and the rate of fluid flow past or through the detector, $V_f$, is given by equation 6.3

$$I_e = K n F V_f^\alpha c$$

(6.3)

Where $K$ is a function of experimental parameters such as the kinematic viscosity of the fluid, diffusion coefficient of the solute, geometry of electrode etc. $F$ is the Faraday and is equal to 96500 coulombs. $n$ is the number of coulombs passed per mole of solute. The exponent, $\alpha$ of the volume flow rate, $V_f$ is dependent on the nature of the convection and the boundary conditions used for solving the equation of convective-diffusional mass transfer (120,121). For example, in a flow-through tubular electrode which operates with low electrolytic efficiency, the limiting current equation is given as

$$I_e = (5.3 \times 10^5) D^{2/3} m L^{2/3} n V_f^{1/3} c$$

(6.4)

where $L$ is the length of the tube in cm, $V_f$ in cm$^3$ sec$^{-1}$, $I_e$ in milliamps and the other terms in their usual units.

The validity of this equation has been confirmed by Blaedel et al.(122). Under conditions of constant flow the area of an eluted chromatographic peak, $Q$ is found by integrating equation 6.3

$$Q = K n F V_f^{\alpha} \int_{t}^{t+\Delta t} c \, dt$$

(6.5)

where $\Delta t$ is the peak width.

The total number of moles eluted, $x$, is given by equation 6.6

\[ x = \int c \, dt \]
\[ x = V_f \int_t^{t+\Delta t} c \, dt \]  
\[ Q = x K n F V_f^{\alpha-1} \]

and a plot of \( \log Q \) against \( \log V_f \) is a straight line of slope \( \alpha - 1 \). For a coulometric detector \( K \) and \( \alpha \) in equation 6.7 are unity and the equation becomes

\[ Q = x n F \]

Hence the sensitivity of \( Q \) to changes in \( V_f \) is eliminated and the need for calibration is removed except for the determination of \( n \).

In this chapter the determination of the characteristics of some post-column platinum and gold microelectrodes is described and the applicability of this form of detector to HSLC discussed.

6.2 Experimental

The 50cm x 2mm i.d. glass column used throughout this series of experiments was dry packed with 37-44 μm Zipax Permaphase ODS support. The injection port, column end fittings and all connecting tubing in contact with the mobile phase were of stainless steel. The pump used was an Orlita DMP 1515, described in chapter four. The mobile phase, 0.1M KCl, was prepared by dissolving the required amount of Analar grade potassium chloride in distilled water. Oxygen, which is reduced at the electrodes under study, was removed by refluxing the liquid for several hours in an atmosphere of nitrogen. Standard solutions of o-nitroaniline were made up in the degassed, nitrogen saturated mobile phase. Samples, generally 1 - 10 μl, were introduced onto the column by injection with a
microsyringe through a rubber septum.

Spherical platinum electrodes were constructed by heating the tip of a platinum wire in a flame until a sphere of the required dimensions had formed. The diameter of the sphere was found by measuring microscope calibrated against a graticule. The platinum wire was heat sealed into a short length of glass capillary and mounted in a glass sleeve as shown in Figure 43. The electrode tip was positioned close under the p.t.f.e. frit at the column exit by the stainless steel adapter. The gold electrode was constructed from gold wire in the same manner, although Araldite epoxy resin was used to seal it into the glass capillary as a leak-free joint could not be produced by heat sealing. The mercury pool anode was positioned as close to the working electrode as possible. Copper wire served to make external contact to both electrodes.

The electrical circuit used is also shown in Figure 43. Voltages applied to the electrodes were selected by the potentiometer circuit. By passing the electrode current through the calibrated variable resistance unit and monitoring the potential variation with a 1 mV potentiometric recorder, the profile of the eluted chromatographic peaks could be recorded. Electrode standing currents were offset using the backing-off facility on the recorder. Peak areas were measured by triangulation i.e. tangents were constructed to the sides of the peak at the points of inflection and extended to meet at a vertex and intersect the baseline. The area enclosed by the triangle is taken as representative of the peak area. The mean volume flow rate through the column at a particular pressure was measured by collecting the eluent over a suitable length of time. The sensitivity of an electrode at any particular flow rate was determined by injecting several samples of standard solution and constructing a plot of peak area (Amp litre) against amount injected (moles). Providing the cell functioned within its linear dynamic range the
plot was a straight line passing through the origin. The sensitivity (Amp litre mole\(^{-1}\)) was given by the gradient.

6.3 Results and Discussion

The construction of a low dead volume micro-cell incorporating a solid electrode, outlined in the preceding section, is particularly easy and acceptable results were produced using the simple circuitry described as the chromatogram in Figure 44a shows. Under the particular operating conditions, noise levels and standing currents were reasonably low and contamination of electrodes due to the accumulation of reaction products was not encountered even at the lowest flow rates used.

Data obtained with four spherical platinum electrodes are summarised in Table 6.1. As expected the sensitivity of this electrode increased with the geometrical surface area. Figure 45 shows a plot of sensitivity against flow rate for the largest of these electrodes, the shape of the plot being typical for this type of cell. Over the range of velocities examined, a plot of \(\log Q/x\) against \(\log V_f\) gave a straight line in agreement with equation 6.7. The value of \(\alpha\) for this electrode is 0.23 (Figure 46). The low volume geometry of the cell produced negligible extra-column band broadening and the agreement in plate heights obtained with this electrode and a photometric detector was excellent at all the velocities examined as Figure 47 shows. Table 6.1 also shows data obtained with a gold electrode of similar construction. Gold has a lower anodic range than platinum but is much more valuable for cathodic studies, having a high hydrogen overpotential. Standing current, sensitivity and noise were all comparable with the platinum electrode of similar diameter.

The obvious disadvantage of the spherical electrode is that the diameter of the electrode cannot be increased indefinitely without an
### TABLE 6.1  Spherical Electrodes

<table>
<thead>
<tr>
<th>Electrode Material</th>
<th>Diameter (mm)</th>
<th>Surface Area (mm²)</th>
<th>Sensitivity (Amp litre mole⁻¹)</th>
<th>Standing Current (μ Amp)</th>
<th>Noise (μ Amp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt</td>
<td>0.29</td>
<td>0.26</td>
<td>2.76 x 10⁻³</td>
<td>2.0</td>
<td>0.007</td>
</tr>
<tr>
<td>Pt</td>
<td>0.83</td>
<td>2.17</td>
<td>2.00 x 10⁻²</td>
<td>0.5</td>
<td>0.005</td>
</tr>
<tr>
<td>Pt</td>
<td>1.12</td>
<td>3.94</td>
<td>3.42 x 10⁻²</td>
<td>1.0</td>
<td>0.005</td>
</tr>
<tr>
<td>Pt</td>
<td>1.39</td>
<td>6.07</td>
<td>6.95 x 10⁻²</td>
<td>1.0</td>
<td>0.003</td>
</tr>
<tr>
<td>Au</td>
<td>1.36</td>
<td>5.81</td>
<td>7.14 x 10⁻²</td>
<td>1.0</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Mobile phase: 0.1 M KCl

Applied voltage: -1.0V

Temperature: ambient

Surface areas are geometrical, calculated from mean electrode diameter.

Sensitivities are for o-nitroaniline at 0.8ml min⁻¹.

Noise levels are averaged short term, peak to peak at 0.8ml min⁻¹.

### TABLE 6.2  Gauze Electrodes

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Sensitivity (Amp litre mole⁻¹)</th>
<th>Standing Current (μ Amp)</th>
<th>Noise (μ Amp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
<td>3</td>
<td>0.002</td>
</tr>
<tr>
<td>2</td>
<td>2.1</td>
<td>4</td>
<td>0.002</td>
</tr>
<tr>
<td>3</td>
<td>3.2</td>
<td>6</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Mobile phase: 0.1 M KCl

Applied voltage: -1.0V

Temperature: ambient

Sensitivities are for o-nitroaniline at 1.5ml min⁻¹.

Noise levels are averaged short term, peak to peak at 1.5ml min⁻¹.
undesirable increase in cell volume. An alternative design of cell was constructed by packing the last few millimetres of the glass column with tightly rolled platinum gauze. This proved to be extremely effective in increasing the sensitivity without any associated increase in peak width due to extra-column dead volume as Figure 44b demonstrates.

The data for three electrodes of this type are summarised in Table 6.2. It would be possible to improve upon the noise levels quoted by introducing additional pulse damping into the system. At high flow rates the pulsations produced by the pump were superposed on the inherent electrode noise. For electrode 3 the limit of detection for o-nitroaniline ($k' = 1.5$) was about 2ng. Figures 48 and 49 show the sensitivity vs. flow rate plots and the $\log Q/x$ vs. $\log V_f$ plots for the electrodes. In the latter figure the slopes of the plots rapidly approach zero at very low flow rates, indicating that the electrodes were working at maximum electrolytic efficiency. This is in agreement with equation 6.8, $n$ having a value of 3. As the flow rate rose to about 0.4 ml min$^{-1}$, only the electrode with the largest surface area, electrode 3, continued to function in this mode. Above this, the efficiency of electrolysis fell quite sharply. At 1 ml min$^{-1}$ the efficiencies for electrodes 3, 2 and 1 were 55%, 38% and 17% respectively. These values may be compared with the 1.7% obtained at this flow rate with the largest of the spherical platinum electrodes (Figures 45 and 46).

The limitations of this simple, inexpensive type of detector are several and refinements can be suggested. Coulometric detectors draw relatively large currents and, for accurate work, the potential at the electrode-solution interface should be held constant. This is
accomplished by supplementing the conventional working and reference electrodes by a third, counter electrode. In this system no current is drawn through the reference electrode which is used solely to monitor the potential of the working electrode. The current is measured by the counter electrode whose potential is controlled to be the same as the reference electrode. A further advantage of the three electrode system is that it allows the use of high resistance solutions including operation with organic solvents (121). In this series of experiments no difficulties were encountered with electrode contamination, but in a real chromatographic system this is unlikely to be the case, particularly if anodic potentials are used. At high mobile phase flow rates it is probable that the electrode is cleaned of adsorbed reaction products by the washing action of the eluent, but at low flow rates the maintenance of a constantly reproducible surface may present problems. Potential cycling, in which the electrode is intermittently polarised to a potential where the adsorbed film is desorbed, would be the most convenient method as this avoids the need for cell demounting. Another approach to this problem is the use of pulse polarography. In this technique the electrode is subjected to short potential pulses with the current only being sampled at the end of the pulse. Thus, any interaction that could occur is eliminated or minimised and the long term stability of the electrode is increased. An added advantage is that pulse polarographic techniques require relatively low concentrations of supporting electrolyte.

With the development and use of solid electrode materials workable over wide potential ranges (123) the coupling of HSLC with electrochemical detection is increasing in popularity, particularly in the biochemical and pharmaceutical fields. Apart from the high sensitivity offered, the
advantage of such detectors lies in the high selectivity that can be attained in those instances where the half-wave potentials of the components to be measured are sufficiently different from each other.
THE DISPERSION OF UNRETAINED SOLUTES IN WIDE BORE COLUMNS

7.1 INTRODUCTION

Throughout the development of gas and liquid chromatography, the field of axial and radial dispersion of unsorbed solute bands in columns packed with impervious spherical particles has come under close scrutiny (54, 69, 124 - 127). In a preceding chapter it was shown that axial band dispersion arises from axial molecular diffusion, a finite rate of equilibration of solute between mobile and stationary phases, and from nonequilibrium within the mobile phase which is generated by the complex flow and velocity patterns within the column. For unsorbed solutes the second source of dispersion is absent and the independent contributions to the plate height can be written as

\[ H = \frac{2 \delta D_m}{u} + \sum i \left[ \frac{1}{2 \lambda_i \bar{d}_p} + \frac{D_m}{\omega_i \bar{d}_p \bar{u}} \right]^{-1} \]  

or in reduced terms

\[ h = \frac{2 \delta}{v} + \sum i \left[ \frac{1}{2 \lambda_i} + \frac{1}{\omega_i v} \right]^{-1} \]  

The first term is the contribution due to axial molecular diffusion. The obstructivity factor, \( \gamma \), generally taken as 0.6 for a bed of impervious spheres (128) allows for the roundabout diffusion path the solute molecules must take to traverse the bed. The summation term arises from the coupling of the flow and diffusive mechanisms of molecular exchange between axial velocity variations over the column cross-section. The full evaluation of this term is difficult because it is necessary to consider contributions to \( h \) from processes occurring on different geometrical scales in the column. In a packing of non-porous particles Giddings (17) distinguished at least four contributions, namely those from transchannel,
short range interchannel, long range interchannel and transcolumn nonequilibrium. The difficulties of assigning values to the geometrical constants $\lambda_i$ and $\omega_i$, however, makes the summation term inadequate. More complex forms of this term have been derived (54) but are unwieldy in practice.

Experiment has shown (54) that the main cause of peak dispersion in columns packed with glass beads using liquid eluents is the existence of transcolumn velocity variations and the slowness of transcolumn equilibration. This has been emphasised by the experiments and theoretical calculations of Littlewood (129) based on the nonequilibrium theory. In view of this, in an effort to determine the dispersion resulting from a non-uniform velocity profile, previous work in gas and liquid chromatography with unsorbed solutes has attempted to clarify the relationship between $h$, $v$ and $\rho$, the column to particle diameter ratio (54,124 , 125 ). In wide columns, $h$ should be dominated by transcolumn effects and it would be expected that only a single term in the summation of equation 7.2 would be important. Experiment shows, however, that even this simplified form does not fit the data. Unfortunately, current theories appear to be unable to describe quantitatively this aspect of band dispersion and resort is generally made to empirical equations (54, 20 ). In spite of this, it is clear that the dispersion of zones caused by uneven flow of eluent through the packing is relaxed by lateral mass transfer. For this reason it is important to consider the mechanism of lateral mass transfer and convenient to define a radial dispersion coefficient, $D_r$.

$$D_r = \frac{1}{2} \frac{d \zeta^2}{dt}$$ (7.3)
where $\sigma_r$ is the standard deviation of the radial concentration profile. A radial plate height $H_r$ may be defined as the rate of increase of the radial variance per unit length of column.

\[
H_r = \frac{d \sigma_r^2}{dL} = \frac{2D_r}{u} \tag{7.4}
\]

In reduced terms

\[
h_r = \frac{H_r}{d_p} \tag{7.5}
\]

Radial dispersion occurs by two mechanisms, viz, obstructed molecular diffusion and anastomosis or stream splitting. The contribution to the radial plate height of the radial component of molecular diffusion is identical to that of the axial component's contribution to the axial plate height. The mechanism of solute dispersion by stream splitting is illustrated in Figure 50A. A stream of eluent in an interparticle channel is confronted by a particle lower down the bed and is forced to divide and go around. Each component of the divided stream then coincides with other fragmented streams. The combined flows suffer the same fate upon meeting other particles in the bed, and so on. From a simple random walk treatment Knox et al. (126) proposed that the radial plate height contribution from anastomosis is given by

\[
h_r = \frac{0.1}{D_3} \tag{7.6}
\]

where $D_3 d_p$ is the number of axial steps taken for each one taken laterally. It was estimated that $D_3$ lay between 0.5 and 1. Hence the dependence of $h_r$ on fluid velocity is given approximately by

\[
h_r = \frac{2\sigma}{v} + 0.15 \tag{7.7}
\]
The equation predicts that at low reduced velocities the dispersion is dominated by obstructed molecular diffusion, while at high reduced velocities, when laminar flow prevails, the reduced radial plate height is virtually constant. This behaviour has been borne out experimentally.

The origin of transcolumn velocity differences is thought to lie in particle fractionation during packing and in the effects of rigid column walls on the packed bed. Knox and Parcher (69), examining the dependence of $h$ on $\rho$ (at a particular value of $v$), found that in the region of $\varphi$ between 6 and 8 there occurred a sharp increase in $h$. Their data are reproduced in Figure 51. They interpreted this effect to be due to a discrete wall layer, about three to four particle diameters wide, in which a severe perturbation of velocity occurs. Hence columns with $\varphi < 6$ are composed almost entirely of the wall layer, while those with $\varphi < 8$ contain a circular core of a random structure surrounded by the wall layer. The authors also showed that columns free of wall effects, termed "infinite diameter", can be constructed by ensuring that the major part of the band is eluted before the radial profile diffuses into the region where wall effects predominate, i.e., if the band is eluted before the base width of the radial profile becomes equal to the column diameter, the condition for a column to be "infinite in diameter" is

$$d_c = 4\varphi_x$$  \hspace{1cm} (7.8)

The elimination of $H$ and $\varphi_x$ between equations 7.4, 7.7 and 7.8 and taking $\varphi = 0.6$ gives

$$\frac{d_c^2}{L} = d_p \left( \frac{19.2}{v} + 2.4 \right)$$  \hspace{1cm} (7.9)
At high velocities this simplifies to

\[ \frac{d_c^2}{L} = 2.4 d_p \tag{7.10} \]

or

\[ \frac{L}{d_c} = 0.4 \phi \tag{7.11} \]

Hence, for given values of \( d_c \) and \( d_p \) the column length beyond which the column would cease to be infinite can be found.

The experimental data of Knox and Parcher showed that for an infinite diameter column, the dependence of \( h \) upon \( v \) was less than for a walled column, indicating that a significant reduction in transcolumn effects had been achieved.

The successful preparation of high efficiency columns utilising small particles is largely attributable to the infinite diameter column principle. In Part II the columns packed with fractions of silica spheres had \( \phi \) values between 200 and 500. In columns of such dimensions it is not clear whether velocity variations extend over the whole column cross section or are confined to a narrow region close to the wall.

The series of experiments reported here were devised to clarify this point and also provide a check on the general validity of equation 7.7.

Using a column of well graded glass beads, the rate of radial dispersion of an unretained solute was measured as a function of mobile phase velocity. This was achieved by centrally injecting the solute at the top of the column bed and recording the radial concentration distribution at that particular linear velocity as it was eluted from the column exit.

Transcolumn velocity variations were measured by establishing, at the top of the column, a thin layer of solute across the cross-section of the packed bed. The solute layer was then eluted and by recording the concentration profile at a series of points across the column exit the mean linear velocity at each point could be found. Concentrations of
solute at particular points across the column exit were measured by a polarographic electrode that could be moved radially from the column centre to the column wall. This method of detection is particularly suitable in this application; By positioning the electrode just beneath the frit at the column exit, the dead volume of the detector is extremely small and the error arising from extra-column zone broadening is minimised. Secondly, the dimensions of the electrode can be made sufficiently small to enable the concentration at particular points to be found with some accuracy. The inherent sensitivity of the technique enables solute concentrations to be kept sufficiently small so that the electrode is operating within its linear dynamic range i.e. the response obtained is directly proportional to the concentration of solute at the electrode.

7.2 EXPERIMENTAL

The moving electrode detector assembly is pictured in Figure 52. The body of the apparatus, constructed of perspex, is clamped to an aluminium base plate which also bears the micrometer screw unit. The top and bottom of the body are drilled out to accept the column and the mercury pool counter electrode respectively. Both are held rigidly in the vertical position by "O"-ring compression seals. Two lengths of glass tubing, acting as electrode bearers, are positioned diametrically opposite to each other in the guides drilled horizontally into the body. The "O"-ring seals are compressed to prevent leakage of mobile phase from the assembly while still allowing free movement of the electrode bearers in the guides. The electrodes themselves pass through p.t.f.e. plugs which press firmly into the ends of the glass carriers. The transverse movement of one electrode across part of the column diameter is precisely controlled by a micrometer screw attached to the free end of the glass.
tube carrier. A guide rod, moving in a slot cut in the base plate, ensures that the electrode remains in the vertical position throughout the length of its travel.

Each electrode was constructed from platinum wire. The end of the wire was heated in a flame until a spherical tip of the required size was formed. The mean diameter of the moving electrode tip was determined by measuring microscope to be 305µm. The mean diameter of the stationary electrode tip was determined as 316µm. The electrodes were sealed into fine glass capillary tubing so that only the tip protruded into the electrolyte. Connecting wires to the external terminals were of platinum sheathed in flexible nylon capillary tubing, so that transverse movement of the electrodes was unimpeded. Both electrodes were positioned in their respective p.t.f.e. plug holders in such a way that the distance between the electrode tip and the column exit frit was less than 0.05mm. The counter electrode, mounted below the platinum electrodes, consisted of a mercury pool concentric with the eluent drain tube. External contact was made with a copper wire immersed in the mercury.

The 77.5cm column used in the experiments was constructed of glass. Araldite epoxy resin was used to attach the stainless steel flange to the column top and also to set the porous p.t.f.e. disc into the column bottom. In the latter case, the resin was used only at two points, clear of the diameter which the electrodes were to traverse, and care was taken to ensure that the frit was flush with the column walls. The mean internal diameter of the column, measured by travelling microscope, was 11.7mm. A fraction of sieved, clean, dry glass beads was used to pack the column by the rotate, bounce and tap method. The mean particle diameter of the fraction was determined by measuring
microscope to be 64μm. The standard deviation from the mean for this fraction was 3μm.

Solute samples of p-nitrophenol, made up in nitrogen saturated mobile phase, were injected by glass microsyringe through a septum directly onto the column. The stainless steel injection port was designed with a tubular guide to ensure that at each injection the needle tip was positioned centrally, just above the packed bed.

The apparatus used throughout the experiments was similar to that described in the previous chapter. The mobile phase, 0.1M KCl was purged with oxygen-free nitrogen and refluxed for several hours before use. An Orlita DMP 1515 (Orlita, Giessen) reciprocating pump was used to deliver the electrolyte to the column head. At the high flow rates used the Bourdon-type gauge was found to be ineffective in smoothing the pulsing flow and additional damping was incorporated into the system. This consisted of a 30cm length of 1/4" i.d. stainless steel tubing, capped at one end and incorporated into the system as an appendix between the pump and pressure gauge. The tube was flushed with oxygen-free nitrogen before fitting. The volume of gas trapped in the appendix was highly effective in absorbing the fluctuation in flow produced by the pump.

The diffusion coefficient of p-nitrophenol in the mobile phase was calculated using the Wilke-Chang equation (76) to be 0.97 x 10\(^{-5}\) cm\(^2\) sec\(^{-1}\).

The detector circuit is outlined in Figure 53. In practice the potentiometer was set to give a potential of -1.0V, with respect to the mercury pool, at each platinum electrode. The parallel connection of these electrodes and their respective calibrated variable resistances enabled a dual channel, 10mV full-scale deflection, potentiometric recorder to monitor the independent currents passing through each
resistance during electrode reaction. Typically, the resistances set were between 1 - 5kΩ, corresponding to recorder full scale deflection of 10 - 2µA.

7.3 PROCEDURE AND CALCULATION OF RESULTS:

(A) Radial concentration profile: The pump was set to the required flow rate and the pressure drop allowed to stabilise. A series of solute samples, each 5µl of p-nitrophenol solution, was injected onto the column at suitably spaced intervals. By manipulation of the micrometer screw the movable electrode was accurately positioned at the centre of the column frit and the complete elution profile of the first injected sample was recorded. Further manipulation of the micrometer screw positioned the electrode 0.5mm toward the column wall and the complete elution profile of the second injected sample was recorded. The process was repeated until axial concentration profiles had been recorded at 0.5mm intervals from the column centre to the column wall. The connection of the other platinum electrode circuit to the second channel of the dual channel recorder provided a simultaneous record of the axial concentration profile of each eluted sample at a fixed point 0.5mm from the column centre. The profiles recorded by the stationary electrode were all Gaussian. Those recorded by the movable electrode only showed signs of asymmetry at points within 1.5mm of the column wall. The concentration at each interval was found from the area of the peak recorded at that point.

The peak areas recorded at the stationary electrode $S_x$, where $x$ is the distance of the movable electrode from the column centre (mm), were found to vary slightly during each series of injections. This was attributed to small differences in the amount of solute introduced onto the column by the syringe. The corresponding peak areas recorded at the moving electrode, $M_x$, were therefore slightly in error. A corrected
peak area $M_x'$ was calculated for each interval by use of equation 7.12

$$M_x' = M_x \frac{S_{\text{mean}}}{\bar{S}_x} \tag{7.12}$$

where $S_{\text{mean}}$ is the mean value of $S_x$ over at least thirty injections.

To simplify the presentation of the results, the peak areas at each interval are expressed as a proportion of the peak area recorded at the column centre, i.e., each value of $M_x'$ is divided by $M_0'$. Thus the radial concentration profile is presented as a plot of proportional concentration, $y$, against $x$, the distance from the column centre.

An example of a typical series of calculations is given in Table 7.1. The procedure was repeated at each mobile phase velocity in order to build up a series of radial concentration profiles. Values of $G_r$ describing each of the profiles were found by fitting each curve to equation 7.13

$$y = \frac{1}{\sqrt{2\pi G_r}} e^{-\frac{x^2}{2G_r^2}} \tag{7.13}$$

(B) Transcolumn velocity variation: In this series of experiments the sample was introduced onto the column by stopping the eluent flow and slowly injecting 30µl of a solution of p-nitrophenol in 1M KCl at a point just above the column packing. The sample, being in a denser medium than the eluent, spread over the column cross-section to form a thin layer covering the top of the packed bed. The flow through the column was then restored and the solute layer eluted down the column. By recording the elution profile at a particular point at the column exit, the mean mobile phase velocity at that point could be calculated. The procedure was repeated until the mean
TABLE 7.1

<table>
<thead>
<tr>
<th>x (mm)</th>
<th>S_x (cm²)</th>
<th>M_x (cm²)</th>
<th>M'_x (cm²)</th>
<th>M'_x/M'_0</th>
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<td>47.86</td>
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<td>27.76</td>
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<td>3.35</td>
<td>0.07</td>
</tr>
</tbody>
</table>

v = 247

Values of S_x, M_x are average values obtained over two or three readings.

S_mean = 44.02

x (mm) : distance of movable electrode from column centre
S_x (cm²) : area of elution profile recorded at fixed electrode, with movable electrode x mm from column centre.
M_x (cm²) : area of elution profile recorded by movable electrode x mm from column centre.
S_mean (cm²) : mean value of S_x over series of readings.

M'_x = M_x \left( \frac{S_{\text{mean}}}{S_x} \right)
mobile phase velocity at 0.5 mm intervals from the column centre to the column wall had been measured. The transcolumn velocity profile, a plot of mean mobile phase velocity against distance from the column centre, was then constructed.

7.4 RESULTS AND DISCUSSION

Figure 54 shows the plot of log h against log v for the column of glass beads used in the series of experiments. The six points correspond to the six mobile phase velocities examined. The plate heights were calculated in the normal way from the Gaussian elution profiles recorded by the polarographic electrode at the centre of the column. As the electrode only sampled those molecules which traversed the central part of the column, the plot is that of a true "infinite diameter column". For comparison, the plot for an infinite diameter column constructed by Knox and Parcher (69) is also shown. The plot lies 0.1 log units below that of this work. This is almost certainly a consequence of the superior packing achieved with the larger, specially graded glass beads. Both plots show that the dependence of log h upon log v is less than is normally encountered with walled columns, the slope of the curves being 0.20 compared with 0.33. It is interesting to note that, by extrapolation, the value of log h at v = 300 is 0.37. This is in excellent agreement with the data of Knox and Parcher (Figure 51) which shows that for columns with \( \phi > 10 \) the value of log h at v = 300 is about 0.35.

Figures 55 to 60 show the radial concentration profiles recorded at the six mobile phase velocities. The curves have been drawn according to equation 7.13. According to equation 7.9, a column of the dimensions used in this series of experiments (\( L = 77.5 \text{cm}, d_p = 64 \mu \text{m}, d_c = 1.17 \text{cm} \)) will be effectively of infinite diameter until v falls below about 50.
The radial concentration profile should, therefore, show increasing divergence from the Gaussian form as \( v \) falls below 50 and \( 4\sigma_r \) exceeds \( d_0 \). At high velocities equations 7.4, 7.5 and 7.7 predict that the radial concentration profile will be characterised by a \( \sigma_r \) value of 2.73 mm. The values of \( \sigma_r \) obtained by experiment, tabulated in Table 7.2, are surprisingly low.

**TABLE 7.2**

<table>
<thead>
<tr>
<th>( u ) (cm sec(^{-1})</th>
<th>v</th>
<th>( \sigma_r ) (mm)</th>
<th>( H_T ) (mm)</th>
<th>( h_T )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.37</td>
<td>247</td>
<td>1.95</td>
<td>0.0049</td>
<td>0.0766</td>
</tr>
<tr>
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<td>149</td>
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<td>0.0049</td>
<td>0.0766</td>
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<td>85</td>
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<td>0.0062</td>
<td>0.0976</td>
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<td>15</td>
<td>2.85</td>
<td>0.0105</td>
<td>0.1637</td>
</tr>
</tbody>
</table>

\( L = 77.5 \) cm
\( dp = 64 \mu m \)
\( D_m = 0.97 \times 10^{-5} \) cm\(^2\) sec\(^{-1}\)

At high velocities the value of \( \sigma_r \) was found to be 1.95 mm and serious divergence from the Gaussian profile only occurs at the lowest velocity examined. Figure 54 shows the plot of log \( h_T \) against log \( v \). The best fit to the experimental data is given by equation 7.14

\[
h_T = \frac{2\sigma}{v} + 0.07 \quad (7.14)\]
i.e. the contribution to the radial plate height due to anastomosis is smaller than expected. In physical terms this means that the value of $\omega_3$ in the stream splitting model (Figure 50A and equation 7.6) probably lies between 1 and 1.5. This suggests that the regularly packed bed model of Figure 50A is not a suitable description of the bed structure of this particular column. It can be envisaged that random packing may also produce, throughout the bed, regions of a more unstable structure, such as that illustrated in Figure 50B. Clearly, in such regions the stream splitting mechanism cannot operate efficiently and the overall radial dispersion is reduced.

The plot of mean mobile phase velocity, $u$, against distance from the column centre, $x$, is shown in Figure 61. Mean mobile phase velocities could be measured to within 1 mm of the wall. At points closer than this the peak shapes were distorted. It was not clear whether this was genuinely a "wall effect" or merely due to extra-column eddies in the flow stream, generated at the walls of the apparatus. However, from the plot a small, but significant, transcolumn velocity variation could be detected. At a point 1 mm from the column wall the mean velocity was found to be 2% greater than at the column centre. Figure 62 shows the elution profiles recorded by the movable electrode at both these positions. Apart from the distinct disparity in elution time, a difference in peak width and symmetry is immediately perceptible. As the data of Figure 63 demonstrates, the width of the eluted solute profile, $w$, increased dramatically as the column wall was approached. This behaviour is repeated in Figure 64 in which the data is derived from a series of point injections at the column centre. The plate heights of the axial elution profiles recorded by the movable electrode are plotted against distance from the column centre. The sharp rise in plate height as the wall is approached
is again in evidence. The form of the transcolumn velocity profile indicates that the geometrical packing mode of the beads is not uniform across the bed; the packing density of the beads being greater at the centre of the column than in the region nearer the walls. The failure of radial dispersion in relaxing the nonequilibrium generated by the velocity inequalities across the column is reflected in the increased axial plate height as the column wall is approached.

The advance of HSLC has depended to a large extent on the successful preparation of high performance columns by a variety of packing methods. The results of this study establish that the movable electrode technique is particularly useful in throwing light upon band dispersion arising from mobile phase processes in such columns. Variation of the parameters $L$, $d_p$ and $d_c$ would yield comprehensive information on any packing method. Furthermore, the scope of the technique is not limited to unretained solutes on impervious spherical supports and a study of the behaviour of retained solutes on porous materials would, no doubt, be fruitful.
PART IV

A SURVEY OF COLUMN PACKING
TECHNIQUES
8.1 Introduction

It has been shown in Chapter 2 that band dispersion along a chromatographic column can be described by several equations of varying sophistication which take into account the contributions to the total broadening due to individual effects. Briefly, these can be listed as solute axial molecular diffusion, non-equilibrium of solute between mobile and stationary phases, and non-equilibrium within the mobile phase itself. For unsorbed solutes the first of these is absent and at high mobile phase velocities the contribution of the second effect is small. Hence the peak broadening due to the third source is of great significance and the minimisation of this effect is of fundamental importance. Velocity differences across the column can be responsible for the major part of the observed plate heights and are caused mainly by inequalities in local packing density and local differences in average particle size. It is clear that the preparation of high efficiency columns requires careful selection of the best method of producing a homogeneously packed bed of material. It has been found experimentally (69) that glass spheres, 480μm in diameter, pack homogeneously in a bed so that their packing characteristics can be taken as ideal. The use of large particles, however, is not desirable, for the theory of chromatography predicts that separation efficiency improves continually with decreasing particle size. In LC the use of small particles cannot be avoided if columns of high efficiency are required.
In order to compare the goodness of packing of various packing methods, the concept of reduced parameters is extremely useful. If all particle size fractions of a particular support packed equally well, the theory shows that for a particular LC system a plot of $h$ vs. $v$ should produce a single curve as particle diameter is varied. This assumes that all the particles in each bed maintain the same configuration and packing density. This is not the case. As the mean particle diameter is lowered below a certain value, the $h$ vs. $v$ plots become successively higher, indicating that the good packing of 480µm beads is not being attained with smaller particles. The reason for this deterioration in performance probably lies in the increasing effect of the surface properties of the particles. It has been postulated that smaller particles are more susceptible to aggregation and bridging, leading to less homogeneously packed beds. The theoretical advantage of using smaller particles is offset by the detrimental band spreading caused by velocity inequalities in the flow, so that as a compromise it has been the practice, until recently, to use particles of a size range that can be reasonably well packed, usually around 40-50µm. It is interesting to note that 'Zipax' particles exhibit a marked improvement in packing properties compared with glass beads of similar size range. This has been attributed to the micro-rough surface of 'Zipax' which significantly reduces the area at points of contact with adjacent particles in the bed.

A variety of techniques exist for packing HSLC columns and it would appear that an optimum packing procedure probably exists for each different type of packing material. In some cases, different size fractions of the same material require different procedures for packing optimum columns. In this particular survey, the packing material used was glass beads, although a few experiments were carried out with
carborundum chips, chosen as an example of an irregular shaped particle.

The packing methods used are listed below:

1. The dry packing material was poured continuously in a slow stream into a funnel fitted to the top of the vertical column. The column was rotated at 60-100 rpm and simultaneously bounced vertically 100 times per minute. The height of the bounce was 1 cm. As the packing level rose inside the column, a glass rod fitted with a rubber sleeve was used to tap the column side at a point just above the packing level.

2. This method was identical to (1) with the exception that the column was not tapped on the side during filling.

3. This method was identical to (1) except that lateral vibration was applied to the column side instead of side-tapping. The vibration was applied at a point just above the level of the packing in the column.

4. An increment of packing material was added while the column was rotated and bounced in the usual manner. A tungsten rod of slightly smaller diameter than the bore of the column was then placed onto the packing top and lateral vibration applied to the column side. Finally, the column was bounced a few times with slight pressure applied down the tamping rod. This cycle was repeated until the column was filled.

5. The packing material was added continuously while the column was vibrated vertically along its axis and simultaneously rotated.

6. The packing was added in increments while the column was rotated about, and vibrated along, its vertical axis. The packing was tamped with a tungsten rod after each incremental addition.
The column was vibrated along its vertical axis during the continuous addition of packing material. As the packing level rose, the column side was tapped at this level.

The column was vibrated along its vertical axis while the packing was added in increments. The packing was tamped with a tungsten rod after each addition.

The packing was slurried with water and forced into the column under 1000 psi. The column was vibrated axially during the process.

The packing was slurried in a mixture of diiodomethane and dichloroethylene, adjusted to the same density as the packing material. The slurry was forced into the column under 1000 psi. The column was vibrated axially during the process.

8.2 Experimental

The glass beads (English Glass Co.) used in the survey were density fractionated by pouring them into a 70% w/w aqueous solution of zinc chloride (density 1.95g ml\(^{-1}\)). The beads that remained floating on the surface were discarded. Those of density greater than 1.95g ml\(^{-1}\) were cleaned by warming in alkaline permanganate solution for two hours before filtering and washing with concentrated hydrochloric acid. The beads were washed with distilled water and finally with acetone before drying in the oven. Size fractionation was carried out by the sedimentation method described in Chapter 4. Carborundum chips were cleaned by washing with moderately concentrated hydrochloric acid, distilled water and acetone. The dry chips were sieved to give the required fractions.

All columns used were 50cm of heavy walled, 2.2mm bore glass tubing fitted with brass end fittings.
Solutes were detected by polarographic dropping mercury electrode detectors of perspex or teflon. To minimise dead volume, the eluent from the column was carried by a short length of capillary bore stainless steel tubing to the detector, where it was delivered directly onto the mercury drop.

The mobile phase throughout the survey was 50% ethanol in 0.1M KCl solution. This was degassed by refluxing and de-oxygenated by a stream of nitrogen.

Unretained solutes were p-nitrophenol and o-nitroaniline injected by microsyringe directly onto the packing top.

High liquid pressures were achieved using an Orlita piston pump (model DMP 1515). Pressures were measured by Bourdon gauge which also helped to damp the pulsating flow delivered by the pump.

An engraving tool (Burgess) was adapted for use in the vibration packing methods. Lateral vibration was carried out by fitting a cork to the head and holding this against the column side. Columns were vibrated along their vertical axis by means of a fitting that directly coupled the column end to the vibrating head.

8.3 Results and Discussion

Table 8.1 shows a summary of the data obtained from the series of experiments. From this survey of column packing methods some general conclusions can be drawn about the packing of glass beads. The most successful technique of all those applied was dry packing by method (1). The importance of side-tapping the column during filling can be seen by comparing the plate heights obtained by methods (1) and (2). The
<table>
<thead>
<tr>
<th>Particle size (μm)</th>
<th>H (mm) at v = 100</th>
<th>h at v = 100</th>
<th>Packing method</th>
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<tbody>
<tr>
<td>30-40</td>
<td>0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.7</td>
<td>(1) dry: rotation, bounce and tapping.</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.3</td>
<td>(2) dry: rotation, and bounce</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.1</td>
<td>(3) dry: rotation, bounce and high amplitude vibration.</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.5</td>
<td>(3) dry: rotation, bounce and medium amplitude vibration.</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.6</td>
<td>(3) dry: rotation, bounce and low amplitude vibration.</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.33</td>
<td>9.5</td>
<td>(4) dry; as (3) plus tamping.</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.33</td>
<td>9.5</td>
<td>(5) dry: rotation and vibration along column axis.</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.37</td>
<td>10.6</td>
<td>(6) dry: as (5) plus tamping at 3cm intervals.</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.42</td>
<td>12.0</td>
<td>(8) dry: as (5) plus tamping at 3mm intervals.</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.31</td>
<td>8.8</td>
<td>(7) dry: vibration along column axis plus tapping.</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.80</td>
<td>22.7</td>
<td>(10) wet: dense slurry plus vibration.</td>
</tr>
<tr>
<td>20-30</td>
<td>0.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.4</td>
<td>(1) dry: rotation, bounce and tapping.</td>
</tr>
<tr>
<td>10-30</td>
<td>0.44</td>
<td>21.8</td>
<td>(1) dry: rotation, bounce and tapping.</td>
</tr>
</tbody>
</table>

Notes:  

a: average of duplicate columns.  
b: very loose packing, resulting in collapse.  
c: average of triplicate columns.
omission of side tapping produced poorer plate heights and poorer reproducibility, indicating that side-tapping must help in consolidation of the packing and in eliminating voids. The lateral vibration technique (methods (3) and (4)) failed to give satisfactory results. The technique may tend to fractionate the particles across the column, giving an inhomogeneous bed which has larger particles near the column wall and smaller particles near the middle. In fact, observations showed the packing to be very loose with gaps appearing in the packing when mobile phase was passed through the column. The amplitude of vibration was varied but did not appear to have any significant effect. Gap formation could be prevented by use of a tamping rod (as described in method (4)) and this produced the best results of the lateral vibration methods. Presumably the fractionation effect was still operating or better performance might have been obtained. This sizing effect would probably not occur with the vertical vibration methods ( (5) to (10) ), although it is not clear whether vertical vibration would impart enough energy to the particles to consolidate the bed without void formation. Tamping at regular intervals would be expected to reduce void formation but the results show that vertical vibration combined with tamping gives poorer results than vertical vibration alone. This can be explained by observing the tamping process during column packing. Although the rod helps in compacting the bed, the top of the packing is disturbed by the tendency of the material to flow up in the narrow space between the rod and the column wall. It was found that if the tamping intervals were decreased from 3cm to 3mm there was an increase in the observed plate height due to the increased disturbance of the bed. The ideal tamping rod should be tight fitting.
in the column bore, although withdrawal of such a rod from the column may cause disturbances in the packing by the vacuum effect.

It is probable that method (1) was successful because the vertical bouncing and side tapping imparted sufficient mechanical energy to the beads to consolidate the packing without void formation, but not enough to cause the sizing of particles across the bed. If this sizing effect is important, a change in plate height should be noted if the width of a particular fraction is varied while keeping the mean particle diameter constant. This was found to be the case experimentally. The average particle diameter was chosen as 25μm and three fractions of varying width prepared. The results are shown in Table 8.2. The narrowest fraction exhibited the lowest plate height as expected, for here the sizing effect would be least important.

Although method (1) produced the best results, the reduced plate height versus reduced velocity plot for the 30-40μm beads shows that the ideal packing configuration of 480μm beads had not been reached. The reduced plate height for 20-30μm beads packed by this method was higher still, demonstrating that the lowering of mean particle diameter was accompanied by a tendency for the beads to settle in a less ordered state. This is shown in Figure 65. As an example of an irregular, non-porous packing material, carborundum chips were sieved and packed by method (1). Like the glass beads, the 37-44μm fraction gave a higher log h vs. log v plot than the 44-53μm fraction, indicating a falling off of homogeneity in packing structure with particle diameter. A wet packing technique (method (9) ) was also tried
TABLE 8.2  Plate heights of unretained solutes on glass beads.  
(mean particle diameter 25 \( \mu \text{m} \)).

<table>
<thead>
<tr>
<th>Particle Size Range (( \mu \text{m} ))</th>
<th>H (mm) at ( v = 100 )</th>
<th>h at ( v = 100 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-40</td>
<td>0.57</td>
<td>22.8</td>
</tr>
<tr>
<td>15-35</td>
<td>0.33</td>
<td>13.2</td>
</tr>
<tr>
<td>20-30</td>
<td>0.29</td>
<td>11.6</td>
</tr>
</tbody>
</table>

Plate heights are the average of duplicate columns.

All columns were packed by Method (1).

TABLE 8.3  Plate heights for unretained solutes on carbonum chips

<table>
<thead>
<tr>
<th>Particle Size Range (( \mu \text{m} ))</th>
<th>H (mm) at ( v = 100 )</th>
<th>h at ( v = 100 )</th>
<th>Packing method</th>
</tr>
</thead>
<tbody>
<tr>
<td>44-53</td>
<td>0.44\textsuperscript a</td>
<td>9.1</td>
<td>(1) dry : rotation, bounce and tapping</td>
</tr>
<tr>
<td>37-44</td>
<td>0.50</td>
<td>10.0</td>
<td>(1) dry : rotation, bounce and tapping</td>
</tr>
<tr>
<td>37-44</td>
<td>0.45\textsuperscript b</td>
<td>9.3</td>
<td>(9) wet : slurry in water</td>
</tr>
<tr>
<td>37-44</td>
<td>0.30\textsuperscript a</td>
<td>6.2</td>
<td>(9) wet : slurry in water see note c</td>
</tr>
</tbody>
</table>

Notes:  
\textsuperscript a : average of duplicate columns.  
\textsuperscript b : very loose packing, resulting in collapse.  
\textsuperscript c : Column packing consolidated by using mobile phase as high pressure 'hammer'.
with these particles and a marked improvement in plate height was noted, as shown in Figure 66 and Table 8.3. The dense solvent slurry technique (method (10)) was applied to the glass beads but here a deterioration in performance occurred. These results support the conclusions of Sie and van den Hoed (130) who found that wet packing techniques were optimum for irregular shaped particles, whereas dry packing was optimum for spherical particles.

Porosities of columns were measured experimentally from pressure drop and flow rate data. Typically, dry packed columns had porosities ranging from 0.36 to 0.41 while those packed by slurry methods gave values in the region 0.50 - 0.54 showing that the wet packing methods gave a more open structure.

An important point noted with the log h vs. log v plots calculated for columns packed with small particles was that the gradients of the plots were higher than expected. The slope of a plot is generally characteristic of the particular material and for non-porous beads the slope is in the range 0.3 to 0.4. However, in this case some plots of gradients approaching unity were found. It was also observed that the eluted peaks were not symmetrical, the deviation from perfect symmetry being measured by the symmetry index, S, where

\[
S = \frac{\text{trailing peak half width}}{\text{leading peak half width}}
\]

The leading and trailing half widths are measured by drawing tangents to the points of inflection of a peak and dropping a perpendicular from the point of intersection to the baseline. The half width is
measured from the point of intersection of this perpendicular with the baseline to the point of intersection of one of the tangents to the baseline.

For some peaks the symmetry index was as high as 3.5 but this was usually found at high mobile phase velocities. It is obvious that considerable error is involved in calculating plate heights based on Gaussian peak profiles using data measured from non-Gaussian peaks. The origin of the skewness is not clear, although non-ideal geometry of the injection port and detector may have contributed.

The phenomenon may also be the result of poor trans-column equilibration. At low mobile phase flow rates trans-column equilibration in narrow bore columns is relatively good, but at high flow rates it becomes poorer. With an uneven packing structure this may well give rise to tailing at higher velocities. It is of interest to note that in wide bore (infinite-diameter) columns, where trans-column equilibration is absent, the opposite effect is observed. At the lower velocities the molecules reach the wall regions where the flow is seriously disturbed and a significant loss in efficiency is observed. In this case the overall effect is to flatten the slope of the log h vs. log v plot (131).
APPENDIX
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Coefficient of flowing mobile phase term in plate height equations.</td>
</tr>
<tr>
<td>$A_s$</td>
<td>Area required by an adsorbed solvent molecule.</td>
</tr>
<tr>
<td>$A_x$</td>
<td>Area required by an adsorbed sample molecule.</td>
</tr>
<tr>
<td>$a_i$</td>
<td>Area required by an adsorbed chemical group i.</td>
</tr>
<tr>
<td>B</td>
<td>Coefficient of longitudinal diffusion term in plate height equations.</td>
</tr>
<tr>
<td>C</td>
<td>Coefficient of mass transfer term in plate height equations.</td>
</tr>
<tr>
<td>$C_m$</td>
<td>Coefficient of mass transfer between the flowing and stagnant parts of the mobile phase in plate height equations.</td>
</tr>
<tr>
<td>$C_s$</td>
<td>Coefficient of mass transfer term arising from stationary phase diffusion in plate height equations.</td>
</tr>
<tr>
<td>c</td>
<td>Solute concentration per unit volume of column packing.</td>
</tr>
<tr>
<td>c</td>
<td>Concentration of electroactive species in the bulk solution.</td>
</tr>
<tr>
<td>$c_m$</td>
<td>Concentration of solute in the mobile phase.</td>
</tr>
<tr>
<td>$c^*_m$</td>
<td>Concentration of solute in the mobile phase at equilibrium.</td>
</tr>
<tr>
<td>$c_s$</td>
<td>Concentration of solute in the stationary phase.</td>
</tr>
<tr>
<td>$c^*_s$</td>
<td>Concentration of solute in the stationary phase at equilibrium.</td>
</tr>
<tr>
<td>D</td>
<td>Effective diffusion coefficient.</td>
</tr>
<tr>
<td>$D_m$</td>
<td>Diffusion coefficient of solute in mobile phase.</td>
</tr>
<tr>
<td>$D_s, D_l$</td>
<td>Diffusion coefficient of solute in stationary phase.</td>
</tr>
<tr>
<td>$D_r$</td>
<td>Radial dispersion coefficient.</td>
</tr>
<tr>
<td>$d_c$</td>
<td>Column diameter.</td>
</tr>
<tr>
<td>$d_l$</td>
<td>Thickness of porous layer, equivalent to particle diameter in a totally porous support.</td>
</tr>
<tr>
<td>$d_p$</td>
<td>Particle diameter.</td>
</tr>
<tr>
<td>$d_s$</td>
<td>Mean diameter of a primary particle in a silica gel.</td>
</tr>
<tr>
<td>$d_s$</td>
<td>Thickness of stationary phase layer.</td>
</tr>
<tr>
<td>F</td>
<td>Faraday, equivalent to 96500 Coulombs.</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>$\Delta G_a^0$</td>
<td>Standard free energy of adsorption.</td>
</tr>
<tr>
<td>$H$</td>
<td>Height equivalent to a theoretical plate.</td>
</tr>
<tr>
<td>$H_r$</td>
<td>Radial plate height.</td>
</tr>
<tr>
<td>$H_d$</td>
<td>Eddy diffusion plate height term arising from diffusion.</td>
</tr>
<tr>
<td>$H_f$</td>
<td>Eddy diffusion plate height term arising from flow.</td>
</tr>
<tr>
<td>$h$</td>
<td>Reduced plate height.</td>
</tr>
<tr>
<td>$h_r$</td>
<td>Reduced radial plate height.</td>
</tr>
<tr>
<td>$I$</td>
<td>Total detector current.</td>
</tr>
<tr>
<td>$I_e$</td>
<td>Limiting current.</td>
</tr>
<tr>
<td>$I_o$</td>
<td>Residual current.</td>
</tr>
<tr>
<td>$J$</td>
<td>Amount of solute carried through unit cross sectional area of the column by the mobile phase in unit time.</td>
</tr>
<tr>
<td>$J^*$</td>
<td>Value of $J$ at equilibrium.</td>
</tr>
<tr>
<td>$K$</td>
<td>Distribution coefficient.</td>
</tr>
<tr>
<td>$K^e$</td>
<td>Value of $K$ at equilibrium.</td>
</tr>
<tr>
<td>$K_{th}$</td>
<td>Thermodynamic equilibrium constant for adsorption.</td>
</tr>
<tr>
<td>$K^0$</td>
<td>Specific permeability.</td>
</tr>
<tr>
<td>$k'$</td>
<td>Column capacity ration</td>
</tr>
<tr>
<td>$k_a$</td>
<td>Apparent first order rate constant for solute adsorption.</td>
</tr>
<tr>
<td>$k_d$</td>
<td>Apparent first order rate constant for solute desorption.</td>
</tr>
<tr>
<td>$L$</td>
<td>Length of column.</td>
</tr>
<tr>
<td>$l$</td>
<td>Random walk step length.</td>
</tr>
<tr>
<td>$M_m$</td>
<td>Molecular weight of mobile phase.</td>
</tr>
<tr>
<td>$M_s$</td>
<td>Molecular weight of stationary phase.</td>
</tr>
<tr>
<td>$M_2$</td>
<td>Molecular weight of solute.</td>
</tr>
<tr>
<td>$M_x$</td>
<td>Area of peak recorded at movable electrode.</td>
</tr>
<tr>
<td>$M_{x}^*$</td>
<td>Corrected peak area recorded at movable electrode.</td>
</tr>
</tbody>
</table>
m  Ratio of adsorbed sample area to that of adsorbed solvent molecule.
N  Number of theoretical plates.
N_{xa}  Mole fraction of sample in the adsorbed phase.
N_{xn}  Mole fraction of sample in the nonadsorbed phase.
n_{xa}  Total number of moles of sample in the adsorbed phase.
n_{xn}  Total number of moles of sample in the nonadsorbed phase.
n_{sa}  Total number of moles of solvent in the adsorbed phase.
n_{sn}  Total number of moles of solvent in the nonadsorbed phase.
n  Number of random walk steps.
n  Number of Coulombs per mole of solute.
n_{m}  Number of solute molecules in the mobile phase.
n_{s}  Number of solute molecules in the stationary phase.
P,\Delta P  Pressure.
P*  Standard pressure.
Q  Area of chromatographic peak.
Q_{i0}  Dimensionless free energy of adsorption of group i on a standard activity surface (α = 1).
q_{m}  Configurational factor for solute diffusion in the mobile phase entrained within a porous particle.
q_{s}  Configurational factor for solute diffusion in the stationary phase.
R  Ratio of solute migration velocity to that of the mobile phase, i.e. the fraction of the solute molecules in the mobile phase at equilibrium.
R_{s}  Resolution.
S  Sensitivity.
S  Packing density of primary particles in a gel.
S_{c}  Specific surface area of a gel calculated from the diameter of the primary particles.
\( S_n \) Specific surface area of a gel obtained by nitrogen adsorption.

\( S^0_x \) Sample adsorption energy on a standard activity surface.

\( S_x \) Area of peak recorded at fixed electrode.

\( S_{\text{mean}} \) Mean value of \( S_x \).

\( T \) Temperature (°K).

\( t_1, t_2 \) Elution distances.

\( t_r \) Elution time of solute.

\( t_0 \) Elution time of unretained solute.

\( t_d \) Solute molecule mean desorption time.

\( t_m \) Mean residence time of solute molecule in mobile phase.

\( t_s \) Mean residence time of solute molecule in stationary phase.

\( \Delta t \) Peak width.

\( u \) Mobile phase linear velocity.

\( u_b \) Solute migration velocity.

\( V \) Volts.

\( V_t \) Molar volume of solute.

\( V_p \) Specific volume of silica gel.

\( V_a \) Volume of an adsorbed solvent monolayer per unit weight of adsorbent.

\( V_m \) Volume of mobile phase.

\( V_s \) Volume of stationary phase.

\( V_R \) Retention volume of a solute.

\( V_g \) Specific retention volume of a solute.

\( V_n \) Net retention volume of a solute.

\( V_f \) Rate of fluid flow.

\( v_2^o \) Molar volume of the pure solute.
\(-m\)  Partial molar volume of solute in the mobile phase.

\(-s\)  Partial molar volume of solute in the stationary phase.

\(V_m\)  Molar volume of pure mobile phase.

\(V_s\)  Molar volume of pure stationary phase.

\(-m_{\infty}\)  Partial molar volume of solute in mobile phase at infinite dilution.

\(-s_{\infty}\)  Partial molar volume of solute in stationary phase at infinite dilution.

\(v\)  Reduced velocity.

\(W\)  Weight of adsorbent.

\(w\)  Base width of peak.

\(w_s\)  Weight of stationary phase in the column.

\(x\)  Distance of movable electrode from column centre.

\(x\)  Total number of moles eluted from column.

\(x_i\)  Mole fraction of solute in phase i.

\(x_s\)  Mole fraction of solute in stationary phase.

\(x_m\)  Mole fraction of solute in mobile phase.

\(\Delta Z\)  Distance between adjacent peak maxima.

\(\alpha\)  Function of the percent water deactivation of an adsorbent.

\(\gamma\)  Obstructive factor for diffusion in the mobile phase.

\(\gamma'\)  Obstructive factor for diffusion in the stationary phase.

\(\gamma_m\)  Solute activity coefficient in mobile phase at infinite dilution.

\(\gamma_s\)  Solute activity coefficient in stationary phase at infinite dilution.

\(\varepsilon_0\)  Eluent polarity parameter for n-pentane.

\(\varepsilon_m\)  Fractional departure from equilibrium in the mobile phase.

\(\varepsilon_s\)  Fractional departure from equilibrium in the stationary phase.

\(\eta\)  Viscosity.

\(\lambda_i\)  Eddy diffusion coefficient for i th velocity inequality.
\( \mu^1 \)  Solute chemical potential in phase 1.

\( \mu^2 \)  Solute chemical potential in phase 1 at standard pressure.

\( \rho_s \)  Density of stationary phase.

\( \rho_m \)  Density of mobile phase.

\( \rho \)  Ratio of column diameter to particle diameter.

\( \sigma_z \)  Standard deviation of the axial Gaussian profile at a distance \( z \) down the column.

\( \sigma_r \)  Standard deviation of the radial concentration profile.

\( \phi' \)  Flow resistance parameter.

\( \psi_2 \)  Association factor.

\( \omega_1 \)  Coefficient of mobile phase plate height contribution for \( i \) th velocity inequality.

\( \Omega \)  Ohms.
REFERENCES


64. P.G. Bird : U.S. Patent; 2, 244, 325 (1941).


87. A. James, J. Ravenhill and R. Scott; ibid., p.197.
97. Laboratory Data Control. 1970. Publicity material. Riviera Beach, Florida, U.S.A.


Figure 1: Relation of stationary and mobile phase concentration profiles to the equilibrium profile.

Figure 2: Stability of silica sols. Relation between gel time and pH.
Figure 3: Components of apparatus for HSIC
Figure 5: Haskel pressure intensifier

Figure 4: Orlita reciprocating pump.
rubber septum
'o' ring
column top fitting
glass column
column bottom fitting
'o' ring

Figure 6: Column fittings
Figure 7: Du Pont photometer

Figure 8: Cecil photometer.
**Figure 9:** Apparatus for dry packing of columns.

**Figure 10:** Apparatus for slurry packing of columns.
Figure 11: Elution record.

tangents drawn at points of inflection

unretained solute

injection mark
Figure 12: log h vs. log v plot for 39μm Zipax in partition mode (ref. 78)
[Figure 13] log h vs. log v plot for 44-53μm Corasil II in partition mode (refs. 77 and 59).
Glass beads coated with a pellicular layer of silica gel (sodium silicate/Acid)
mobile phase:  n-hexane
stationary phase:  0.5% BOP

$d_p$: 40.5 μm    $D_m$: 3.3 x 10^{-5} cm² s⁻¹

dry packed.

Figure 14: Pellicular silica gel: log h vs. log v.
Figure 15: 37 - 44μm Pellicular silica gel, 0.5% BOP
Mobile phase: n-hexane. L: 41cm
Glass beads coated with a pellicular layer of silica gel (sodium silicate/acid)

Mobile phase: n-hexane
Stationary phase: 0.4% BOP
\( d_p = 40.5 \mu m \)

\( D_m = 3.3 \times 10^{-2} cm^2 s^{-1} \)

dry packed.

Toluene \( k' = 0 \) 0.8 0

O-t-butyl phenol \( k' = 0.36 \) 1.1 \( 1.8 \times 10^{-2} \)

O-cresol \( k' = 1.4 \) - -

P-cresol \( k' = 25 \) 1.2 \( 3.4 \times 10^{4} \)

**Figure 16:** Pellicular silica gel: \( \log h \) vs. \( \log v \).
Figure 17: 37 - 44μm Pellicular silica gel, 0.4% BOP mobile phase: n-hexane. L: 50cm.
Glass beads coated with a pellicular layer of silica gel (sodium silicate/Acid)

*mobile phase: n-hexane*

*stationary phase: 0.4% BOP*

\[ d_p: \quad 40.5 \text{ \mu m} \]

\[ D_m: \quad 3.3 \times 10^{-5} \text{cm}^2\text{s}^{-1} \]

dry packed

---

<table>
<thead>
<tr>
<th>Substance</th>
<th>( k' )</th>
<th>( A )</th>
<th>( C )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>( k' = 0 )</td>
<td>0.8</td>
<td>0</td>
</tr>
<tr>
<td>O-t-butyl phenol</td>
<td>( k' = 0.37 )</td>
<td>1.0</td>
<td>0.7 \times 10^{-2}</td>
</tr>
<tr>
<td>2,4-xyleneol</td>
<td>( k' = 0.82 )</td>
<td>1.2</td>
<td>1.0 \times 10^{-2}</td>
</tr>
</tbody>
</table>

---

Figure 18: Pellicular silica gel: log \( h \) vs. log \( v \).
Glass beads coated with two pellicular layers of silica gel (sodium silicate/Acid)
mobile phase: n-hexane
stationary phase: 0.6% BOP
dp: 40.5μm
Dm: 3.3 x 10^-5 cm^2 s^-1
dry packed

Figure 19: Pellicular silica gel: log h vs. log v.
1. toluene
2. 3, methyl 6, t-buty phenol
3. o-t-buty phenol
4. 2,4 xylenol

Figure 20: 37 - 44μm. Pellicular silica gel, 0.6% B0P mobile phase: n-hexane. L: 44 cm.
Glass beads coated with two pellicular layers of silica gel. (Stober, Fink and Bohn silica sol).

- Mobile phase: n-hexane
- \( d_p: 40.5 \mu m \)
- \( D_m: 3.3 \times 10^{-5} \text{cm}^2\text{s}^{-1} \)

Dry packed.

**Figure 21:**

Pellicular silica gel: log h vs. log v.

- 0.5% BOP ○ toluene \( k'=0 \)
- 0.5% BOP Δ o-cresol \( k'=152 \)
- 0.5% BOP ● o-cresol \( k=0.85 \)

After hydrothermal treatment: 0.5% BOP ○ toluene \( k'=0 \)

0.5% BOP ● o-cresol \( k=0.85 \)
Log 0

**mobile phase:** n-hexane

**dp:** 40.5\(\mu\)m

**\(D_m:** 3.3\(\times\)10\(^{-5}\) cm\(^2\)s\(^{-1}\)

**dry packed.**

---

**single thin pellicular layer of silica gel (0.3\% BOP):**
- toluene \(k' = 0\)
- 2,4 xylenol \(k' = 0.62\)
- acetophenone \(k' = 0.47\)
- 3-methyl,6-tert-butyl phenol \(k' = 1.6\)

**single thick pellicular layer of silica gel (1.0\% BOP):**
- toluene \(k' = 0\)
- acetophenone \(k' = 0.47\)

---

**Figure 22:** Pellicular silica gel (Bechtold and Snyder silica sol): log \(h\) vs. log \(v\).
Figure 23:
Pellicular silica gel
1% BOP
mobile phase: n-hexane
L: 50cm
Glass beads coated with a pellicular layer of silica gel (Bechtold and Snyder silica sol).

- mobile phase: n-hexane
- stationary phase: 1% BOP
- $d_p$: 40.5 $\mu$m
- $D_m$: $3.3 \times 10^{-5} \text{cm}^2\text{s}^{-1}$
- dry packed

**Figure 24:** Pellicular silica gel: $\log h$ vs. $\log v$. 
Figure 25: 37 - 44 μm Pellicular silica gel, 1% BOP
mobile phase: n-hexane L: 40cm

1: toluene
2: o,t-butyl phenol
3: 2,4 xylenol

1 minute
Glass beads coated with a pellicular layer of silica gel (commercially available 14nm silica sol).

Mobile phase: n-hexane
Stationary phase: 1% BOP
$d_p$: 40.5μm
$D_m$: $3.3 \times 10^{-5} \text{cm}^2\text{s}^{-1}$

dry packed.

Figure 26: Pellicular silica gel: log $h$ vs. log $v$.  

- Toluene $k'=0$ 0.95 0.02x10^2
- 2,4 xylenol $k'=1.78$ 1.80 4.2x10^2
Figure 27: Commercially prepared silica sol: Effect of silica concentration on gel time.
Glass beads coated with a pellicular layer of silica gel (commercially available 14nm silica sol.)

- Mobile phase: n-hexane
- Stationary phase: 1.5% BOP
- \( d_p = 40.5 \mu m \)
- \( D_m = 3.3 \times 10^{-5} \text{cm}^2\text{s}^{-1} \)
- Dry packed.

**Figure 28:** Pellicular silica gel: \( \log h \) vs. \( \log v \).
Totally porous spheres prepared from Bechtold and Snyder silica sol,
mobile phase: methylene chloride
\( \bar{d}_p: 21 \, \mu m \),
\( D_m: 2.7 \times 10^{-5} \text{cm}^2\text{s}^{-1} \)
\( \phi': 1648 \)
dry packed.

Figure 29: Porous silica gel spheres in adsorption mode: \( \log h \) vs. \( \log v \).
Totally porous spheres prepared from Bechtold and Snyder silica sol.
mobile phase: methylene chloride
d$_p$: 16µm
D$_m$: $2.7 \times 10^{-5}$cm$^2$s$^{-1}$
dry packed.

Figure 30: Porous silica gel spheres in adsorption mode: log h vs. log v.
Figure 31: 21μm silica gel spheres. Adsorption mode. Mobile phase: methylene chloride. L: 12.5cm
Porous spheres prepared from 13-14nm silica sol.

\( d_p: \quad 40.5\mu m \)

\( D_m: \quad 2.7 \times 10^{-5}\text{cm}^2\text{s}^{-1} \) (methylene chloride)

\( 3.8 \times 10^{-5}\text{cm}^2\text{s}^{-1} \) (n-hexane)

dry packed.

**Figure 32**: Porous silica gel spheres in adsorption mode: log h vs. log v.
Porous spheres prepared from 13-14 nm silica sol.

d_{p}: 25.5 \mu m

D_m: 2.7 \times 10^{-5} \text{cm}^2 \text{s}^{-1} \text{ (methylene chloride)}.

3.8 \times 10^{-5} \text{cm}^2 \text{s}^{-1} \text{ (n-hexane)}

\phi': 1/27

dry packed

Figure 33: Porous silica gel spheres in adsorption mode: log h vs. log v.
Porous spheres prepared from 13-14nm silica sol.

\( d_p: 12.5 \mu m \)

\( D_m: 2.7 \times 10^{-5} \text{cm}^2\text{s}^{-1} \) (methylene chloride)

\( 3.8 \times 10^{-5} \text{cm}^2\text{s}^{-1} \) (n-hexane)

\( \phi': 1019 \)

dry packed.

**Figure 34**: Porous silica gel spheres in adsorption mode: log \( h \) vs. log \( v \).
Figure 35: log h vs. log v plot for 44-53μm Porasil 250 in adsorption mode.
Mobile phase: methylene chloride

d_p: 12µm

D_m: 2.7 x 10^{-5} cm^2 s^{-1}

 denomination: 1524

dry packed

Figure 36: Spherisorb silica gel spheres, "S 10 W", in adsorption mode: log h vs log v.
Figure 37: Silica gel spheres. Adsorption mode.
mobile phase: n-hexane.
Porous spheres prepared from 7-9nm silica sol.
mobile phase: methylene chloride
\(d_p\): 20\(\mu\)m
\(D_m\): \(2.7 \times 10^{-5}\text{cm}^2\text{s}^{-1}\)
\(\phi\): 1353
dry packed.

Figure 38: Porous silica gel spheres in adsorption mode: log \(h\) vs. log \(v\).
Porous spheres prepared from 7-8nm silica sol.

Mobile phase: methylene chloride

\[ d_p = 12\mu m \]

\[ D_m = 2.7 \times 10^{-5} \text{cm}^2\text{s}^{-1} \]

\[ \phi' = 886 \]

Slurry packed.

Figure 39: Porous silica gel spheres in adsorption mode: \( \log h \) vs. \( \log v \).
Figure 40: Pore size distribution data for silica gel prepared from 13-14nm sol.
Figure 41: Pore size distribution data for gel prepared from 7-8nm silica sol.
Figure 42: Pore size distribution data for gel prepared from Bechtold and Snyder silica sol.
Figure 43: Details of detector circuit, platinum gauze electrode and spherical electrodes.
1: p-nitrophenol
2: ethyl nitrate
3: iodomethane
4: 1,3,5 trinitrobenzene
5: nitrobenzene

Figure 44b: Platinum gauze electrode
Figure 44a: Spherical platinum electrode

Polarographic electrode detection. 37-44μm Zipax ODS
Mobile phase: 0.1 M KCl
spherical platinum electrode
diameter: 1.39 mm
solute: o-nitroaniline
mobile phase: 0.1 M KCl
applied voltage: -1.0 V

Figure 45: Sensitivity vs. Flow rate plot for spherical platinum electrode.
Figure 46: Spherical platinum electrode, diameter 1.39 mm.
Zipax Permaphase ODS (37-44μm)

Mobile phase: 0.1 M KCl

L = 50 cm

$k' = 1.5$ α-nitroaniline

$k' = 0$ p-nitrophenol

Figure 47: log h vs. log v plots for photometric and electrolytic detectors
Figure 48: Platinum gauze electrodes. Sensitivity vs. Flow rate plots.
Figure 49: Platinum gauze electrodes.
The mechanism of solute dispersion by stream splitting.
Figure 51: $\log h$ (at $v = 300$) vs. $\log \epsilon$
Figure 52: Moving electrode detector assembly.

- column
- platinum electrodes
- mercury pool
- copper wire
- nylon sleeve
- eluent outlet
- electrode terminals
- micrometer
Figure 53: Detector circuit.
Figure 54: log $h$ vs. log $v$ for 64 µm glass beads.
Figure 55: Radial concentration profile.
Figure 56: Radial concentration profile.
Figure 57: Radial concentration profile.
Figure 58: Radial concentration profile
Figure 59: Radial concentration profile

\( v = 29 \)
\( \sigma_r = 240 \text{ mm} \)
Figure 60: Radial concentration profile.

\( \nu : 15 \)

\( \sigma_r : 2.85 \text{ mm} \)
Figure 61: Plot of mean mobile phase velocity, $u$, against distance from the column centre, $x$. 
**Figure 62**: Elution profiles recorded by fixed electrode and movable electrode at column centre and near column wall.
Figure 63: Variation of peak width at points across the column.

Figure 64: Variation of axial plate height at points across the column.
Figure 65: log h vs. log v plot for fractionated glass beads.
Figure 66: log h vs. log v plot for fractionated carborundum chips.
PELLICULAR SILICA GEL PREPARED
FROM SODIUM SILICATE
PELLICULAR SILICA GEL PREPARED FROM BECHTOLD & SNYDER SILICA SOL
PELLICULAR SILICA GEL
PREPARED FROM 13 nm SILICA SOL
× 185

× 4800

× 9600
POROUS SILICA GEL SPHERES PREPARED FROM 13 nm SILICA SOL

EM 15

x 4800

EM 16

x 19200
POROUS SILICA GEL SPHERES PREPARED FROM 7nm SILICA SOL
× 440

× 1680

× 17600
POROUS SILICA GEL SPHERES PREPARED FROM BECHTOLD & SNYDER SILICA SOL