

STUDIES IN HIGH SPEED LIQUID
CHROMATOGRAPHY

by

Gordon J. Kennedy, B.Sc.

Thesis submitted for the Degree of
Doctor of Philosophy

University of Edinburgh

1973



ACKNOWLEDGEMENTS

The author wishes to express his thanks to Dr. John H. Knox for his enthusiastic encouragement during the course of this work,

to Professor Charles Kemball for the provision of laboratory and library facilities,

to the University of Edinburgh for the award of the Sir David Baxter Scholarship during the course of the work,

to Mrs. Sandra Carter for typing this thesis

and to past and present research students and members of the staff of the Chemistry Department for their advice, encouragement and friendship.

The history of chromatography is surveyed with particular emphasis on the course of, and the factors influencing, the renaissance of liquid chromatography which has occurred during the past decade.

The thermodynamics of the partition and adsorption modes of LC is outlined. Equations are given which relate the relative retention of two closely related solutes in partition LC to the molar volumes of the eluent and solutes, the difference in the cohesive energy densities of the solutes and the ratio of their critical temperatures. Equations are presented which relate the specific retention volume of a solute in adsorption LC to the adsorbent capacity and activity, an eluent parameter and additive contribution from the groups present in the solute molecule, composed of adsorption energy terms, second order corrections to these latter terms due to neighbouring groups and group volume terms.

The kinetics of LC is reviewed. The development of the random walk theory is outlined. The application of this theory to the prediction of the plate height contributions arising from longitudinal molecular diffusion, adsorption - desorption kinetics, diffusion controlled kinetics and mobile phase dispersion is discussed. The plate height theories developed by van Deemter et al., Keulemans and Kwantes, and Jones are briefly outlined. The application of the generalised nonequilibrium theory to the prediction of the plate height contributions arising from step-wise

kinetics, diffusional mass transfer and transcolumn velocity variations is discussed.

Studies of the performance of the diatomaceous earths, Chromosorb^R W, Chromosorb^R P and Phase Separation Universal^R, and of Zipax^R in partition LC, and of Porasil^R, Corning CPG^R, and Corasil^R in both partition and adsorption LC are reported. For each support in each mode the variation of $\log h$ (where h is the reduced plate height) with $\log v$ (where v is the reduced velocity) is reported for an unretained, or lightly retained, solute and one, or more, moderately retained solutes. The $\log h$ vs $\log v$ curves found for unretained, or lightly retained, solutes are lower for the surface layered materials, Zipax^R and Corasil^R, than those for the porous Porasil^R and Corning^R CPG materials. These latter materials give lower curves than the diatomaceous earths. The plate height contributions from intraparticle diffusion are within the range predicted by the nonequilibrium theory. The dependence of h upon K' , the column capacity ratio, is shown to be small for all the supports investigated, with the exception of Corasil^R I and Corasil^R II.

The data obtained are shown to fit the empirical equation

$$h = 2\gamma/v + Av^n + Cv$$

where γ is the obstructive factor for diffusion and A , C and n are constants. The first term, significant when v is less than 10,

accounts for the dispersion due to longitudinal molecular diffusion. The second term is used to approximate the more complicated dependence on v of the mobile phase contributions to h , predicted by the coupling theory of eddy diffusion. n is shown to depend on K' . A is shown to be weakly dependent on K' and to depend strongly on the quality of packing achieved in a given column. The third term accounts for the contributions to h arising from the stationary phase and stagnant regions of the mobile phase. C depends on the nature of the support and the configuration of the stationary phase.

The dependence of solute retention volumes on the difference in adsorption free energies between the solute and the eluent is examined for a number of solute-eluent pairs in adsorption chromatography on Corning CPG^R 75. The retention volumes of isotopically labelled samples of three eluents were determined and it is shown that, where the solute adsorption free energy is less than that of the eluent, the solute retention volume is less than that of an isotopically labelled mobile phase sample.

CONTENTS

	<u>Page</u>
Acknowledgements	i
Summary	ii - iv

CHAPTER 1

<u>Introduction</u>	1 - 19
1.1 Historical Survey	1
1.2 The Renaissance of Liquid Chromatography	4
1.3 The Development of Liquid Chromatography	9

CHAPTER 2

<u>Thermodynamics of Liquid Chromatography</u>	20 - 38
2.1 Thermodynamics of Liquid Chromatography	22
2.2 Thermodynamics of Partition Chromatography	24
2.3 Thermodynamics of Adsorption Chromatography	29

CHAPTER 3

<u>The Kinetics of Liquid Chromatography</u>	39 - 85
3.1 Classification of Chromatographic Methods	40
3.2 Theoretical Plate Model	40
3.3 The Random Walk Model	42
3.4 Generalised Nonequilibrium Theory	62

CHAPTER 4

<u>Equipment and Experimental Procedure</u>	86 - 106
4.1 General Features of Chromatographic Equipment	86
4.2 Preparation and Packing of the Columns	90
4.3 Detectors	92
4.4 The Experimental Procedure	100
4.5 The Calculation of the Results	102

CHAPTER 5

<u>Performance of Various Supports in High Performance Liquid Chromatography</u>	107 - 139
5.1 Introduction	107
5.2 Experimental	115
5.3 Results	124
5.4 Discussion	127

CHAPTER 6

<u>Retention Volume Determinations</u>	140 - 147
6.1 Introduction	140
6.2 Experimental	144
6.3 Results and Discussion	145

CHAPTER 7

<u>Conclusions</u>	148 - 152
7.1 The Contributions of this Work	148
7.2 Future Developments	150

	<u>Page</u>
<u>APPENDIX</u>	153 - 164
List of Symbols	153 - 159
References	160 - 164
Figures	
Reprints of Two Publications	

CHAPTER 1

INTRODUCTION

1.1. Historical Survey

Liquid chromatography was the first of the chromatographic techniques currently employed in modern analytical chemistry to be discovered. The discovery was made by the botanist Tswett (1) who, in 1906, extracted pigments from green leaves with petroleum ether and eluted the extract through a column containing calcium carbonate. The separation of the components of the plant extract during elution down the column resulted in the development of a series of coloured bands with intervening colourless zones. Tswett named the process chromatography. The term has since been extended beyond the description of separations of molecules which absorb visible light to embrace a much wider group of separation methods, which may be defined to include

"any method of separation of the components of a mixture which depend upon the repeated distribution and redistribution of the molecules of the mixture between a flowing or 'mobile' phase and a fixed or 'stationary' phase."

Chromatography only emerged as a widely applied analytical technique after its rediscovery in 1931 by Kuhn, Lederer and Winterstein (2). A further important advance was the invention of liquid-liquid chromatography, based, according to the above definition, on the partition of the molecules of a mixture

between two liquid phases, by Martin and Synge (3) in 1941; this method was rapidly adopted. The latter invention paved the way for paper chromatography (4), and thin layer chromatography (5). Martin and Synge also suggested that the mobile liquid in the liquid-liquid technique might be replaced by a gas. Martin and James (6) put this suggestion to the test in 1952 and their analysis of fatty acids by partition between nitrogen and silicone oil, distributed on a diatomaceous earth support, heralded the beginning of a period of explosive growth in the number and range of applications of this newly discovered technique of elution gas-liquid chromatography. While all three modes of chromatographic development, viz., displacement, frontal analysis and elution are applicable to liquid as well as to gas chromatography the use of all three was largely pioneered in the field of gas chromatography.

The displacement technique introduced by Tiselius (7), Claesson (8) and Phillips (11) was first used successfully in gas-solid systems (9, 10, 11, 12). A small sample of the mixture to be analysed is introduced into a column packed with an adsorbent. A carrier gas stream which contains a constant concentration of a strongly adsorbed vapour, the displacer, is passed through the column. The components of the mixture are displaced from the adsorbent and are carried down the column, each component being displaced by the next most strongly adsorbed sample component. The displacement development of a chromatogram is shown in

Figure 1. In this technique the column requires to be regenerated by heating to desorb the displacer before it can be used for another analysis.

The frontal analysis method is a modification of adsorption chromatography in which a dilute solution of the mixture to be analysed is constantly forced through an adsorbent column at a constant initial concentration in the carrier fluid stream. The least strongly retained component emerges from the column first and each succeeding component leaves the column mixed with the preceding components. Figure 2 shows schematically the development of frontal analysis.

In elution analysis a discrete sample of a mixture is sharply introduced into the column and the mixture is swept through the column by an inert carrier fluid. The rates of migration of components of the mixture depend upon their relative distribution coefficients between the carrier fluid and the stationary phase. The components are thus eluted in the mobile phase from the column with characteristic retention volumes. The need for column regeneration is avoided in this mode of operation. The development of an elution chromatogram is shown in Figure 3. Most notable among the advantages of the elution mode of chromatographic development are the reproducibility of the results obtained and the versatility with regard to the combination of phases used. The work to be described in this thesis is

entirely concerned with elution chromatography which will be discussed in more detail below; the displacement and frontal methods will receive no further attention.

In the two decades following the work of Martin and James elution gas chromatography was confirmed as an extremely versatile analytical tool in all branches of chemistry and biochemistry. Other chromatographic techniques were, at least temporarily, in eclipse. Major contributions during this time were made, in gas-liquid chromatography, by James and Martin (13), James and Phillips (14), Ray (15) and Glueckauf (16), and, in gas-solid chromatography, by Creemer and Mueller (17), Zhukhovitskii et al., (18), Ray (15) and Patton, Lewis and Kaye (19).

1.2. The Renaissance of Liquid Chromatography

The predominance of gas chromatography among chromatographic methods in the 1950s and 1960s may be attributed to two major factors:

- a) technological developments giving rise, most importantly, to a range of versatile and highly sensitive detectors, and
- b) a continuously developing theoretical background elucidating the requirements for still higher limits of practical performance.

In the former category, the development of the flame ionization detector by Harley, Nel and Pretorius (20), and McWilliam and Dewar (21, 22), and of the argon detector by Lovelock (23 - 26),

represented landmarks in the detection of very small concentrations of solutes in chromatographic effluents.

In the latter category, an early attempt to develop a theory of chromatography was made by J.N. Wilson (27) in 1940. Martin and Synge (3) in 1941 outlined the plate theory of chromatography which was improved by De Vault (28) and Weiss (29). H.C. Thomas (30) in 1948 made an outstanding contribution in the field of chromatographic theory. Perhaps the crucial factor which led to the theoretical understanding of gas chromatography outstripping that of liquid chromatography at this time was the adoption of the former technique by the petroleum industry for the analysis of oils and related products and the consequent involvement of chemical engineers in its development. The latter group were probably more orientated towards theoretical elucidation than the organic and biochemists who were the major users of liquid chromatography, though Martin's intuitive deduction of some of the factors affecting column performance is evidence of a clear insight into chromatographic mechanism. The theories developed by Lapidus and Amundsen (31) and Glueckauf (16) formed the basis of the well known treatment of van Deemter (32) in 1956. More recent developments have been made by Golay (33), Khan (34, 35) and by Giddings, the foremost contributor to the field of chromatographic theory (36 - 40).

The interaction of these two factors led to gas chromatography becoming, by the late 1960s, one of the most frequently used techniques in the analytical chemist's armoury; the two influences have had a similarly crucial role in the renaissance of liquid chromatography. Additionally, liquid chromatography complements the range of applicability of gas chromatography in one very important respect.

Thus, since gas chromatographic elution volumes are related to the solute vapour pressure (strictly the solute fugacity) at the temperature of the elution, analysis of involatile compounds must be carried out at increasingly higher temperatures to ensure elution in reasonable times. An upper limit to the temperature of analysis is set, either by the volatility or thermal stability of the stationary phase, or by the thermal stability of the solutes themselves. This limitation has only been partially bypassed. Davison et al. (41) first suggested the combination of pyrolytic techniques with gas chromatography for the identification of polymers. Janak (42,43) applied this method to the identification of the structure of other nonvolatile organic substances. Giddings et al. (44) used gas pressures of up to 2,000 atmospheres to take advantage of the solvent power of dense gases. However the technological problems of handling gases at such pressures must rule out this development for routine use. Resort had to be made to classical liquid chromatography, in which, typically, columns

were packed with 200 micron particles and solvents were delivered by gravity feed, for the analysis of involatile materials. Analysis times in these conditions were typically hours or even days.

Giddings (37,40) first identified the ratio of the rates of diffusive and of flow transport as the crucial parameter governing the performance of all chromatographic systems and thus realised that the theory developed for gas chromatography was, in all essentials, applicable to liquid chromatography also. The theory indicated that performance comparable to gas chromatography could be attained in liquid chromatography and that, to achieve this goal, particle diameters must be reduced by at least one order of magnitude and supports should permit rapid mass transfer between stationary and mobile phases. A further requirement for high performance liquid chromatography was the development of highly sensitive detectors. Progress has been rapid in each of these three areas.

Thus the work to be described below employed particles of 50 microns diameter; already it is becoming usual to work with particles down to 10 microns in diameter. Although the theory predicts that optimum performance will be attained by using particles of about 1 micron diameter the foreseeable limits of detector sensitivity and geometry suggest that the practically optimal particle diameter may be around 5 microns. The use

of particles in this size range requires the use of column inlet pressures of 1,000 - 5,000 p.s.i..

The most efficient support material so far produced is 'Zipax'. This material consists of particles with a solid glass core coated with silica microspheres about 200 nm in diameter. The stationary phase is distributed as a thin film on the microsphere layer and is easily accessible by diffusion from mobile phase. Porous silica spheres with a high surface area and controlled pore sizes have also recently been developed. These supports can tolerate a much higher stationary phase loading than 'Zipax' without overloading. The latter are likely to become important in the development of preparative scale liquid chromatography.

The third factor involved in the resurgence of liquid chromatography has been the introduction of high sensitivity detectors. The most widely used detectors are the refractive index detector and the ultra-violet absorption photometer. The concentration sensitivity of the RI detector is about 1 in 10^6 . The detection limit of the ultra-violet photometer varies with the extinction coefficient of the solute; the limit for a compound with a molar extinction coefficient of 10^4 is about 1 in 10^9 . This sensitivity is approached by the polarographic detector. Wire transport detectors have a sensitivity of about 1 in 10^6 though this has recently been improved.

An important result of these theoretical and technological advances has been a unification of the practice of liquid chromatography. Modern supports are equally suitable for use in adsorption, partition and gel-permeation chromatography. The range of analytical problems susceptible to attack by modern high speed liquid chromatography appears to span the whole of chemistry and biochemistry and the gamut of molecular weights from mononuclear aromatics to high polymers.

1.3. The Development of Liquid Chromatography

Since its discovery by Tswett (1) and its rediscovery by Kuhn, Lederer and Winterstein (2) the practice of liquid chromatography underwent remarkably little change until the late 1960s. Typically during this period analytical columns were packed with particles of about 200 microns diameter, column diameters ranged from 1 to 5 cm, solvents were delivered by gravity feed and typical analysis times were hours or even days. Detection of the components of a mixture depended on the collection of fractions for subsequent analysis by titrimetric or more recently, by spectro photometric methods. Thus Martin and Synge (3) in their classical paper in 1941 reported the use of a 1 cm diameter column packed with silica gel in analysing mixtures of acetylated amino acids. To make the individual solute bands visible an indicator solution was included in the gel during its preparation.

In the regions of the column where the concentration of the amino acids was high the indicator underwent a colour change. In this way the progress of the separation could be followed and the bands corresponding to the mixture components collected for analysis by titration. Though detection methods have advanced many analyses are still carried out by a technique essentially identical to that used by Martin and Synge, and their technique did not differ markedly from the methods used by Tswett in his analysis of plant pigments in 1906. Martin and Synge did, however, point out that shorter analysis times could be achieved "by using very small particles and a high pressure difference across the length of the column."

A notable feature of the development of liquid chromatography in this period was the proliferation of techniques employed in the various fields where LC was used. In the absence of a theoretical framework to unify the practical advances made in the different areas of application there was little information transfer, and, until the emergence of such an all-embracing theoretical framework in the mid 1960s, suggestions which led to better performance were slow to be adopted. The theoretical guidelines had first to be outlined. Thus Martin et al. (4) in 1944 had introduced paper chromatography. Kirchner et al. (5) reported the use of thin layer chromatography in 1951 though, as early as 1938, the Russians Izmailov and Schraiber (45) had reported the separation

of the components of various tinctures described in the Russian Pharmacopoeia by adsorption on layers of alumina and other adsorbents on microscope slides. Also in 1951, Mainhard and Hall (46) in the United States had reported the use of adsorbent coated glass plates for the separation of inorganic ions. Quantitative analysis by either of these techniques is laborious involving reaction of the zones with some suitable reagent to give a product which can be measured by densitometry. The practice of the different modes of column liquid chromatography also tended to become compartmentalised because of the different support materials and detection methods required. Thus the analysis of amino acids by ion exchange chromatography developed as a distinct field without reference to other areas of chromatography; the methods of preparation and packing of the ion exchange resins used were entirely different from the technology used in adsorption chromatography. As will be shown below modern developments have led to a convergence in the technology of all modes of LC though the field of amino acid analysis is still comparatively isolated by the specialised detection method used. This method depended on the discovery by Ruheman (47) that the compound ninhydrin reacts with most amino acids to produce the intensely coloured compound diketohydrindylidene-triketohydrindamine. The culmination of development in this field was the description of the

automatic analysis procedure of Spackman et al. in 1958 (48).

As early as 1947 Mayer and Tompkins (49) had suggested that, since equilibration is faster in small resin particles, analysis times could be decreased by a reduction in particle size. Hamilton (50) re-iterated this conclusion in 1958, again in the context of ion exchange chromatography, and made quantitative estimates of diffusion coefficients on the basis of the van Deemter equation. It seems probable that, if this suggestion had been widely adopted and developed, the use of high column inlet pressures and small particles to achieve fast analysis might have emerged one, or even two, decades earlier. However, at that time, the barriers between fields and between theory and practice had still to be breached in LC and the problems of continuous monitoring of effluents from LC columns had still to be attacked.

In the early 1960s, then, the practice of liquid chromatography had seen no dramatic development since the introduction of the method. Information transfer between the different areas of LC practice had been inhibited by the separate development of techniques and terminologies in each without the common language of a unified theory to emphasise the underlying similarities of the various methods and, consequently, the identical requirements which were prerequisites of improved performance throughout LC practice. This was in marked contrast to the situation in GC where a well-developed theory was playing a crucial role in its advance. An

equally vital role has been played by theory in the development of LC from 1964 onwards.

Prior to these theoretical developments comparisons of performance in LC were largely empirical; performance comparable to that achieved in GC was not considered a practicable goal. This situation was to undergo dramatic change with the theoretical developments of the late 1950s and the 1960s which showed that GC and LC could be directly compared and indicated the direction of progress required in LC in order that comparable column performance to that current in GC be attained. LC performance could now be evaluated relative to a theoretically predicted optimum.

Giddings (37,40) was the first to establish clearly that GC and LC performance data could be compared directly if they were expressed in terms of reduced variables viz. the reduced plate height and reduced velocity as defined below. This enabled the fairly advanced theoretical background developed for GC to be applied in its entirety to LC. The inter-relation of pressure drop, particle size, mobile phase velocity and column efficiency to analytical performance was soon elucidated. It was now clear that, as a result of the very small diffusion coefficients in liquids (a factor of 10^4 - 10^5 smaller than gas phase diffusion coefficients), the optimum particle diameter for use in LC would be much less than for GC. The actual diameter used depends on a choice of the other

operating parameters; present day work typically employs particles of 20 to 50 microns diameter in columns about 1 m. long. Although, in principle, the same resolution might be achieved by using a column 5 mm long packed with 0.5 micron diameter particles in 1/100 th of the time present day technology is inadequate to solve the problems associated with such small column volumes. The use of 2 to 5 micron diameter particles probably represents a realistic medium term goal. The problem of optimising the several operating variables has been examined in detail by Knox and Saleem (51). Another consequence of the very much lower diffusion coefficients in LC is that the design of the support particle geometry is much more crucial to column performance than in GC. To achieve elution times for unretained solutes around one minute with such columns inlet pressures must be in the region 1,000 to 5,000 p.s.i.. Column diameters in such work have been 1 to 5 mm. In consequence the column volumes are small, detector volumes must be comparably small to avoid undue extra-column dispersion and sample sizes must be small to avoid overloading.

The design of highly sensitive detectors remains one of the major problems for the future development of LC. Each of the currently available detection methods has certain disadvantages; none has the combination of sensitivity and versatility offered by

flame ionisation detection in GC. Among currently available methods u-v detection is the most sensitive but suffers the limitation that it is only applicable to molecules with appreciable molar extinction coefficients. The introduction of commercial instruments of high sensitivity operating over a range of wavelengths broadens the scope of this method. Solute transport detectors are probably in principle the most versatile instruments. The only restriction on the nature of the compounds to be detected is that they must be considerably less volatile than the mobile phase used to elute them. Current models as yet have rather low sensitivities. Liquid scintillation counting is only applicable in restricted circumstances and has not yet been developed for the continuous monitoring of a low flow rate of liquid. To be fast rather high levels of radioactivity are required.

The principles of operation of the other detectors available can be briefly outlined. The fluorimeter detector measures the fluorescent energy from a solute which has been excited by u-v radiation; the mobile phases used must not absorb significantly either the exciting or the emitted wavelength. Polarographic detection of metal ions, nitro compounds, amino acids, ketones and inorganic anions has been reported (52). The heat of adsorption detector (53, 54) is based on the measurement of the temperature change which occurs when solutes adsorb and desorb at a surface.

The differential refractometer detector monitors the difference in refractive index between a reference mobile phase stream and the column effluent. Detectors based on the measurement of conductivity, dielectric constant, density and other bulk properties have been described; all are very temperature sensitive and of low detection sensitivity.

The other important technological frontier of liquid chromatography is the design of support materials. It can readily be deduced from the theory of LC that rapid mass transfer between the two phases and within the stationary phase is essential for the achievement of high separation efficiencies. Golay (55) first suggested that the rate of mass transfer could be increased by the use of support particles with a thin porous outer layer surrounding an impervious core. Knox et al. (56) emphasised the importance of distributing the stationary phase in a thin uniform layer easily accessible to the mobile phase. Halasz and Horvath (57) were among the first to apply this suggestion and achieved significantly faster ion exchange separations using layered particles in which the exchange was confined to a thin surface layer. The development of surface layered particles was then taken up by Kirkland who published high performance LC data obtained with spherical siliceous particles with a porous surface layer of controlled thickness and pore size and a solid impervious core (58). The next advance in support design was to replace the porous

surface layer by a layer of microspheres. The resultant support material, marketed under the trade name Zipax^R, which has a surface layer of microspheres of 200 nm diameter, has been used by Kirkland (59) to pack columns with plate heights as small as 5 particle diameters at the optimum flow rate. The work to be described in Chapter 5 below shows that this performance can be further improved. At present it appears that Zipax^R is the most efficient support for use in analytical columns and the material is now available with ion exchange surface layers also. The major disadvantage of Zipax^R is that only very light loadings of stationary phases can be used (about 1%) without seriously hindering the very good mass transfer characteristics of the surface. There are indications that this difficulty can be bypassed by the use of recently developed porous supports.

Two types of porous support suitable for use in high performance LC were investigated in this work. Porasil^R is a material consisting of silica spheres of large surface area and controlled pore sizes. The material is currently available with mean pore sizes ranging from 70 to 1500 Å. As is shown in Chapter 5 this material can be packed in columns to give plate heights which are twice as large as those obtained with comparable fractions of Zipax^R. These materials can be used for adsorption chromatography as well as for partition and gel permeation chromatography. Corning CPG^R is a two phase borosilicate

glass leached by acid to produce a porous glass of uniform pore size. Though the particles of this latter material are irregular the plate heights obtained are very similar to those obtained with Porasil^R. As the drive towards the use of smaller particles in LC proceeds the performance differences between these materials and Zipax^R will narrow. Meanwhile these high surface area materials can tolerate much higher loadings at stationary phases without serious deterioration of their mass transfer characteristics. They thus appear to have potential for immediate application to preparative scale LC. Finally the possibility of coating these particles with a layer of ion exchange resin is being investigated. This would provide an ion exchange particle for use at the high inlet pressures now typical of modern LC practice. While the performance of such a material might be somewhat poorer than that of the Zipax^R material its capacity would be much larger.

In the immediate future it seems probable that supports of these two types, porous and surface layered, will form the basis of LC practice, the superior performance of Zipax^R type materials being complemented by the greater versatility and larger capacity of the porous materials. It seems probable that the next generation of chromatographic supports, in the 1 to 5 microns diameter range, will be more similar to the porous supports in current use. The problems of the preparation and packing of

particles of this size range are now being attacked. Progress will then become limited by detector technology.

During this period it seems likely that partition, adsorption, ion exchange and gel permeation chromatography will come to be based on the porous silica supports and to employ the same detection methods. The unification of LC practice will then be complete. Perhaps the most valuable advance would be a substantial improvement in the performance of the solute transport type of detector. Progress beyond this towards the best performance attainable giving analysis times of a few seconds using sub-micron diameter particles will involve considerable advances in detection sensitivities and in the techniques of packing very small columns. Perhaps the most valuable contribution of theory to practice is the elucidation of these ultimate goals.

CHAPTER 2THERMODYNAMICS OF LIQUID CHROMATOGRAPHY

Chromatographic resolution of the solutes of a mixture requires that the centres of the solute zones are separated by the column by a distance greater than the sum of their half-widths. The zone widths generated by passage through the column are dependent on the kinetics of the mass transfer processes occurring in the column. These dynamic processes are described in Chapter 3 and their effects on the widths of solute zones are examined quantitatively. The ratios of the distances between the point of injection and the zone centres depend on the thermodynamics of the system; they are equilibrium properties of the particular solutes and combination of phases used. Quantitatively, separation is discussed in terms of the resolution, defined as

$$R_s = \Delta z / 4\sigma \qquad 2.1$$

where Δz is the distance between adjacent peak maxima and σ is the standard deviation of the Gaussian concentration profile generated by the dynamic column processes. In what follows we take flow in the column to be in the z direction and the point of injection of the sample as the origin. An average value for σ must be used in evaluating the resolution since the two peaks will differ in their standard deviations.

Δz is proportional to the difference in the velocities of migration of the solutes. The velocity of migration of a

particular solute may vary from zero, corresponding to complete sorption, to the mobile phase velocity, corresponding to complete absence of sorption. The migration velocity of a solute is a fraction R of the mobile phase velocity where R is defined as

$$R = \frac{\text{Rate of movement of band}}{\text{Rate of movement of mobile phase}} \quad 2.2$$

If the probability that a molecule of a given solute species is in the mobile phase is r then the time fraction spent by the molecule in the mobile phase will approach r as the total time spent in the column becomes large. Since net motion in the flow direction is only achieved during residence in the mobile phase the molecule moves with this fraction r of the mobile phase velocity and r may be identified with R , as defined by Equation 2.2. Furthermore, if a large number of solute molecules are considered, the fraction of molecules in the mobile phase at equilibrium will equal r .

Consequently R may be defined as

$$R = \frac{\text{number of solute molecules in the mobile phase at equilibrium}}{\text{total number of solute molecules in the column}} \quad 2.3$$

While, as indicated in Chapter 3, there is always local perturbation of the equilibrium by the column dynamics, the value of R averaged over the whole band is unaffected and is dependent only on the nature of the solute, the mobile phase and the stationary phase. The relation of R to the thermodynamics of the solute-column system will now be examined for partition and adsorption

chromatography.

2.1 Thermodynamics of Liquid Chromatography

The distribution coefficient is defined as

$$K = \frac{\text{solute concentration in the stationary phase}}{\text{solute concentration in the mobile phase}} \quad 2.4$$

In the discussion of adsorption chromatography the volume of the adsorbed phase is assumed to be the volume of a monolayer of solvent molecules and concentrations are expressed in terms of this volume.

R can be related to the retention volumes

$$R = V_m / V_R = V_m / (V_n + V_m) \quad 2.5$$

V_R is defined as the volume of mobile phase passing any cross-section of the column between the time of injection of the solute and the time when half of the symmetrical elution band has emerged from the column. V_m is the retention volume defined in this way for an unretained solute. V_n is the net retention volume defined as

$$V_n = V_R - V_m \quad 2.6$$

R = fraction of solute molecules in the mobile phase at equilibrium

$$= \frac{\text{amount of solute in unit length of mobile phase}}{\text{amount per unit length of mobile phase} + \text{amount per unit length of stationary phase}}$$

$$= \frac{c'_m v_m}{c'_m v_m + c'_s v_s} \quad 2.7$$

where c'_m and c'_s are concentrations of the solute in the mobile and stationary phases, respectively, and v_m and v_s are the volumes of the phases per unit length of column.

From Equations 2.5, 2.6 and 2.7, and the definition of K it is clear that, if the column is homogeneous,

$$V_R = V_m + KV_s \quad 2.8$$

$$\text{and } V_n = KV_s \quad 2.9$$

where V_s is the total volume of the stationary phase in the column. Since the compressibility of liquids is of the order of 10^{-4} atm^{-1} the retention volume in liquid chromatography may be calculated directly from the retention time and the measured flow rate.

In general, the ratio K will be a function of the solute concentration. Only as the solute concentration approaches zero does K become independent of concentration. In the latter circumstances the isotherm relating the concentrations of the solute in the two phases is linear. When the sorption isotherm is linear the solute concentrations in the two phases are in constant ratio and the distribution coefficient can be related to the thermodynamic functions describing the sorption equilibrium. The theoretical development proceeds to relate K to the chemical potentials of the solutes and thus to the free energy changes accompanying adsorption or partition.

The final step is the attempt to predict, from the molecular parameters of the solutes, the values of the adsorption energies

or the activity coefficients required to calculate the distribution coefficients in given chromatographic systems. Because of the differences between the continuum models of partition and the discrete site models of adsorption the developments of the thermodynamics of the two modes of chromatography, although entirely equivalent, are couched in somewhat different terms corresponding to the differences in the conceptual models employed and are best considered independently.

2.2. Thermodynamics of Partition Chromatography

According to Martire and Locke, (60,61) the thermodynamics of liquid-liquid chromatography can be considered in terms of two binary solutions at equilibrium at the column pressure P and temperature T . For either of the phases the solute activity coefficient at T and P , $\gamma_2(T, P)$ can be written as

$$RT \ln \gamma_2(T, P) = \mu_2^i(T, P) - \mu_2^{i'}(T, P) \quad 2.10$$

where $\mu_2^i(T, P)$ is the solute chemical potential in the actual chromatographic solution in phase i and $\mu_2^{i'}(T, P)$ is the chemical potential the solute would have if the solution were ideal. The latter can be related to the mole fraction, x_2^i , of solute in the solution by

$$\mu_2^{i'}(T, P) = \mu_2^{o,i}(T, P) + RT \ln x_2^i \quad 2.11$$

where $\mu_2^{o,i}$ is the chemical potential of the pure liquid solute at T and P . With reference to a standard pressure, P^*

$$\mu_2^{o,i}(T,P) = \mu_2^{*,i}(T,P^*) + (P - P^*) v_2^o \quad 2.12$$

where v_2^o is the molar volume of the pure solute, which is independent of pressure in the range of LC operating pressures.

$$\text{The equilibrium condition is } \mu_2^s(T,P) = \mu_2^m(T,P) \quad 2.13$$

where the superscripts s and m refer to the stationary and mobile phases respectively.

Combining Equations 2.10 - 2.13

$$\mu_2^{*,m}(T,P^*) - \mu_2^{*,s}(T,P^*) = \frac{RT \ln x_2^s \gamma_2^s(T,P)}{x_2^m \gamma_2^m(T,P)} \quad 2.14$$

If the pure solute is chosen as the solute standard state then

$$\lim_{x_2^i \rightarrow 1} \mu_2^i = 1 \quad 2.15$$

$$\text{and } \mu_2^{*,m}(T,P^*) = \mu_2^{*,s}(T,P^*) \quad 2.16$$

The solute activity coefficient at the standard pressure P^* is related to that at any pressure P by

$$RT \ln \gamma_2^i(T,P) = RT \ln \gamma_2^i(T,P^*) + (\bar{v}_2^i - v_2^o)(P - P^*) \quad 2.17$$

where $\bar{v}_2^i$ is the partial molar volume of the solute in solution i.

Therefore

$$RT \ln (x_2^s / x_2^m) = RT \ln [\gamma_2^m(T,P^*) / \gamma_2^s(T,P^*)] + (P - P^*)(\bar{v}_2^m - \bar{v}_2^s) \quad 2.18$$

The distribution coefficient, as defined above, may be written as

$$K = \frac{n_2^s V_m}{n_2^m V_s} \quad 2.19$$

where n_2^s and n_2^m are the number of moles of solute in the stationary and mobile phases, respectively, at equilibrium and V_s and V_m are the volumes of the two phases in the column. In the dilute solutions used in LC $n_2^i = x_2^i n^i$ is the number of moles of phase i present.

$$\text{Thus, } K = \frac{x_2^s n^s V_m}{x_2^m n^m V_s} = \frac{X_2^s v_m^o}{x_2^m v_s^o} \quad 2.20$$

where v_m^o and v_s^o are the molar volumes of the pure mobile and stationary phases, respectively. If it may be assumed that the solute molecules interact only with solvent molecules and not with each other, an assumption valid at the concentrations used in LC,

then

$$\ln K = \ln \frac{\gamma_2^{m,\infty} (T, P^*) v_m^o}{\gamma_2^{s,\infty} (T, P^*) v_s^o} + \frac{P - P^*}{RT} (\bar{v}_2^{m,\infty} - \bar{v}_2^{s,\infty}) \quad 2.21$$

where $\gamma_2^{m,\infty}$ and $\gamma_2^{s,\infty}$ are the solute activity coefficients at infinite dilution in the mobile and stationary phases, respectively, and $\bar{v}_2^{i,\infty}$ are the corresponding partial molar volumes at infinite dilution.

Where $V_g = V_n / w_s = K / \rho_s$ (w_s is the weight of stationary phase in the column and ρ_s is its density), P^* , the standard pressure = 1 atm. and $\bar{P}$ is the mean column pressure

$$\ln V_g = \ln \frac{\gamma_2^{m,\infty} (T,1) M_m}{\gamma_2^{s,\infty} (T,1) M_s \rho_m(T)} + \frac{\bar{P} - 1}{RT} (\bar{v}_2^{m,\infty} - \bar{v}_2^{s,\infty}) \quad 2.22$$

where M_m and M_s are the molecular weights of the mobile and stationary phases, respectively, and $\rho_m(T)$ is the eluent density at the column temperature.

At usual LC pressures the second term on the right side of Equation 2.22 is negligible and the specific retention volume, V_g , is given, to a good approximation, by

$$V_g = \frac{\gamma_2^{m,\infty} M_m}{\gamma_2^{s,\infty} M_s \rho_m} \quad 2.23$$

at constant temperature.

The relative retention, α , of two solutes a and b is

$$\alpha = \frac{(V_g)_a}{(V_g)_b} = \frac{\gamma_a^{m,\infty} \gamma_b^{s,\infty}}{\gamma_b^{m,\infty} \gamma_a^{s,\infty}} \quad 2.24$$

It has been shown (62) that these solute activity coefficients can be expressed as the product of two contributions, a configurational, or athermal, contribution and a thermal contribution arising from intermolecular interactions in the solution.

$$\text{Thus } \ln \gamma_2 = \ln \gamma_2^{\text{th}} + \ln \gamma_2^{\text{ath}} \quad 2.25$$

While the athermal contribution arises solely from entropic effects the thermal contribution contains both enthalpic and entropic components. The Flory-Huggins expression (63, 64) may be used

to evaluate the athermal contribution to the activity coefficient

$$\ln \gamma_2^{\text{ath}} = \ln(1/r) + (1 - (1/r)) \quad 2.26$$

where r can be approximated as the ratio of the molar volumes of solvent (component 1) to solute (component 2). The thermal contribution to the activity coefficient of a solute 2, has been related to that of a reference solute, 2', in the same solvent under the same conditions (65).

$$\text{Thus } \ln \gamma_2^{\text{th}} = \ln \gamma_{2'}^{\text{th}} + \frac{v_{2'}^0}{RT} \left[\frac{E_1}{v_1^0} - \frac{E_{2'}}{v_{2'}^0} \right] \left[\frac{T_2^*}{T_{2'}^*} - 1 \right] \quad 2.27$$

where (E_1/v_1^0) and $(E_{2'}/v_{2'}^0)$ are the cohesive energy densities of the solvent and reference solute, respectively, v_1^0 and $v_{2'}^0$ are their molar volumes, and T_2^* and $T_{2'}^*$ are the critical temperatures of the solute and reference solute, respectively.

The treatment is only applicable if the reference solute is closely related in solution behaviour to the solutes of interest.

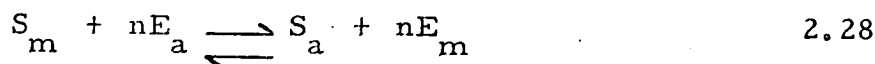
The athermal contribution to the relative retention is increased by divergence of the molar volumes of the stationary and mobile phases and of the two solutes a and b , particularly for solutes a and b of small molar volumes. The thermal contribution to the relative retention is favoured by large differences in the cohesive energy densities of the two phases. The latter contribution is also increased by an increase in the fractional difference of the critical temperatures of the two solutes. Martire and Locke (61)

have demonstrated correlations based on this theoretical background for aromatic hydrocarbons in the system acetonitrile/squalane. Their data for saturated hydrocarbons in the same system confirm that the theory is inapplicable where solvent-solvent interactions are stronger than solute-solvent interactions.

Keller, Karger and Snyder (66) have attempted to extend the solubility parameter treatment to polar solutes and solvents and to partition and adsorption chromatography. Their treatment uses the assumption that interaction energies in solution can be expressed as the sum of independent contributions from dispersion and polar interactions. They additionally include an independent contribution arising from hydrogen bonding. These three components of the solubility parameter are analysed in detail. The authors indicate that they do not anticipate that these developments will lead to the calculation of retention parameters directly from estimated solubility parameters but rather that they will lead to improved solvent selection on the basis of the availability of the component solubility parameters.

2.3. Thermodynamics of Adsorption Chromatography

According to Snyder (67, 68) the adsorption of a polyatomic molecule, S, from solution in an eluent, E, can be represented by the following chemical reaction analogue.



where the subscripts m and a refer to the mobile and adsorbed phases, respectively. n is the number of eluent molecules displaced by the adsorption of one solute molecule.

In the region of isotherm linearity where the solute concentration is small the lateral interactions of each solute molecule in the adsorbed phase will be with eluent molecules only and the energies of these interactions can be grouped with the site energies since they are independent of solute concentration.

The isotherm for adsorption of S on a set of homogeneous sites i is then

$$K_i = \frac{\theta_i}{c_m (1 - \theta_i)^n} \quad 2.29$$

where θ_i is the fraction of the sites i occupied by S molecules, c_m is the solution concentration of S and K_i is the thermodynamic equilibrium constant for the reaction 2.28 on the sites i. Equation 2.29 reduces to the Langmuir isotherm when $n = 1$ and is virtually intractable for other cases.

θ , the fraction of sites, or of the total adsorbent surface, covered by S, is given by

$$\theta = \int_{-\infty}^{\infty} \theta_i N_i dF_i \quad 2.30$$

in which $N_i dF_i$ is the fraction of sites with free energy between F_i and $F_i + dF_i$. F_i is the dimensionless free energy of sites of set i, defined as $-\log K_i$. The overall distribution coefficient for all sites, K_c , is obtained by combining Equation 2.29, with $n = 1$, and Equation 2.30, and rearranging

$$K_c = \int_{-\infty}^{\infty} \frac{N_i dF_i}{\exp(F_i) + \theta/K_c} \quad 2.31$$

The limiting value of K_c as θ approaches zero, K_o , is the linear distribution coefficient and is equal to θ/c_m . The latter coefficient is related to the distribution coefficient K , defined by Equation 2.4. thus

$$K_o = v_m^o K \quad 2.32$$

where v_m^o is the molar volume of the eluent.

To investigate quantitatively the requirements for linearity of the isotherm it is necessary to solve Equation 2.31 for K_c as a function of the solute concentration. In order to evaluate the integral it is necessary to assume or determine the distribution function for F_i . The simplest case to consider is a rectangular distribution function where $N_i = 1/p$ for values of F_i between zero and $-p$ and is elsewhere equal to zero.

$$\text{Then} \quad \int_{-\infty}^{\infty} N_i dF_i = 1 \quad 2.33$$

$$\text{Equation 2.31 gives} \quad K_o = 1/p \int_{-p}^0 \exp(-F_i) dF_i \quad 2.34$$

$$\text{and} \quad K_c = \int_{-p}^0 \frac{dF_i}{\exp(F_i) + \theta/K_c} \quad 2.35$$

When these two equations are combined the resulting equation reduces, in the limit of large p and small θ , to

$$K_c/K_o = \frac{\theta p}{\exp(\theta p) - 1} \quad 2.36$$

A value of the ratio K_c/K_o arbitrarily close to unity may be chosen as a criterion of linearity. It may then be readily deduced from Equation 2.36 that linear capacity is inversely proportional to p , the width of the site free energy distribution. This is a general feature of all site energy distributions, that the linear capacity decreases with increasing spread of the site energies. The changes in linear capacity produced by water deactivation of adsorbents can be predicted on the basis of these arguments using an assumed or experimentally determined site free energy distribution function.

When the sorption isotherm is linear throughout the elution of a solute the solute retention volume, V_R , is given by

$$V_R = V_m + KV_a \quad 2.37$$

where V_m is the total volume of the mobile phase and V_a is the total volume of the adsorbed phase in the column. The equivalent retention volume, V_o , is defined as

$$V_o = V_n/W \quad 2.38$$

where V_n is the net retention volume, as defined by Equation 2.6, and W is the total weight of adsorbent in the column.

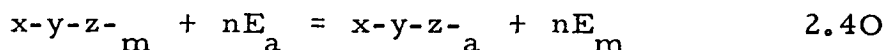
The equivalent retention volume is then related to distribution coefficient K_o by Equation 2.39

$$V_o = V_c K_o / v_m^o \quad 2.39$$

where V_c is the adsorbent capacity, defined as the volume of adsorbed phase per unit weight of adsorbent. Equation 2.32 can

be used to relate V_o to the distribution coefficient, K , as previously defined.

The solute S of reaction 2.28 can be considered as consisting of groups $x-y-z$. The reaction 2.28 can be represented as



The thermodynamic equilibrium constant for the reaction is then related to the free energy change which, for reaction 2.40, can be expressed as

$$\Delta F_1 = (F_{x-y-z})_a + n(F_E)_m - (F_{x-y-z})_m - n(F_E)_a \quad 2.41$$

where $(F_i)_j$ is the partial molar free energy of group i in phase j .

To a first approximation a linear free energy relationship may be assumed to hold between the free energies of the groups x , y , and z which are present in the solute molecule S .

$$\text{Thus } (F_{x-y-z})_j = (F_x)_j + (F_y)_j + (F_z)_j \quad 2.42$$

The volume of each group is equivalent to some fraction of the molar volume of the eluent and the adsorption equilibrium for the group i if the molecule S may be represented thus

$$i_m + \delta_i E_a = i_a + \delta_i E_m \quad 2.43$$

where δ_i is the ratio of the partial molar volumes of the group i of the solute molecule and the eluent E . The free energy change associated with the adsorption of individual groups i can then be expressed as

$$(\Delta F_2)_i = (F_i)_a + \delta_i(F_E)_m - (F_i)_m - \delta_i(F_E)_a \quad 2.44$$

$$\text{It follows that } \Delta F_1 = \sum_i (\Delta F_2)_i \quad 2.45$$

where the summation is over all the groups i which occur in the solute molecule. If the parameter Q_i is defined as $-(\Delta F_2)_i$, then

$$\log V_o = \log V_c + \sum_i Q_i \quad 2.46$$

The structural group free energy terms, Q_i , decrease regularly with increasing water deactivation of the adsorbent and an approximately linear free energy relationship seems to hold between Q_i and a function α of the percent water deactivation.

$$Q_i = \alpha Q_i^o \quad 2.47$$

where $0 \leq \alpha \leq 1$ and Q_i^o is the value of Q_i for $\alpha = 1$

This relationship is consistent with the view that water deactivation merely removes active sites but does not give rise to sites with different characteristics. The following equation describes, to a first approximation, the relationship of the specific retention volume to the structure of the solute and the activity of the adsorbent.

$$\log V_o = \log V_a + \alpha \sum_i Q_i^o \quad 2.48$$

The influence of a change of eluent on V_o will now be examined.

In terms of reaction 2.43 and Equation 2.44 the contributions to the free energy change from individual groups i and eluent molecules E arise from solvation forces in the mobile phase and from solvation and adsorption forces in the adsorbed phase, since, in general, only the parts of adsorbed molecules shielded from the

mobile phase will be unsolvated. To a first approximation the energies of solvation can be disregarded by comparison with the energies of adsorption. Thus equation 2.44 can be approximated as

$$(\Delta F_2)_i \doteq F_i + \delta_i F_E \quad 2.49$$

where the free energy terms F_i and F_E may be identified with the bond energies of the bonds between the adsorbent surface, and the individual group i and the eluent molecule, respectively. In terms of equation 2.49 the effect of a change of eluent from a to b on $(\Delta F_2)_i$ is given by

$$(\Delta F_2)_{ib} - (\Delta F_2)_{ia} = \delta_i (F_a - F_b) = \delta_i \epsilon_{ab} \quad 2.50$$

where the subscripts a and b for the left hand side terms refer to the group adsorption equilibria in eluents a and b respectively and F_a and F_b are the adsorption energies of eluent molecules a and b respectively. The term ϵ_{ab} is proportional to the difference in adsorption energies per volume of the eluents a and b ; δ_i is determined by the volume of the group i . Thus the relative retention volume of a solute for a given adsorbent using eluents a and b is given by

$$\log (V_{oa} / V_{ob}) = \epsilon_{ab} \sum_i \delta_i \quad 2.51$$

where the summation is over all the groups of the solute molecule. The values of δ_i may be estimated from tabulated atomic contributions. The values of ϵ_{ab} can be referred to a single

eluent. Snyder (94) has tabulated values for both parameters and has referred the values of the latter parameter to n-pentane as zero. Since the adsorption energy of the eluent is expected to change with adsorbent activity in the same manner as that of the solute, ϵ_{ab} should vary with a as does Q_i . ϵ_{ab} should be related to its value ϵ_{ab}^0 where a is equal to 1 thus

$$\epsilon_{ab} = \epsilon_{ab}^0 a \quad 2.52$$

Finally the effect of the residue of the solute molecule on the specific retention volume of the individual group i must be considered.

The effect of other groups j in the same molecule as the group i can be expressed, to a first approximation, by an additive contribution to the group adsorption energies. These contributions are tabulated as q_j , the change in the adsorption energy of a group i due to the presence of the group j . The values of q_j for the same chemical group in different positions relative to the group i of interest will, in general, be different. The two major effects contributing to the q_j 's are, firstly, modification of the electronic structure of the group i by the presence of the group j , and, secondly, steric interference by the group j with the interaction of the group i with the adsorbent surface. The q_j 's may be expected to show the same dependence on the adsorbent activity as the primary adsorption energy terms, the Q_i^0 's.

Thus the specific retention volume of a solute may be related to the solute molecular structure, the adsorbent activity and the eluent strength by the equation

$$\log V_o = \log V_c + a \left[\sum_i Q_i^o + \sum_j q_j^o - \epsilon^o \sum_i \delta_i \right] \quad 2.53$$

where ϵ^o is the value of ϵ_{ab}^o , as defined earlier, for the given eluent b referred to n-pentane as eluent a. The summations involved in this equation should only include contributions from groups present in the solute molecule which are confined to the adsorbed phase when the solute molecule is adsorbed. A number of reservations should be made with regard to the general applicability of equation 2.53.

That the free energy of the solute can be approximated as a linear free energy relationship is the key assumption underlying the entire treatment. The precision of the predictions will depend on the validity of assuming constant group free energy contributions corrected for the interactions with other groups of the molecule by constant second order terms. The application of the equation in practice will be limited by the availability of published values of both parameters. The assumption that solvation energy terms are negligible relative to adsorption energy terms may not be universally valid. When such terms are significant, and this may often be the case when specific interactions with eluents are sought in order to achieve separations, then the equation will not hold.

Finally the identification of adsorption free energies with the bond energies of adsorption neglects entropy contributions to the free energies. In cases such as o-alkyl substituted aromatics, where the rotational degree of freedom of the alkyl group about the bond to the aromatic nucleus is hindered or lost by adsorption of the molecule, the effect on the Q_i^0 term of the ortho substituent can be included in the q_j^0 terms for o-alkyl substituents. Where the entropy term dominates the free energy of adsorption of the whole solute molecule then the retention volume will depend on the entropy change of the adsorption reaction more strongly than on the enthalpy change and the treatment outlined here is no longer valid.

CHAPTER 3THE KINETICS OF LIQUID CHROMATOGRAPHY

In the course of elution through a chromatographic column the concentration distributions of the solutes originally introduced into the column by sample injection are broadened, firstly in the mobile phase, by the interaction of molecular diffusion with the velocity inequalities generated in any cross section of the column by the complex flow pattern and secondly, for retained solutes, by a finite rate of mass transfer between stationary and mobile phases and by diffusion in the stationary phase. The band widths generated by these processes in the column are, as indicated earlier, the second parameters determining, along with the thermodynamically governed separations of band maxima, the resolution attainable in any chromatographic system. The theories proposed to treat these dispersive processes fall into two categories, viz. plate and rate theories; only the latter yield information on the effect of kinetic parameters such as diffusion and the rate of inter-phase mass transfer on solute band broadening.

Prior to a detailed consideration of these theories it is useful to classify chromatographic methods according to their general thermodynamic and kinetic features.

3.1. Classification of Chromatographic Methods

Chromatographic systems may be classified according to the ideality of the kinetics and according to whether the distribution isotherm is linear or non-linear. In ideal chromatography all equilibria are assumed to be instantaneously attained, the mobile phase flow is assumed to be uniform and it is assumed that diffusion does not occur in either of the two phases. Linearity of the distribution isotherm implies that the ratio of the concentrations of a solute in the two phases is independent of the magnitude of the concentration. Application of these two criteria gives rise to four possible classes of chromatographic method; only linear non-ideal chromatography will be considered. Linear non-ideal conditions correspond to the conditions used in practical elution chromatography.

3.2. Theoretical Plate Model

The plate theory, introduced by Martin and Synge (3) in 1941, was the first theory to describe the development of a solute concentration profile under the influence of nonequilibrium and when the isotherm was linear. According to this model a chromatographic column is characterised by a parameter, H , the height equivalent to a theoretical plate (HETP) or plate height. By analogy to a distillation column the plate height is the length of a section of column such that the eluent solution issuing from it is in equilibrium with the mean concentration of solute within the section.

The subsequent development of this theory involves the assumption of complete equilibrium within a plate of solute distribution between stationary and mobile phases. The number of plates in the column is then simply the column length divided by H.

Mayer and Tompkins (49) interpreted the plate model in terms of intermittent rather than continuous flow. Glueckauf (16) replaced this discontinuous flow treatment which leads to a binomial concentration distribution by a continuous flow model which leads to a Poisson distribution. After a sufficiently large number of equilibration steps all the plate models predict that the concentration profile will approach Gaussian form as concluded by Martin and Synge.

$$\text{i.e.} \quad c = Ae^{-\Delta z^2 / 2\sigma_z^2}$$

where Δz is the distance from the peak maximum and σ_z is the standard deviation of the band in the column. The number of plates in a length $Z = L$, is then

$$N = (\sigma_z^2 / L)^{-1}$$

where L is the column length.

Though Martin and Synge deduced on the basis of intuitive arguments that H should be proportional to the flow velocity and the square of the particle diameter, d_p , the plate theory per se cannot yield information on such relationships. Giddings (37) has criticised the theory for this irrelevance to the details of the

kinetic phenomena of the chromatographic process and also for its assumptions of plate wide equilibrium and of the discrete nature of the plates. Despite these criticisms the theory provides the parameter H which is universally used to characterise band spreading. H is best defined as the rate of increase with distance of the variance of the Gaussian concentration profile, i.e.

$$H = \frac{d\sigma_z^2}{dz} \quad 3.1$$

which, for a uniform column, is equivalent to

$$H = \frac{\sigma^2}{L} \quad 3.2.$$

3.3. The Random Walk Model

Although the wide applicability of the random walk model to the kinetics of chromatography was not exhaustively reviewed until Giddings' treatment in 1965 (37) the model serves as a convenient introduction to the rate theories of chromatography in general. The major advantage of the random walk treatment is its simplicity. Thus the theory yields expressions for the dependence of plate heights on flow velocity, particle diameter, diffusion coefficients, rate constants and stationary phase dimensions which are essentially correct after a number of steps sufficiently large to generate a Gaussian profile. These conclusions are obtained more simply from this theory than from

the more rigorous treatments. In the treatment of processes such as the coupling of flow generated and diffusive dispersion where the underlying physical processes are enormously complicated the random walk model provides the only tractable approach. The major disadvantage of the theory is its inexactness. The dynamic processes of chromatography, which involve mainly continuous changes, are treated in this theory in terms of discrete steps. The numerical inexactness of the theory's predictions can be traced to the necessarily arbitrary assignments of the numbers and lengths of steps involved in the column processes.

The Development of the Model: In a one-dimensional random walk a molecule may be supposed to take steps of length l along the x -axis with an equal probability of moving in the $+x$ or the $-x$ direction at each step. Then after n steps, where n is a large number, the probability that the molecule will be k steps from the origin of its displacements is

$$p(k) = (2/\pi n)^{\frac{1}{2}} \exp(-k^2/2n) \quad 3.3$$

This is a Gaussian probability distribution with a standard deviation of

$$\sigma = l(n)^{\frac{1}{2}} \quad 3.4$$

The correspondence of this model with any process of molecular motion is established by taking the root-mean-square average length of the molecular displacements as the step length l . In the following account a more useful interpretation of the above is that,

if a large number of molecules execute a many-step random walk from the same origin, then the resulting concentration profile is described by the Gaussian distribution.

From Equation 3.4 it is clear, firstly, that σ is directly proportional to the step length l , and, secondly, that σ increases proportionally to $n^{\frac{1}{2}}$. Thus, since the separation of zone maxima during elution down a column increases proportionally to the distances migrated by the zones and these distances are proportional to n , the number of steps taken, while the zone widths only increase proportionally to $n^{\frac{1}{2}}$, resolution, as defined by Equation 2.1, increases proportionally to $n^{\frac{1}{2}}$. It is often more convenient to cast Equation 3.4 in the form

$$\sigma^2 = l^2 n \quad 3.5$$

where σ^2 is the variance of the distribution. As shown by Equations 3.1 and 3.2 H , the plate height, is directly proportional to the variance.

If two factors contribute to the variance then the variance due to the combined operation of the two factors may be related to the variances due to the separate operation of the two factors thus,

$$\sigma_{xy}^2 = \sigma_x^2 + \sigma_y^2 + 2\rho_{xy}\sigma_x\sigma_y \quad 3.6$$

where ρ_{xy} , the correlation coefficient of the variables x and y , clearly lies between 0 and 1. The correlation coefficient ρ_{xy} is zero only if the step lengths and probabilities for displacements by

the two processes are unaffected by superposition of the processes.

While the rigorous prediction of the correlation coefficients has proved intractable many of the processes contributing to band broadening in chromatographic systems may be regarded as independent of each other and hence give additive contributions to the total variance, according to Equation 3.6 with $\rho_{xy} = 0$, i.e.

$$\sigma_{xy}^2 = \sigma_x^2 + \sigma_y^2 \quad 3.7$$

and, more generally, in the case of k contributing component processes the total variance is given by

$$\sigma^2 = \sum_{j=1}^k \sigma_j^2 \quad 3.8$$

where σ_j^2 is the variance generated by the j th process. The total plate height in these circumstances is given by

$$H = \sum_{j=1}^k H_j \quad 3.9$$

where H_j is the plate height contribution of the j th process.

The random walk model can represent, among other processes, molecular diffusion. The dispersion of molecules by this mechanism is usually described by Equation 3.10

$$\sigma^2 = 2Dt_D \quad 3.10$$

where σ^2 is the mean square distance from the origin of the diffusion or the variance of the resulting Gaussian concentration distribution, D is the diffusion coefficient and t_D is the time of

diffusion. Zone spreading in chromatography has often, because of this analogy, been characterised by an effective diffusion coefficient which, in terms of Equation 3.10, is just the factor relating the measured variance of the zone to the elution time.

In the application of both the random walk and the effective diffusion analogies to zone spreading all dispersive factors should be referred to the zone centre as origin. The random walk model will now be applied to the estimation of the variances due to some of the dispersive processes occurring in a chromatographic column.

Longitudinal Molecular Diffusion: The dispersion due to this mechanism may be estimated from Equation 3.10. The diffusion time t_D is the time spent by a solute molecule in the mobile phase while migrating the distance L , the column length. This time, t_m , is given by $t_m = L/v$, where v is the mobile phase velocity. The calculated value of σ^2 is $2D_m t_m = 2D_m L/v$, where D_m is the diffusion coefficient of the solute in the mobile phase. In a packed column the tortuous and constricted path followed by the diffusing molecules reduces D_m by a factor γ , the obstructive factor. Thus the plate height contribution from this source is given by

$$H = 2\gamma D_m / v \quad 3.11$$

Knox et al. (56,69) have carried out the theoretical treatment of the obstructive factor and have measured it experimentally for several materials. Another experimental study of this factor has

been made by Bennet and Bolch (70).

A significant plate height contribution may arise from longitudinal molecular diffusion in the stationary phase. The value of σ^2 is here given by $2D_s t_s$, where D_s is the diffusion coefficient of the solute in the stationary phase and t_s is the time spent in the stationary phase. The ratio of t_s/t_m is $(1-R)/R$, where R is the fraction of solute in the mobile phase, and thus $\sigma^2 = 2D_s t_m (1-R)/R$. Therefore

$$H = \frac{2\gamma_s D_s (1-R)}{v R} \quad 3.12$$

where γ_s is the obstructive factor for diffusion in the stationary phase.

Adsorption-Desorption Kinetics: The random walk model may readily be applied to the evaluation of the plate height contribution arising from the adsorption and desorption of solute molecules if these processes obey first order kinetic laws. To evaluate this contribution it is necessary to determine the average distance by which a molecule is advanced or retarded relative to the centre of the solute zone by a single desorption or adsorption. The second parameter required is the number of such events occurring during migration through the column.

Times $t_a = 1/k_a$ and $t_d = 1/k_d$ are taken as the average times which a molecule spends in the mobile and stationary phases

before its stay is terminated by adsorption or desorption, respectively. k_a and k_d are the first order rate constants governing adsorption and desorption, respectively. If v is the average velocity of the mobile phase then the molecule migrates a distance vt_a between successive adsorptions and a total of L/vt_a steps will be required to migrate the column length L . Thus $n = 2L/vt_a$ since each adsorption is followed by a desorption. Since the zone centre is moving with a velocity of Rv it will move a distance Rvt_a as the desorbed molecule moves a distance vt_a . Thus the required step length is

$$l = vt_a - Rvt_a = vt_a(1-R) \quad 3.13$$

From Equation 3.5 $\sigma^2 = 2(1-R)^2 vt_a L$

and $H = 2(1-R)^2 vt_a$

and, since $t_a/t_d = R/(1-R)$, $H = 2R(1-R)vt_d \quad 3.14$

or $H = 2K'(1+K')^{-2}vt_d \quad 3.15$

where $R = 1/(1+K')$.

It follows that, with v and t_d constant, H has a maximum at

$R = \frac{1}{2}$ ($K' = 1$), and that, with constant K' and v , H is minimised

by increasing the desorption rate constant. Equations 3.14 and

3.15 also indicate the linear dependence of the plate height contribution on the mobile phase velocity.

Diffusion Controlled Kinetics: Diffusion controlled kinetic processes may originate either in the stationary phase or in the mobile phase; the relative importance in any given case will depend on the magnitude of the R value and the ratio of the diffusivities in the two phases. The process of diffusion controlled desorption is qualitatively similar to the previously discussed case of kinetic control. However, while the former desorption time was governed by a first order constant, the diffusional desorption time is the mean time required for a molecule of solute to diffuse a certain distance, the average depth of the stationary liquid film, and is thus determined by the diffusion coefficient of the solute in the stationary phase. Thus, if the depth of the stationary phase is d_s , then the mean desorption time t_d is approximately the average diffusion time t_D , obtained from Equation 3.10, required to cover a distance d_s .

Thus
$$t_d = d_s^2 / 2D_s$$

and substitution of this value of t_d in Equation 3.14 gives

$$H = 2R(1-R)d_s^2 v / D_s \quad 3.16$$

or
$$H = 2K' / (1+K')^2 \cdot d_s^2 v / D_s$$

This equation is identical, apart from the numerical constant, to that obtained by van Deemter et al., (32), Golay (33), Jones (71) and others. The more rigorous nonequilibrium treatment to be

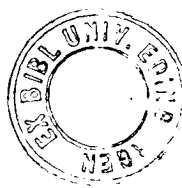
discussed below indicates that the factor 2 obtained here should be replaced by a configuration factor q , the value of which depends on the precise shape of the stationary phase pools. Equation 3.16 shows that the plate height contribution from this source can be reduced by reducing the mean depth of the stationary phase film and by increasing the value of D_s , the solute diffusion coefficient in the stationary phase. This contribution to H is also directly proportional to v , the mobile phase velocity.

Mobile Phase Dispersion: According to Equations 3.7 and 3.8 the superposition of several random walks will give rise to a total variance of the resulting concentration distribution which is just the sum of the variances which would have been generated by the successive operation of each of the processes in isolation, provided that the random walk processes are all mutually independent. Equation 3.9 indicates that the plate height contributions in such circumstances are also additive. However, as indicated by Equation 3.6, when the random processes are not mutually independent this simple result is no longer valid.

Consideration of the flow through any cross-section of a packed column suggests that, although there is a mean linear velocity of flow through the cross-section, the wide variation in the dimensions of the available flow channels resulting from the complex packing structure will give rise to a similarly wide range

of local flow velocities. Thus some solute molecules will be advancing relative to the zone centre while other solute molecules will be retarded. In other cross-sections distant by at least a few particle diameters a similar situation will prevail but different sets of the solute molecules will be undergoing advance or hindrance. This process appears to have certain qualitative similarities to the process of molecular diffusion and might thus be treated using the random walk analogue. This source of zone broadening would occur in the absence of molecular diffusion since, even if locked in a single streamline during its passage through the column, a molecule will, after a distance of many particle diameters, have experienced the spectrum of prevailing mobile phase velocities. This phenomenon is known as eddy diffusion. The classical theory of eddy diffusion only considers the termination of these velocity biases by this flow mechanism.

However the exchange of molecules between regions of different velocities can occur by lateral diffusion as well as by longitudinal flow transport. The application of the random walk analogue to diffusion has already been outlined. These two exchange mechanisms interact in such a way that each tends to reduce the step lengths which would have occurred by the operation of the other mechanism in isolation so that, as will be shown below, the total plate height contribution is not the sum of the contributions calculated separately but is less than either of



these individually calculated contributions. The coupling theory of eddy diffusion treats the interaction of these two mechanisms of termination of velocity biases. The treatment is simplified if the velocity inequalities are grouped into five categories which are approximately independent. This approach is as suggested by Giddings (37).

The five categories of velocity inequalities are:

1. The transchannel effect which arises from the velocity gradient between the walls and the centres of individual flow channels.
2. The transparticle effect which arises from the velocity difference between the essentially stagnant mobile phase within the pores of a porous packing and the flowing mobile phase in the interparticle space.
3. The short-range interchannel effect which is due to velocity differentials between large open channels caused by irregularities in the packing and the narrower channels in the adjacent regions of closer packing.
4. The long-range interchannel effect which arises from differences between adjacent units consisting of both wide and narrow channels. It is these units individually which contribute the short range effect.
5. The transcolumn effect which arises from velocity differences between the outer and inner regions of the column.

To apply the random walk theory to these effects an effective step length must first be defined. If it is assumed that a molecule must diffuse a distance $\omega_a d_p$ to reach one velocity extreme from another then, according to Equation 3.10, the average "exchange" time t_e is given by

$$t_e = \omega_a^2 d_p^2 / 2D_m \quad 3.17$$

where D_m is the solute diffusion coefficient in the mobile phase. The step length l is the distance lost or gained with respect to the mean due to temporary residence in one of the extremes. This distance is $\Delta v t_e$ where Δv is the difference between the extreme and the mean velocity. Δv will be some multiple of the mean velocity v i.e., $\Delta v = \omega_\beta v$ where ω_β , like ω_a , depends on the particular effect under consideration. The step length is thus

$$l = \omega_\beta v t_e \quad 3.18$$

which can be considered as the product of the distance $S = v t_e$ which a molecule is carried without severe velocity changes, and $\omega_\beta = \Delta v / v$, the fraction of that distance which is gained or lost with respect to the average.

$$\text{Thus} \quad l = \omega_\beta S \quad 3.19$$

The number of steps is then simply

$$n = L/S = L/v t_e \quad 3.20$$

and the plate height contribution is

$$H = \omega_\beta^2 S \quad 3.21$$

Substitution of $S = vt_e$ and using equation 3.17 for t_e gives

$$H = \frac{\omega_a^2 \omega_\beta^2}{2} \frac{d_p^2 v}{D_m} \quad 3.22$$

which for a given effect, i , may be written

$$H = \omega_i d_p^2 v / D_m \quad 3.23$$

The sum of the ω_i parameters, i.e. $\sum_i \omega_i$, should yield the experimentally observed value of ω .

If S is considered to be given by

$$S = \omega_\lambda d_p \quad 3.24$$

where ω_λ is a structural parameter, usually close to unity, and this expression is substituted in equation 3.21 then

$$H = \omega_\beta^2 \omega_\lambda d_p \quad 3.25$$

For a given category of velocity inequality i this may be written as

$$H = 2\lambda_i d_p \quad 3.26$$

$$\text{where } \lambda_i = \omega_\beta^2 \omega_\lambda / 2 \quad 3.27$$

and both ω_β and ω_λ are evaluated for the particular velocity inequality category i . Giddings' estimates for the parameters

ω_a , ω_β , ω_i , ω_λ , λ_i and the consequent plate height contributions are tabulated for the five categories of velocity inequality in Table 3.1.

Table 3.1

<u>Velocity inequality</u>	ω_a	ω_β	ω_i	ω_λ	λ_i	$H_i(d_p)$
Transchannel	0.17	1	0.01	1	0.5	1
Transparticle	0.5	1	0.1	10^4	10^4	10^4
Short range interchannel	1.25	0.8	0.5	1.5	0.5	1
Long range interchannel	10	0.2	2	5	0.1	0.2
Transcolumn	$m/2$	0.1	$0.001m^2$	$5m^2$	$0.02m^2$	$0.04m^2$

The transcolumn estimates only refer to effects occurring in straight columns and not to the transcolumn effects arising from bending or coiling of the column. m in these estimates is the number of particle diameters in one column diameter.

While the classical theory of eddy diffusion, as outlined, assumes that the solute molecules are locked into fixed streampaths and thus take displacement steps of about one particle diameter in fact these steps may be more or less reduced in length and hence increased in number by diffusive exchange of molecules between flow streampaths. Flow variations will also act to reduce the step length of a diffusion controlled random walk. The balance of the two mechanisms will be shown to depend on the ratio of the rate of diffusive transport to the rate of flow transport. If, for simplicity,

the situation where only one type of velocity inequality operates is considered the above interaction may be examined quantitatively.

The velocity persistence span may be written as

$$S = L/(n_f + n_D) \quad 3.28$$

where n_f and n_D are the numbers of exchanges by the flow and diffusive mechanisms respectively.

From Equation 3.21
$$H = \omega_\beta^2 L/(n_f + n_D) \quad 3.29$$

Equation 3.20 shows that $n_D = L/S_D = L/vt_e$ where S_D is the persistence length associated with the diffusive mechanism.

Using Equation 3.17 for t_e ,

$$n_D = 2LD_m/v\omega_a^2 d_p^2 \quad 3.30$$

and
$$n_f = L/S_f = L/\omega_\lambda d_p \quad 3.31$$

Substitution of these into Equation 3.29 and rearrangement of the equation gives

$$H = \frac{1}{1/2\lambda_i d_p + D_m/\omega_i v d_p^2} \quad 3.32$$

Since diffusive exchange leads to

$$H_D = \omega_i d_p^2 v/D_m \quad 3.33$$

and flow exchange leads to

$$H_f = 2\lambda_i d_p \quad 3.34$$

Equation 3.32 is equivalent to

$$H = \frac{l}{1/H_f + 1/H_D} \quad 3.35$$

The total plate height is given by

$$H = \sum_i \frac{l}{1/2\lambda_i d_p + D_m / \omega_i v d_p^2} \quad 3.36$$

in which the summation contains a term for each velocity inequality.

These equations are very different from the flow-diffusion contributions to the plate height predicted by the classical theory of eddy diffusion viz.,

$$H = H_f + H_D$$

and predict that the value of H is less than either H_f or H_D , approaching the smaller of them from below as the difference between them increases i. e. the plate height is governed by the faster exchange mechanism. Thus at high velocities the dependence of Equation 3.34 is approached and at low velocities the relation approaches that of Equation 3.33.

The characterisation of the mobile phase velocity independently of the nature of the mobile phase involves the definition of a reduced velocity, v ,

$$v = d_p v / D_m \quad 3.37$$

which expresses the ratio of v , the rate of flow transport, to the term D_m / d_p , the rate of diffusive transport in terms of particle

diameters. A similarly universal measure of the plate height involves the definition of a reduced plate height, h ,

$$h = H/d_p \quad 3.38$$

which expresses the plate height as a number of particle diameters. In terms of these reduced parameters Equation 3.36 may be re-written in the form

$$h = \sum_i \frac{1}{1/2\lambda_i + 1/\omega_i \nu} \quad 3.39$$

Over a rather limited range of reduced velocities this equation can be simplified somewhat. Thus Giddings suggests that over the range of ν from 0.5 to 20, the contributions to the reduced plate height from effects 1, 2 and 5 continue to increase with velocity and over this range their dependence on ν is approximately linear. The contribution from effect 4 can be written as $h = 2\lambda_4$. Only for the contribution of effect 3 need the coupling dependence of Equation 3.36 be used. For the range of reduced velocities chosen the total plate height can be represented as

$$h = \omega_1 + \omega_2 + \omega_5 \nu + 2\lambda_4 + 1/(1/2\lambda_3 + 1/\omega_3 \nu) \quad 3.40$$

Further, the plate height contribution due to longitudinal diffusion has been shown to be inversely proportional to flow velocity and this term may be abbreviated as B/ν . Also, sorption-desorption

kinetics and diffusion controlled stationary phase processes gave rise to contributions which may be written as $C_k v$ and $C_s v$ respectively. Those mobile phase terms which, over the limited range under consideration, are considered to be constant can be written as A and those which are directly proportional to velocity can be written as $C_m v$. These can be grouped with the other Cv terms.

$$\text{Overall, } H = A + B/v + Cv + \sum \frac{1}{1/A + 1/C_m v} \quad 3.41$$

Over a wider range of reduced velocities the A term and the C_m component of the C term will not show a simple velocity dependence and the appropriate coupled terms must be re-inserted in the summation of equation 3.41.

In reduced parameters

$$h = 2\gamma(1 + \beta_s)/v + \Omega v + \sum_i (1/2\lambda_i + 1/\omega_i v) \quad 3.42$$

where $\beta_s = (1 - R)\gamma_s D_s / R \gamma D_m$

and the first term describes the influence of longitudinal diffusion in both phases. The Ωv term is the stationary phase mass transfer term. It is composed of kinetic and stationary phase diffusion terms. All the Ω terms can be written

$$\Omega = CD_m / d_p^2$$

Throughout this description of the random walk model the effects of variations in the parameter R have been largely

neglected. Since the treatment is approximate and variations in R do not affect the dependence of the plate height on the variables considered very markedly this simplification is reasonable.

The most fruitful refinement of statistical concepts as applied to chromatographic kinetics has perhaps been the development of stochastic treatments by Giddings and Eyring (75), Giddings (76), Benyon, Clough, Crooks and Lester (77) and McQuarrie (78). These approaches require the precise definition of the kinetic mechanisms. The progress of a single molecule down the column is considered. According to the definition of the mechanism the probability of the molecule reaching the small element δz of the column length from z to $z + \delta z$ from the point of injection is calculated and the resulting probability density function may be found. The chief disadvantage of the approach is that its rigour leads to intractable mathematics unless the kinetic mechanisms are comparatively simple; its major advantage is that the limitation of the long-time approximation, which restricts other treatments, is bypassed. Zone asymmetry and multiple zones arising from slow reactions are two examples of cases where the long-time approximation can no longer be made and the stochastic approach is the only valid treatment.

Historically the material-conservation equations formed the basis of the earliest rigorous approach to the kinetics of

chromatography by Thomas (30). From 1952 and the work of Lapidus and Amundson (31) the task of developing equations of practical value in the design of efficient chromatographic columns proceeded using both the material-conservation approach and statistical concepts similar to those underlying the development of the random walk theory as outlined above. This approach was used by van Deemter, Klinkenberg, Sjenitzer and Zuiderweg (32,72) and the equation

$$H = 2\lambda d_p + \frac{2\gamma D_m}{v} + \frac{8}{\pi^2} \frac{K'}{(1 + K')^2} \frac{d_s^2}{D_s} v \quad 3.43$$

is known as the van Deemter equation.

Keulemans and Kwantes (73,74) put this equation in the simple form

$$H = A + B/v + Cv \quad 3.44$$

Following a suggestion by Jones that there should be two mobile phase diffusion terms Equation 3.44 was modified to the form

$$H = A + B/v + C_s v + (D_m + E_m) v \quad 3.45$$

Later Jones (71) derived the following plate height equation, known as Jones' extended HETP equation.

$$H = 2\lambda d_p + 2\gamma D_m/v + 2/3(K'/(1 + K')^2)(d_s^2/D_s)v + c_1(K'^2/(1 + K')^2)(d_m^2/D_m)v + c_2(d_p^2/D_m)v + 2\phi(c_1 c_2)^{1/2}(K'/(1 + K'))(d_p d_m/D_m)v$$

$$\text{often written as } H = A + B/v + C_s v + C_1 v + C_2 v + C_3 v \quad 3.46$$

The first three terms of this equation are identical to those of the

van Deemter equation except that C_s differs in the numerical coefficient $2/3$ instead of $8/\pi^2$. The C_1 term represents resistance to mass transfer in the mobile phase, C_2 is the velocity distribution term and C_3 is a correlation term accounting for the interaction between C_1 and C_2 . The major barrier to the extension of this approach is the difficulty of calculating the correlation coefficient.

3.4. Generalised Nonequilibrium Theory

As a chromatographic zone moves through a given section of the column the solute concentration first increases to a maximum and then decreases back to zero. In the leading part of the zone the stationary phase solute concentration at any given point lags below the value it would take if it were in equilibrium with the mobile phase concentration at that point. In the trailing part of the zone the stationary phase concentration is higher than the value corresponding to equilibrium with the adjacent mobile phase concentration. These departures from equilibrium occur because, as the kinetic processes re-establish equilibrium at each point within the zone at a less than infinite rate, the enrichment and depletion of the mobile phase concentration in the leading and trailing parts of the zone respectively caused by the mobile phase flow continuously maintains these departures from equilibrium. The relation of the stationary and mobile phase concentration profiles to the equilibrium profile is shown in Figure 4. As a

zone of a retained solute migrates down a column the mean zone velocity is a fraction R of the mobile phase velocity where R is the equilibrium fraction of the solute molecules which are in the mobile phase. Individual solute molecules, while desorbed, move with the mobile phase velocity; the mean velocity Rv can be interpreted as the velocity averaged over all the solute molecules, adsorbed or desorbed. Since, as indicated above, nonequilibrium leads to a continuous variation throughout the zone of the fraction of solute molecules in the mobile phase, if the zone is considered as a large number, n , of elementary sections, a value R_n may be assigned to each such elementary slice where R_n is defined as the local fraction of solute molecules in the mobile phase. R_n will then decrease continuously from the leading to the trailing edge of the zone. The mean velocity of solute molecules within each slice of the zone will be $R_n v$. The velocity of each slice will then be higher than the velocity of the immediately following slice and the velocity Rv , corresponding to the equilibrium distribution between the phases, will only be associated with the central slice. There will thus be a net flux of solute outwards from the zone centre. Also it may be concluded from the above that the extent of dispersion due to the effects of nonequilibrium will increase with increasing fractional departure from equilibrium. The nonequilibrium theory attempts to quantify this flux induced by nonequilibrium and to

relate it to the observed plate height.

The assumed symmetry of the distribution of the local mass transfer rates is equivalent to the assumption that the kinetic processes considered are equivalent to diffusion processes and this assumption is made throughout the following treatment. The latter assumption means that the concentration profiles will approach Gaussian form after sufficiently long times. The nonequilibrium theory is thus subject to the long-time assumption. It will be found that the development of the theory is simplified if the fractional departures from equilibrium are small. Since chromatographers have long realised that practical chromatographic systems must operate close to equilibrium this assumption corresponds to normal practice.

The Nonequilibrium Parameters: According to the theory developed by Giddings (37) the solute concentration, c , the number of moles per unit volume of the column, may be divided into two parts, c_m and c_s , the mobile and stationary phase concentrations. Thus $c = c_m + c_s$ and, at equilibrium, these will be designated c_m^* and c_s^* .

$$c_s^*/c_m^* = K' = R/(1 - R)$$

Equilibrium departure terms ϵ_m and ϵ_s may be defined as

$$c_m = c_m^* (1 + \epsilon_m) \quad 3.47$$

$$c_s = c_s^* (1 + \epsilon_s) \quad 3.48$$

which give

$$\epsilon_m = \frac{c_m - c_m^*}{c_m^*} = \frac{\Delta c_m}{c_m^*} \quad 3.49$$

and

$$\epsilon_s = \frac{c_s - c_s^*}{c_s^*} = \frac{\Delta c_s}{c_s^*} \quad 3.50$$

The theory will be largely framed in terms of the ϵ and c^* parameters.

Nonequilibrium in the two phases relates the two sets of parameters

thus:

$$c_m^* \epsilon_m + c_s^* \epsilon_s = 0 \quad 3.51$$

so that either ϵ term may be written in terms of the other

$$\epsilon_s = -c_m^* \epsilon_m / c_s^* = -R \epsilon_m / (1 - R) \quad 3.52$$

$$\text{since } c_m^* / c_s^* = R / (1 - R)$$

The application of the nonequilibrium theory to the evaluation of the C terms of the plate height equation will now be outlined.

Stepwise Kinetics: Consider an arbitrary number of discrete states, A_i , A_j , A_k , etc., which can be reached by solute molecules through first order interconversion processes. These can be represented as



in which the k's are first order rate constants. The downstream velocity of a molecule in the i th state is v_i . The overall

concentration of solute in state i , referred to unit volume of column is c_i and the sum of all these concentrations is c

$$\text{i.e.} \quad c = \sum_i c_i \quad 3.54$$

At equilibrium the state i contains the fraction X_i^* of the total number of solute molecules

$$X_i^* = c_i^* / c \quad 3.55$$

and under nonequilibrium conditions each concentration is related to its equilibrium value c_i^* through the departure from equilibrium term ϵ_i .

$$c_i = c_i^* (1 + \epsilon_i) \quad 3.56$$

The balance of equilibrium expression, by analogy with Equation 3.51, is

$$\sum c_i^* \epsilon_i = \sum X_i^* \epsilon_i = 0 \quad 3.57$$

The mean zone velocity is given by

$$V = \sum X_i v_i \quad 3.58$$

Only mobile phase states contribute terms to this summation.

1. Mass Transfer and Nonequilibrium: The mass transfer term for state i may be obtained as a sum over all reaction paths by which its concentration may be increased or decreased

$$s_i = \sum_j k_{ji} c_j - c_i \sum_j k_{ij} \quad 3.59$$

At equilibrium this expression becomes

$$0 = \sum_j k_{ji} c_j^* - c_i^* \sum_j k_{ij} \quad 3.60$$

If each concentration is written in the form of Equation 3.56

$$s_i = \sum_j k_{ji} c_j^* - c_i^* \sum_j k_{ij} + \sum_j k_{ji} c_j^* \epsilon_j - c_i^* \epsilon_i \sum_j k_{ij} \quad 3.61$$

the first two terms of which correspond to Equation 3.60 and are thus equal to zero.

Thus

$$s_i = \sum_j k_{ji} c_j^* \epsilon_j - c_i^* \epsilon_i \sum_j k_{ij} \quad 3.62$$

The principle of microscopic reversibility gives the equilibrium relationship

$$k_{ji} c_j^* = k_{ij} c_i^* \quad 3.63$$

and substitution of this into the first term on the right of Equation 3.62 gives

$$s_i = \sum_j k_{ij} c_i^* \epsilon_j - c_i^* \epsilon_i \sum_j k_{ij} \quad 3.64$$

which is equivalent to

$$s_i = c_i^* \sum_j k_{ij} (\epsilon_j - \epsilon_i) \quad 3.65$$

2. Mass Transfer and Flow: The equation of mass conservation for solute in state i is

$$\frac{\partial c_i}{\partial t} = s_i - v_i \frac{\partial c_i}{\partial z} \quad 3.66$$

where longitudinal diffusion has been ignored as a solute transport mechanism. Application of the near-equilibrium approximation gives

$$s_i = \frac{\partial c_i^*}{\partial t} + v_i \frac{\partial c_i^*}{\partial z} \quad 3.67$$

The term $\partial c_i^* / \partial t$ can be converted to a distance derivative by noting that the zone is moving with a mean velocity V and thus

$$\frac{\partial c_i^*}{\partial t} = -V \frac{\partial c_i^*}{\partial z} \quad 3.68$$

The final expression for s_i is then

$$s_i = (v_i - V) \frac{\partial c_i^*}{\partial z} \quad 3.69$$

3. The ϵ Values: Combination of Equations 3.64 and 3.69 gives

$$c_i^* (\sum_j k_{ij} \epsilon_j - \epsilon_i \sum_j k_{ij}) = (v_i - V) \frac{\partial c_i^*}{\partial z} \quad 3.70$$

and since c_i^* is proportional to the overall concentration c this can be written as

$$\sum_j k_{ij} \epsilon_j - \epsilon_i \sum_j k_{ij} = (v_i - V) \frac{\partial \ln c}{\partial z} \quad 3.71$$

If there are n states then the n equations of the form of Equation 3.71 may be solved along with the balance of nonequilibrium expression, Equation 3.57, to yield the values of the n ϵ terms.

4. The Plate Height Expression: The solute flux through any given cross section of the column is the sum of the fluxes of solute in each state.

$$J = \sum_i c_i v_i = \sum_i c_i^* (1 + \epsilon_i) v_i \quad 3.72$$

The equilibrium fraction of solute in state i is X_i^* so that

$$J = c \sum_i X_i^* (1 + \epsilon_i) v_i \quad 3.73$$

The component of J leading to zone spreading is

$$\Delta J = c \sum_i X_i^* \epsilon_i v_i \quad 3.74$$

From the form of the set of equations for the ϵ terms it can easily be shown that each ϵ is proportional to $\partial \ln c / \partial z$. Equation 3.74 shows ΔJ to be proportional to c . Thus ΔJ is proportional to

$$c \frac{\partial \ln c}{\partial z} = \frac{\partial c}{\partial z}, \quad \text{the concentration gradient showing that this}$$

source of dispersion is an effective diffusion process with

coefficient D . J may be written as $-D \frac{\partial c}{\partial z}$, and D is then given

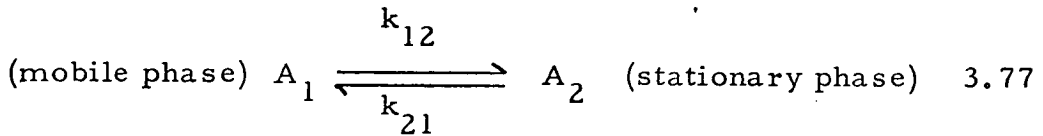
$$\text{by} \quad D = - \frac{\sum_i X_i^* \epsilon_i v_i}{\partial \ln c / \partial z} \quad 3.75$$

The plate height is then

$$H = 2D/V = \frac{-2 \sum_i X_i^* \epsilon_i v_i}{V \partial \ln c / \partial z} \quad 3.76$$

which can be evaluated once the ϵ values have been obtained.

The simplest application of the above is to one-site adsorption kinetics. This can be represented as



where k_{12} and k_{21} are the adsorption and desorption rate constants respectively. X_1^* is equal to R and X_2^* is $1-R$.

The zone velocity V is Rv where v is the mobile phase velocity.

The v_i 's are $v_1 = v$ and $v_2 = 0$.

The set of equations corresponding to Equation 3.71 consists here of two equations

$$\begin{aligned} -k_{12} \epsilon_1 + k_{12} \epsilon_2 &= E_1 \text{ where } E_1 = (1 - R)v \partial \ln c / \partial z \\ \text{and } X_1^* \epsilon_1 + X_2^* \epsilon_2 &= 0 \end{aligned} \quad 3.78$$

Only ϵ_1 , which is associated with a finite velocity, is required.

$$\epsilon_1 = \frac{\begin{vmatrix} E_1 & k_{12} \\ 0 & X_2^* \end{vmatrix}}{\begin{vmatrix} -k_{12} & k_{12} \\ X_1^* & X_2^* \end{vmatrix}} = \frac{E_1 X_2^*}{k_{12}(X_1^* + X_2^*)} \quad 3.79$$

and, since $X_2^* / (X_1^* + X_2^*) = 1 - R$

$$\epsilon_1 = \frac{-(1 - R)^2 v \partial \ln c}{k_{12} \partial z} \quad 3.80$$

$$\text{Thus } H = 2(1 - R)^2 v / k_{12} \quad 3.81$$

and, since $R / (1 - R) = k_{21} / k_{12}$

$$H = 2R(1 - R)v / k_{21} \quad 3.82$$

This treatment has also been applied to the more complex cases of multi-site adsorption and the adsorption of complex molecules.

Diffusional Mass Transfer: Here the important parameters refer to small regions of the two phases rather than to regions of the column. (Primed symbols will be used to indicate reference to

local conditions. e.g. c'_s is the local concentration per unit volume of the stationary phase).

The balance of nonequilibrium expression is obtained by equating the sum total of solute as it actually exists with that found at equilibrium

$$\sum \int c'_i dV_i = \sum \int c_i^* dV_i \quad 3.83$$

where V_i is the volume of phase i per unit volume of column and dV_i is the differential element of V_i . Since $c'_i = c_i^*(1 + \epsilon'_i)$, this expression becomes

$$\sum c_i^* \int \epsilon'_i dV_i = 0 \quad 3.84$$

The integral, $\int \epsilon'_i dV_i$, equals $\bar{\epsilon}'_i V_i$ and thus

$$\sum c_i^* \bar{\epsilon}'_i V_i = 0 \quad 3.85$$

Since $\bar{\epsilon}'_i = \epsilon_i$ and $c_i^* V_i = c_i^*$

$$\sum c_i^* \epsilon_i = \sum X_i^* \epsilon_i = 0 \quad 3.86$$

The mean velocity of the zone is given by

$$V = \frac{\sum \int c_i^* v'_i dV_i}{\sum \int c_i^* dV_i} \quad 3.87$$

$$\text{or by } V = \sum X_i^* \bar{v}_i \quad 3.88$$

1. Mass Transfer and Nonequilibrium: The rate of accumulation of solute within a phase is given by

$$s_i' = D_i \nabla^2 c_i' \quad 3.89$$

where ∇^2 is the Laplacian operator. Since $c_i^{*'} is a constant$

$$\nabla^2 c_i' = c_i^{*'} \nabla^2 \epsilon_i' \quad 3.90$$

and, thus,
$$s_i' = D_i c_i^{*'} \nabla^2 \epsilon_i' \quad 3.91$$

2. Mass Transfer and Flow: The equations are developed analogously to the case of adsorption kinetics, overall parameters being replaced by local parameters.

The initial mass conservation equation is

$$\frac{\partial c_i'}{\partial t} = s_i' - v_i' \frac{\partial c_i'}{\partial z} \quad 3.92$$

and the final equation is

$$s_i' = (v_i' - V) \partial c_i^{*'} / \partial z \quad 3.93$$

3. The ϵ Term: Equations 3.91 and 3.93 can be combined and rearranged to give

$$\nabla^2 \epsilon_i' = \frac{(v_i' - V)}{D_i} \frac{\partial \ln c}{\partial z} \quad 3.94$$

The evaluation of ϵ in given circumstances depends on integration of this equation subject to the approximate boundary conditions.

4. The Calculation of the Plate Height: The solute flux through a unit area normal to the flow in unit time is given by

$$J = \sum \int c_i' v_i' dA_i \quad 3.95$$

where A_i is the fraction of the cross-sectional area occupied by phase i. In terms of equilibrium departure this becomes

$$J = \sum c_i^* \int v_i' dA_i + \sum c_i^* \int \epsilon_i' v_i' dA_i \quad 3.96$$

where only the second term contributes to zone spreading

$$\Delta J = \sum c_i^* \int \epsilon_i' v_i' dA_i \quad 3.97$$

$$\text{Since } c_i^*/c_i^* = 1/A_i \text{ and } c_i^* = X_i^* c \quad c_i^* = X_i^* c/A_i \quad 3.98$$

and substituting this into Equation 3.97 gives

$$\Delta J = c \sum X_i^* (1/A_i) \int \epsilon_i' v_i' dA_i \quad 3.99$$

Substituting this into the expression for zone diffusion yields

$$D = - \frac{\sum X_i^* (1/A_i) \int \epsilon_i' v_i' dA_i}{\partial \ln c / \partial z} \quad 3.100$$

The plate height $H = 2D/V$ is given by

$$H = \frac{2 \sum X_i^* (1/A_i) \int \epsilon_i' v_i' dA_i}{V \partial \ln c / \partial z} \quad 3.101$$

Diffusion in the Stationary Phase: The general approach is to find the stationary phase departure term through the integration of Equation 3.94 and to relate this to the mobile phase departure term through the boundary conditions applicable to the integration. The direct integration of Equation 3.94 over the whole of the stationary phase, which would be impossible except in rather simple cases, is circumvented by the use of the general

combination law.

In the application of this law it is assumed that the stationary phase can be divided into small units whose boundaries must have an open area of contact with the mobile phase but must be elsewhere impermeable to solute exchange. It is expected that, due to surface tension, the stationary phase lies mainly in the pores and cavities of the support, thus fulfilling these criteria to a good approximation. These units are the smallest elements of the stationary phase which can be treated independently. The treatment is as follows.

The nonequilibrium parameter θ is defined as

$$\theta = \frac{(\epsilon'_s - \epsilon_m)D_s}{Rv \partial \ln c / \partial z} \quad 3.102$$

At points where ϵ'_s is zero θ takes the value θ_o where

$$\theta_o = \frac{-\epsilon_m D_s}{Rv \partial \ln c / \partial z} \quad 3.103$$

and $H = 2Rv\theta_o/D_s \quad 3.104$

The balance of nonequilibrium expression is here

$$\epsilon_m R = -\bar{\epsilon}'_s (1 - R) \quad 3.105$$

The quantity $\bar{\epsilon}'_s$ may be found also by solving Equation 3.102 for ϵ'_s and averaging

$$\bar{\epsilon}'_s = \epsilon_m + \bar{\theta} (Rv/D_s) \partial \ln c / \partial z \quad 3.106$$

where $\bar{\theta}$ is the mean value of θ averaged over the total mass of the stationary phase in the small region.

Using Equation 3.103 $\epsilon'_s = (\bar{\theta} - \theta_o)(Rv/D_s) \partial \ln c / \partial z$ 3.107

and Equation 3.105 becomes

$$\bar{\epsilon}'_s = \frac{R\theta_o}{1-R} \frac{Rv}{D_s} \frac{\partial \ln c}{\partial z} \quad 3.108$$

Combining Equations 3.107 and 3.108 gives

$$\theta_o R = (\bar{\theta} - \theta_o)(1-R) \quad 3.109$$

$$\text{or } \theta_o = \bar{\theta}(1-R) \quad 3.110$$

$$\text{and now } H = 2R(1-R)v\bar{\theta}/D_s \quad 3.111$$

Since $\bar{\theta}$ is an average over the total stationary phase volume within a unit volume of the column it may be written as

$$\bar{\theta} = \sum_j (V_j/V'_s) \bar{\theta}_j \quad 3.112$$

where V_j is the volume of unit j , V'_s the stationary phase volume per unit column volume, and the summation is over all the units into which the stationary phase may be divided.

Thus

$$H = \frac{2R(1-R)v}{D_s} \sum_j (V_j/V'_s) \bar{\theta}_j \quad 3.113$$

This plate height is made up of a sum of terms representing a contribution from each unit of stationary phase.

The basic equation for the ϵ terms, Equation 3.94, takes the

following form for the stationary phase.

$$\nabla^2 \epsilon'_s = -(Rv/D_s) \partial \ln c / \partial z \quad 3.114$$

and, since $V = Rv$ and $v_s = 0$,

$$\nabla^2 \theta = \frac{D_s \nabla^2 \epsilon'_s}{Rv \partial \ln c / \partial z} \quad 3.115$$

Combining these two equations gives

$$\nabla^2 \theta = -1 \quad 3.116$$

where the boundary conditions are

$$\partial \theta / \partial w = 0 \quad 3.117$$

at the closed surface, and

$$\theta = 0 \quad 3.118$$

at the open surface. w is normal to the bounding surface.

These equations can often usefully be expressed in terms of dimensionless parameters $\hat{x} = x/d_j$ and $\hat{\theta}_j = \theta_j/d_j^2$ where d_j is the maximum depth of the unit from its open surface.

The plate height equation can now be written in terms of $\hat{\theta}_j$

$$H = \frac{2R(1-R)v}{D_s} \sum_j \hat{\theta}_j \frac{V_j}{V_s} d_j^2 \quad 3.119$$

$$\text{or} \quad H = \sum_j q_j \frac{V_j}{V_s} R(1-R) \frac{d_j^2 v}{D_s} \quad 3.120$$

where q_j is a configuration factor.

The latter form emphasises the dependence of H on the square of the unit depth, the mobile phase velocity and the inverse of the stationary diffusion coefficient.

The simplest application of this formulation is to the plate height contribution from a uniform liquid film. The whole film can here be treated as the unit. In terms of dimensionless parameters

$$\nabla^2 \hat{\theta}_j = -1$$

and for this case the equation takes the form

$$\partial^2 \hat{\theta} / \partial \hat{x}^2 = -1 \quad 3.121$$

$$\text{which integrates to } \hat{\theta} = -\hat{x}^2/2 + g_0 \hat{x} + g_1 \quad 3.122$$

At the closed surface $\partial \hat{\theta} / \partial w = -\hat{x} + g_0$, and must be zero.

Since $\hat{x} = 0$, $g_0 = 0$.

At the open surface $\hat{x} = 1$ and $\hat{\theta}$ must equal zero. From

$$\text{Equation 3.122 } g_1 = \frac{1}{2}.$$

$$\text{Thus } \hat{\theta} = -\hat{x}^2/2 + \frac{1}{2} \quad 3.123$$

$$\text{and the mean value of this is } \bar{\theta} = \int_0^1 \hat{\theta} d\hat{x} = 1/3 \quad 3.124$$

The configuration factor is thus $2/3$ and the plate height is given

by

$$H = 2/3 R(1 - R) \frac{d_f^2 v}{D_s} \quad 3.125$$

where d_f is the film thickness.

Diffusion in the Mobile phase: In a two phase system where the average velocity in the mobile phase is v , the local velocity is v'_m and the mean zone velocity is Rv the equation for the local departure term becomes

$$\nabla^2 \epsilon'_m = \frac{v'_m - Rv}{D_m} \frac{\partial \ln c}{\partial z} \quad 3.126$$

The plate height expression then becomes

$$H = - \frac{\frac{2}{A_m} \int \epsilon'_m v'_m dA_m}{v \frac{\partial \ln c}{\partial z}} \quad 3.127$$

Since the integral is equal to the mobile phase cross-sectional area A_m times the average of the product $\epsilon'_m v'_m$ the expression reduces to

$$H = - \frac{2 \overline{\epsilon'_m v'_m}}{v \frac{\partial \ln c}{\partial z}} \quad 3.128$$

The plate height calculated from the above equation for the mobile phase is always (apart from the transcolumn effect) approximately proportional to the particle size, d_p , squared and the velocity, v , and inversely proportional to the mobile phase diffusion coefficient, D_m .

$$H = \omega d_p^2 v / D_m \quad 3.129$$

where ω is essentially constant. This is the form of dependence derived from the random walk treatment given earlier. In order

to obtain this from the nonequilibrium Equation 3.128 it must be assumed that all diffusion and flow processes can be scaled directly to the particle diameter and that the relative orientation of particles and packing density remain constant despite changes in d_p , v or D_m .

The following dimensionless variables may be defined

$$\hat{\epsilon}'_m = \epsilon'_m D_m / v d_p^2 \partial \ln c / \partial z, \quad \hat{x} = x/d_p \quad 3.130$$

where x is essentially a radial coordinate and thus

$$\hat{\nabla}^2 = d_p^2 \nabla^2$$

$$\text{so that } \hat{\nabla}^2 \hat{\epsilon}'_m = (\hat{v}'_m / v) - R \quad 3.131$$

The relative velocity v_m/v remains constant with changes in v and D_m and, since the flow processes are assumed to occur on a scale proportional to d_p , this ratio is a function of the dimensionless coordinate x/d_p .

$$\text{Thus } \hat{\nabla}^2 \hat{\epsilon}'_m = f(\hat{x}) \quad 3.132$$

$$\text{so that } \hat{\epsilon}'_m = \hat{\epsilon}'_m(\hat{x}) \quad 3.133$$

Replacing ϵ'_m in Equation 3.128 by $\hat{\epsilon}'_m$ by use of Equation 3.130 gives

$$H = -2(\overline{\hat{\epsilon}'_m v_m / v}) v d_p^2 / D_m \quad 3.134$$

Since both $\hat{\epsilon}'_m$ and the relative velocity v_m/v are functions only of $\hat{x}$ the averaging procedure yields a constant.

$$\text{Thus } \omega = -2 \left(\frac{\hat{e}'_m v'_m}{v} \right) \quad 3.135$$

and is constant with changes in v , d_p or D_m . The dependence suggested in Equation 3.129 is thus verified.

This treatment may be applied to zone dispersion in a capillary column. The local velocity is here given by

$$v'_m = 2v(1 - r^2/r_c^2) \quad 3.136$$

where r is the distance from the column axis and r_c is the column radius.

After fairly straight-forward solution of the nonequilibrium equations the plate height is found to be

$$H = (6R^2 - 16R + 11)r_c^2 v / 24D_m \quad 3.137$$

A form equivalent to Equation 3.137 was first derived by Golay (33).

The theory has also been applied to transcolumn velocity variations. Giddings has used an approach which is applicable to any radially symmetric velocity profile. A very general profile of this type gives v , the mobile phase in terms of the expansion

$$v = \bar{v} \sum G_n (r/r_c)^n \quad 3.138$$

where $\bar{v}$ is the mean velocity averaged over the column cross section, r_c is the column radius, r the variable distance along the radial axis and the G_n 's are the expansion coefficients. Then the differential equation for equilibrium departure may be written

in the form

$$\nabla^2 \epsilon = \frac{v - \bar{v}}{\gamma D_m} \frac{\partial \ln c}{\partial z} \quad 3.139$$

where ϵ is the overall, rather than local, equilibrium departure in a given region of the column, measuring the total departure from equilibrium in both phases.

After integration and evaluation of the integration constants the plate height is given by

$$H = \frac{\bar{v}_r^2}{\gamma D_m} \frac{G_n G_m}{(n+2)} \left[\frac{2}{(m+4)} - \frac{4}{(n+2)(n+m+4)} - \frac{n(n+6)}{(m+2)(n+2)(n+4)} \right] \quad 3.140$$

and can be obtained in terms of the G_n coefficients.

Littlewood (79) in applying the theory to trans column effects assumed velocity profiles of the general form

$$v(x, y) = v_o [1 + bf(x, y)] \quad 3.141$$

v_o is a base velocity and b is a dimensionless constant. When the profiles are assumed to expand as the column diameter i.e. when $f(r)$ can be written as $f(r/R)$, where R is the column diameter, the plate height contribution is of the form

$$H = \text{Constant} \frac{b^2 R^2}{Dg(b)} \quad 3.142$$

where D is the total transverse diffusion coefficient, given by $\gamma D_m + 0.05v d_p$, and $g(b)$ is a weak function of b . Thus the plate height contribution increases with the square of the column radius

for these profiles.

Knox and Parcher (80) applied the theory to a column with a distinct wall layer. The velocity profile can be represented as

$$f(r) = 1 + \exp(-R/ad_p)[\exp(-R/ad_p) - \exp(-r/ad_p) - \exp(r/ad_p)] \quad 3.143$$

The two dimensionless scale factors b and a of Equations 3.141 and 3.143 are measures of the velocity extremes across the column cross section and the effective thickness of the wall layer, respectively. The wall effect is assumed to decay exponentially towards the centre of the column, and, for large values of the column to particle diameter ratio, ρ , there is a distinct layer within a few particle diameters of the wall in which the velocity is significantly greater than that in the centre of the column.

The velocity at any point on the cross section of the column is given by

$$\bar{v} = v_o + (bv_o/R) \int_0^R f(r)dr \quad 3.144$$

and the plate height contribution is

$$H = \frac{(ad_p)^2 b^2}{3 \gamma D} \frac{(2R^2 + 9Rad_p + 12a^2 d_p^2)x^2 - (16R^2 + 24a^2 d_p^2)x + (2R^2 - 9Rad_p + 12a^2 d_p^2)\bar{v}}{R(1-b) + abd_p - x(Rb + abd_p)^2} \quad 3.145$$

where $x = \exp(-2R/ad_p) = \exp(-\rho/a)$

The treatment indicates that the plate height contribution has a maximum around $\rho = 2$, and becomes independent of the column

diameter at high values of ρ .

Giddings has made two other notable extensions of the application of the theory to mobile phase phenomena. In the earlier discussion of the application of the nonequilibrium theory to mobile phase diffusional mass transfer leading from Equation 3.92, the mass conservation equation, to Equation 3.94 for the equilibrium departure terms, explicit account was not taken of the exchange of solute between fast and slow flow regimes by the erratic variation in the velocity of each streampath; only diffusional mass transfer was considered.

Using Equation 3.92 in the form

$$s'_m = \frac{\partial c'_m}{\partial t} + v'_m \frac{\partial c'_m}{\partial z} \quad 3.146$$

and since $c'_m = c_m^* (1 + \epsilon'_m)$

the first term on the right may be written as

$$\frac{\partial c'_m}{\partial t} = c_m^* \frac{\partial \epsilon'_m}{\partial t} + (1 + \epsilon'_m) \frac{\partial c_m^*}{\partial t} \quad 3.147$$

Since ϵ'_m does not change rapidly with time at a given point and is in any case small the first term on the right is negligible compared to the second. Since ϵ'_m is small compared to unity the equation reduces approximately to

$$\frac{\partial c'_m}{\partial t} = \frac{\partial c_m^*}{\partial t} = -Rv \frac{\partial c_m^*}{\partial z} \quad 3.148$$

The second term on the right of Equation 3.146 contains the term

$\partial c'_m / \partial z$ which is given by

$$\frac{\partial c'_m}{\partial z} = c_{m'}^* \frac{\partial \epsilon'_m}{\partial z} + (1 + \epsilon'_m) \frac{\partial c_{m'}^*}{\partial z} \quad 3.149$$

where ϵ'_m in the final term is negligible but $\partial \epsilon'_m / \partial z$ is not.

$$\text{Thus} \quad \frac{\partial c'_m}{\partial z} = c_{m'}^* \frac{\partial \epsilon'_m}{\partial z} + \frac{\partial c_{m'}^*}{\partial z} \quad 3.150$$

Equation 3.146 reduces to

$$s'_m = (v'_m - Rv) \frac{\partial c_{m'}^*}{\partial z} + v'_m c_{m'}^* \frac{\partial \epsilon'_m}{\partial z} \quad 3.151$$

and combining this with Equation 3.91 gives

$$\nabla^2 \epsilon'_m = \frac{(v'_m - Rv)}{D_m} \frac{\partial \ln c}{\partial z} + \frac{v'_m}{D_m} \frac{\partial \epsilon'_m}{\partial z} \quad 3.152$$

This equation differs from Equation 3.94 in containing the final term which may be regarded as the term required to allow for eddy diffusion.

More recently Giddings (38, 39) has applied the non-equilibrium theory to a model which contains some of the features of velocity variation along the streampaths of fluid flow in a granular medium. The model consists of two parallel stream-paths one of which has a higher velocity than the other. The velocity is assumed to be uniform over the cross section of each channel. The system is divided into cells of a fixed length.

Diffusion is assumed to occur both within and between channels. After a cell length the velocity bias may change and is assumed to change discontinuously. P is the probability that no switching of the velocity bias occurs between two adjacent cells. Using the nonequilibrium theory Giddings was able to relate the plate height for the model to the flow velocity and to s , the correlation parameter between adjacent cells, defined as $s = 2P - 1$. A decrease in the correlation parameter leads to a decrease in the plate height. Limiting plateaux occur in the reduced plate height - reduced velocity curves for all values of s ; for values of s less than -0.268 the curves show a high velocity maximum. While the random walk model predicts an asymptotic high velocity value for the reduced plate height this is the first model to predict a subsequent decline. Many compilations (e.g. 56) of experimental data show evidence of such a decline at high reduced velocities and the effect could be potentially very significant for practical analytical operations. When the correlation parameter is set equal to $+1$ and the cell length is increased the predictions of the theory approach the experimental data for a capillary bundle model. The treatment indicates that care must be taken in the use of cell models to ensure that the cell boundary conditions do not introduce a degree of correlation which is not justified by the real column packing configuration. This development represents the first advance beyond a random walk treatment of eddy diffusion.

C H A P T E R 4

EQUIPMENT AND EXPERIMENTAL PROCEDURE

4.1. General Features of Chromatographic Equipment

Figure 5 indicates the essential features of a chromatographic system.

The main components of the system are:-

1. A solvent supply
2. A pump
3. A pre-column
4. An analytical column
5. A detector
6. A suitable device to amplify and record the detector signals

In the experiments to be described below solvents were purified before use by passage through an alumina column to remove any aromatic species which may have been present as impurities and which would have been detected by the ultra-violet photometer. The solvents were degassed by refluxing for at least one hour before being used. Solvent was delivered to the piston pump described below by gravity feed and passed through a 10 micron teflon sinter (2.5 cm diameter, 3 mm thick) between the reservoir and the pump. The pressure intensifier system is provided with a reservoir; the solvent is filtered in the line after the pump.

The pumps used were:-

a) A Model DMP 1515 (Orlita, Geissen)

Figure 6 is a schematic of this pump. Between the pump cylinder in which the working plunger and regulating plunger operate and the diaphragm head there is a connecting passage, interrupted by a diaphragm. The cylinder and the passage are filled with a hydraulic fluid with good lubricating properties which is separated from the liquid to be pumped by the diaphragm. During the pressure stroke the hydraulic fluid enclosed in this section is forced against the diaphragm which in turn displaces the liquid to be pumped. A regulating plunger, positioned opposite the working plunger and constantly driven by the crank, controls the volume of liquid introduced by the suction stroke. During the suction stroke the working plunger moves backward followed by the spring-loaded regulating plunger which remains in contact with the working plunger until it is retained by a stop in the adjustable setting member. The working plunger then separates from the regulating plunger and sucks up the quantity of liquid to be pumped during the rest of the stroke. During the pumping stroke this quantity of liquid and the spring-loaded regulating plunger which projects into the pump cylinder are displaced by the working plunger. By adjusting the screw control of the regulating plunger the swept volume may be varied

continuously from zero to its maximum corresponding to a delivery rate of about 10 ml per minute for the 3 mm plunger model used in this work. This pump can be used against back pressures up to 3,000 p.s.i.. Long term pressure stability was excellent at pressures above 50 p.s.i. but became poorer at lower pressures. This method of pumping also gives rise to pressure pulsing.

b) A Haskel pressure intensifier (Haskel Engineering and Supply Co.)

Figure 7 is a schematic of this pump. The essential elements of this pump are the large area pneumatic piston which is driven by gas pressure from an air cylinder and the small area liquid piston. The latter is rigidly coupled to the pneumatic piston and acts on the liquid to be pumped. During the return stroke the liquid chamber is rapidly refilled through a non-return inlet valve from the pump reservoir. The volume of the chamber upon which the small piston acts is about 70 ml. The pressure amplification factor of the model used was about 20, delivering pressures up to 3,000 p.s.i.. The long term pressure stability of this pump was also excellent at pressures above 50 p.s.i. but deteriorated at lower pressures. This method of pumping gives pulse-free solvent delivery.

Other methods of pumping available include pumps of the screw-driven syringe type and a simpler pneumatic pump in which a plastic bottle or stainless steel or teflon bellows is placed inside

an airtight container and gas pressure is applied from an air cylinder to the inside of the gas container and the outside of the liquid container. The pressures delivered are limited by the strength of the container and the pressure of the gas supply.

In the work reported here, to deliver solvent at pressures below 50 p.s.i., a long thick-walled tube was inserted into the line, filled with degassed solvent and pressurised directly by an air cylinder. In this way it was possible to deliver solvent at pressures down to 5 p.s.i. with good pressure stability over a period of several hours.

Pressures were measured by Bourdon gauges (Negretti and Zambra, London) which also damped pressure fluctuations arising when the piston pump was used. Pressure fluctuations at the column inlet were estimated to be less than $\pm 2\%$ of the total pressure.

For liquid-liquid chromatography in which the analytical column packing was coated with a film of the stationary liquid phase the solvent was first passed from the pump through a pre-column. This column, 1 m long and about 1/8" i.d., was packed with large diameter diatomaceous earth particles coated with a high loading of the stationary phase present in the analytical column. In this way the solvent was saturated with the stationary phase and stripping of the stationary phase from the analytical column was

avoided. Though the flow resistance of the pre-column was low it served as an additional pulse damper. The use of pre-columns is unnecessary for adsorption chromatography, the only other mode of chromatography studied in this work. From the pre-column the solvent was passed through the analytical column and the detector. These elements of the system are described in detail below. Connections in the system were made using 1/8" o.d. thick-walled nylon tubing which withstands pressures of up to 2,500 p.s.i. and brass couplings (Wade Couplings Ltd., Birmingham, England).

4.2. Preparation and Packing of the Columns

The columns were constructed from 2 mm i.d., 8 mm o.d. capillary glass tubing which withstands pressures of 1,500 p.s.i. Column inlet and outlet fittings were constructed in brass. The inlet fitting is shown in Figure 8. The glass tubing was sealed into the fittings by Araldite^R epoxy resin. The outlet end of the column was sealed with a porous teflon^R plug to contain the packing. The primary aim of this design is to minimise the dead volume of the system between the injection point and the detector since any dead volume will lead to dispersion of peaks and dead volume unswept by the solvent will, in addition, give rise to peak asymmetry. Thus in sealing the glass tubing into the brass end fitting the glass was carefully ground so that it was flush with

the surface of the brass flanges. Thus when the detector connection fitting was fitted the ends of the glass were in contact with its surface with no intervening space. It was also important to ensure that the connections to the detector were of small volume.

Columns were packed using a mechanical device shown schematically in Figure 9. The packings were carried in a stream of air from a small vessel into a funnel attached to the column inlet flange. This method ensured as far as possible that the particles did not stick together as they were added to the column. The column was simultaneously rotated at about 60 r.p.m. and bounced about 180 times per minute. The height of the bounce was about 1 cm. The column was tapped with a light, hard object at the level of the accumulating bed. The packing was not tamped. 50 cm columns were packed by this method in about 15 minutes. The procedure is adapted from that of Sie and van den Hoed (81) and is very similar to that found to be optimal by Kirkland (59). Plate height reproducibility of about 10% from column to column was found for unretained solutes on four columns packed with Porasil^R (Figure 16). Although each element of the method viz., rotating, bouncing and lateral tapping was found to contribute to its efficiency the method has the advantage that its efficiency was not found to be particularly sensitive to the rates of any of the three perturbations.

Column packings of the Zipax^R, Corasil^R, Corning^R and Porasil^R materials were 44 - 53 microns diameter fractions sieved from batches received from the suppliers using sieves supplied by Endecotts (Filters), London. Chromosorb^R W was used in a number of particle diameter ranges. 80 - 100 mesh material was sieved from a nominally 80 - 100 mesh batch. Smaller particle sizes of this material were obtained by grinding the 80 - 100 mesh material and sieving out the desired fraction. The small mesh fractions of Chromosorb^R P and Phase Separation Universal^R were similarly obtained from commercially supplied large mesh material. The physical properties of the supports used are summarised in Table 4.1 below.

For partition chromatography the support to be coated was accurately weighed and placed in a flask. Batches of about 5 g were used. The appropriate weight of the stationary liquid phase was dissolved in a suitable solvent (β, β' -oxy-dipropionitrile in acetone in this work) and the particles to be coated were slurried in this solution. The solvent was then removed by slow evaporation on a rotary evaporator. This procedure was believed to produce an even film of stationary phase on the particle surface. For adsorption chromatography the supports were heated for at least an hour before use at 200°C in vacuo.

4.3. Detectors

Table 4.1

Physical Properties of Supports

<u>Material</u>	<u>Description</u>	<u>Pore Size</u> $\text{\AA}^{\circ}$	<u>Pore Volume</u> $\text{cm}^3 \text{g}^{-1}$	<u>Surface Area</u> $\text{m}^2 \text{g}^{-1}$
Porasil PS60	Spherical particles; regular pore size silica gel	<100	0.85	350
Porasil PS250	"	100-200	0.85	200
Corning CPG75	Porous glass chips; silica; regular pore size distribution	70	0.45	300
Corning CPG240	"	230	0.82	70
Corasil I	Fine pore silica gel probably layered on glass spheres	<50	0	7(300) ^a
Corasil II	As Corasil I but with double layer	"	0	14(300) ^a
Zipax	5 layers of 200nm silica spheres on larger glass spheres	1000	0	0.5(15) ^b
Chromosorb W	Diatomaceous earth calcined with alkaline flux)	5-100x 10^4 (mainly)	2.8	1-3.5
Chromosorb P	Diatomaceous earth (mixed with binder, calcined at higher temperatures)	0.005-5x 10^4 (mainly)	1.15	4-6
Phase Separation Universal	Highly silanised properties similar to diatomaceous earths.	- ^c	- ^c	- ^c

^a The specific area of the silica gel layer is about $300 \text{ m}^2 \text{g}^{-1}$, the other figure refers to the material as a whole.

^b The specific area of 200 nm silica spheres is $15 \text{ m}^2 \text{g}^{-1}$, the other figure refers to the material as a whole.

^c There is no data available for this material.

An outline is given of the operation of a wire transport detector which was used in earlier experiments not reported here. Ultra-violet photometric detection was used in all the plate height determinations reported in Chapter 5 below. Liquid scintillation counting of ^{14}C was used in some of the retention volume determinations reported in Chapter 6 below. Both of these methods will be discussed in detail; a brief summary of other detection methods currently available and their principal limitations will also be given below.

Ultra-violet detectors have sensitivity ranges down to 0.01 absorbance units f.s.d. ($\pm 1\%$ noise) and it is thus possible to detect compounds with molar extinction coefficients of 10^4 at concentrations as low as 1 in 10^9 . Figure 10 shows an outline of an ultra-violet detector. The detectors of this type used were:-

a) Du Pont Model 410

This is a monochromatic instrument employing a low-pressure mercury source which produces 254 nm radiation. The development of this monitor and its specifications have been described by Kirkland (82). In this instrument the light from the source is first collimated then split by the semi-transparent mirror into two beams. One beam then passes through the sample cell while the other is focussed on a reference photocell. The sample beam is focussed on a second photocell. The arrangement does not require a reference cell. With solvent flowing the full scale

sensitivity is 0.01 absorbance units ($\pm$ 0.0002 A.U. short term noise). The maximum long term drift over 12 hours is 0.001 A.U. without control of the ambient temperature. The upper limit of linear operation is 1.2 - 2.0 A.U. The sample cells have a light path length of 1 cm and a volume of 8 microlitres.

b) Varian u-v photometer.

This instrument also uses a 254 nm low-pressure mercury source but differs from the Du Pont instrument in using a reference cell. The specifications are similar. The most sensitive range is 0.005 A.U. (f.s.d. $\pm$ 0.0002 A.U. noise) and the drift is 0.0004 A.U. per hour. The optical path length is 1 cm and the cell volume is 8 microlitres.

c) Cecil Instruments CE 212 photometer

This is a variable wavelength instrument which employs a deuterium lamp emitting radiation of 220 to 400 nm wavelength. The instrument has only recently been developed to monitor a flowing liquid stream and its exact performance specifications have not been determined. The optical path is 1 cm long and the cell volume is 8 microlitres.

For ultraviolet detection the mobile phase must be transparent to the wavelengths employed while the solutes to be detected must absorb significantly. When these conditions apply this is the most sensitive detection method currently available.

Wire transport detectors operate by removing the mobile phase before the detection process. Figure 11 shows a schematic of the model used (Pye Unicam, Pye Instruments Ltd., Cambridge, England). The column effluent flows through the coating block in which a small fraction is collected on the moving wire. This sample is carried by the wire into the first oven, the evaporator oven, whose temperature is set so that the mobile phase is completely evaporated. The residual non-volatile solute adhering to the wire is then carried into the second oven whose temperature is generally in the range $600^{\circ} - 700^{\circ}\text{C}$. In this oven the solute is pyrolysed and the products of pyrolysis are then carried in a gas stream into the flame ionisation detector. The f.i.d. signal is amplified and recorded in the normal way. Despite the very high sensitivity of the f.i.d. the overall sensitivity of the instrument only permits detection of concentrations of about 1 in 10^5 . This sensitivity loss can be attributed to two factors, inefficient coating of the wire and noise resulting from the wire transport mechanism. Thus in the model used the wire only collects about 1% of the total column effluent. The noise resulting from the erratic motion of the wire probably accounts for a loss of a further order of magnitude in sensitivity.

Van Dijk (83) has recently reported an improvement in this sensitivity by a factor of 20 to 50 by spraying the column effluent

onto the wire instead of the block coating method used in the Pye instrument. It has been suggested that the wire might be replaced by an absorbent band to increase the fraction of the effluent which is collected; the problems appear to be, firstly, the achievement of even coating of the band and, secondly, the rapid cleaning of the band. The transport wire is readily cleaned by pyrolysis in the cleaning oven. A recently reported modification of this procedure (84) replaces the pyrolysis by combustion of the solute in a stream of oxygen. The carbon dioxide produced is reduced to methane which is then detected by flame ionisation.

In addition to its low sensitivity solute transport methods are restricted to systems where the mobile phases are considerably more volatile than the solutes to be detected. This latter restriction is not a major limitation.

Liquid scintillation counting was used to count the soft beta emission from ^{14}C -labelled compounds. A Beckman LS-133 liquid scintillation counter was used to detect ^{14}C -labelled samples of ethanol, ethyl acetate and carbon tetrachloride eluted in ethanol, ethyl acetate and carbon tetrachloride, respectively. In this method discrete samples of the column effluent ranging from 15 to 150 microlitres were added to 15 ml batches of a "scintillation cocktail". The cocktail contained naphthalene which is electronically excited by the emitted beta electrons; energy

transfer then follows to diphenyloxazole, also present in the cocktail, and the fluorescence emission of the latter molecule, which occurs at a longer wavelength than that associated with the de-excitation of naphthalene, is detected by appropriate photocells. This method has not been developed for continuous monitoring of small volume flow rates and would require rather high levels of radioactivity for rapid detection of low concentrations.

The fluorimeter detector measures the fluorescent emission from a solute which has been excited by u-v radiation. Many compounds of biological importance fluoresce in the visible region after excitation by u-v light. In the operation of a fluorimeter the light from a u-v source is filtered and focused on the cell. The fluorescence may either be detected parallel to or at right angles to the incident beam. The exciting wavelength is then blocked by a filter and the intensity of the emitted energy is measured by a photocell. A reference cell may be used to cancel the fluorescence from the mobile phase. The mobile phase should not absorb radiation at the exciting or the fluorescing wavelength.

Polarography measures the current between a polarisable and a nonpolarisable electrode as a function of the applied voltage. The polarographic detector as used in chromatography employs a constant voltage applied between the electrodes which results in oxidation or reduction of the solute. The current which flows is

then measured continuously and recorded as a function of time. Metal ions, nitro compounds, amino acids, ketones and inorganic anions have all been detected in column effluents by this technique (52). Three types of polarisable electrode have been described viz. the dropping mercury electrode, the carbon impregnated silicone electrode and the platinum electrode. This method requires that the column effluent conducts an electric current and that the solutes react at the electrode.

The heat of adsorption detector (53, 54) is based on the measurement of the heat change occurring when a solute molecule is adsorbed and then desorbs at a surface. The measured signal is a double peak which is not easily related to the concentration of the solute species involved. This type of detector requires precise temperature control. The differential refractometer detector measures the refractive index difference between a reference mobile phase cell and an analytical cell. Again temperature control must be very precise and the sensitivity of the instrument is limited by the comparatively small differences between the refractive indices of most organic compounds. Detectors based on the measurement of conductivity, dielectric constants, density and other bulk properties have been described. All bulk property detectors are very temperature sensitive and most are not sufficiently sensitive for the requirements of modern

high speed liquid chromatography.

4.4. The Experimental Procedure

The columns used were straight sections of glass capillary tubing, prepared and packed as described previously. The column packing was de-aerated by flushing with the mobile phase. The solutes to be used were made up in 1 - 10% solutions in the mobile phase liquid. Reduced plate height vs. reduced velocity curves were determined for an unretained component and at least one retained component on each of the column packings used.

Before any of the data used in the reported curves (Chapter 5 below) were collected the column was flushed with the mobile phase until the retention volumes of the retained components became constant. The desired mobile phase pressures were selected by adjustment of the hand-screw of the piston pump or the air pressure to the pressure intensifier and were allowed to stabilise before the injections of the test solutes were made. The sample sizes used in the plate height determinations reported in Chapter 5 and in the determinations of retention volumes of u-v absorbing solutes reported in Chapter 6 ranged from 1 to 5 microlitres. The retention volumes of compounds which are not u-v absorbers were determined from the double peak produced by the u-v detector when relatively large concentrations of compounds whose refractive indices differ from that of the mobile phase flow through the detector cell. This effect arises from the presence of multiple

optical paths through the detector cell. The relative intensities detected by the photocells from these paths depend on the variation of the refractive index of the liquid present within the cell at any given time. Detection of compounds by this effect requires injection of samples ranging from 20 to 50 microlitres of the pure solutes. The sample size required for detection depends on the difference between the refractive indices of solute and solvent.

Injections were made using glass microsyringes which withstood the highest pressures used in the work (1,000 p.s.i.). The injections were made through 6 mm diameter, 3 mm thick rubber septa directly into the column packing. The injection head used is shown diagrammatically in Figure 8. This technique gave reproducible injections even at the highest pressures used. Care was taken to inject into the packing to avoid any dispersion of the solute sample before it reached the packed bed. The column effluent passed through low-volume connections made with 0.005" bore stainless steel capillary and Swagelok^R couplings to the detector.

For the detection of the ^{14}C labelled samples a teflon tip was fitted to the end of the steel capillary outlet. The drop volume delivered from this tip was determined for each mobile phase used at a constant flow rate. This enabled correlation of the retention volume with the number of drops of eluent required

to elute the peak maxima. The effluent was added directly from the teflon tip, drop-wise, to the vials of "scintillation cocktail". These samples were counted until the error in the counts per minute reading had fallen to a sufficiently low value for unambiguous identification of the sample containing the maximum solute concentration. The other detection systems used monitored the column effluent continuously.

The detector signals were presented on a Servoscribe^R potentiometric recorder. The shortest retention times measured were about 30 seconds so that the response time of the detection and recording systems can be neglected.

4.5. The Calculation of the Results

Figure 12 shows the elution record of a single injection of a solute. The measurements derived from this record are indicated and will be referred to immediately below.

a) Peak Symmetry

Chromatographic theory shows that under ideal conditions sharply injected solutes which are fully resolved from each other should elute from the column as Gaussian concentration distributions. Any measurements relating to the kinetics of chromatography e.g. the determination of plate heights, can only be reliably and simply interpreted if the data are derived from peaks which approximate closely to Gaussian symmetry. The

data reported in Chapter 5 below were derived from peaks whose symmetry satisfied two tests.

Firstly the baseline width, w_0 , of a peak can be determined by drawing tangents to the peak at the points of inflection and producing these to intersect with the baseline. The distance between the two points of intersection is w_0 and is equal to 4 standard deviations for a peak which is exactly Gaussian. Using this value of w_0 a value may be calculated for the width at its half-height, $w_{\frac{1}{2}}$, of a Gaussian distribution with a standard deviation of the measured $w_0/4$. This calculated value of $w_{\frac{1}{2}}$ can then be compared with the measured width of the peak at its half-height. Data are only reported for peaks where these two values agree to within 5%.

Alternatively a symmetry index may be defined as

$$\text{Symmetry index} = \frac{\text{Trailing half width, } w_t}{\text{Leading half width, } w_l}$$

The leading and trailing half widths are obtained by producing the tangents to the points of inflection until they intersect and dropping a perpendicular from this point of intersection to the baseline.

The distances from the point of intersection of the perpendicular with the baseline to the points of intersection of the tangents to the leading and trailing edges of the peak, respectively, with the baseline are the required half widths. Data are only reported for

peaks with symmetry indices between 1.00 and 1.10.

b) Reduced Velocity

The reduced velocity of a given solute in a given mobile phase liquid is defined by Equation 4.1.

$$\text{Reduced velocity, } v_r = \frac{v d_p}{D_m} \quad 4.1$$

where v is the mobile phase linear velocity, d_p is the particle diameter and D_m is the diffusion coefficient of the solute in the mobile phase. v is determined from the recorder chart speed and the retention distance t_R measured from the elution record. d_p is taken as the mean of the smaller and larger sieve openings which define the fraction used. Most of the supports used were 44 - 53 micron fractions and d_p was thus taken as 48.5 microns.

D_m was calculated for all the solutes used in the determinations of plate heights from the Wilke-Chang equation (85), Equation 4.2,

$$D_m = \frac{7.4 \times 10^{-8} (\psi_2 M_2)^{0.5} T}{\eta V_1} \quad 4.2$$

where η is the viscosity of the solvent in centipoises, M_2 is the molecular weight of the solvent, V_1 is the molar volume of the solute and ψ_2 is an association factor which takes the values 1.0, 2.6, 1.9 and 1.5 for non-polar solutes, water, methanol and ethanol, respectively. T is the absolute temperature. D_m is given in units

of $\text{cm}^2 \text{sec}^{-1}$. The calculated values for all the solutes used in the plate height determinations, in all of which cyclohexane was used as the mobile phase, were found to lie close to $1.2 \times 10^{-5} \text{ cm}^2 \text{sec}^{-1}$ and this value was assumed for all solutes in the plots of reduced plate height vs. reduced velocity. This introduces negligible error.

c) Reduced Plate Height

The reduced plate height is defined by Equation 4.3.

$$\text{Reduced plate height, } h, = H/d_p \quad 4.3$$

where d_p is the particle diameter and H is the plate height for the column and is defined by Equation 4.4

$$H = L(w_o/4t_R)^2 \quad 4.4$$

where L is the column length, w_o is the previously defined baseline width of the peak and t_R is the retention distance of the peak maximum.

d) Retention Volume

The retention volume of a peak is defined as the volume of mobile phase required to elute 50% of an injected sample of a single solute. For peaks of Gaussian symmetry 50% of the injected sample has been eluted when the peak maximum elutes. Thus the retention volumes of solutes can be determined from the retention distance t_R , the recorder chart speed and the volume flow rate of the mobile phase.

In the experiments using scintillation counting to determine retention volumes the retention volume is found by counting the drops which elute from the time of injection of the solute until collection is started in vials of the cocktail. After counting it is known which drop contained the maximum concentration and, knowing the drop volume, the retention volume can be directly obtained.

CHAPTER 5PERFORMANCE OF VARIOUS SUPPORTS IN HIGH
PERFORMANCE LIQUID CHROMATOGRAPHY5.1. Introduction

Many early experimental studies of the efficiencies of gas chromatographic columns were interpreted in terms of the "classical" theories of peak dispersion. The most widely known of these are due to van Deemter et al. (33), Golay (34) and Jones (71). All of these treatments regard contributions to the overall plate height as additive and give rise to equations of the form of Equation 5.1

$$H = 2\lambda d_p + 2\gamma D_m / v + \omega d_p^2 v / D_m \quad 5.1$$

This may be written in terms of reduced parameters as

$$h = 2\lambda + 2\gamma / v + \omega v \quad 5.2$$

However, as indicated earlier, Giddings pointed out that diffusive and flow dispersion are coupled so that their contributions to the plate height are not additive. The combined contribution of the two mechanisms to H can be represented thus

$$H = \sum_{i=1}^n (1/2\lambda_i d_p + D_m \omega_i d_p^2 v)^{-1} + 2\gamma D_m / v \quad 5.3$$

or, in terms of reduced parameters

$$h = \sum_{i=1}^n (1/2\lambda_i + 1/\omega_i v)^{-1} + 2\gamma / v \quad 5.4$$

where the summations are carried over each type of velocity inequality.

Using liquid eluents to achieve high reduced velocities Knox (86) produced the first clear evidence that the coupling equations correctly described the data. He also showed that, while an equation of the form of Equation 5.4 fitted his data much more closely than equations of the form of Equation 5.2, a good fit to the data could only be achieved by replacing the summation of Equation 5.4 with an integration thus

$$h = \iint \frac{f(\lambda, \omega) d\lambda d\omega}{1/2\lambda + 1/\omega\nu} + 2\gamma/\nu \quad 5.5$$

where $f(\lambda, \omega)$ is a weighting factor such that

$$\iint_0^\infty \frac{f(\lambda, \omega) d\lambda d\omega}{1/2\lambda + 1/\omega\nu} = 1$$

Equation 5.5 loses the simplicity of the summed form of Equation 5.4 where separate summation terms are identified with each of the previously described categories of velocity inequalities. The values of λ and ω are not easily predicted. Knox's data also confirm Giddings' suggestion (40) that Equation 5.5 may be factorised into two terms where one term describes the trans-column contribution and the other accounts for the contributions arising from the other categories of velocity inequalities.

The second term of Equation 5.5 represents the contribution of axial molecular diffusion to the plate height viz.,

$$h = 2\gamma/\nu \quad 5.6$$

where γ is the obstructive factor for molecular diffusion. The value of γ has been taken as 0.6 for randomly packed impervious spheres and 0.8 for diatomaceous earths (69,70). For highly porous materials such as Porasil^R and Corning CPG^R γ is likely to be between 0.8 and 1.0.

According to Giddings' nonequilibrium theory (37) contributions to the plate height will arise as follows. The contribution which arises from the balance of nonequilibrium in the stationary phase where the mechanism of equilibrium is diffusion has the form of Equation 5.7

$$h = q \frac{K'}{(1 + K')^2} \frac{\bar{d}_s^2}{d_p^2} \frac{D_m}{D_s} \nu \quad 5.7$$

where q is a configurational factor relating to the geometry of the stationary phase, K' is the column capacity ratio, $\bar{d}_s^2$ is the mean square film thickness of the stationary phase and D_s is the diffusion coefficient of the solute in the stationary phase.

The contribution arising from adsorption on a one-site surface is given by Equation 5.8

$$h = \frac{2K'}{k_d(1 + K')^2} \frac{D_m}{d_p^2} \nu \quad 5.8$$

where k_d is the desorption rate constant.

The contribution arising from equilibration by diffusion in the stagnant part of the mobile phase in a porous support is given by Equation 5.9

$$h = q' \left[1 + \frac{aK'}{(1 + K')} \right]^2 v \quad 5.9$$

where q' and a are configurational factors for the mobile phase.

The contribution arising from nonequilibrium in the streaming part of the mobile phase is, as indicated above, more complex in its dependence on the reduced velocity since the flow and diffusive mechanisms cooperate to reduce it from the values predicted by the classical additive theories. Since, as pointed out by Knox (86), none of the theoretical expressions advanced to account for this contribution are entirely satisfactory the data reported below will be correlated and discussed in terms of an empirical equation.

A general expression for the plate height contributions from the streaming mobile phase can be written in the form

$$h = 2\gamma/v + f(v, \rho) \quad 5.10$$

where ρ is the column to particle diameter ratio. In the present work ρ is greater than 20 and the plate height is independent of the column diameter. Equation 5.11 was proposed by Huber(87) and was found by Knox and Parcher to fit their data.

$$h = (1/2\lambda + 1/c'' v^{n''})^{-1} \quad (n'' \leq 1) \quad 5.11$$

n'' may be best regarded as an empirical parameter. Thus, while Huber used $n'' = 0.5$, the latter authors fitted their data using $n'' = 0.33$. For a range of ν covering less than two orders of magnitude Equation 5.11 is well approximated by Equation 5.12

$$h = c'\nu^{n'} \quad (n' < 1) \quad 5.12$$

where n' is found to lie between 0.25 and 0.35.

The contributions to the total reduced plate height can be grouped into three terms which differ in their dependence on ν , the reduced velocity. The term due to axial diffusion is proportional to $(\nu)^{-1}$; the terms arising from mass transfer between the stationary phase or stagnant regions of the mobile phase and the streaming regions of the mobile phase are linear in ν and the terms arising from the various categories of velocity inequalities which prevail in different regions of the streaming mobile phase are proportional to some fractional power of ν . Thus the overall reduced plate height can be represented by Equation 5.13

$$h = (2\gamma/\nu) + A\nu^n + C\nu \quad 5.13$$

where n lies between 0.25 and 0.35 for unsorbed solutes.

The first term of this equation is negligible when ν is more than 10. When ν is greater than 10^3 the experimental plate height curves for unsorbed solutes reach a limiting plateau with increasing ν and at still higher reduced velocities decline as ν increases.

Since both Equations 5.12 and 5.13 include a mobile phase term which depends on a positive power of ν both will cease to fit the

data as v increases above 10^3 . Equations of the form of Equation 5.12 which do not include the leading term of Equation 5.13 will, in addition, cease to approximate the data as the reduced velocity falls below 10. The dependence of h upon v over a fairly narrow range of velocities between 10 and 10^3 may be reasonably approximated by Equation 5.14

$$h = dv^m \quad 5.14$$

where m may lie between 0.25 and 0.8.

This equation, however, can only be regarded as a useful adjunct to short range extrapolation of experimental data since it has no theoretical foundation.

Equation 5.13 corresponds more closely to the theoretical constructs discussed in Chapter 3. As v is decreased the second and third terms which depend on positive powers of v will decrease and at sufficiently low values of v the plate height will be given by the first term of the equation. Thus at low reduced velocities the plate height curve is expected to reach a minimum and then increase as the reduced velocity is further decreased. Figure 13 shows data for 163 micron diameter Chromosorb W particles which exhibit this behaviour. These data show a minimum plate height of less than 2 particle diameters occurring at a reduced velocity of about 3, in good agreement with theoretical predictions. That Equation 5.13 correctly predicts the asymptotic behaviour of the

reduced plate height at very low reduced velocities is confirmed by these data and by the more extensive compilation for Zipax^R by Done and Knox (88), shown in Figure 24.

The terms contributing to the coefficient C of Equation 5.13 can be derived from the nonequilibrium theory and explicit expressions for some of them are given in Equations 5.7, 5.8 and 5.9. The evaluation of the third term of Equation 5.13 requires estimates of the configurational factors applicable to the particular system. Ultimately, with increasing v , this term will dominate the plate height as predicted by Equation 5.13 and the plate height will become linearly dependent on the reduced velocity.

The second term of Equation 5.13 is the least satisfactory. As shown in Equations 5.3 and 5.4 the total plate height contribution arising from mobile phase nonequilibrium can be attributed to discrete additive contributions from each of five categories of velocity inequality. Since velocity inequalities in the column cover continuously a wide range this arbitrary categorisation represents a simplification and one which, as Knox showed, is not justified by the experimental data. When the summation of Equation 5.4 is replaced by the second term of Equation 5.13 the constants A and n which emerge cannot be simply related to the mobile phase processes occurring in the column. The range of reduced velocity over which the approximation is valid is unlikely to be larger than from 10 to

10^3 .

In the interpretation of the experimental data according to Equation 5.13 it is found that the term Av^n arising from mobile phase processes is the major term in the range of reduced velocity from 10 to 100. In this region of the curve the dependence of h on v is very close to the form

$$h = Av^n$$

where A and n are as used in Equation 5.13. In fitting the experimental data to Equation 5.13 both A and n are found to differ for different supports and for different values of K' , the column capacity ratio. For columns where C is significant the third term of Equation 5.13 becomes important at reduced velocities above 100.

The experimental data are presented as plots of $\log h$ vs. $\log v$. The gradients of these plots at any value of $\log v$ represent the instantaneous value of m in Equation 5.14. Over the range of $\log v$ from 0.5 to 3 where most of the data lie these plots are close to linearity i.e. the approximation of Equation 5.14 holds fairly well over this range. As is shown below the data are better fitted by Equation 5.13 since the plots show a consistent trend to higher values of n as the reduced velocity increases.

The positions of the plots for unsorbed solutes relative to the plot for a column packed with large diameter particles of the

same support indicate the quality of packing achieved with small diameter particles. It is usually assumed that particles of 200 microns diameter or larger can be readily packed to give the best packing possible for a given material so that the potential for improvement in the packing of smaller diameter material is indicated by a comparison of the plots for unsorbed solutes. A comparison of plots for solutes of different K' values on a given support can reveal the relative importance of equilibration in the stationary phase and in stagnant regions of the mobile phase. Since, according to Equation 5.7, the stationary phase contribution is zero for $K' = 0$ and for $K' = \text{infinity}$ and passes through a maximum where $K' = 1$ while, according to Equation 5.9, the stagnant mobile phase contribution increases as K' increases from zero to infinity any decrease in h as K' rises above unity indicates the importance of equilibration in the stationary phase. For surface layered materials where the contribution to h from stagnant regions of the mobile phase will be minimal the dependence of h upon K' will yield information on the mechanism of equilibration in the stationary phase.

5.2. Experimental

The columns were 40 to 50 cm lengths of 2 mm i.d., 8 mm o.d., straight glass tubing prepared and packed as described in Chapter 4. The specifications of the columns are given in Table 5.1. β, β' oxydipropionitrile (BOP) was used as the stationary

phase in all the partition chromatography experiments. For each column plate heights were determined at a number of mobile phase velocities covering a range of 2 - 3 orders of magnitude. Cyclohexane was used as the mobile phase for all plate height determinations. Plate height measurements were made for an unretained solute and at least one retained solute on each support used. Both partition and adsorption chromatography was carried out on the Porasil^R, Corasil^R and the Corning^R (CPG) materials. Where the same solute is used in both adsorption and partition modes, coating with BOP, a polar stationary phase, is seen to reduce K' . Each reported plate height value refers only to peaks within the previously defined symmetry limits and is the mean of two concordant determinations. The data obtained for column 32 are shown as an example in Table 5.2. The K' values of the solutes used on the various supports are shown in Table 5.3. Chlorobenzene was used as the unretained solute throughout. It is, however, slightly retained by adsorption on silica. Its K' value on CPG^R 75 is 0.15 and, since this support has the highest surface area of all the supports examined, its K' is probably less than this value on the other supports. The value of 0.15 or less will not substantially affect the conclusions reached in the discussion.

From the data plots of $\log h$ vs. $\log v$ were constructed for each solute-support system. These were then fitted to Equation

5.13 to obtain the values of the parameters A, C and n. These are tabulated in Table 5.4. Values of the parameters obtained from the data of Knox and Parcher for large diameter glass beads, from Kirkland's data (58) for Gaschrom^R P and from the more extensive compilation of data for Zipax^R by Done and Knox are included for comparison.

Table 5.1
Specifications of the Columns

<u>Material</u>	<u>Particle Diameter</u> range (microns)	<u>Mean Particle</u> <u>Diameter</u> (microns)	<u>Length</u> (cm)	<u>Column</u> <u>Diameter</u> (mm)	<u>Stationary</u> <u>Phase</u>
Chromosorb W	149 - 177	163	101.8	5	-
	44 - 74	59	50	2	-
	37 - 44	40	48	2	-
	37 - 44	40	46	2	4% w/w
Chromosorb P	44 - 74	59	40	2	-
	44 - 74	59	40	2	4% w/w
Phase Separation Universal	44 - 74	59	50	2	4% w/w
Porasil 60	44 - 53	48.5	43	"	-
	"	"	43	"	4% w/w
Porasil 250	"	"	43	"	-
	"	"	48	"	4% w/w
Corning GPG 75	"	"	48	"	-
	"	"	48	"	4% w/w
Corning CPG 240	"	"	43	"	-
	"	"	43	"	4% w/w
Corasil I	"	"	43	"	-
	"	"	43	"	1% w/w
Corasil II	"	"	48	"	-
	"	"	48	"	1% w/w
Zipax	"	"	48	"	"

Table 5.2

Experimental Data for Column 32Results:- chlorobenzene

Run	Baseline width w_o (cm)	Retention distance (cm)	Retention time (mins)	log h (mean)	log ν (mean)
1	0.62	4.95	1.65	0.95	2.26
	0.61	4.72	1.57		
2	1.50	10.95	0.91	1.00	2.51
	1.42	10.67	0.89		
3	0.91	5.80	0.48	1.14	2.78
	0.93	5.80	0.48		
4	0.93	7.86	2.62	0.89	2.04
	0.94	8.02	2.67		
5	0.59	5.32	5.32	0.84	1.75
	0.57	5.05	5.05		
6	0.81	8.50	8.50	0.69	1.56
	0.70	7.57	7.57		
7	1.74	21.66	21.66	0.53	1.10
	1.92	25.25	25.25		

Results:- topanol

Run	Baseline width w_o (cm)	Retention distance (cm)	Reduced plate height, h	log h (mean)
1	1.27	6.91	18.72	1.27
	1.20	6.61	18.26	
2	3.28	15.52	24.75	1.40
	3.29	15.18	26.03	
3	2.18	8.29	38.32	1.58
	2.16	8.30	37.53	
4	1.92	11.30	16.00	1.19
	1.92	11.56	15.29	

Table 5.2 cont'd.

Run	Baseline width w_o (cm)	Retention distance (cm)	Reduced Plate height, h	log h (mean)
5	1.12	7.75	11.57	1.06
	1.05	7.32	11.40	
6	1.36	11.62	7.59	0.91
	1.22	9.80	8.59	
7	3.10	29.65	6.06	0.75
	3.30	34.34	5.12	

Table 5.3Solute Retention Ratios

<u>Material</u>	<u>Solute</u>	<u>Retention Mode</u>	<u>K'</u>
Chromosorb W	2-phenyl ethanol	Partition	1.8
Chromosorb P	2-phenyl ethanol	Partition	0.8
Phase Separation Universal	"	"	1.4
Porasil 60	acetophenone	Adsorption	0.45
	benzylaldehyde	Partition	0.75
Porasil 250	acetophenone	Adsorption	0.5
	nitrobenzene	Partition	0.45
Corning CPG 75	acetophenone	Adsorption	6.0
	2,4-dimethyl 6-tert butyl phenol	Partition	1.0
	(topanol)		
Corning CPG 240	acetophenone	Adsorption	4.0
	topanol	Partition	0.4
Corasil I	2-tertbutyl phenol	Adsorption	2.5
	topanol	Partition	0.4
Corasil II	topanol	Adsorption	0.8
	topanol	Partition	0.5
	nitrobenzene		0.6
	2,4 dinitrotoluene		3.2
	2-nitroaniline		11.5
Zipax	3-phenyl propanol	Partition	0.4

Notes:-

Chlorobenzene was used throughout as an unsorbed solute. However, it is slightly retained on the high surface area silica supports. This correction is discussed elsewhere. Cyclohexane was used as the mobile phase throughout. The K' values refer to ambient temperatures.

Table 5.4Experimental Parameters for the Plate Height Equation

$$\text{Equation } h = 2\gamma/\nu + A\nu^n + C\nu$$

Assumed values: γ for non porous materials = 0.6

γ for porous materials = 0.9

<u>Material</u> (d_p microns)	<u>K'</u>	<u>A</u>	<u>C</u>	<u>n</u>
Chromosorb W (163)	0	0.78	1.6×10^{-2}	0.33
Chromosorb W (37 - 44)	0	3.24	1.7×10^{-2}	0.33
	1.8	4.71	3.0×10^{-2}	0.4
Chromosorb W (44 - 74)	0	3.40	2.1×10^{-2}	0.33
Phase Separation Universal (44 - 74)	0	6.55	3.1×10^{-2}	0.33
	1.4	7.62	3.5×10^{-2}	0.4
Chromosorb P (44 - 74)	0	6.03	1.1×10^{-2}	0.33
	0.8	9.07	1.2×10^{-2}	0.4
Gaschrom P ^a (58)	1.1	1.36	0.94×10^{-2}	0.4
	1.8	1.70	1.2×10^{-2}	0.4
Porasil PS6O and PS25O (44 - 53)	~ 0	1.46	3.6×10^{-2}	0.33
	0.4 - 0.8	1.72	5.0×10^{-2}	0.33
Corning CPG75 (44 - 53)	~ 0	1.18	2.9×10^{-2}	0.33
	6.0^b (adsorption)	1.85	6.0×10^{-2}	0.4
Corning CPG24O (44 - 53)	~ 0	1.40	1.7×10^{-2}	0.33
	4.0^b (adsorption)	1.51	3.4×10^{-2}	0.4

Table 5.4 cont'd.

<u>Material</u>	<u>K</u>	<u>A</u>	<u>C</u>	<u>n</u>
Corasil I (44 - 53)	O	1.36	0.1×10^{-2}	0.33
	2.5^b (adsorption)	1.77	4.5×10^{-2}	0.4
Corasil II	O	0.98	0.4×10^{-2}	0.33
	0.8^b (adsorption)	1.57	4.7×10^{-2}	0.4
Zipax (44 - 53)	O	0.77	0.30×10^{-2}	0.33
	0.4	0.95	0.32×10^{-2}	0.4
(37 - 44) ^c	O	0.77	O	0.3
(85 - 130) ^c	0.9	0.95	0.32×10^{-2}	0.4
Glass beads ^d (480)	O	0.37	O	0.33

Notes:-

- ^a Kirkland (58)
- ^b These solutes gave the highest plate heights
- ^c Done and Knox (88)
- ^d Knox and Parcher (80)

5.3. Results

The $\log h$ vs. $\log v$ plots for all the systems examined are shown in Figures 13 - 23. The curves are drawn using the values of A, C and n tabulated in Table 5.4. Figure 13 shows the curves for unsorbed solutes on several columns packed with some diatomaceous earth materials. These include batches of 37 to 44 microns and 44 to 74 microns diameter. The lowest curve shows the data obtained for 80 - 100 mesh Chromosorb W (average $d_p = 163$ microns). It is clear that there is a large performance deficit between all the packings of smaller diameter particles and the large particle packing.

Figure 14 shows the data obtained from 53 - 63 micron diameter Chromosorb P for an unretained and a moderately retained solute. Data obtained by Kirkland (58) for the same material are shown for comparison; the performance is considerably better than was attained in this work. Comparison with Figure 13, however, indicates that poor packing still limits the performance of the small particles.

Figure 15 shows the data for retained solutes as well as for inert solutes on 40 micron diameter Chromosorb W and on Phase Separation Universal 59 micron diameter particles. Comparison of the unsorbed solute data with Figure 14 indicates that a better packing has been achieved with Chromosorb W than with Chromosorb

P or the Phase Separation material. This comparison also suggests a somewhat smaller dependence of performance on K' for Chromosorb P than for either of the other two materials. This is confirmed quantitatively and will be discussed below.

Figure 16 shows data for Porasil^R PS6O and PS25O in both adsorption and partition modes. The data are derived from four columns, two of which are packed with particles bearing 4% w/w stationary phase. The very close agreement between the four sets of data for inert solutes indicates the reproducibility of the packing technique. The dependence of h on K' is clearly small. The flattening of the plots at low reduced velocities reflects the dispersive effect of longitudinal molecular diffusion, represented by the first term of Equation 5.13. Figure 16 includes some data obtained by Little, Horgan and Bombaugh (89) which are very similar to those determined in this work. The data obtained by de Vries et al. (90) also shown for comparison, were obtained in 1967 and serve to show how far the technique of packing small particles has advanced since then. Only for particles of 200-250 microns diameter do their data coincide with this more recent work.

Figures 17 and 18 show the data obtained for lightly and moderately retained solutes in both adsorption and partition modes using Corning^R CPG 75 and CPG 240. This material is made by leaching two phase borosilicate glass which has been submitted to

a controlled cooling procedure (91). The process gives a porous silica of very uniform pore size and its physical structure is probably similar to that of Porasil^R which is a spherical silica gel subjected to heat and hydrothermal treatment (92). The dependence of h upon K' is greater than for Porasil^R but the two materials perform almost identically with retained solutes.

Figures 19 and 20 show data for retained and unretained solutes in adsorption and partition chromatography on Corasils^R I and II and Figure 21 shows data for solutes of different K' in partition chromatography on Corasil^R II. Figure 22 shows more extensive data on the variation of h with K' in adsorption chromatography on Corasil^R II compiled by Done (95). The most striking feature of all these data is the marked dependence of h upon K' . These materials consist of impervious cores with one (Corasil^R I) or (Corasil^R II) surface layers of silica gel. The unretained solute data is very similar to that obtained for Zipax^R and shown in Figure 23 while the retained solute data is close to the data for retained solutes on Porasil^R and Corning^R CPG.

Figure 23 shows data obtained for sorbed and unsorbed solutes in partition chromatography on Zipax^R. Figure 24 shows a more extensive set of data obtained on this material by Done and Knox (88). This latter set shows both the low reduced velocity minimum in h and the subsequent increase of h as the velocity is further reduced. There is some indication of a flattening of the

unsorbed solute data towards the theoretically predicted plateau at high reduced velocities. The data obtained in this work are in good agreement both with this latter set and with those recently reported by Kirkland (59). These results confirm that, for materials of this size range, the lowest plate heights and thus the highest performance can be attained with Zipax^R.

5.4. Discussion

The number of data points used to construct each of the log h vs. log v curves is not sufficient to determine the parameters A , C and n of Equation 5.13 with high accuracy. However, since Equation 5.13 has the best theoretical foundation of the widely used empirical equations, interpretation of the data in terms of these parameters seems to be justified. It seems preferable, however, to regard the data as being consistent with the equation using the tabulated values of the parameters rather than as providing positive evidence that this equation gives the best fit to the real systems. This reservation applies more strongly to the mobile phase term of Equation 5.13 and thus to the values found for the parameters A and n . As suggested earlier, the stationary phase term C rests on firmer theoretical foundations.

At reduced velocities above 10 the first term of Equation 5.13 is negligible and, for the moderate values of C found in this work, the third term only becomes significant as the reduced

velocity approaches 100. In the intervening region the dominant contribution to the plate height arises from the second term of Equation 5.13, the mobile phase term. In fitting the data to Equation 5.13 n may be regarded as an arbitrarily fixed parameter leaving only A and C to be varied or, alternatively, n may be regarded also as an adjustable parameter. A consideration of the data obtained in this work in the range of reduced velocity from 10 to 100 suggested that a better fit could be obtained by varying n and thus varying the gradients of the log-log plots in this range. The data for all unsorbed and lightly sorbed solutes are well fitted by the value $n = 0.33$. However, as shown in Figure 24 for the data of Done and Knox, the curve drawn using the value $n = 0.30$ fits the data for the unsorbed solutes better than the curve using $n = 0.33$. The curves drawn through the data for the solute of lowest (or zero) K' in Figures 13 - 23 use the value $n = 0.33$. For more strongly retained solutes the data seem to be better fitted using the value $n = 0.4$ and the curves for these solutes have been drawn with $n = 0.4$. It seems probable that unsorbed solutes can best be described by values of n between 0.30 and 0.33 while sorbed solutes have n values ranging from 0.33, at low K' values, to 0.4 at values of K' giving a maximum stationary phase contribution.

The interpretation of the variation of n with K' and with the

nature of the support is difficult. It could be plausibly suggested that a mobile phase term is present which is independent of K' and, consequently, that a fixed optimum value of n should be isolable which should depend on the nature of the support and the quality of the packing but not on K' . Giddings (93) however, has predicted that the mobile phase terms are weakly dependent on K' . It seems that this dependence might be reflected in both A and n when the second term of Equation 5.13 is used to approximate the coupled form of the mobile phase terms. If the departure of the gradients of the log-log plots for solutes of moderate values of K' from those of the unsorbed solute curves was to be attributed to the influence of the stationary phase term of Equation 5.13, which is linear in v , then the most marked improvements in the fitting of the experimental data by variation of n should be found for the plots of data for retained solutes on supports where the values of C are particularly high. However, as shown in Figure 24, the data for solutes with $K' = 0.9$ on Zipax^R are very well fitted by the curve drawn with $n = 0.4$. The values of C for this support are particularly low and the stationary phase term does not contribute significantly to the plate height at reduced velocities of less than 300. Thus the fitting of this set of data is the best evidence available for a genuine dependence of n on K' . Though, as is discussed below, the term Av^n shows large variations between

packings of different quality the value of n seems to be independent of the quality of the packing and, for unsorbed solutes, only dependent on the nature of the support. Though the interpretation of the dependence of n on K' is difficult it would be valuable to confirm it by the fitting of more extensive sets of data since the Av^n term is significant over the range of practical operation.

Though A is, like n , a composite parameter referring to the whole of the mobile phase and thus cannot be related in detail to the column kinetics it is much more readily related to the overall column performance than n was shown to be. While n seemed to be dependent on K' and not on the quality of the packing A is dependent on both parameters. Giddings showed that the mobile phase contribution to the plate height is proportional to

$$(1 - 0.15/(1 + K)) \quad \text{for porous supports}$$

and to $(1 - 0.30/(1 + K))$ for non-porous supports

For the data presented here both A and n appear to exhibit a weak dependence on K' which is compatible with this weak dependence of the total mobile phase contribution. The major source of variation in A arises from the very large variations in the total mobile phase contribution due to great differences in the quality of packings achieved with the different supports used. A is thus the most useful single parameter characterising the packing of columns. The A term for retained solutes is in no case more than twice the

corresponding unsorbed solute A term, in agreement with the theoretical predictions.

The A terms found for the diatomaceous earth supports are very much larger than those found for the silica based supports while the A term for Kirkland's data on Gaschrom P is of comparable magnitude to the values found for the silica supports in this work. Thus, in general, the packing quality achieved for these latter supports was much better than for the diatomaceous earth materials although it appears from Kirkland's results that these can be packed with comparable efficiency. The reproducibility of packing of the diatomaceous earth materials was found to be poorer than the reproducibility attained with the silica supports. The A term of 0.78 for the 235 microns diameter Chromosorb W^R confirms the earlier suggestion that comparison with packings of large particles of the same support indicates the deficiency of packing of the smaller particles. This A term is in fact close to that obtained for Zipax^R. Comparison of the values of A quantifies the performance deficit. The A terms found for the silica supports are broadly similar except that the term for Zipax^R is substantially lower than the others. This indicates that the packing achieved with this support is better than that achieved with the other supports.

The factors affecting the packing of these materials are not clear. It appears that the packing achieved with the Corning CPG^R

materials is as good or marginally better than that attained with Porasil^R. This is contrary to the widely held belief that spherical particles pack more efficiently than irregular particles. It is possible that irregular particles do not give rise to regions of close packing similar to those occurring in randomly packed arrays of approximately spherical particles. A large plate height contribution in packings of spherical particles arises from velocity inequalities between regions of close packing and regions of approximately cubic packing. If only one mode of packing occurs in random arrays of irregular particles then the mobile phase plate height contribution would be less than for the spherical particles. This possibility might be illuminated by permeability data on the two types of packings. The very low A term for Zipax^R conflicts with the suggestion, however. The A value of 0.4 found by Knox and Parcher for 480 microns diameter glass spheres seems to be the lowest value which can be achieved by random packing of spheres. There remains considerable scope for improvement in the methods of packing of small particles.

The parameter C describing the stationary phase and stagnant mobile phase contributions to the plate height is the most amenable of the three parameters A, C and n of Equation 5.13 to interpretation. The small values of C reported for the pellicular materials Zipax^R and Corasil^R are subject to large errors since,

where C is of this magnitude, the term C only contributes significantly to the total plate height at the highest reduced velocities used in the present study. An accurate determination of C would require an extension of the data to reduced velocities of 3×10^3 at least. The data of Done and Knox (88) for Zipax^R which extend to somewhat higher reduced velocities indicate that C is probably not significantly different from zero for unsorbed solutes on this material and this conclusion may extend to the data for unsorbed solutes on Corasil^R also. The C terms for the unsorbed solutes on all the other supports studied and for the retained solutes on all the supports, except Zipax^R, are large enough to be determined fairly accurately from data in the range of reduced velocities studied.

For unretained solutes C has a significant value only on porous materials. The nonequilibrium theory predicts that the plate height contribution from porous spheres will be given by Equation 5.15

$$h = C_v = \frac{[(1 - \phi) + \phi(K'/(1 + K'))]}{30(1 - \phi)\gamma'} \quad 5.15$$

where ϕ is the fraction of mobile phase in the interparticle space and is around 0.5 for most materials. γ' is the obstructive factor for diffusion in the stagnant mobile phase inside a particle, and for highly porous materials like Porasil^R and CPG^R is probably between 0.6 and 1.0. Substituting these values in Equation 5.15

gives $C = 1.5 \text{ to } 2.5 \times 10^{-2}$ for unretained solutes and $C = 3 \text{ to } 5 \times 10^{-2}$ for strongly retained solutes.

The values of C determined for all the size ranges of Chromosorb^R W and for CPG^R 240 for unsorbed solutes fall within the predicted range. The value determined for unretained solutes on Chromosorb^R P is in good agreement with the C value for the lightly sorbed data found for Gaschrom^R P by Kirkland but both values are significantly lower than those found for the white forms of the diatomaceous earths. The ratios of the values for the pink and white materials is in reasonable agreement with the ratio expected from the published values for the pore volumes per gram of the materials.

The low value of C for unsorbed solutes on CPG^R 240 relative to the values found for both the Porasil^R supports and for CPG^R 75 suggests that the value of the intraparticle obstructive factor is lower for this material and suggests that narrow pores have been eliminated. CPG^R 75 which has a much lower intraparticle void volume than either CPG^R or Porasil^R and, as with Chromosorb^R P, might be expected to exhibit a low value of C , in fact, is found to have a much larger value than the larger pore CPG^R material. Thus it seems that intraparticle diffusion is relatively hindered in this material. This conclusion appears to hold for both the Porasil^R materials also. It seems likely that

the internal structures of these porous silicas, except that of the large pore CPG^R particles, contain significantly tortuous and constricted channels. The relatively high C value for the Phase Separation Universal^R material may also be due to restricted intraparticle diffusion.

The values of C found for retained solutes on all the porous supports except Chromosorb^R P are in close agreement with the predicted range of values. The latter support appears to exhibit a very low dependence of C upon K' . The C term found for CPG^R 75 is again higher than that found for CPG^R 240 and since the value of the function $K' (1 + K')^{-2}$ is similar for the solutes involved, the plate height contribution due to partition kinetics will be similar and the difference must again be attributed to a more open internal structure in the large pore size material.

Application of Equation 5.15 to the surface layer of the pellicular supports suggests that the C term should be negligible for unsorbed solutes and very small for retained solutes. This is true of Zipax^R. Although the C terms found for unsorbed solutes on Corasil^R are close to zero the terms for retained solutes are comparable to the C terms for retained solutes on the porous supports. Three effects rule out restricted molecular diffusion as the cause of this strong K' dependence of C. Firstly, the unsorbed solute would diffuse into the silica gel layer and be

subject to the dispersive effects of any purely diffusive mechanism occurring in this layer. The near-zero C values for these solutes conflict with this. Secondly, the data of Figure 21 for the variation of h with K' show that the stationary phase contribution to h has a maximum value at a finite value of K' confirming that a stationary phase kinetic effect is involved. Thirdly, while the unsorbed solute C terms are quite different for Corasil I and Corasil II and correspond to the difference expected for diffusion in a thicker surface layer on the latter material, the retained solute C terms show no such difference. In addition, the C term appears to be affected by solute structure for solutes of similar K' values. A characterisation of the gel layer, which might permit interpretation of these results, has not yet been published. In agreement with theoretical predictions, however, the C term for a solute with $K' = 0.9$ on Zipax^R is very much smaller than the terms found for retained solutes on any of the other supports confirming the excellent mass transfer characteristics of the stationary phase layer on this support.

Thus it has been shown that the fitting of the $\log h$ vs. $\log v$ curves to Equation 5.13 and the evaluation of the values of the parameters A , C and n of the equation has yielded much information on the packing quality of supports and their mass transfer characteristics. While previously these plots have been fitted to straight lines and characterised in terms of their position and their

overall gradient it appears that fitting to Equation 5.13 gives more detailed information on column performance. Thus the results of this work strongly suggest that the parameter n of Equation 5.13 is related only to K' and is not sensitive to the mobile phase flow pattern. Fitting of the data to straight lines gives rise to an overall gradient parameter, sensitive to mobile phase effects as well as to variation in K' since it is influenced by the third term of Equation 5.13, the stationary phase mass transfer term, which is linear in v . It now appears that only the parameter A , as evaluated here, is dependent on mobile phase flow effects. This parameter, which is only weakly dependent on K' , is most strongly dependent on the quality of packing as reflected in the column performances at intermediate values of the reduced velocity. Finally the parameter C is most strongly affected by the variation of h at higher values of the reduced velocity. This parameter is the most completely interpreted in terms of current theories and can be predicted precisely after the specification of the appropriate configuration factors.

In the immediate future it seems that the parameters A and n will remain largely empirical. The rather narrow range of variation of n , which could be valuably confirmed by examination of larger sets of data, should render possible its estimation with fair precision in most practical circumstances. The parameter A was

shown to reflect the variation in the efficiency of packing. Only this parameter differed markedly between the poorly performing columns of small diatomaceous earth particles and the efficient columns packed with pellicular and porous supports of the same particle diameters and with large diameter diatomaceous earth material. Thus the evaluation of this parameter, which can be based with fair accuracy on a plate height measurement at a single reduced velocity in the appropriate range, characterises the quality of packing achieved. The C term may be composed of several components. Thus, as discussed, the contribution from diffusion in the intraparticle space of a porous support depends on the value of K' for the solute involved, on the value of ϕ , the fractional interparticle volume, and on γ' , the intraparticle obstructive factor. The contribution from sorption kinetics depends on K' and the desorption rate constant. The contribution from diffusion in the stationary phase layer depends on the layer thickness and the solute diffusion coefficient in the layer as well as on K' . Many of these parameters on which the various contributions to C depend seem to be amenable to quite accurate estimation by chromatographic and other methods. Such an attempt to calculate C exactly, combined with the confidence that the first term of Equation 5.13 represents the situation at low reduced velocities well, would enable the Av^n term of the equation, the

term due to the mass transfer processes in the streaming regions of the mobile phase and the most intractable term of the equation, to be isolated.

CHAPTER 6RETENTION VOLUME DETERMINATIONS6.1. Introduction

The precise definition of the term "unretained" as applied to solutes is of considerable importance in the determination of accurate values of K' , the column capacity ratio, since the volume available to the mobile phase in the column is taken to be the retention volume of an unretained solute. Also, as shown in Chapters 3 and 5, the use of solutes which are confined to the mobile phase facilitates the separate examination of dispersion processes in the two phases. In the particular case of adsorption chromatography the assumption which appears to be implicit in most previous work is that the total mobile phase volume of the column is available to all solute molecules and that, while molecules whose free energies of adsorption are greater than the free energy of adsorption of mobile phase molecules have values of R which are less than unity, molecules whose free energies of adsorption are less than that of the mobile phase molecules have the same retention volumes as labelled mobile phase molecules. The dependence of the retention volumes of these latter species on the magnitude of their adsorption free energies does not seem to have been examined. The work reported below is a preliminary investigation of this dependence.

If an unretained solute is defined as a solute for which the value of R , as defined in Equation 2.2, is unity then the rate of movement of the solute band must equal the rate of movement of the mobile phase. The solute molecules must move with the same average velocity in the flow direction as the mobile phase molecules. Thus the solute molecules must have the same probability as the mobile phase molecules of occupying any region of any phase present in the column. In partition chromatography the probability of finding solute molecules in the stationary phase is higher than that of finding molecules of the mobile phase in these regions. Conversely, in gel permeation chromatography, the probability of finding the high molecular weight molecules to which the technique is applied in certain regions of the column, viz. the pores of the solid support, is lower than the probability of finding mobile phase molecules in these locations. In both these modes of chromatography separation occurs by virtue of differences in these probabilities for the different solute species. It will be shown that, in adsorption chromatography, there are regions where the probability of finding certain solute species is lower than the probability of finding mobile phase molecules i.e. certain solute species have smaller retention volumes than labelled mobile phase molecules.

In a column packed with an adsorbent the surface of the

adsorbent exerts attractive forces on molecules in the adjacent regions of the mobile phase and the action of these forces causes a shell of molecules to become associated with the surface. (A similar situation will obtain at any interface in chromatographic system but the adsorptive forces are, in general, weaker than those at an adsorbent surface.) The molecules of this shell can be regarded as associated with distinct sites on the adsorbent surface. These sites exhibit a spectrum of adsorption free energies. The mean adsorption energy depends on the species adsorbed and on the nature of the adsorbent while the form of the site energy distribution depends on the latter factor and on the previous treatment of the material. The molecules constituting the surface layer constantly exchange with the molecules of the bulk mobile phase; the mean residence time for a molecule in the shell is related to the mean adsorption energy. The volume of this adsorbed layer is a significant fraction of the total mobile phase volume for columns packed with high surface area adsorbents.

In the presence of a given concentration of a solute species in the mobile phase an equilibrium concentration of the solute is established in the surface layer. At constant temperature, pressure and solute concentration in a given mobile phase, the equilibrium concentration is a function of the difference in adsorption free energy between the molecules of the solute and those of the

mobile phase. When the adsorption free energy of the solute is much larger than that of the mobile phase the equilibrium fraction of solute molecules in the mobile phase is low and R , the ratio of the migration velocity of the solute to the mobile phase velocity, is close to zero. When the solute is an isotopically labelled sample of the mobile phase species the difference in adsorption energies is expected to be very small and the equilibrium concentrations of the solute in the bulk and adsorbed phases would be expected to be very nearly equal. When the adsorption energy of the solute is smaller than that of the mobile phase the equilibrium concentration of solute in the mobile phase is expected to be larger than its concentration in the adsorbed layer and thus its retention volume is expected to be smaller than that of the isotopically labelled mobile phase sample. The situation is somewhat analogous to gel permeation chromatography; there are regions of the column where the probability of finding a solute molecule is lower than that of finding a mobile phase molecule. As the difference in adsorption energies becomes large the probability of finding a solute molecule in the adsorbed layer tends to zero and such a solute will have a retention volume less by the volume of the adsorbed layer than the retention volume of a labelled mobile phase sample.

The importance of evaluating column performance for a solute which is confined to the mobile phase in the interpretation of

plate height data will be apparent from the discussion in Chapters 3 and 5. It is probably general practice to accept as an unretained solute the solute of lowest retention volume in a test group of compounds. This procedure, when applied in conjunction with u-v detection, is likely to restrict the compounds tested to aromatic and substituted aromatic compounds. It is likely that all compounds of these classes have higher retention volumes than saturated hydrocarbons in adsorption chromatography. The experiments reported here indicate that the volume available to the mobile phase in the column can be reliably determined by determining the retention volume of a sample of isotopically labelled mobile phase molecules.

6.2. Experimental

The column used in all these experiments was a 48 cm length of 2 mm i.d., 8 mm o.d., straight glass tubing packed, as described in Chapter 4, with 44 - 53 microns Corning CPG^R 75 particles. This support has the highest surface area of all those investigated and was thus expected to show the largest retention volume differences. Three mobile phases were investigated, viz. carbon tetrachloride, ethyl acetate and ethanol. The solutes were detected by one of three methods, viz. u-v absorption, refractive index changes which give rise to a double peak signal from the u-v detector and liquid scintillation counting of ¹⁴C labelled samples.

The solute retention volumes were obtained from the chart record, as described in Chapter 4, for the solutes detected by the first two methods.

In order to identify peak maxima by liquid scintillation counting it was necessary to collect samples of 10 to 20 drops of the column effluent in the initial runs for a particular solute. These samples were then counted for a sufficient time to determine unambiguously the sample of maximum activity. In the succeeding runs this part of the effluent volume was collected in samples of one drop. The counting of these samples determined the retention volume to an accuracy corresponding to the drop volume (10 - 15 microlitres). By arranging to collect drops of smaller volume the concentration vs. time record obtained will approach the continuous form obtained from conventional detectors e.g. u-v monitors, as shown in Figure 12. Figure 25 shows the form of the detection record obtained by this method. As indicated, by drawing tangents at the points of inflexion it is possible to estimate plate heights from these records, as described in Chapter 4.

6.3. Results and Discussion

The specific retention volume of a solute is related to the molecular structure of the solute, the adsorbent activity and the eluent strength by Equation 2.53,

$$\log V_o = \log V_c + a \sum_i Q_i^o + \sum_j q_j^o - \epsilon_i^o \sum_i \delta_i$$

where the individual terms have been defined and discussed in Chapter 2 above.

In these experiments, where the aim was to establish qualitatively the trend of retention volumes of solutes whose adsorption energies are close to, or less than, that of the eluent, no attempt was made to measure the activity of the adsorbent used. The results are reported as net retention volumes and are not referred to unit mass of adsorbent as required for direct comparison with Equation 2.53. The reported net retention volumes are based on an assumed value of zero for the elution of n-pentane by ethanol; the value predicted by Equation 2.53 is $0.033V_c$ when α is unity.

Table 6.1 shows the net retention volumes V_n found for the listed solute-eluent pairs. The data reported verify that solutes whose adsorption energies are less than that of the mobile phase molecules have lower retention volumes and, thus, lower probabilities of residence in the adsorbed layer, than the mobile phase molecules. Furthermore the data indicate that the probability of residence in the adsorbed layer falls as the difference between the adsorption energies of the two species increases. While the data qualitatively follow the trend predicted by Equation 2.53, with the exception of the results for chlorobenzene, the quantitative correlation is poor. More detailed investigation

would require that the adsorbent activity be measured in order to make quantitative correlations of retention volume data with Equation 2.53.

Table 6.1

Measured Retention Volumes

Eluent	Solute	V_n/cm^3
carbon tetrachloride	n-pentane	0.02
carbon tetrachloride	carbon tetrachloride	0.12
carbon tetrachloride	chlorobenzene	0.13
ethyl acetate	n-pentane	0.0
ethyl acetate	carbon tetrachloride	0.03
ethyl acetate	chlorobenzene	0.07
ethyl acetate	ethyl acetate	0.14
ethyl acetate	nitromethane	0.15
ethanol	n-pentane	0.0
ethanol	carbon tetrachloride	0.02
ethanol	chlorobenzene	0.10
ethanol	acetone	0.19
ethanol	nitromethane	0.16
ethanol	ethanol	0.31

CHAPTER 7CONCLUSIONS7.1. The Contributions of This Work

In the development of high performance liquid chromatography the packing of increasingly efficient columns and the correlation of column performance data in terms of equations of increasing universality are goals of central importance. As long as the ultimate goal of a priori prediction of column performance from a knowledge of the properties of the mobile and stationary phases and the support remains to be realised it will be important to establish the validity of available empirical equations in correlating column performance data. In the achievement, in practice, of improved column performance the efficient packing of small particles is essential.

The work described in this thesis represents a contribution both to the efficient packing of columns using particles of 40 - 60 microns diameter and to the experimental verification of an empirical reduced plate height - reduced velocity equation.

As has been discussed in Chapter 5 above this work has shown that 40 - 60 microns diameter particles of both the surface layered and the totally porous silica types can be packed reproducibly to give reduced plate heights of 2 - 5 at optimum reduced velocities. It has been confirmed that, using particles of

this size range, the surface layered particles investigated, viz., Zipax^R and Corasil^R, give substantially lower reduced plate height-reduced velocity curves for unretained solutes than those found for the totally porous silica materials investigated, viz., Porasil^R and Corning CPG^R. These latter materials, in turn, give lower curves than the diatomaceous earth supports. It has further been shown that the magnitudes of the plate height contributions from intraparticle diffusion are within the theoretically predicted range.

With the stationary phase loadings used in this work the dependence of the reduced plate height on K' , the column capacity ratio, is small both for the totally porous materials and for Zipax^R. While the loading of 1% used with the latter materials is close to the maximum possible without impairing stationary phase mass transfer, the loadings of 4% used with the totally porous silica-based supports can probably be substantially increased without impairment of efficiency. The dependence of h upon K' was also found to be small for adsorption chromatography on the porous silica supports. Corasil^R, however, exhibits a strong dependence of h upon K' in both adsorption and partition modes. In general, for particles of the sizes investigated, Zipax^R gives the highest column efficiencies while the silica-based supports give larger capacity. Figures 26 - 31 show some of the separations achieved in the course of this work.

It has been shown that the data obtained in this work are well represented by the empirical reduced plate height - reduced velocity equation, Equation 5.13. Further work directed to the accurate determination of the values of the parameter C for the commonly used chromatographic supports and to the establishment of the dependence of the parameter n on K' would be of value since use of Equation 5.13 would then permit the prediction of the reduced plate height - reduced velocity relationship over the range of normal operation from the experimental determination of the parameter A for the given column. The determination of A can be made from one plate height measurement in the appropriate range of reduced velocity, i.e. the range where the Av^n term is dominant in Equation 5.13.

Finally, the work described in Chapter 6 above has provided a more precise definition of the term, unretained, as applied to chromatographic solutes and has indicated the importance of determinations of column void volumes using isotopically labelled mobile phase samples.

7.2. Future Developments

Considerable progress is being made in the packing of particles in the 5 - 20 microns diameter range to give reduced plate heights close to those obtained for 50 micron diameter particles in this work. Much of this work now relates to supports of the totally porous type. As indicated in Chapter 1 above, it is

expected that the performance difference between these materials and the surface layered packings will narrow as the particle diameters are decreased. Reduced plate heights of 6 - 10 have been achieved recently by Done and Knox (95) using 20 micron alumina, by Kirkland (96) using 5 - 10 micron silica microspheres and by Majers (97) using 5 - 10 micron silica gel. It seems probable that optimum packing of particles in this size range will soon be achieved.

De Stefano and Beachell (98) have recently investigated the improved column performance arising from the "infinite diameter" effect in columns packed with 37 - 75 micron diameter Porasil. In a column which is sufficiently wide that only a small fraction of a centrally injected sample reaches the wall the band broadening due to the transcolumn velocity inequalities is eliminated. It seems likely that this effect will be useful in improving the performance of columns packed with particles of 20 microns diameter and smaller. The performance enhancement due to this effect will also be of value in the use of wide bore columns for high efficiency preparative scale LC. The higher capacities of the totally porous supports render them more suitable for this latter application than the low capacity, surface layered supports.

Further development of the chemical bonding of stationary phases to the surfaces of supports is to be expected; as yet only

Zipax^R is available with bonded stationary phases. Bonding of ion exchange materials to the high surface area silica supports would give much higher ion exchange capacity than is given by Zipax.

The silica based, controlled pore size particle may thus become the most efficient support for all modes of liquid chromatography.

When the problems of packing 2 - 5 microns diameter particles of this type have been overcome optimum performance possible with current detector technology will probably have been achieved.

APPENDIXList of Symbols

A	Coefficient of mobile phase term in plate height equations
A, A_i	Eddy diffusion contributions to the plate height
A_i	Fraction of column cross-sectional area occupied by phase i
B	Coefficient of longitudinal diffusion term in plate height equations
b	Dimensionless constant indicating the relative magnitude of transcolumn velocity variations
C	Coefficient of mass transfer terms in plate height equations
C_m	Coefficient of the sum of the plate height contributions which are directly proportional to the mobile phase velocity, in plate height equations
C_s	Coefficient of the term arising from stationary phase diffusion in plate height equations
C_k	Coefficient of the term arising from adsorption-desorption kinetics in plate height equations
c_i'	Solute concentration in phase i , referred to unit volume of phase i
c_m', c_s'	Values of c_i' for the mobile and stationary phases, respectively
c_i^{*}	Equilibrium value of c_i'
c	Solute concentration, referred to unit volume of the column
c_i	Solute concentration in state i , referred to unit volume of the column
c_m, c_s	Values of c_i for the mobile and stationary phases, respectively

c_i^*	Equilibrium value of c_i
D	Diffusion coefficient or effective diffusion coefficient
D_m, D_s	Diffusion coefficients in the mobile and stationary phases, respectively
d_s	Depth of the stationary phase
d_j	Depth of the unit, j , of the stationary phase
d_p	Particle diameter
d_c	Column diameter
F_i	Dimensionless free energy of adsorption of sites i
ΔF_1	The free energy change due to adsorption of the solute
$(F_i)_j$	Partial molar free energy of the group i of the solute molecule in phase j
$(\Delta F_2)_i$	Free energy of adsorption of the group, i , of the solute molecule
GC	Gas chromatography
H	Height equivalent to a theoretical plate
H_j	Contribution to H from the j th process
h	Reduced plate height
J	Solute flux through a column cross-section
ΔJ	Component of J arising from nonequilibrium
K'	Column capacity ratio
K	Distribution coefficient (stationary phase concentration/ mobile phase concentration)
K_i	Equilibrium constant for adsorption on sites i
K_c	Overall equilibrium constant for adsorption on all sites

K_o	Limiting value of K_c at low solute concentration
k_a, k_d	First order rate constants governing adsorption and desorption, respectively
k_{ij}	First order rate constant for transition from state i to state j
LC	Liquid chromatography
L	Column length
l	Step length of random walk
M_2	Molecular weight of the mobile phase
n	Number of random walk steps
n	Exponent of v in the mobile phase term of empirical reduced plate height-reduced velocity equation
n_2^s, n_2^m	Numbers of moles of solute in the stationary and mobile phases, respectively
n^i	Number of moles of phase i
P	Pressure
P^*	Standard pressure
$\bar{P}$	Mean pressure in column
Q_i	Free energy of adsorption term for group i
Q_i^o	Value of Q_i when adsorbent is fully activated
q_j	Second order adsorption free energy term correcting Q_i for the presence of group j
q_j^o	Value of q_j when the adsorbent is fully activated
q	Stationary phase configuration factor
q_j	Configuration factor for the unit j of the stationary phase
R	Ratio of solute migration velocity to the mobile phase

velocity; equivalently, the fraction of the solute molecules in the mobile phase at equilibrium

S	Distance moved by solute molecule with approximately constant velocity
s_i	Rate of solute accumulation in state i
s_i'	Local value of s_i
T	Temperature ($^{\circ}\text{K}$)
t_R	Retention distance of peak (from chart record)
t_e	Exchange time between flow streams of different velocity
t_a, t_d	Mean times of adsorption and desorption, respectively
t_m, t_s	Times spent by solute molecule in mobile and stationary phases, respectively
t_D	Time for which diffusion occurs
V	Mean zone velocity
V_g	Specific retention volume
V_m	Retention volume of an unretained solute
V_R	Retention volume of a retained solute
V_n	Net retention volume
V_o	Equivalent retention volume
V_a	Total volume of adsorbed phase in column
V_c	Volume of adsorbed phase per unit weight of adsorbent
V_s	Volume of stationary phase in column
V_i	Volume of phase i per unit volume of column
V_j	Volume of the unit j of the stationary phase
V_s'	Volume of the stationary phase per unit volume of column

v	Mobile phase linear velocity
v_i	Velocity of solute in state i
v_i'	Local value of v_i
v_m^o, v_s^o	Molar volumes of mobile and stationary phases, respectively
v_2^o	Molar volume of pure solute
$\bar{v}_2^i$	Partial molar volume of solute in solution i
W	Weight of adsorbent in column
w_o	Baseline width of solute peak
$w_{1/2}$	Width, at half-height, of solute peak
w_s	Weight of stationary phase in column
X_i	Fraction of solute in state i
X_i^*	Equilibrium value of X_i
x_2^i	Mole fraction of solute in solution in phase i
α	Relative retention of two solutes
α	Function of the percent water deactivation of an adsorbent
γ	Obstructive factor for diffusion
γ_s	Stationary phase obstructive factor
γ_2^m, γ_2^s	Solute activity coefficients in the mobile and stationary phases, respectively
$\gamma_2^{m,\infty}, \gamma_2^{s,\infty}$	Solute activity coefficients at infinite dilution in the mobile and stationary phases, respectively
$\gamma_2^{th}, \gamma_2^{ath}$	Thermal and athermal contributions, respectively, to the solute activity coefficient
δ_i	The ratio of the partial molar volume of the group i of the solute molecule to the molar volume of the mobile phase

ϵ	Equilibrium departure term
ϵ_m, ϵ_s	Mobile and stationary phase equilibrium departure terms, respectively
ϵ'_m, ϵ'_s	Local values of ϵ_m and ϵ_s
$\hat{\epsilon}_m, \hat{\epsilon}_s$	Dimensionless equilibrium departure terms
ϵ_{ab}	Eluent polarity parameter, proportional to the difference in adsorption energies of eluents a and b
ϵ_{ab}^o	Value of ϵ_{ab} for fully activated adsorbent
ϵ^o	Value of ϵ_{ab}^o when n-pentane is reference eluent
θ_i	Fraction of sites i occupied by solute molecules
θ	Fraction of all sites occupied by solute molecules
$\theta, \theta_o, \theta_j$	Nonequilibrium parameters
$\hat{\theta}_j$	Dimensionless nonequilibrium parameter
λ, λ_i	Eddy diffusion coefficients
μ_2^i	Solute chemical potential in solution in phase i
$\mu_2^{i'}$	Solute chemical potential in ideal solution in phase i
$\mu_2^{o,i}$	Chemical potential of pure solute
$\mu_2^{*,i}$	Chemical potential of pure solute at standard pressure
v	Reduced velocity
ρ_m, ρ_s	Densities of the mobile and stationary phases, respectively
ρ_{xy}	Correlation coefficient of variables x and y
ρ	Column to particle diameter ratio
σ	Standard deviation of zone, in length units
Ω	Dimensionless nonequilibrium term, $= CD_m/d_p^2$

ω	Coefficient of mobile phase plate height contribution, $H = \omega d_p^2 v / D_m$
ω_i, ω_l	etc. Values of ω for particular processes in the mobile phase
ω_a	Ratio of distance between velocity extremes to d_p
ω_β	Ratio of difference between velocity extremes to the mean velocity
ω_λ	Ratio of distance of velocity persistence distance, S , to d_p

REFERENCES

1. M. Tswett: Ber.deut.botan.Ges., 24, 316, 384, (1906).
2. R. Kuhn, E. Lederer and A. Winterstein: Hoppe-Seyler's Z. physiol. Chem. 197, 141, (1931).
3. A.J.P. Martin and R.L.N. Synge: Biochem. J., 35, 1358, (1941).
4. R. Consden, A.H. Gordon and A.J.P. Martin: Biochem.J. 38, 224, (1944).
5. J.C. Kirchner, J.M. Miller and G.J. Keller: Anal. Chem. 23, 420, (1951).
6. A.T. James and A.J.P. Martin: Biochem.J., 50, 679, (1952).
7. A. Tiselius: Arkiv.Kemi. Mineral Geol. 143, 5, (1940); A16, 11, (1943).
8. S. Claesson: ibid., 23A, 133, (1946).
9. S. Claesson: ibid., 24A, 7, (1946).
10. W.L. Turner: Nat. Petrol News, 35, 234, (1943).
11. C.S.G. Phillips: Disc. Faraday Soc. 7, 241, (1949).
12. J.Janak: Chem. Listy, 47, 464, 817, 1184, (1953).
13. A. T. James and A.J.P. Martin: Analyst, 77, 952, (1952).
14. D.H. James and C.S.G. Phillips: J. Chem.Soc. 1600, (1953); 3446, (1956).
15. N.H. Ray: J. Appl. Chem. 4, 21, 82, (1954).
16. E. Glueckauf. Trans. Faraday Soc. 51, 34, 1540, (1955).
17. E. Creemer and R. Mueller: Z. Electrochem. 55, 217, (1951).
18. A.A. Zhukhovitskii, O.V. Zolotoreux, V.A. Sokolov and N. M. Turkel'taub: Doklady Akad. Nauk., U.S.S.R. 77, 435, (1951).
19. H.W. Patten, J.S. Lewis and W.I. Kaye: Anal. Chem., 27, 170, (1955).

20. J. Harley, W. Nel and V. Pretorius: *Nature*, 181, 177, (1958).
21. I.G. McWilliam and R.A. Dewar: *ibid.*, 182, 1664, (1958).
22. I.G. McWilliam and R.A. Dewar in "Gas Chromatography 1958", D.H. Desty, Ed., Acad. Press, N. Y. (1958), p. 192.
23. J.E. Lovelock: *J. Chromatog.*, 1, 35, (1958).
24. J.E. Lovelock: *Nature*, 181, 1460, (1958).
25. J.E. Lovelock: *ibid.*, 182, 1063, (1958).
26. J.E. Lovelock in "Gas Chromatography 1960", R.P.W. Scott, Ed., Butterworths, Washington, D.C., 1960, p. 16.
27. J.N. Wilson: *J. Amer. Chem. Soc.*, 62, 1583, (1940).
28. D. deVault: *ibid.*, 65, 532, (1943).
29. J. Weiss: *ibid.*, 65, 297, (1943).
30. H.C. Thomas: *Ann. N. Y. Acad. Sci.* 49, 161, (1948).
31. L. Lapidus and N.R. Amundsen: *J. Phys. Chem.* 56, 984, (1952).
32. J.J. van Deemter, F.J. Zuiderweg and A.A. Klinkenberg: *Chem. Eng. Sci.* 5, 271, (1956).
33. M.J.E. Golay in "Gas Chromatography 1958", D.H. Desty, Ed, Acad. Press, New York, (1958), p. 36.
34. M.A. Khan: *Nature*, 186, 800 (1960).
35. M.A. Khan in "Gas Chromatography 1962", M. van Swaay, Ed., Butterworths, Washington, D.C., 1962, p. 3.
36. J.C. Giddings: *J. Chem. Phys.* 31, 1462, (1959).
37. J.C. Giddings: "Dynamics of Chromatography", Part 1, Marcel Dekker, Inc., New York, N. Y., 1965.
38. J.C. Giddings and P.D. Schettler: *J. Phys. Chem.*, 73, 2577, (1969).
39. J.C. Giddings and P.D. Schettler: *ibid.*, 73, 2582, (1969).

40. J.C. Giddings: Anal. Chem., 35, 2215, (1963).
41. W.H.T. Davidson, S. Sloney and A.L. Wragg: Chem. and Ind. (London) 1954, 1356.
42. J. Janak: Nature, 185, 684, (1960).
43. J. Janak: Coll. Czech. Chem. Comm. 25, 1780, (1960).
44. J.C. Giddings: J. Chromatog. Sci., 7, 276, (1969).
45. N.A. Ismailov and M.S. Schraiber: Formatsiya, 3, 1, (1938).
46. J.E. Mainhard and N.F. Hall: Anal. Chem., 21, 185, (1949).
47. S. Ruheman J. Chem. Soc. 17, 1438, 2025 (1910).
48. D.A. Spackman, W.H. Stein and S. Moore: Anal. Chem. 30, 1190, (1958).
49. S.W. Mayer and E.R. Tompkins: J. Amer. Chem. Soc., 69, 2866, (1947).
50. P.B. Hamilton: Anal. Chem., 30, 914, (1958).
51. J.H. Knox and M. Saleem: J. Chromatog. Sci., 7, 614, (1969).
52. J.G. Koen, J.F.K. Huber, H. Poppe and G. den Boef: ibid., 8, 192, (1970).
53. M.N. Monk and D.N. Ravel: ibid., 7, 48, (1969).
54. T.B. Davenport: J. Chromatog., 42, 219, (1969).
55. M.J.E. Golay in "Gas Chromatography 1960", R.P.W. Scott, Ed., Butterworths, Washington, D.C., 1960, p. 139.
56. D.S. Horne, J.H. Knox and L. McLaren: Separation Science, 1, 531, (1966).
57. I. Halasz and C. Horvath: Anal. Chem., 36, 1178, (1964); 36, 2226, (1964).
58. J.J. Kirkland: J. Chromatog. Sci., 7, 7, (1969).
59. J.J. Kirkland: ibid., 10, 129, (1972).

60. D.C. Locke and D.E. Martire: Anal.Chem., 39, 921, (1967).
61. D.E. Martire and D.C. Locke: ibid., 43, 68, (1971).
62. A.J. Ashworth and D.H. Everett: Trans. Faraday Soc. 56, 1609, (1960).
63. P.J. Flory: J.Chem.Phys. 10, 51, (1942).
64. M.L. Huggins: Ann. N.Y. Acad.Sci., 43, 1, (1942).
65. G.R. Luckhurst and D.E. Martire: Trans. Faraday Soc., 65, 1248, (1968).
66. R.A. Keller, B.L. Karger and L.R. Snyder in "Gas Chromatography 1970", R. Stock, Ed., Inst. of Petroleum, London, 1970, p125.
67. L.R. Snyder: J.Chromatog., 5, 430, (1961).
68. L.R. Snyder: ibid., 6, 22, (1961).
69. J.H. Knox and L. McLaren: Anal.Chem., 36, 1477, (1964).
70. E.R. Bennet and W.E. Bolch: ibid., 43, 55, (1971).
71. W.L. Jones: ibid., 33, 829, (1961).
72. A.A. Klinkenberg and F. Sjenitzer: Chem.Eng.Sci., 5, 258, (1956).
73. A.I.M. Keulemans and A. Kwantes in "Vapour Phase Chromatography", D.H. Desty, Ed., Butterworths, London, 1956, p15.
74. A.I.M. Keulemans in "Gas Chromatography 1961", Reinhold, N.Y., 1961.
75. J.C. Giddings and H. Eyring: J.Phys.Chem., 59, 416, (1955).
76. J.C. Giddings: J.Chem.Phys., 26, 159, (1957).
77. J.H. Benyon, S. Clough, D.A. Crooks and G.R. Lester: Trans. Faraday Soc. 54, 705, (1958).
78. D.A. McQuarrie: J.Chem.Phys., 38, 437, (1963).

79. A.B. Littlewood in "Gas Chromatography 1964", A. Goldup, Ed., Inst. of Petroleum, London, 1965, p77.
80. J.H. Knox and J.F. Parcher: Anal. Chem., 41, 1599 (1969).
81. S.T. Sie and N. van den Hoed: J. Chromatog. Sci., 7, 257, (1969).
82. J.J. Kirkland: Anal. Chem., 40, 391, (1968).
83. J.H. van Dijk: J. Chromatog. Sci., 10, 31, (1972).
84. R.P.W. Scott and J.G. Lawrence: ibid., 8, 65, (1970).
85. C.R. Wilke and P. Chang: Amer. Inst. Chem. Engr. J., 1, 264, (1955).
86. J.H. Knox: Anal. Chem., 38, 253, (1966).
87. J.F.K. Huber in "Advances in Chromatography 1969", A. Zlatkis, Ed., Preston Technical Abstracts Co., Evanston, Ill., 1969, p283.
88. J.N. Done and J.H. Knox: J. Chromatog. Sci., 10, 606, (1972).
89. J.N. Little, D.F. Horgan and K.J. Bombaugh: ibid., 8, 625, (1970).
90. A.J. deVries, M. lePage, R. Beau and C.L. Guillemin: Anal. Chem., 39, 935, (1967).
91. A.M. Filbert and M.L. Hair: J. Chromatog. Sci., 7, 72, (1969).
92. Pechiney St. Gobain, French Patent, 1, 493, 240, (1967).
93. J.C. Giddings: Anal. Chem., 34, 1186, (1962).
94. L.R. Snyder: "Principles of Adsorption Chromatography", Marcel Dekker Inc., N.Y., 1968.
95. J.N. Done: unpublished results.
96. J.J. Kirkland: J. Chromatog. Sci., 10, 593, (1972).
97. R.E. Majers: Anal. Chem., 44, 1722, (1972).
98. J.J. deStefano and H.C. Beachell: ibid., 10, 654, (1972).

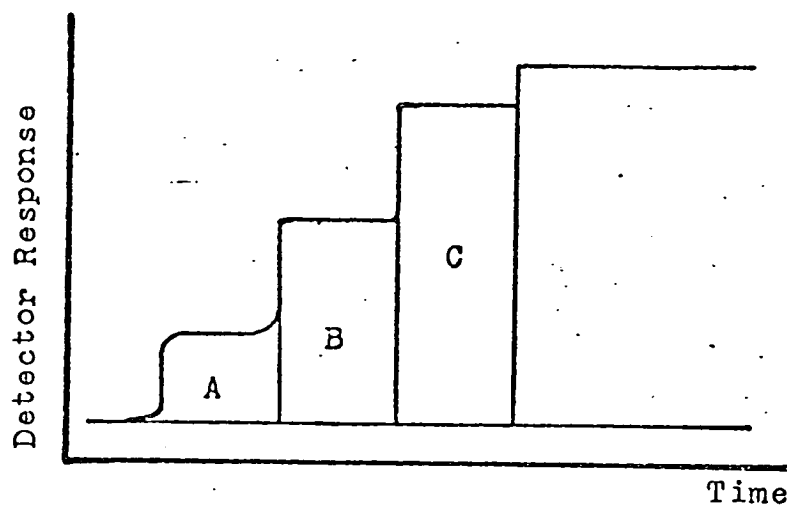


Figure 1: Displacement development of a 3-component mixture

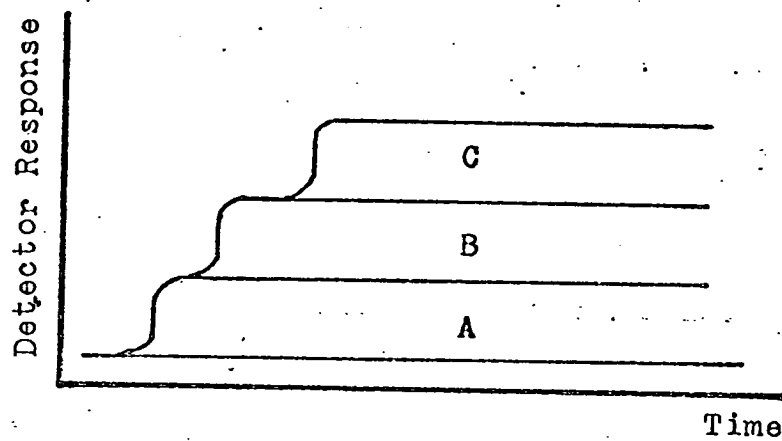


Figure 2: Frontal analysis of a 3-component mixture

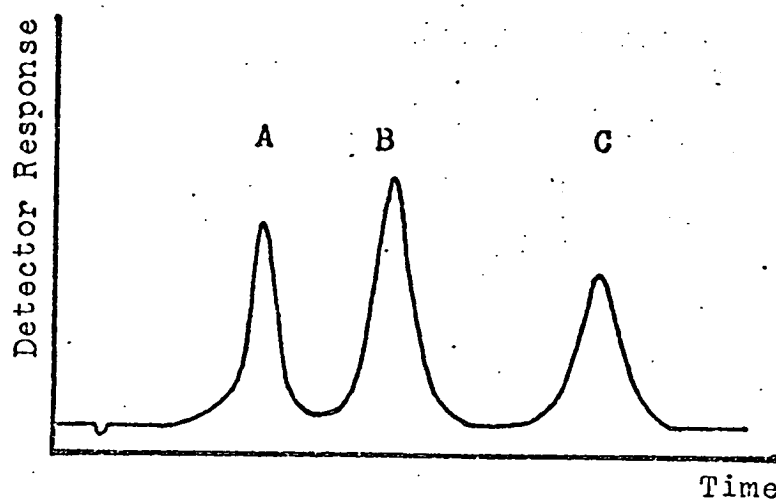


Figure 3: Elution analysis of a 3-component mixture

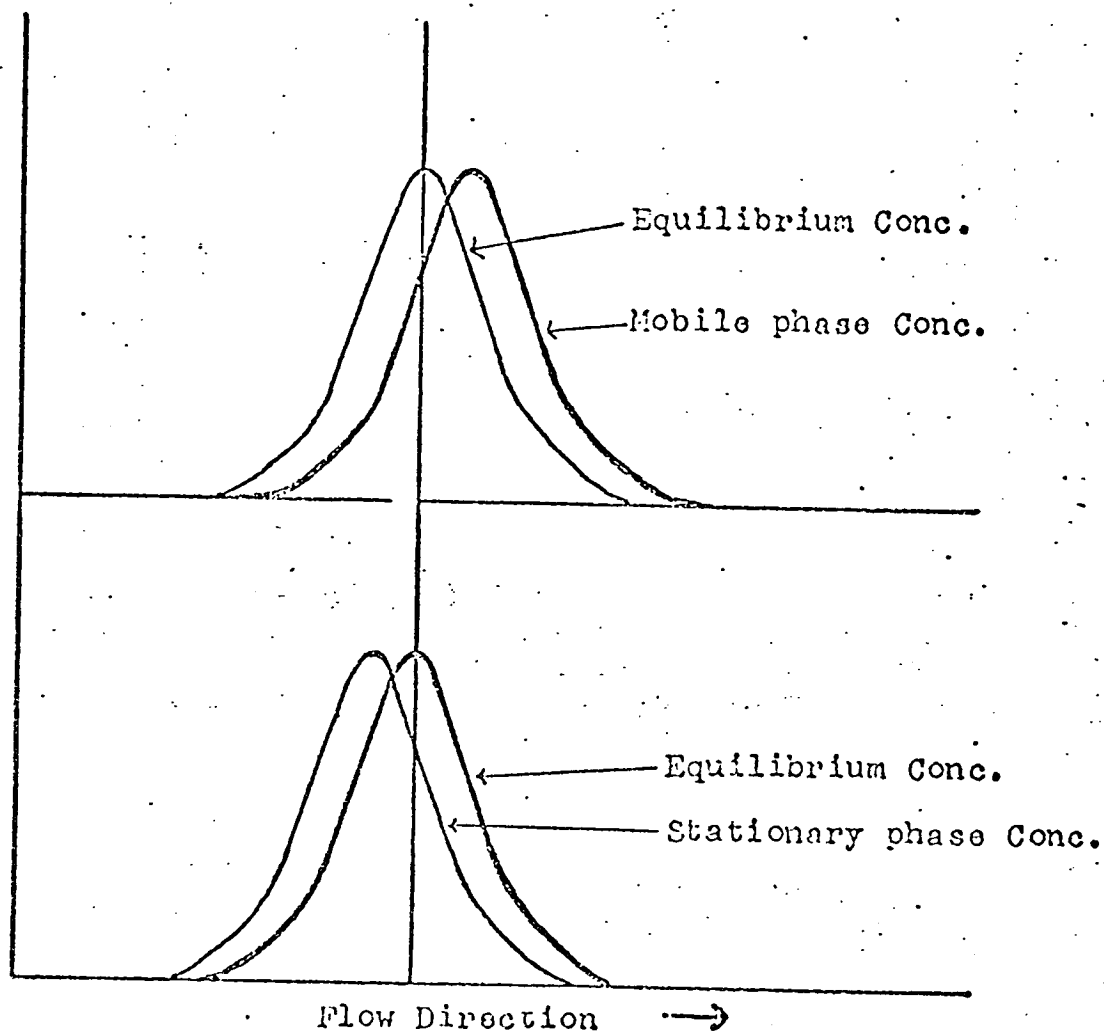


Figure 4: Relation of Stationary and mobile phase concentration profiles to the equilibrium profile.

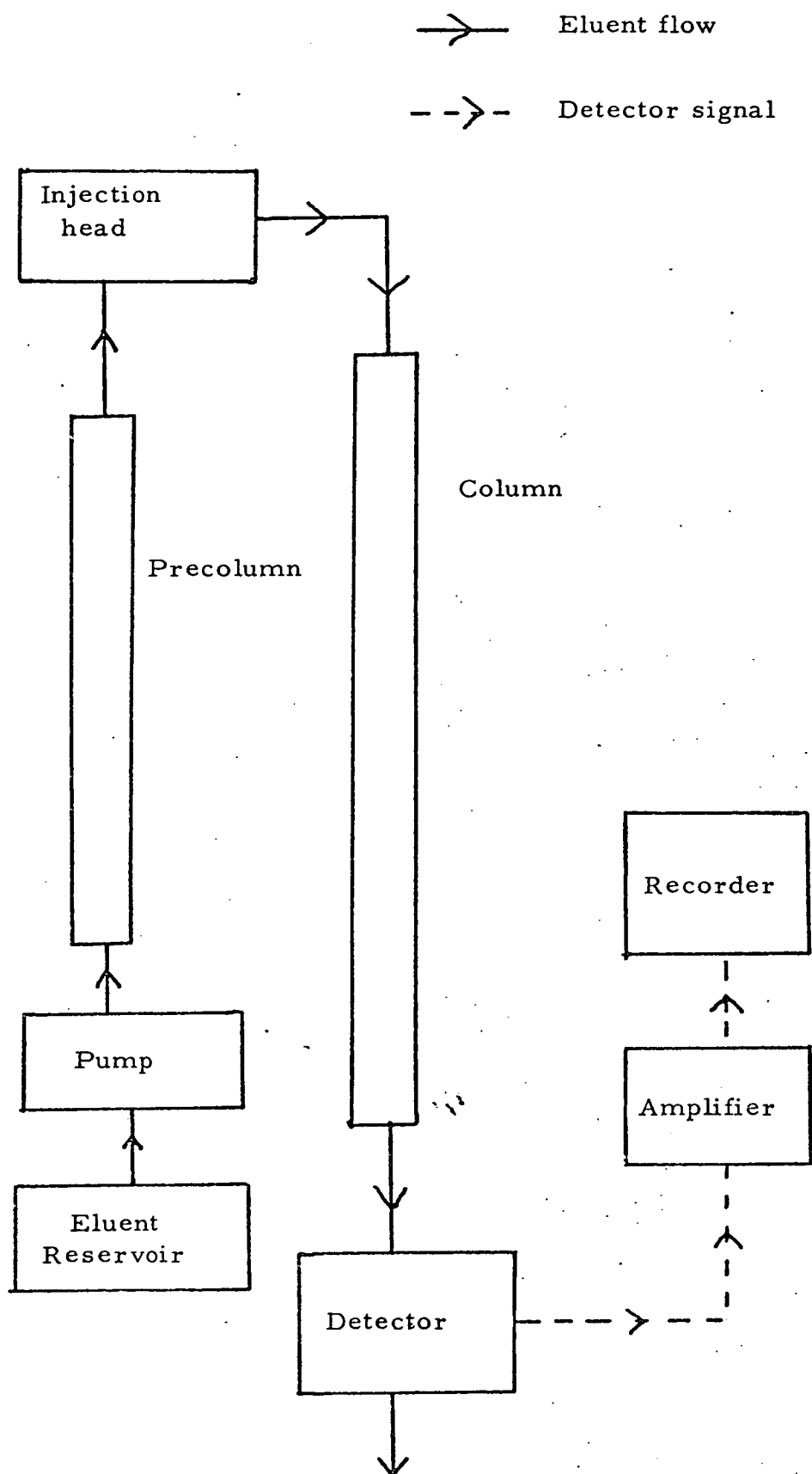


Figure 5: Schematic of Chromatographic system

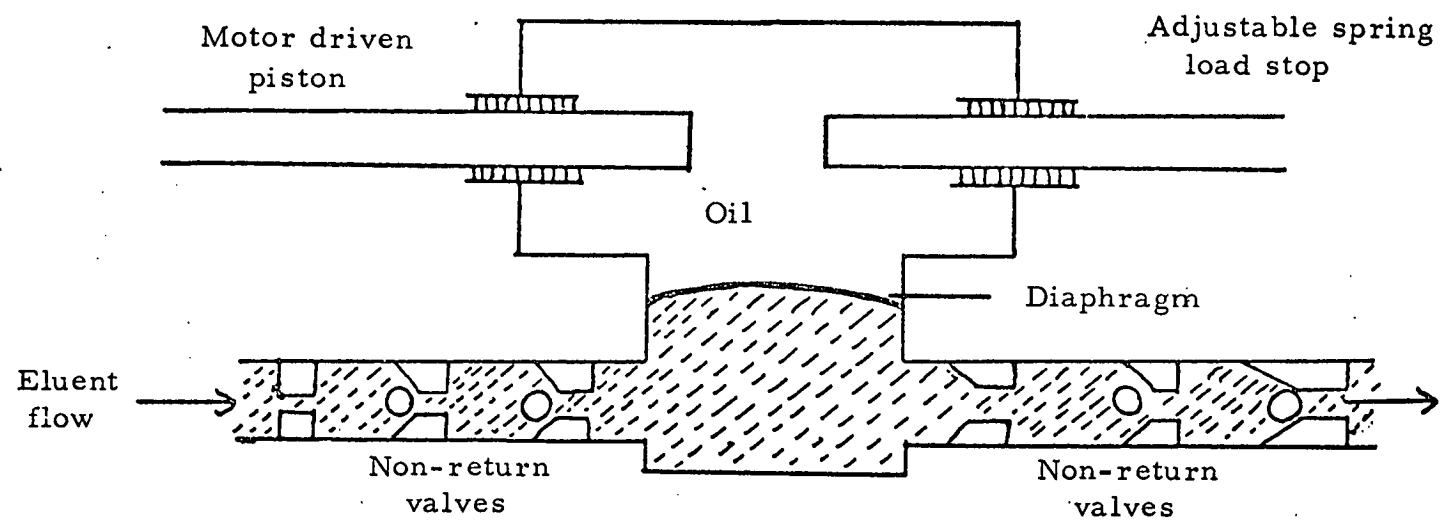


Figure 6: Schematic of Orlita pump

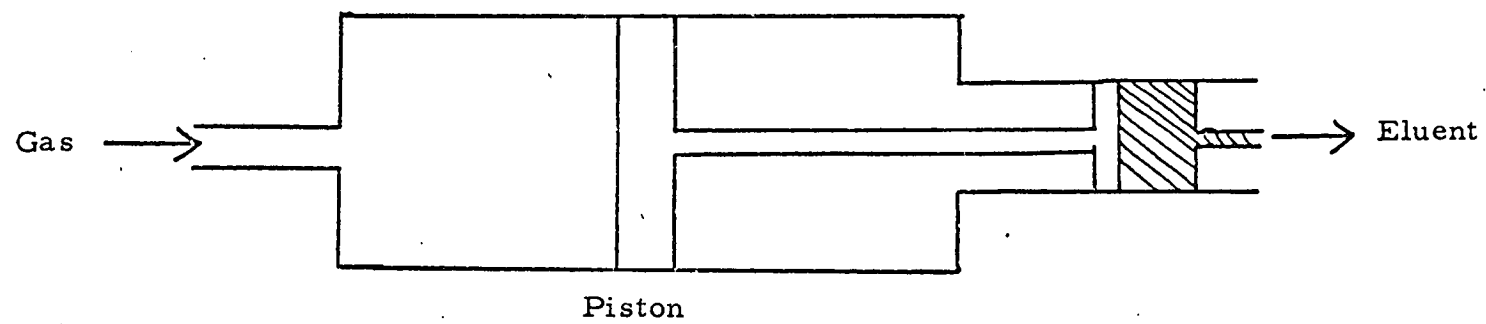


Figure 7: Schematic of Haskel pressure intensifier

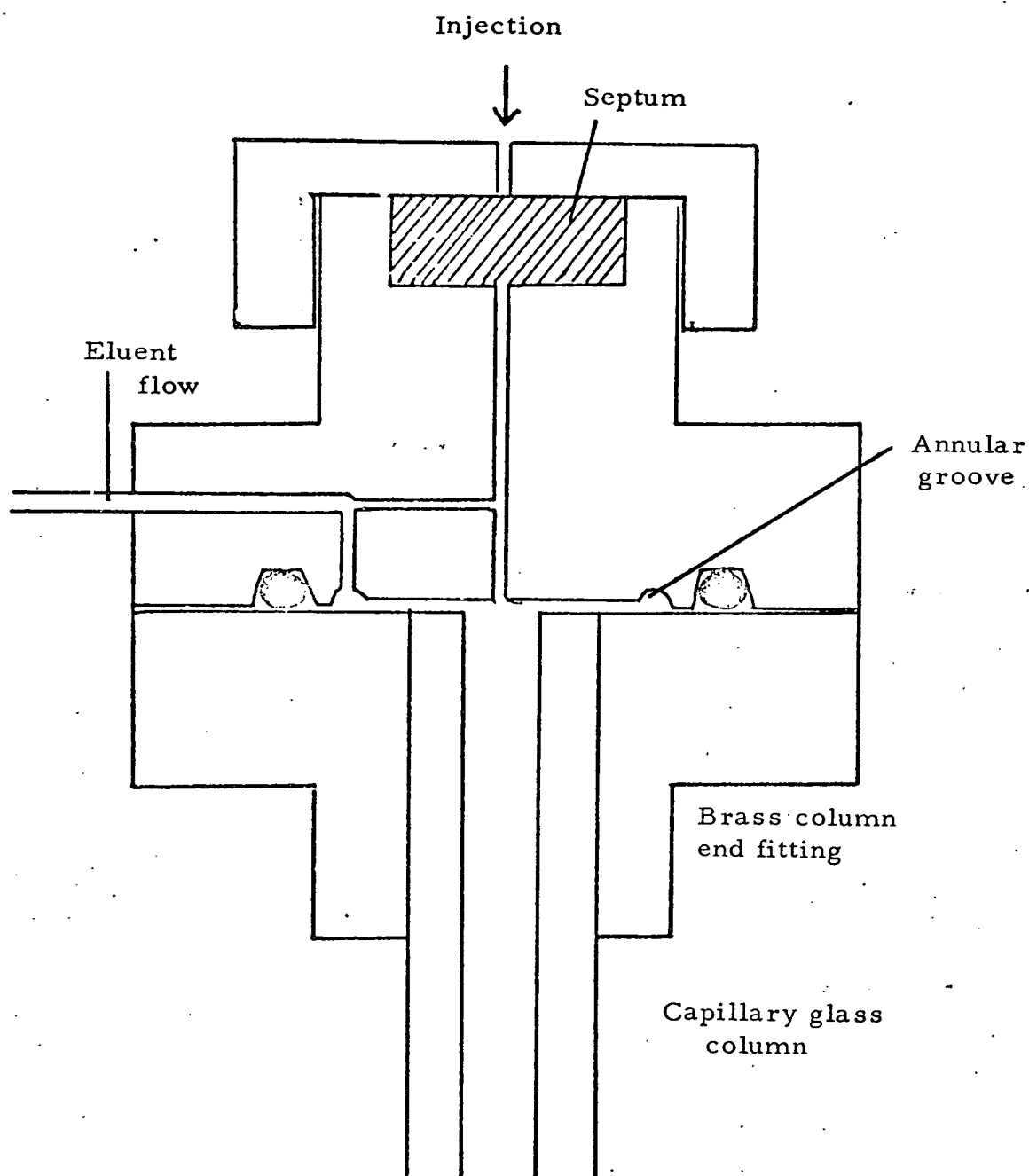


Figure 8: Schematic of injection head and column end fitting

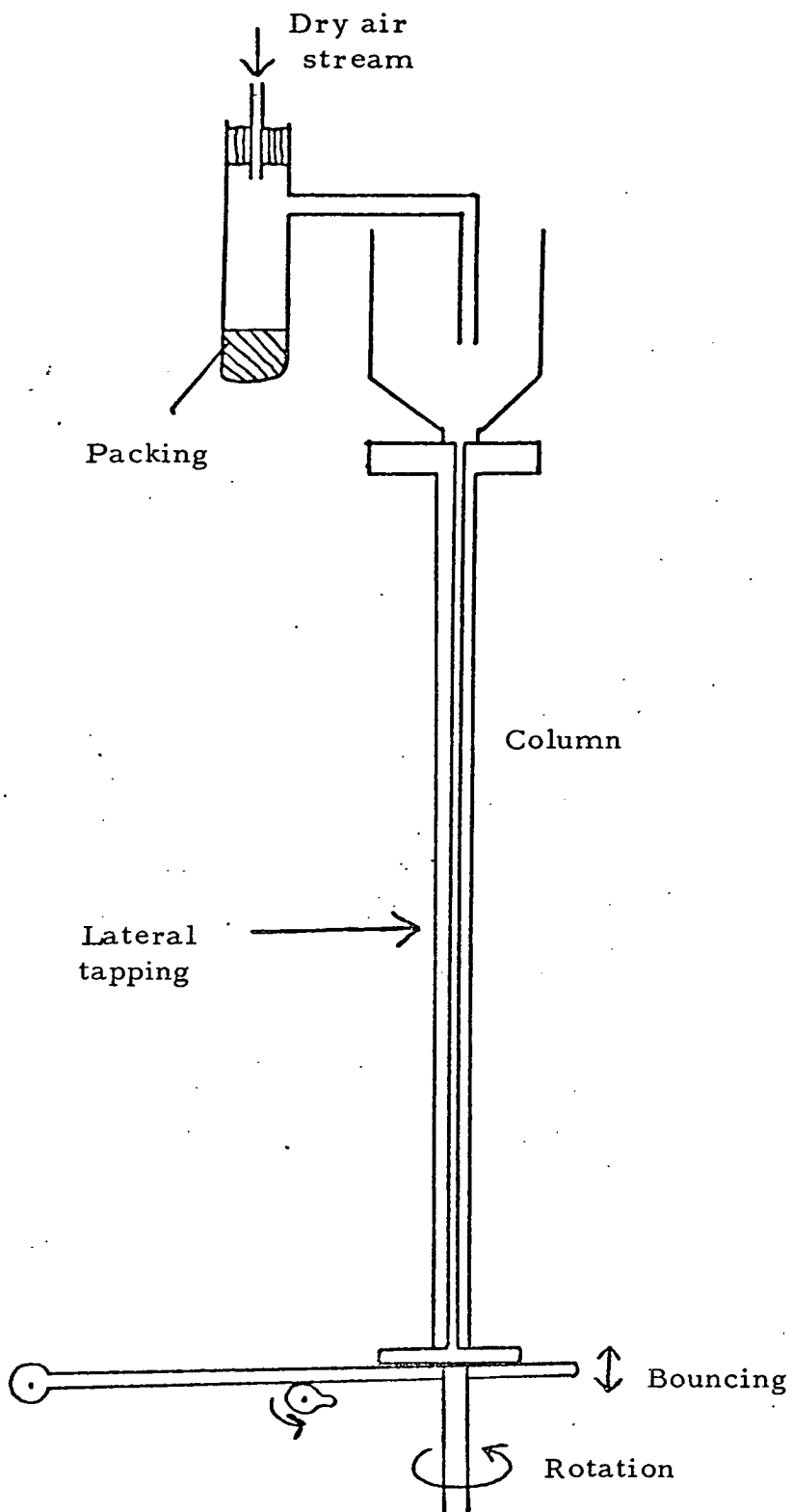


Figure 9: Column packing device

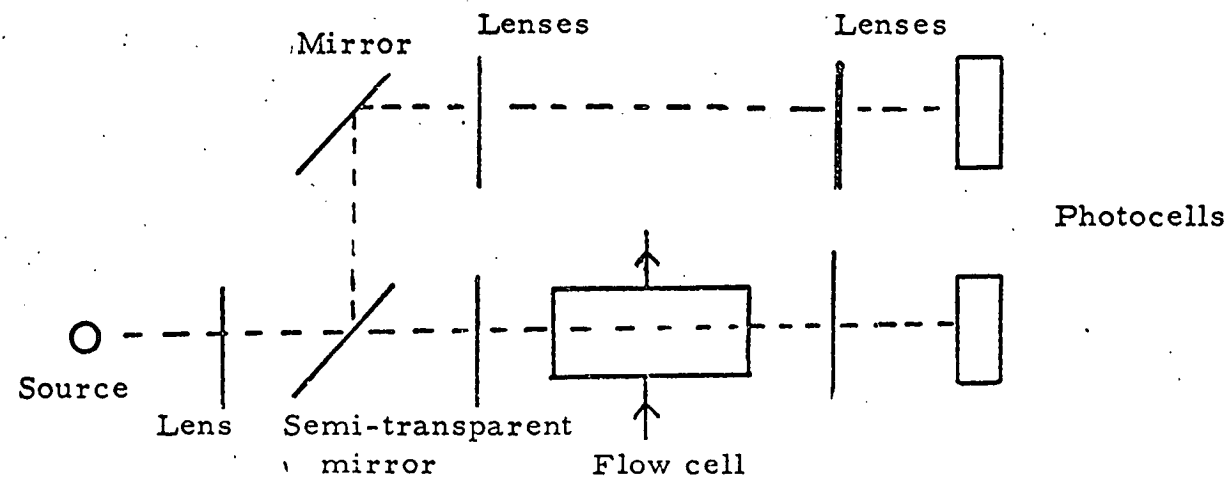


Figure 10: Schematic of u-v detector

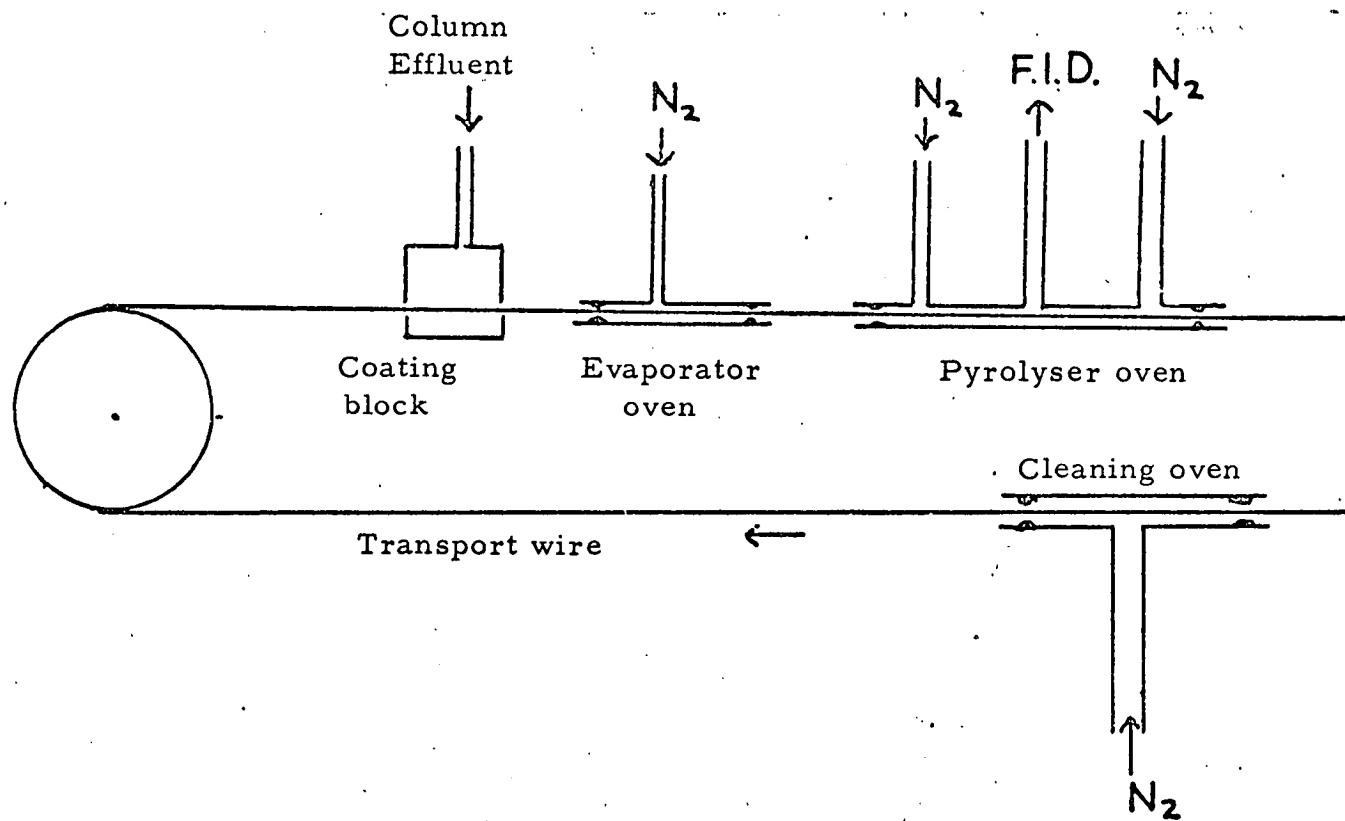


Figure 11: Schematic of wire transport detector

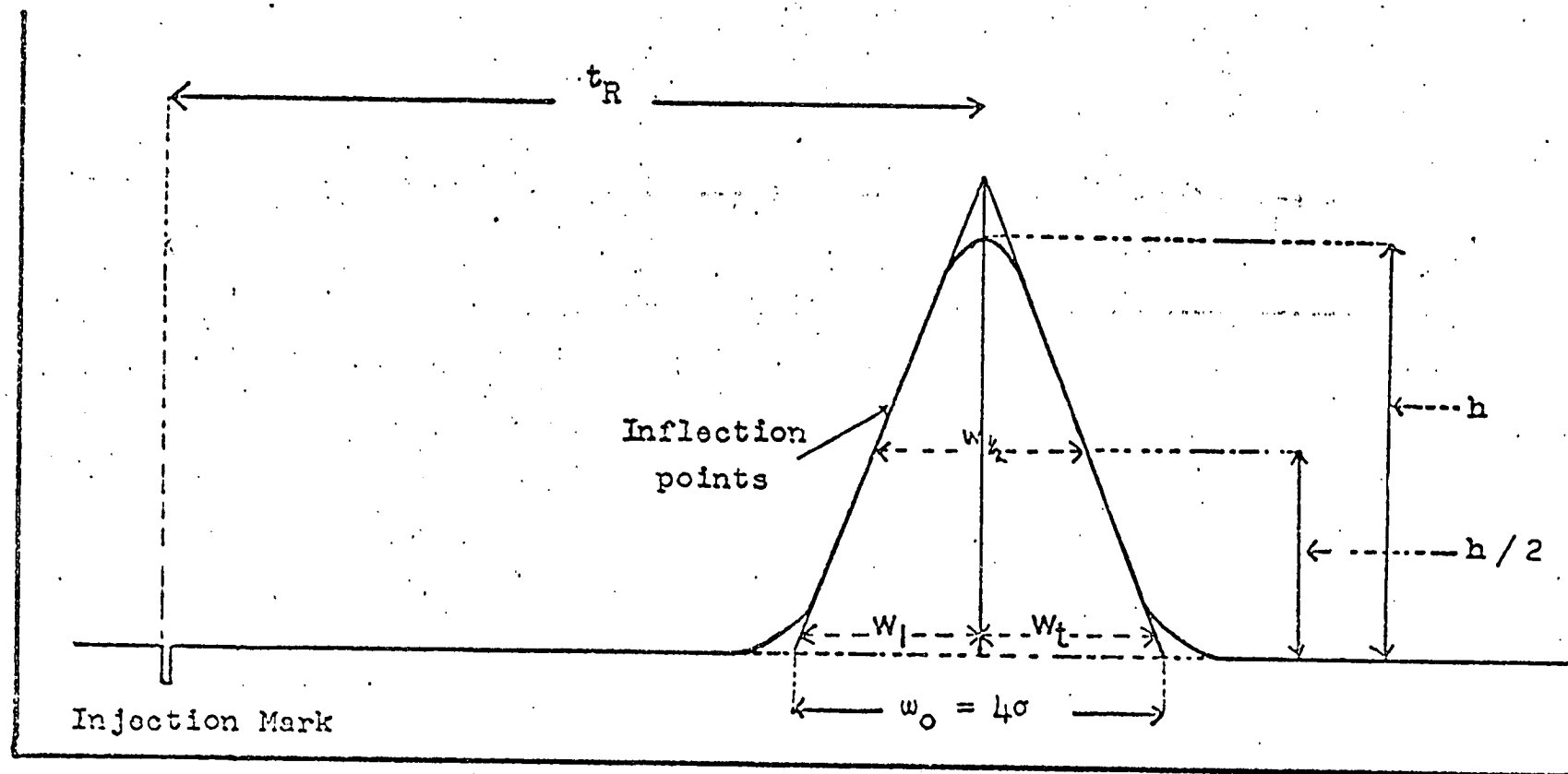


Figure 12: Elution record for injection of one solute

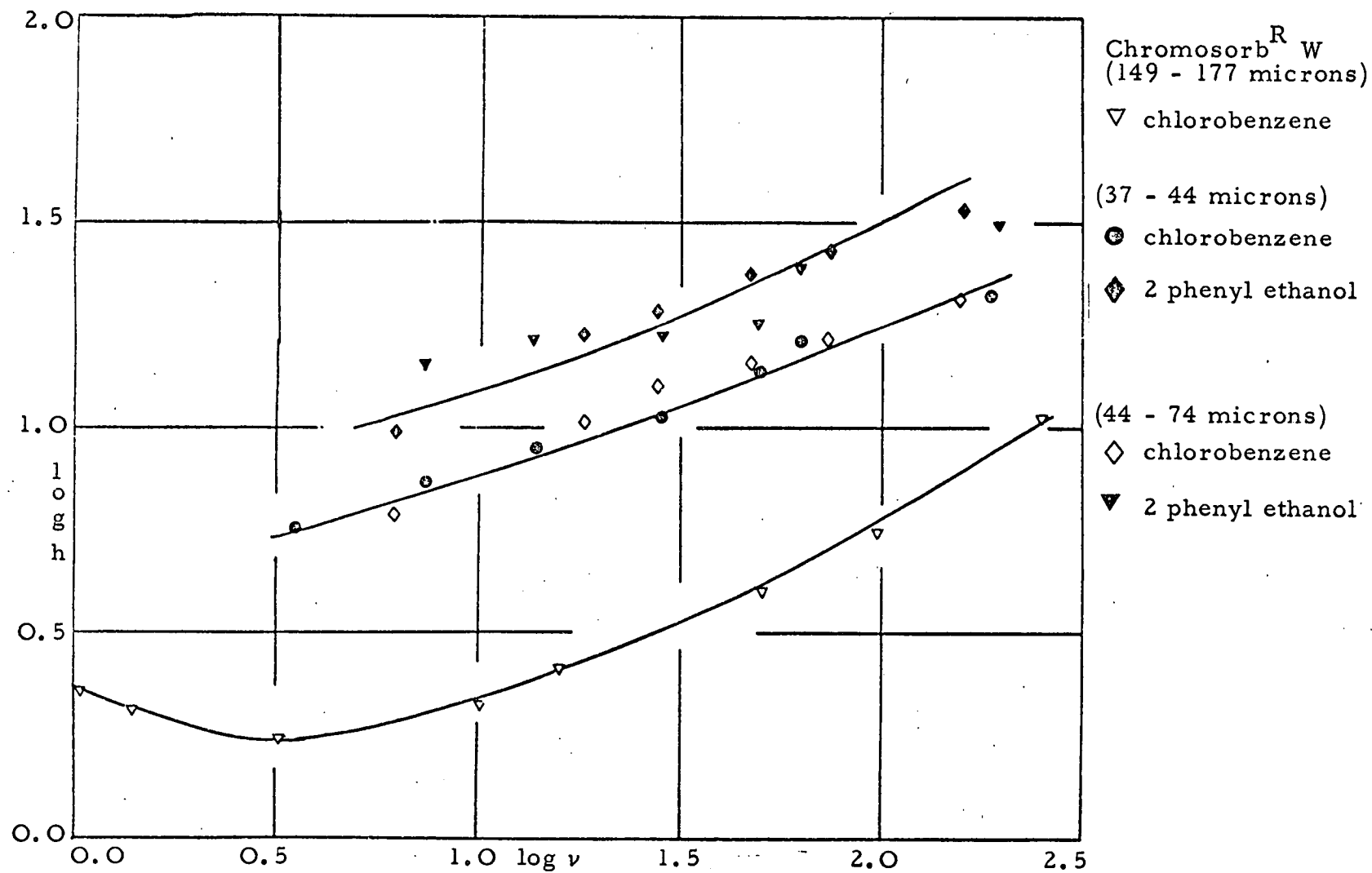


Figure 13: log h vs log ν, Chromosorb^R W

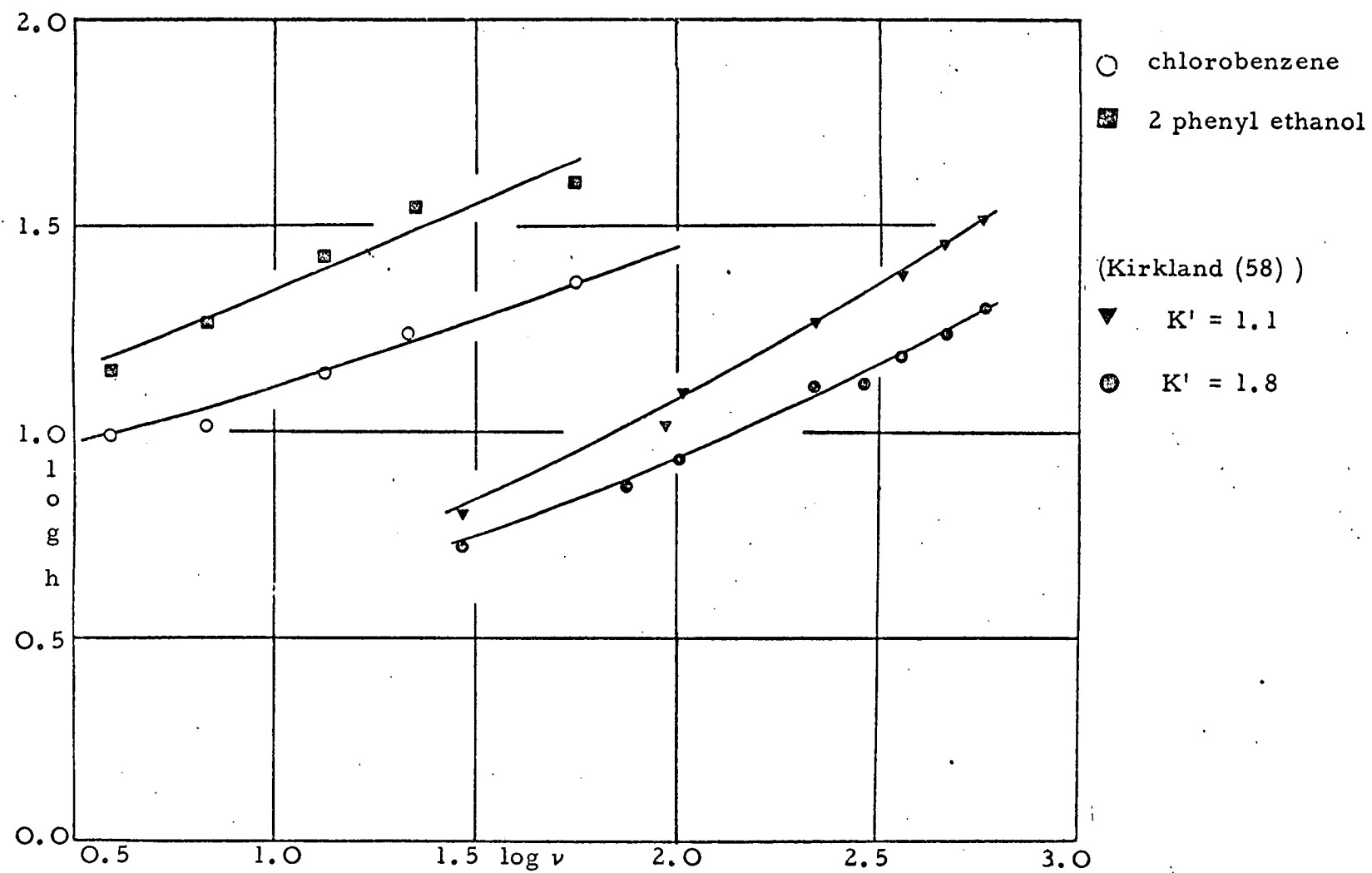


Figure 14: log h vs. log ν , Chromosorb^R P

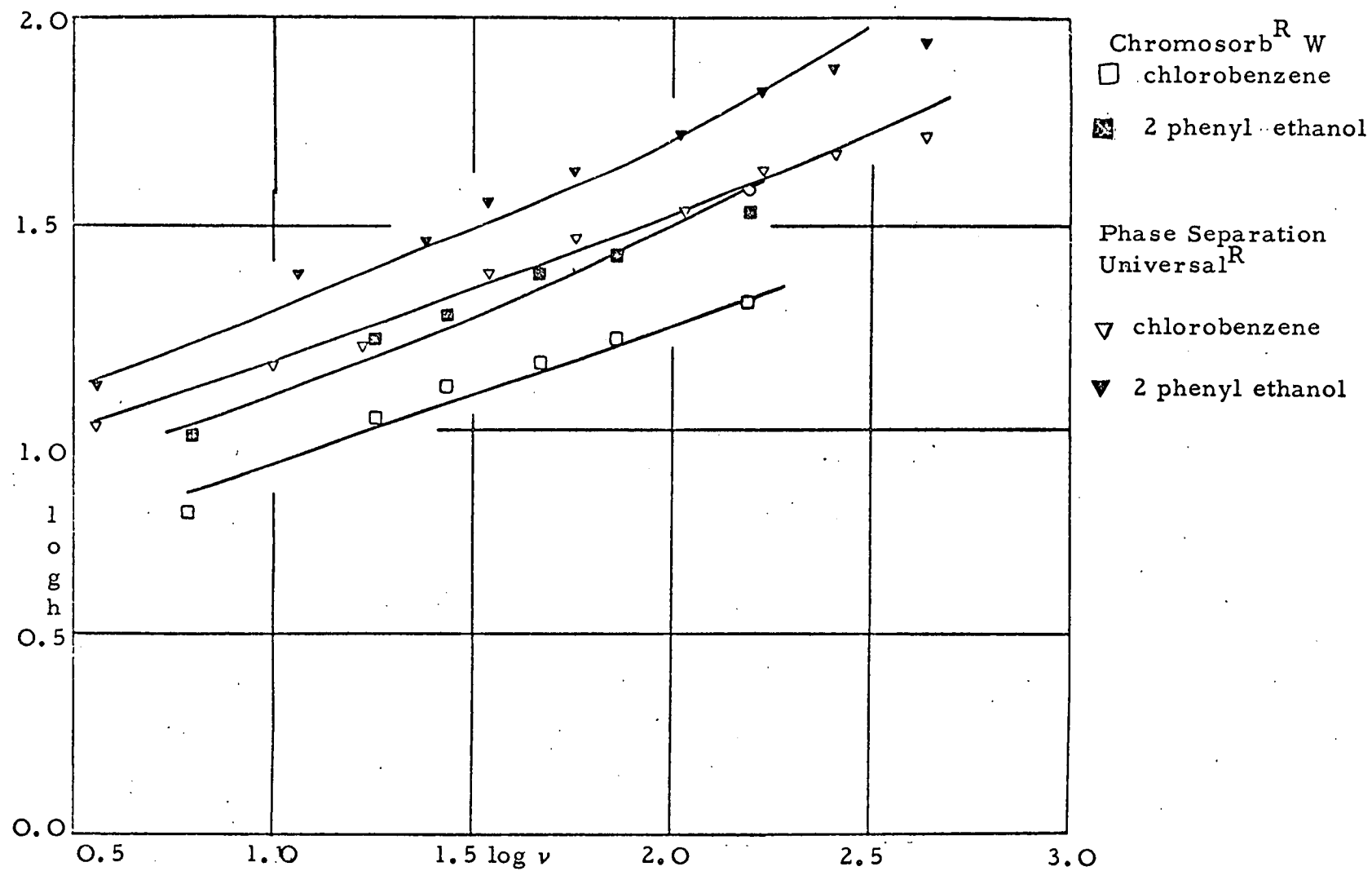


Figure 15: $\log h$ vs. $\log \nu$ Chromosorb^R W and Phase Separation Universal^R

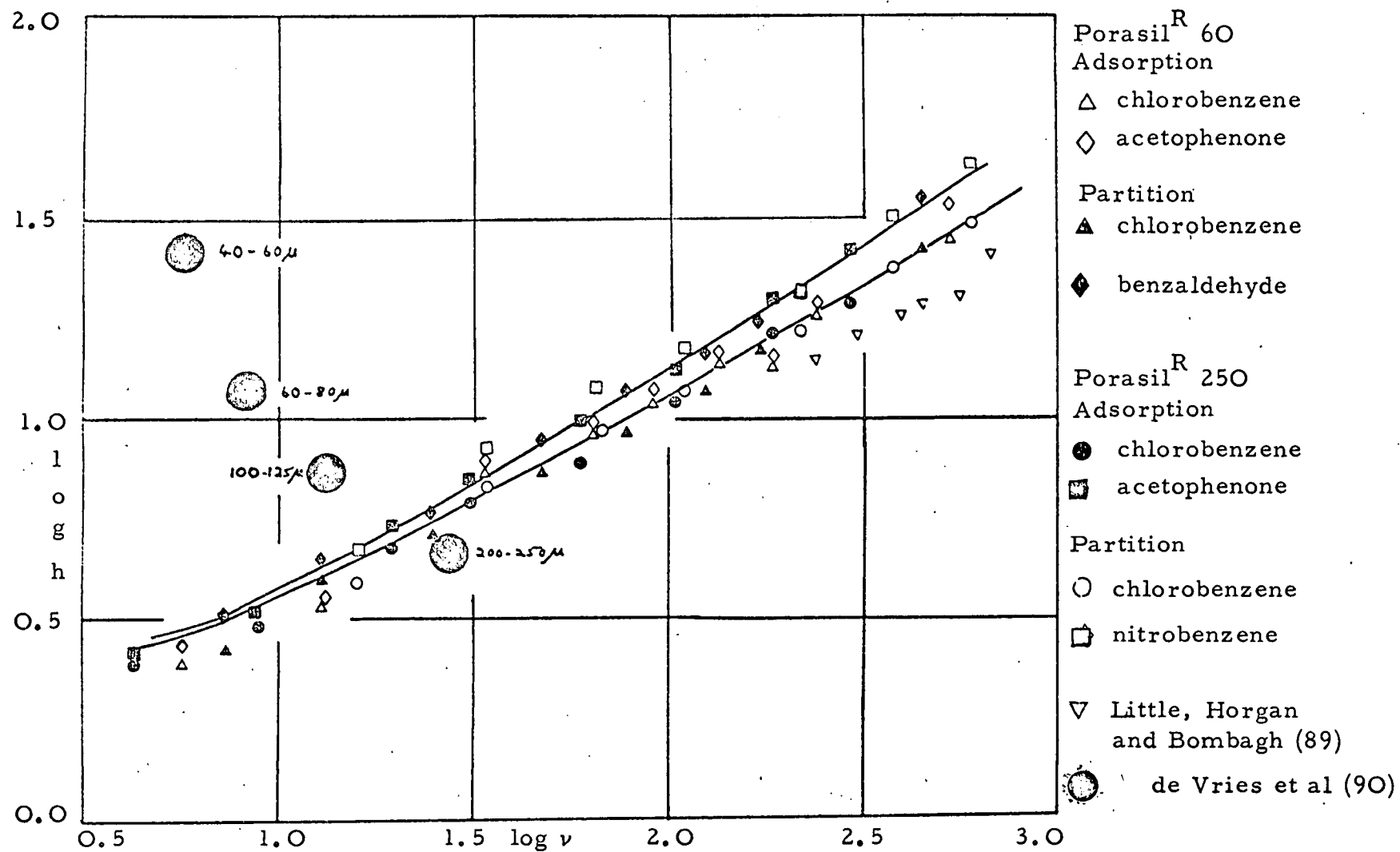


Figure 16: Porasil^R PS 60 and PS 250 (44 - 53 microns) $\log h$ vs $\log v$

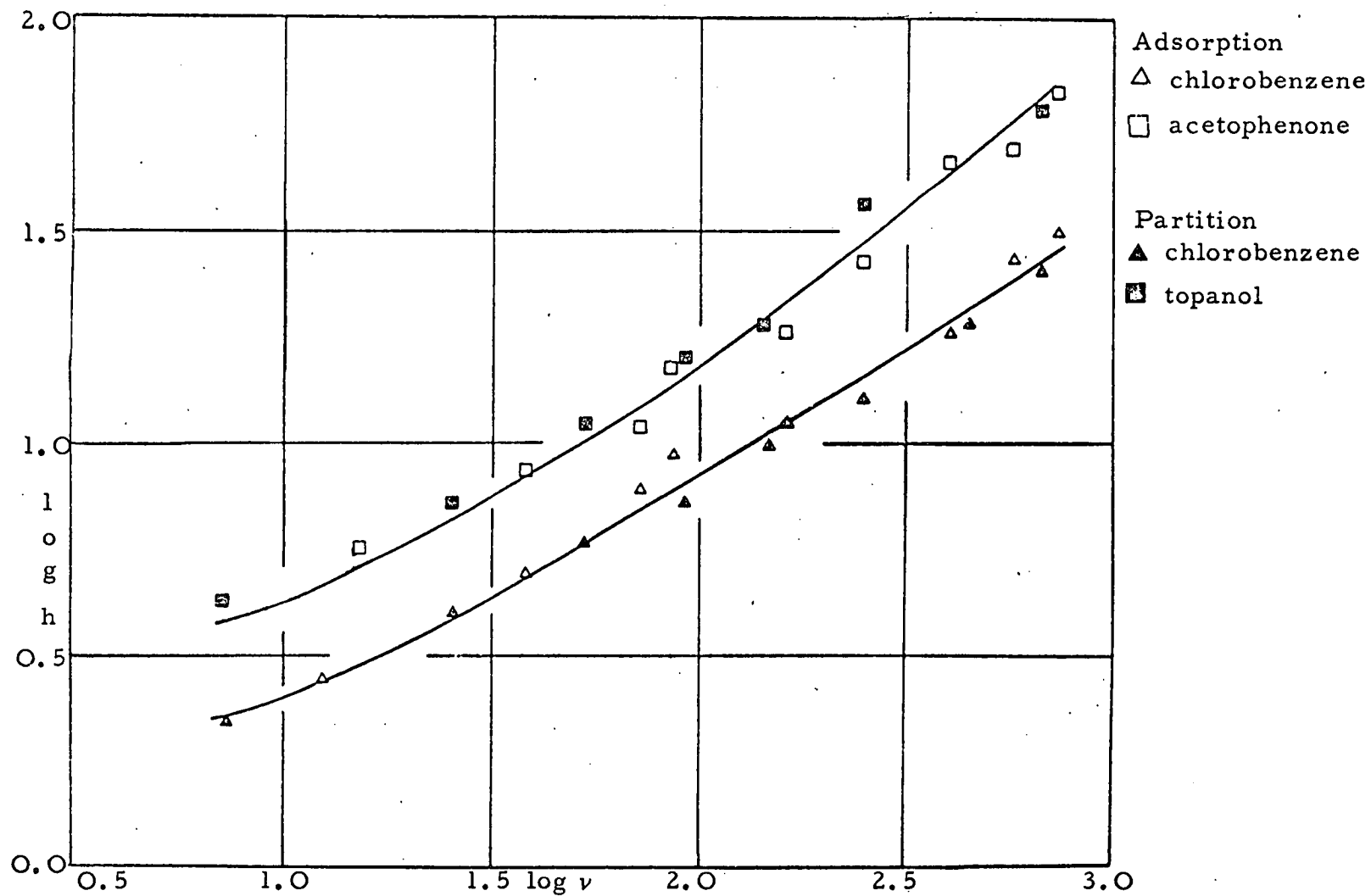


Figure 17: $\log h$ vs $\log \nu$, Corning^R CPG 240

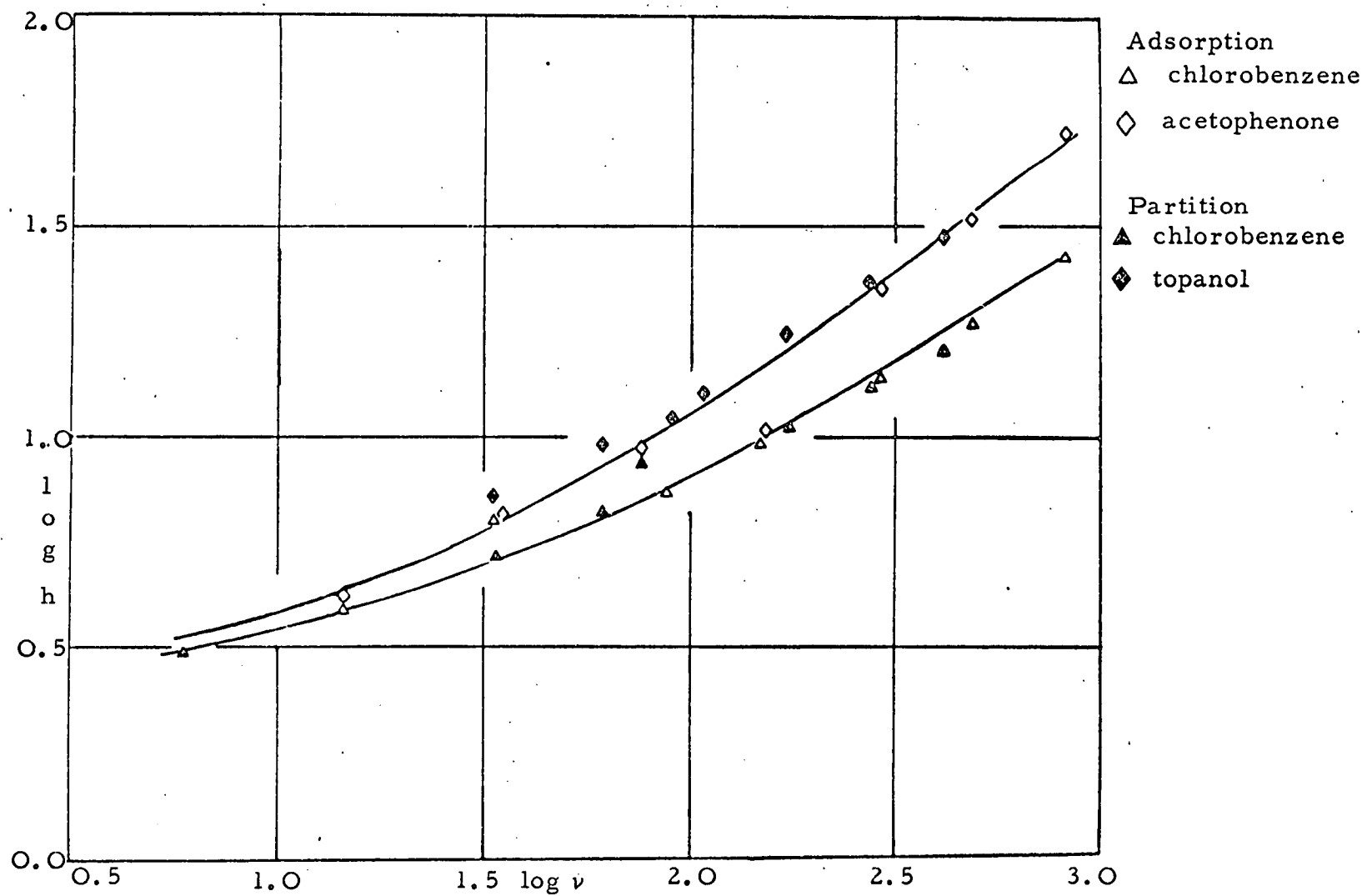


Figure 18: $\log h$ vs. $\log \nu$, Corning^R CPG 240

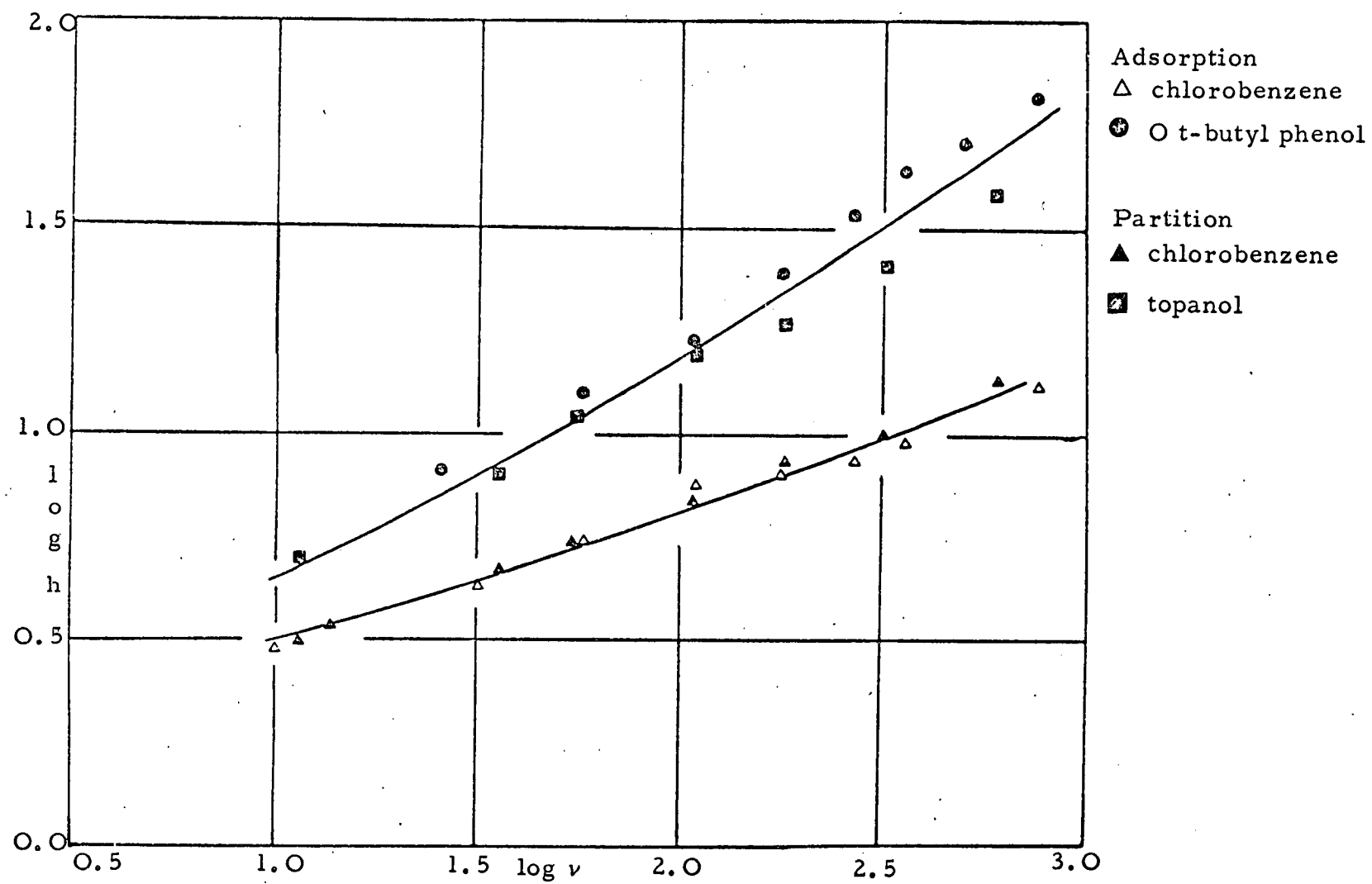


Figure 19: log h vs. log ν , Corasil^R I

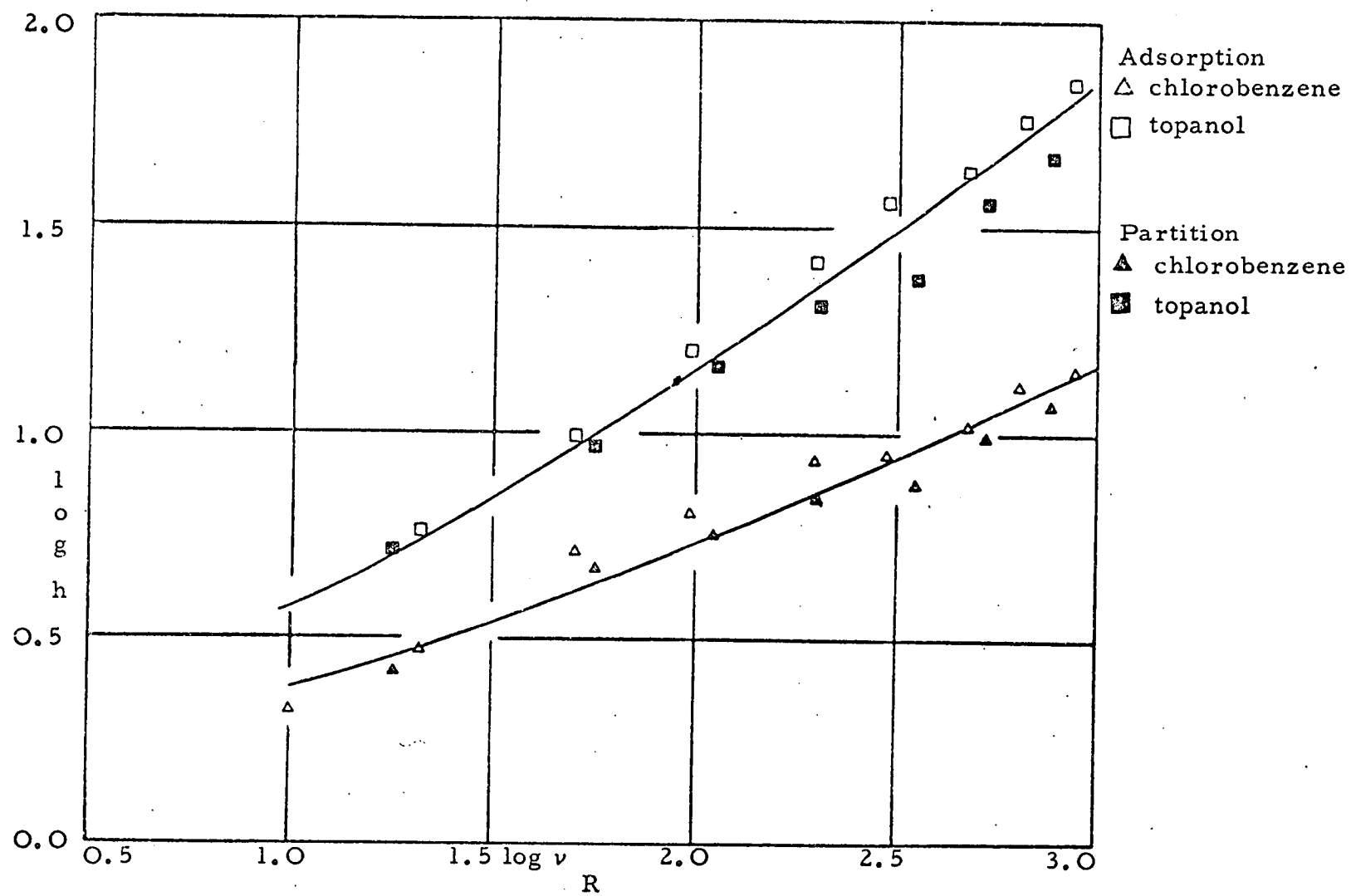


Figure 2O: log h vs. log ν , Corasil II

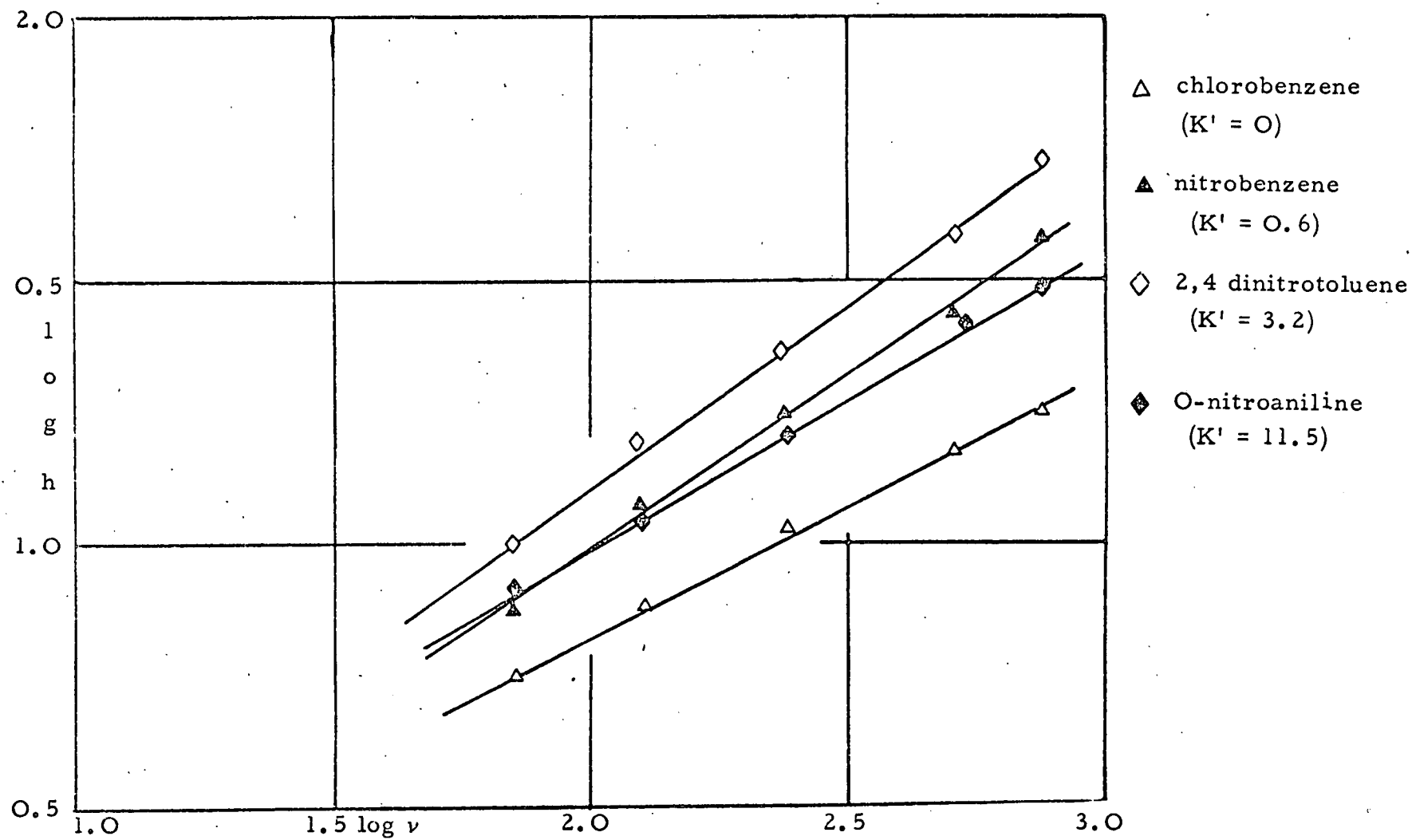


Figure 21: $\log h$ vs. $\log v$, Corasil II (partition, 1% BOP)

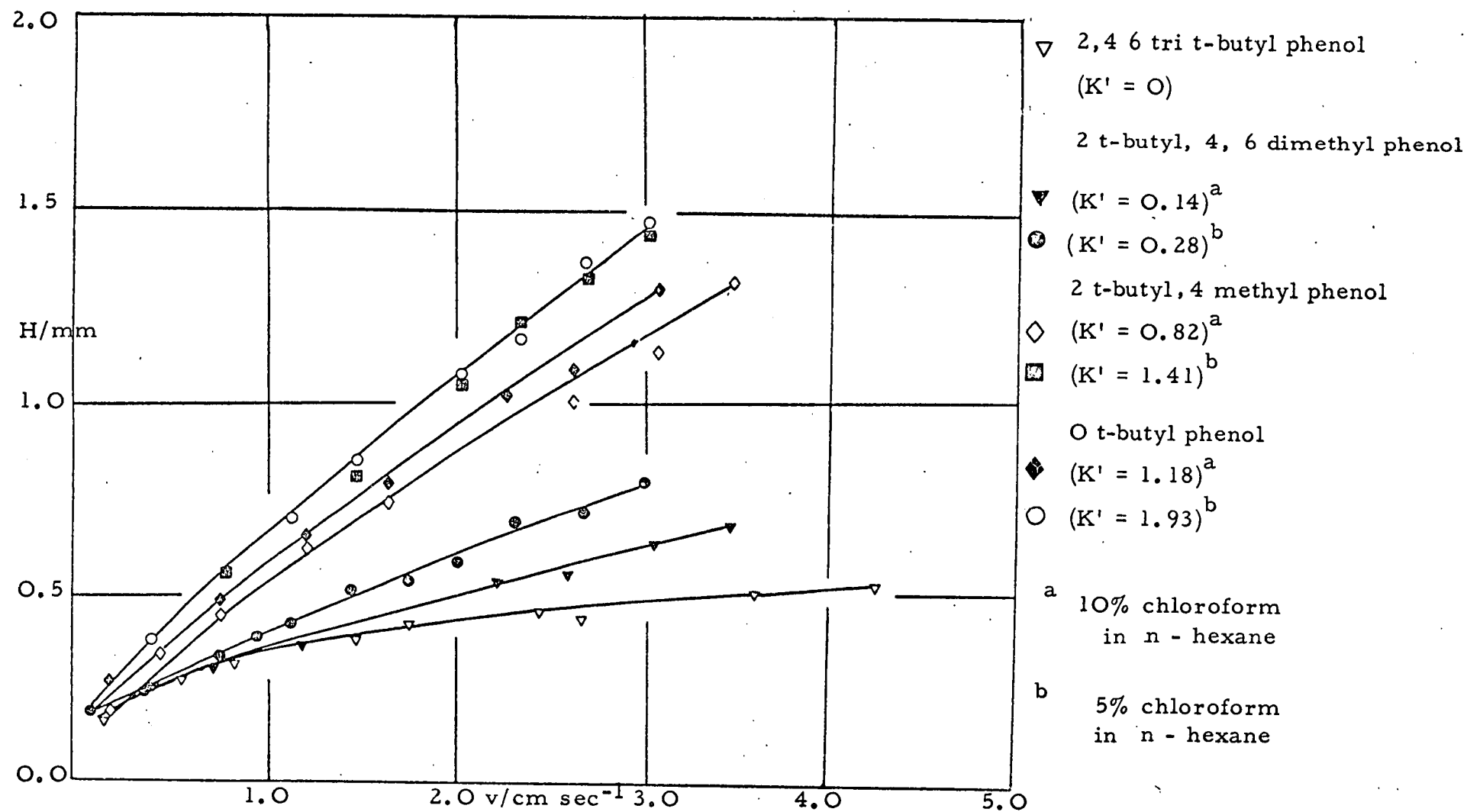


Figure 22: H vs v Corasil^R II (44 - 53 microns)

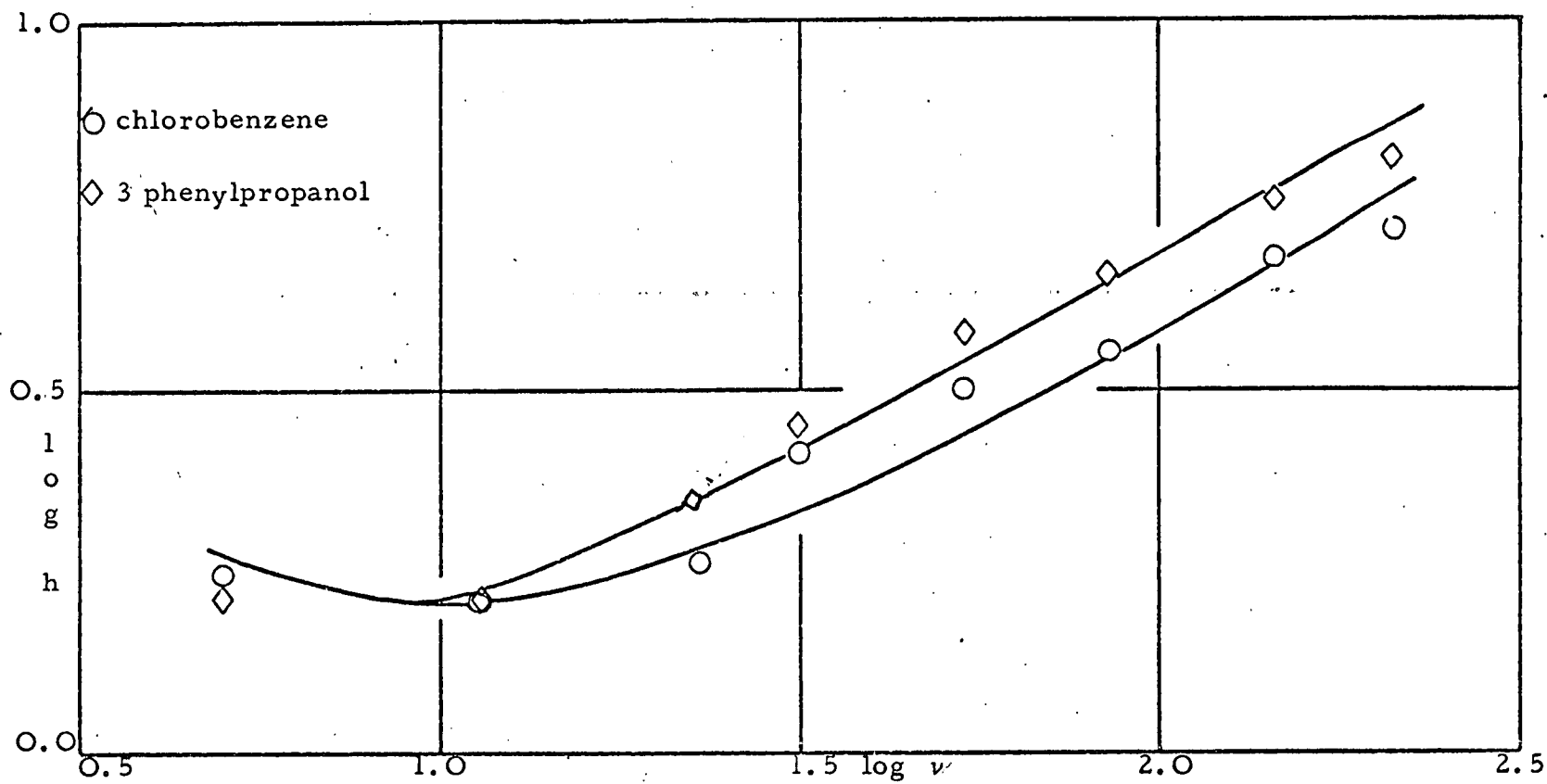


Figure 23: log h vs log ν , Zipax^R (partition)

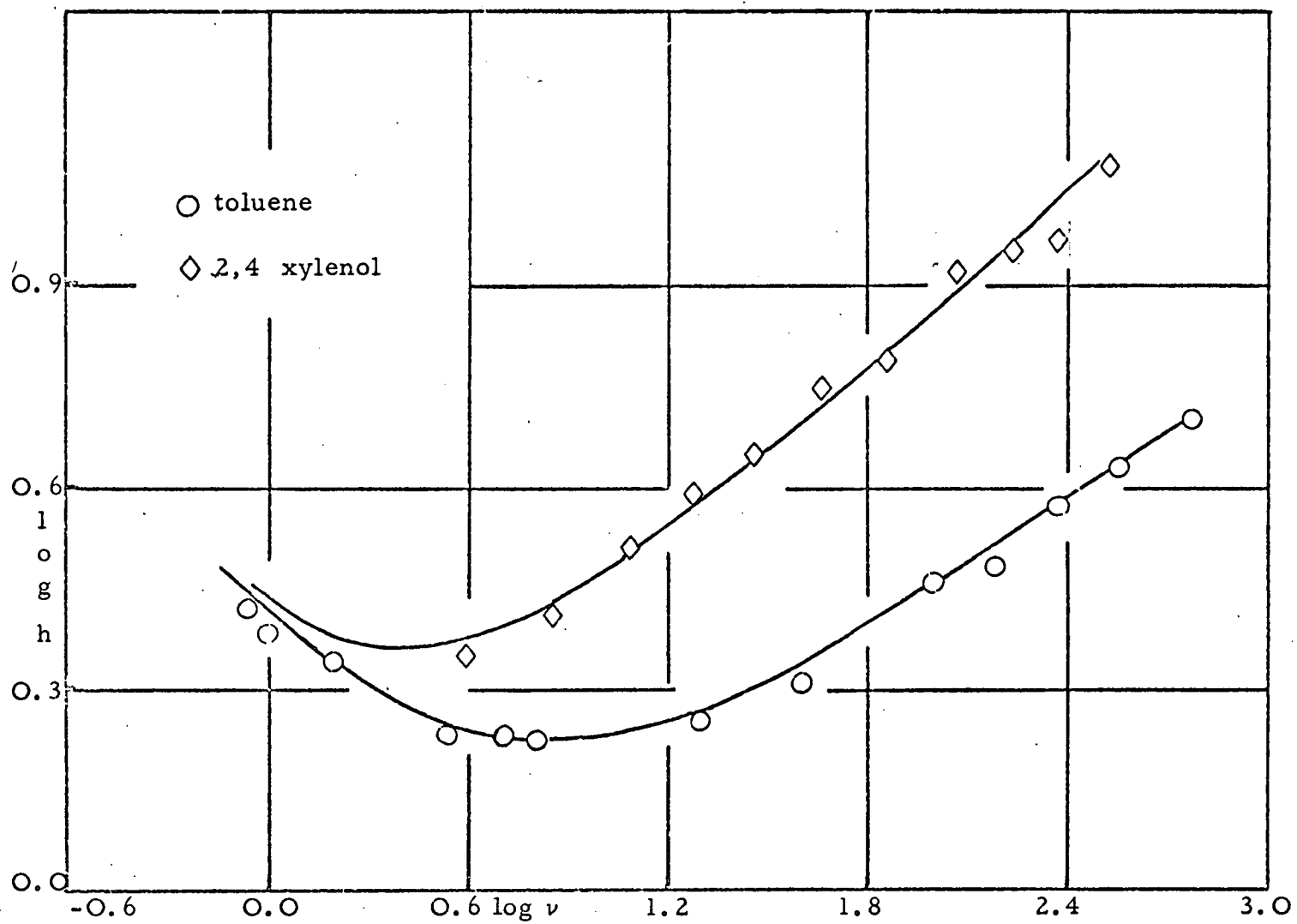


Figure 24: $\log h$ vs. $\log \nu$, Zipax^R, 1% w/w BOP. (Done and Knox (88)).

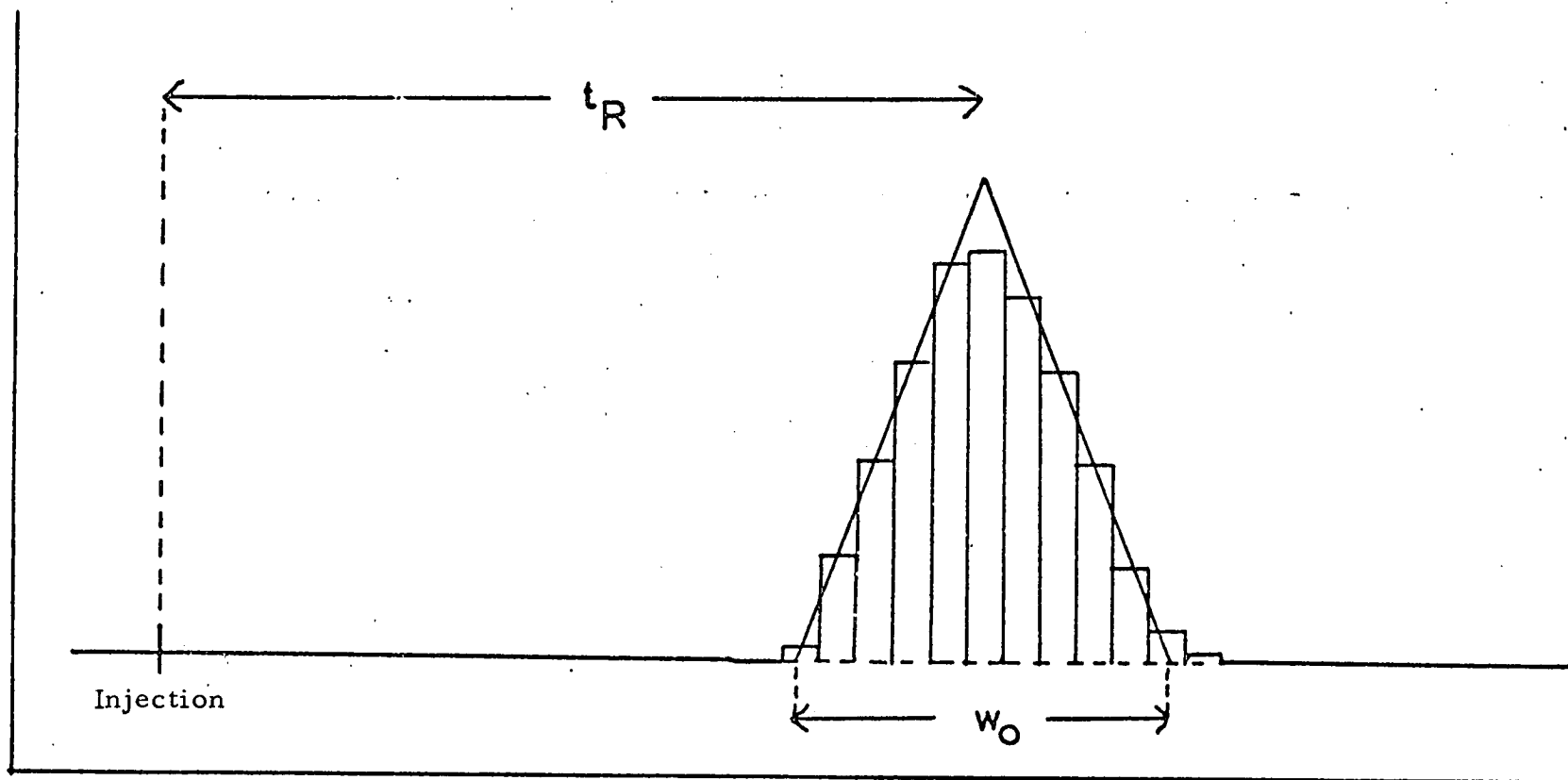


Figure 25: Elution record from scintillation counting

- 1 nitrobenzene
- 2 2,4 dinitrotoluene
- 3 o-nitroaniline

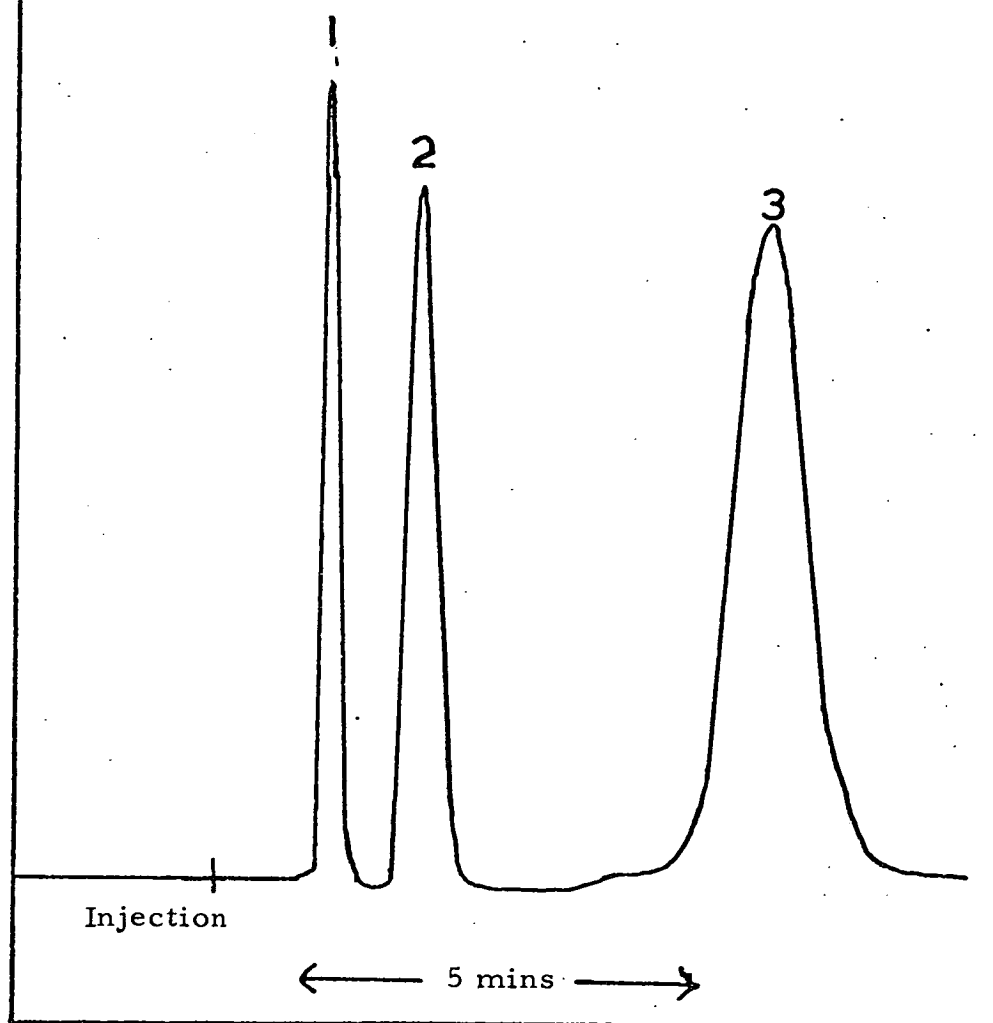


Figure 26: 44 - 53 microns Porasil^R PS 60 4% BOP
Mobile phase : cyclohexane

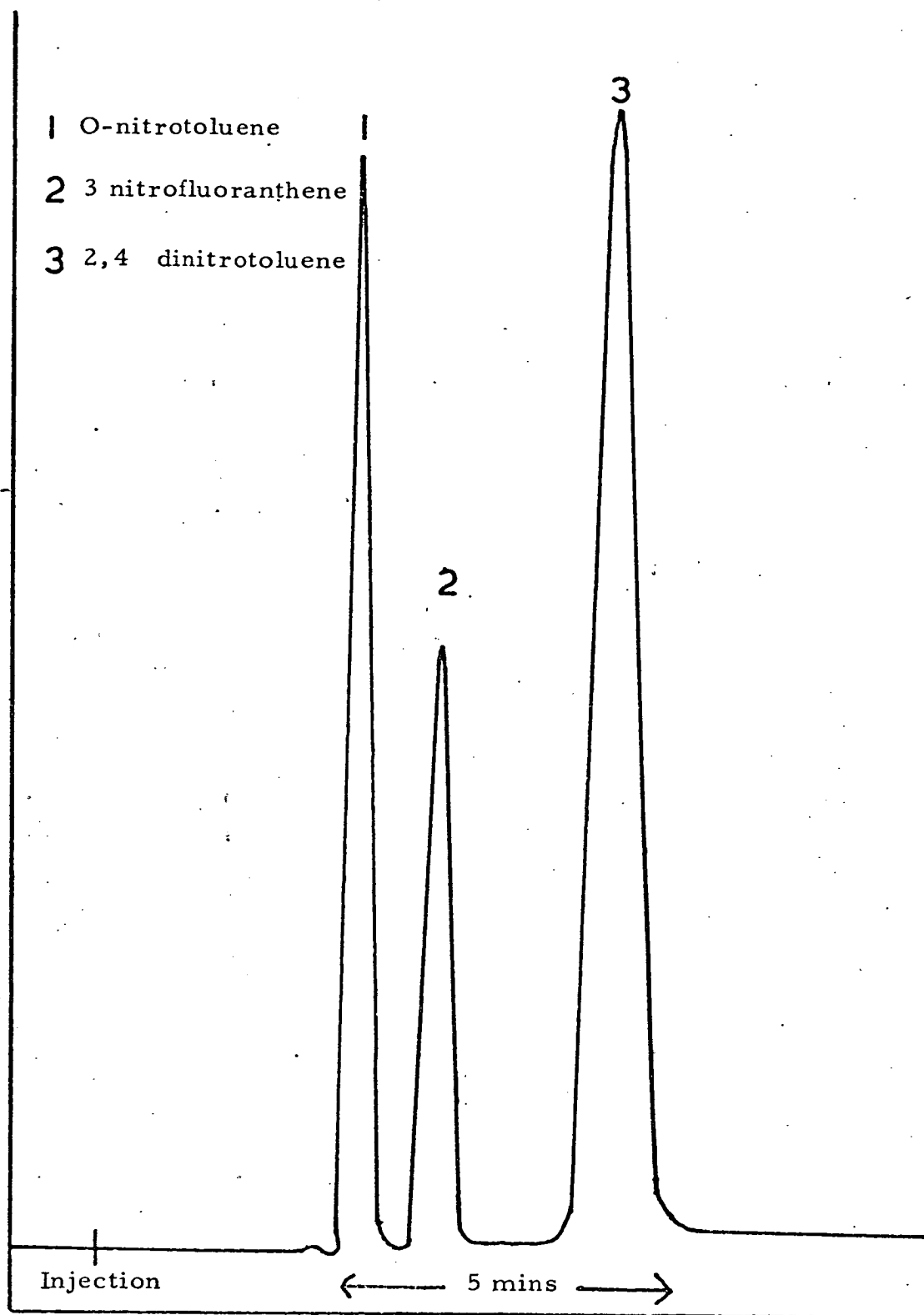


Figure 27: 44 - 53 microns Corning CPG^R 240, 4% BOP
Mobile phase : cyclohexane

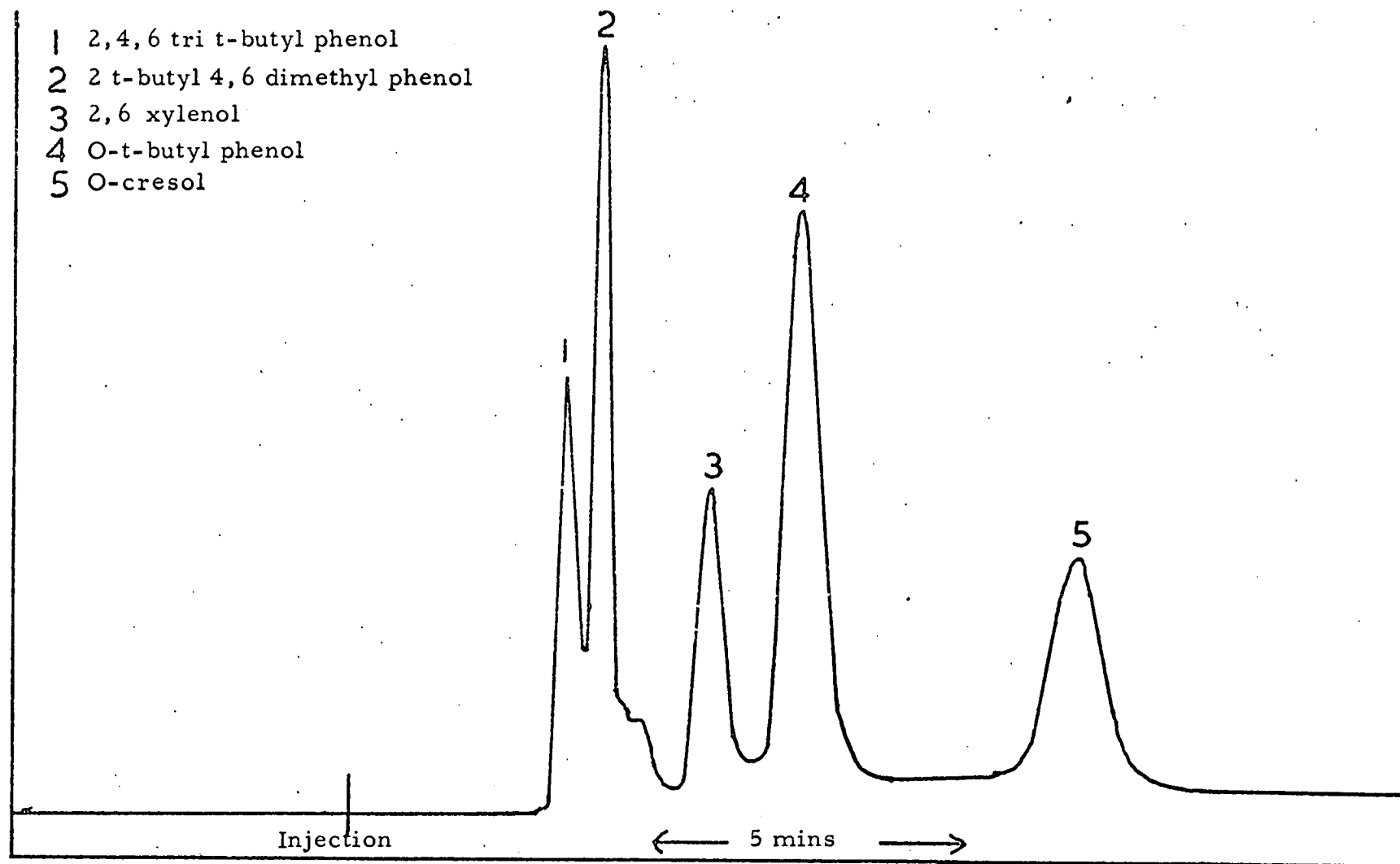


Figure 28: 44 - 53 microns Corasil^R I. Mobile phase: cyclohexane (Adsorption)

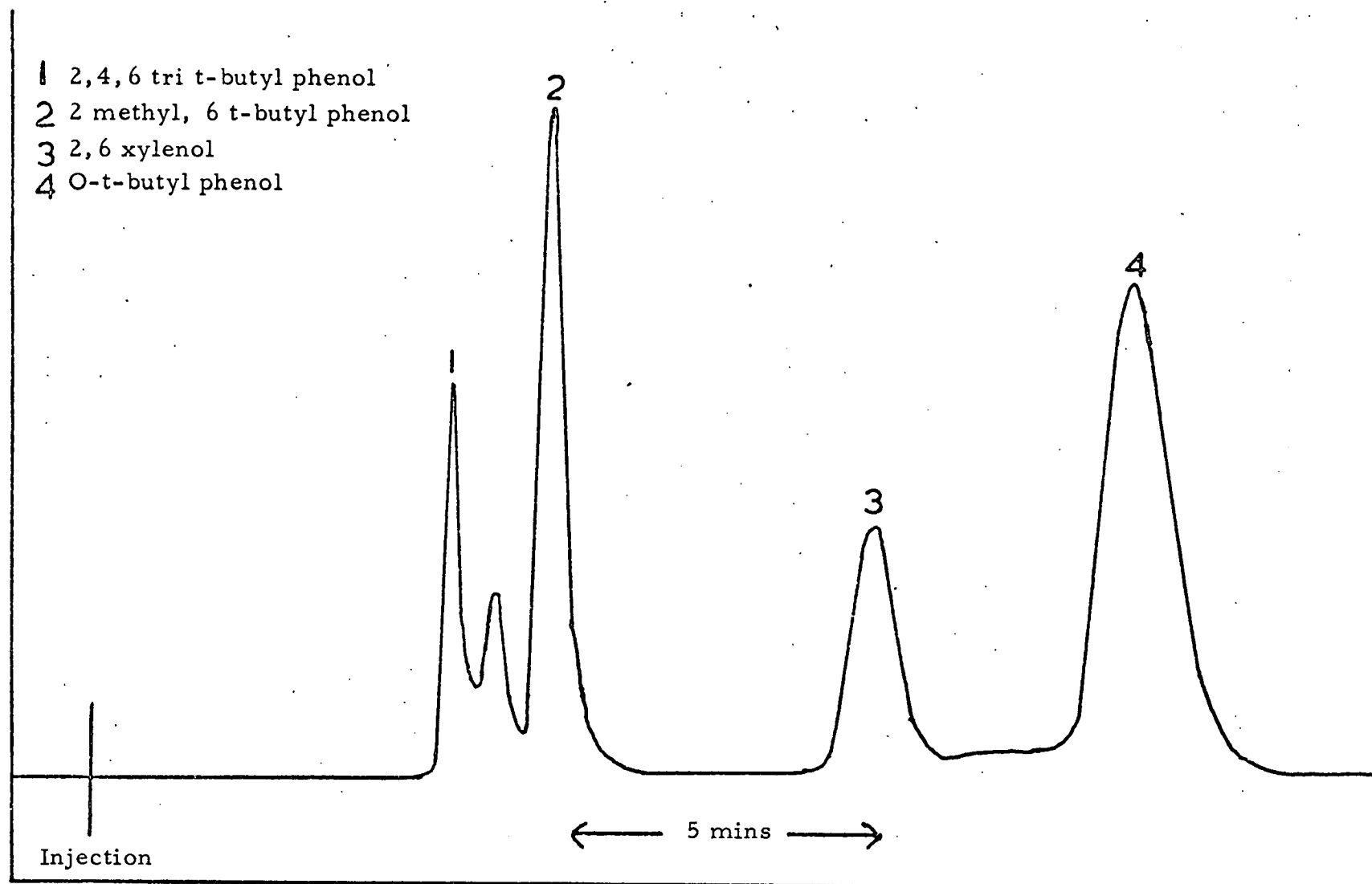


Figure 29: 44 - 53 microns Corasil^R II. Mobile phase : cyclohexane (Adsorption)

- 1 nitrobenzene
- 2 2,4 dinitrotoluene
- 3 O-nitroaniline

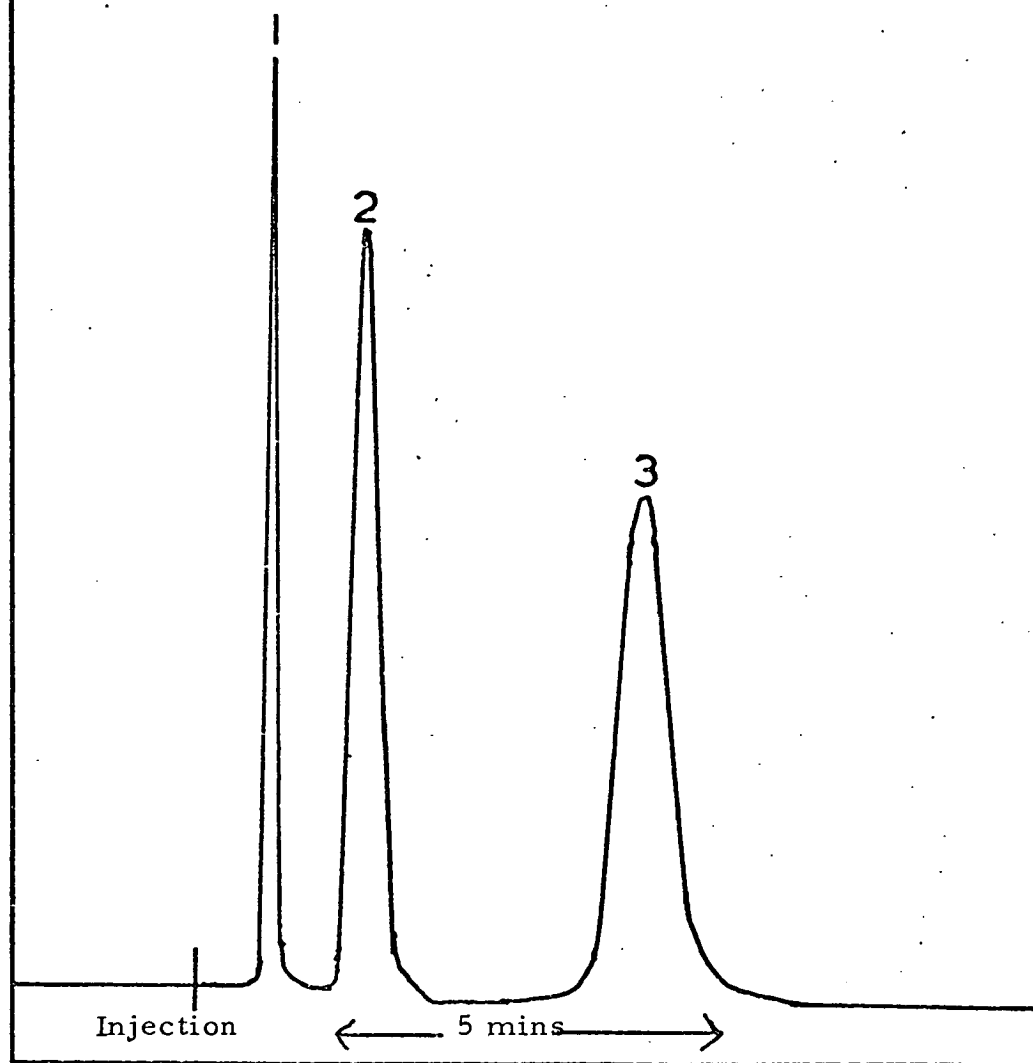


Figure 3O: 44 - 53 microns Corasil^R II, 1% BOP
Mobile phase : cyclohexane

- 1 nitrobenzene
- 2 2,4 dinitrotoluene
- 3 O-nitroaniline
- i unidentified impurity

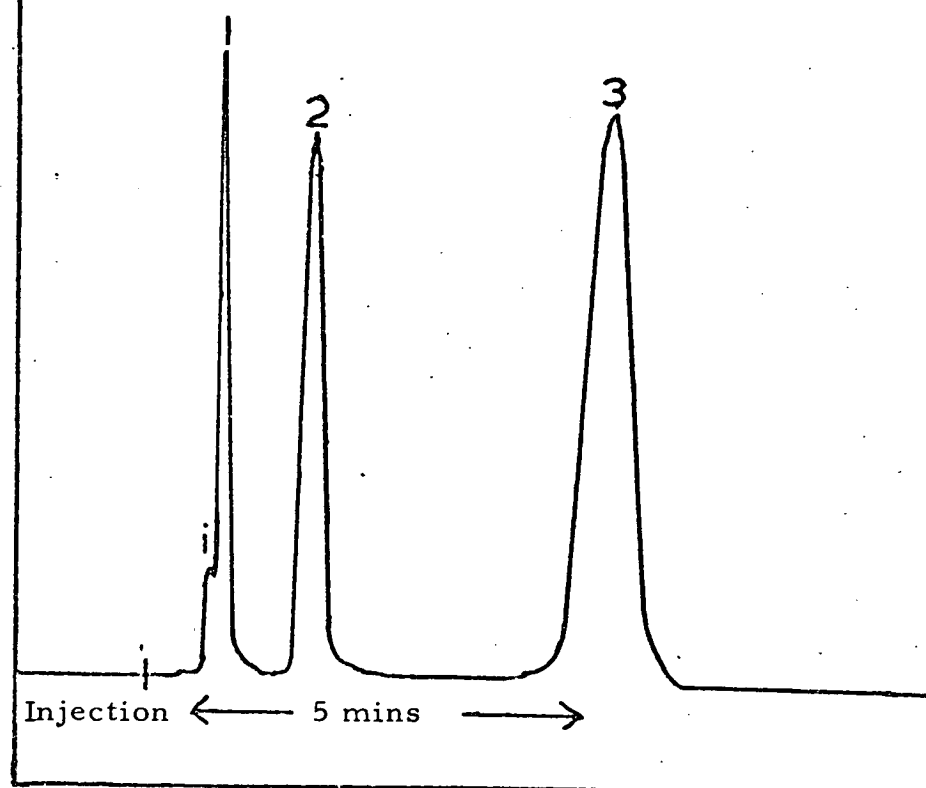


Figure 31: 44 - 53 microns Zipax^R, 1% BOP
Mobile phase : cyclohexane

Revolution Liquid Chromatography

J. N. DONE, G. J. KENNEDY & J. H. KNOX

Department of Chemistry, University of Edinburgh

Liquid chromatography can now be performed in minutes rather than hours or days with a sensitivity and ease of operation previously associated only with gas chromatography. The new techniques promise to be of immense importance for the development of chemistry, biochemistry and medicine.

Liquid chromatography (LC), the original form of chromatography devised by Tswett¹, did not gain widespread recognition until it was rediscovered by Lederer and co-workers in about 1930 (ref. 2). It was extended by Martin and Synge³ who invented liquid-liquid partition chromatography in 1941. This, in its turn, paved the way for paper chromatography⁴, thin-layer chromatography⁵ and gas chromatography^{6,7} (GC).

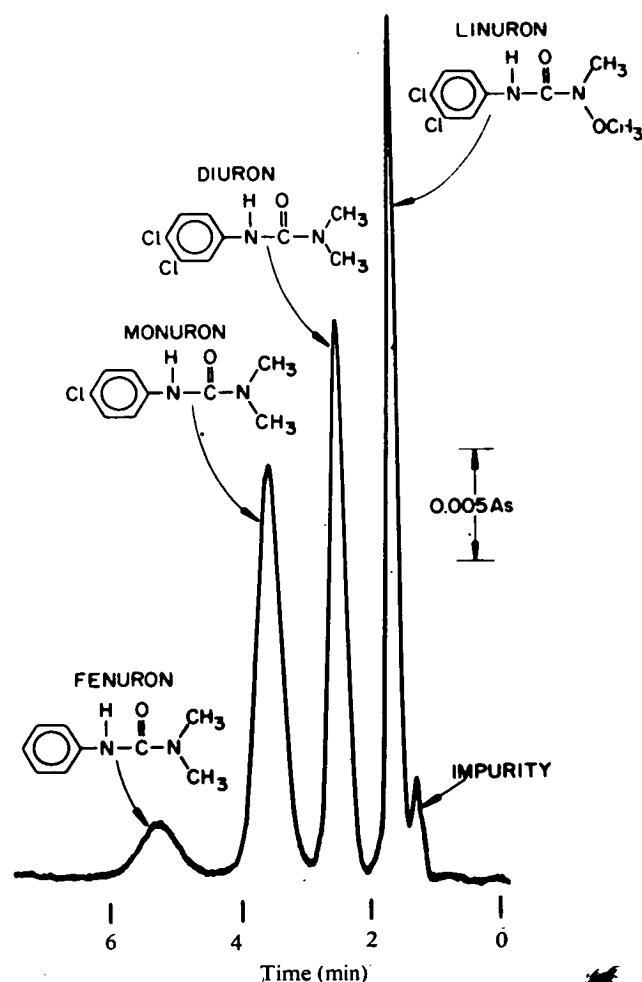


Fig. 1 Fast liquid chromatogram of urea herbicides, from ref. 12. Conditions: 1 m x 2 mm column packed with 40 μ m 'Zipax' coated with 1% $\beta\beta'$ oxydipropionitrile (BOP); mobile phase, dibutyl ether; sample, 0.7 μ g of each material; detector, ultraviolet photometer.

Gas chromatography was an immediate success because of the simplicity of the equipment and the wide range of its applicability. The technique and theory of the method developed rapidly, whereas, by contrast, the theory of liquid chromatography remained more or less static, at the stage reached by Martin and Synge³. Giddings^{8,9} was one of the first to recognize that the theory developed for gas chromatography was equally applicable with minor modifications to liquid chromatography. He showed that the performance which had previously satisfied liquid chromatographers could be greatly improved by paying attention to certain key features. Indeed, the nub of the problem in LC was the appreciation of the interrelation between pressure drop, particle size, eluent velocity, and column efficiency. The theory indicated that by using high pressures and very small particles LC could rival GC both in speed and resolving power. Although the value of using small particles had been appreciated by Hamilton in connexion with ion exchange chromatography¹⁰, and by Snyder for adsorption chromatography¹¹, their ideas were not generally assimilated and it was left to those who had received their principal training in gas chromatography to make the most notable advances. Particular credit must go to Kirkland of du Pont¹² who first showed how high speed separations could be achieved in a few minutes by using specially prepared layered particles about 30 μ m in diameter and pressures up to 3,000 pound inch⁻². The emergent bands were detected by a high sensitivity ultra-violet photometer. Fig. 1 shows one of the first high speed liquid chromatograms published by Kirkland.

An important benefit of the application of the theory was a unification of the practice of LC. Modern equipment for HPLC is similar for all forms of liquid chromatography, and is equally usable for adsorption, partition and gel chromatography. Only for the chromatography of amino-acids on ion exchange resins is special equipment required, and this stems more from the special detection system and the slowness of the method than from any chromatographic peculiarities.

Equipment

Present equipment for HPLC (see, for example, ref. 13) mirrors many features familiar to gas chromatographers, and present trends are towards systems with the same versatility, ease of operation, cheapness and capacity for automation.

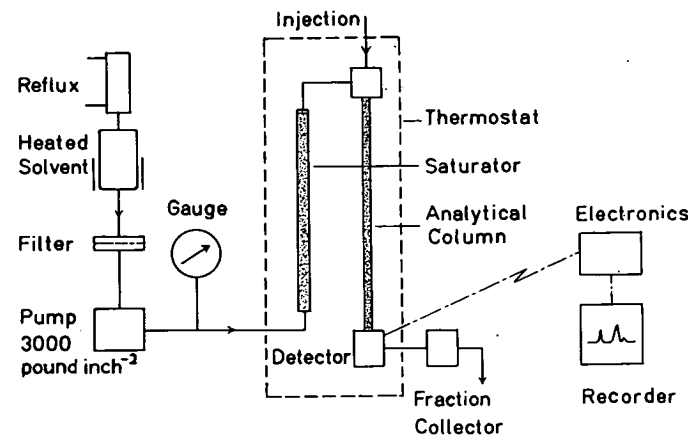


Fig. 2 Outline of HPLC equipment.

Fig. 2 outlines the essential parts. Degassed eluent is pumped at a pressure up to 3,000 pound inch⁻² through a column containing a finely divided and well graded partitioning material in the size range 10 to 50 μm . To handle the high inlet pressures, columns and fittings are usually of stainless steel, and packing materials are strong and rigid. Most are based on glass or silica. Columns are about 2 mm in diameter and 50 to 100 cm long. Flow rates are about 20 mm s⁻¹ so that unretained materials are eluted in about 1 min as is the practice in gas chromatography. When the partitioning agent is slightly soluble in the mobile phase, as in liquid-liquid partition chromatography, a pre-column or saturator must be placed before the analytical column to prevent stripping of the stationary phase.

Samples may be injected either by an injection valve or more often by a microsyringe: a typical injection might be 1 to 10 μl . of a 1% solution of an unknown mixture. Surprisingly, 5 μl . syringes, widely used in gas chromatography, can readily withstand pressures of 5,000 pound inch⁻² without shattering. The chief limitation of syringe injection is not the syringe itself but the rubber septum through which the syringe needle is inserted. When the pressure exceeds about 1,500 pound inch⁻² the septum must be protected.

The most decisive feature of current technology in LC is the use of highly sensitive post-column detectors. Like GC detectors, those used in HPLC produce an electrical signal proportional to the concentration of the eluted solute. Because column volumes are small, detector volumes must also be small, typically 10 μl . The most popular detectors are the refractive index (RI) monitor¹⁴ and the ultraviolet absorption photometer¹⁵. The RI monitor compares the refractive indices of the eluent and the pure mobile phase. Because it monitors a bulk property it is responsive to all substances to some extent, but it has the inevitable drawback of being somewhat insensitive. The best RI monitors will discriminate to about 10⁻⁷ RI units, but because the change in RI from one pure substance to another is of the order of 0.1 the concentration sensitivity is about 1 in 10⁶. Ultraviolet detectors, on the other hand, are much more sensitive but are limited to substances which absorb (for example, aromatic and carbonyl compounds). The limit of detection of a substance with a molar extinction coefficient of about 10⁴ is about 1 in 10⁹. As in GC, sensitivity has generally to be bought at the expense of generality but, of course, in many applications selectivity is highly desirable, and very high sensitivities may be expected in such cases.

There are many other principles on which detection may be based. Polarography¹⁶, for example, has been used successfully for the analysis of pesticides and is capable of a on a pellet of adsorbent, is somewhat less sensitive. In sensitivity of about 1 in 10⁸. The micro-adsorption detector¹⁷, which uses the heat released by adsorption of eluted solutes transport detectors¹⁸ column effluent is collected on a wire or

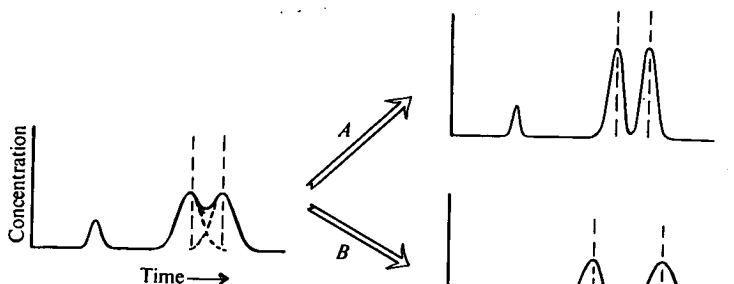


Fig. 4 Improvement of resolution. A, By making peaks narrower; B, by increasing peak separation. Initial peaks represent unretained solutes, and full vertical lines the moments of injection.

band which first passes through a low temperature oven where the solvent (necessarily volatile) is removed. Any involatile solute remains on the wire which next passes to a reactor where the solute is either pyrolysed or oxidized. The gaseous products from the reactor pass to a sensitive GC detector. Existing transport detectors are no more sensitive than RI monitors, but they can probably be greatly improved. They possess considerable versatility because they can be made specific for different elements including, of course, carbon.

Key to the Revolution

The achievement of high performance in chromatography requires an understanding of how resolution can be controlled and improved—for what every practising chromatographer wants is better resolution, in a shorter time, at a lower cost, and with less expense of effort.

Resolution in chromatography is defined as

$$\text{Resolution} = \frac{\text{Difference in times of peak maxima}}{\text{Mean peak width at base}} = \frac{(t_2 - t_1)}{\frac{1}{2}(w_1 + w_2)} \quad (1)$$

and the concept is illustrated in Fig. 3 by a separation of two sulphur heterocyclic isomers with a resolution of 2.8. A resolution of unity implies almost base-line separation. Incomplete resolution, a common problem, may be improved either by making the individual peaks narrower without changing their relative retention times, or by increasing the peak separation without changing their widths as shown in Fig. 4.

To increase separation without changing peak widths requires a change in the relative partition ratios of the substances between the mobile and stationary phases. This means changing the thermodynamic properties of the column. To obtain sharp peaks, chromatography must be carried out in very nearly perfect equilibrium conditions⁹,

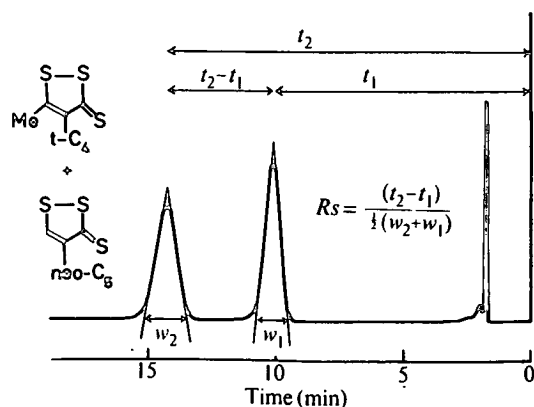


Fig. 3 Calculation of resolution. Separation of heterocyclic sulphur isomers on 'Corasil II' by adsorption chromatography; mobile phase, 1% CCl₄ in hexane.

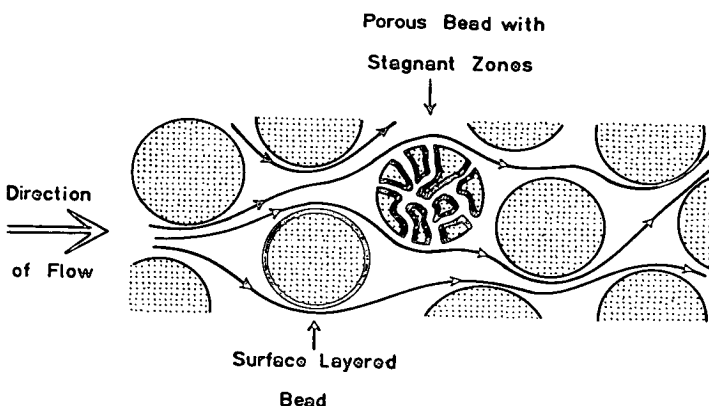


Fig. 5 Two dimensional representation of the flow regimes in a packed column, for porous and surface layered particles. —→, Flow lines; hatched borders, stationary phase; stippled areas, support structure.

and it is thus not possible to influence retention ratios by changing parameters such as the flow rate, particle size and column length. Changes in relative retentions can be obtained only by changing the stationary phase and/or the mobile phase. Although determination of the optimum combination of phases is important in any chromatographic study, the search for the ideal combination can be fruitless if the kinetic features of the equipment are poor, so that peaks are wide and elution times long. It is therefore vital to examine how resolution can be improved by making peaks narrower.

There are two sources of band spreading in any form of chromatography. The first is slow axial diffusion, and the second is the disturbance of equilibrium caused when one fluid flows over another fluid or over a solid. Only the second source of spreading is important in liquid chromatography as currently practised. In discussing flow-generated dispersion, three distinct regimes within any column may usefully be defined (Fig. 5); these are the stationary phase which is permanently held by the support (or may actually be the support), the stagnant part of the mobile phase which fills the interstices of the support, and the free flowing part of the mobile phase in the interparticle space. Within the flowing part, there is a whole spectrum of fluid velocities both across the column and along the column. The band spreading which velocity variations within and between these regions tend to produce can be countered in two ways; first, by molecular diffusion across the direction of flow, and second, by the tortuous nature of the flow itself, for no velocity, however extreme, is maintained for more than a few particle diameters downstream of any point in the column. The dispersion is generally reported in terms of quantity called the "height equivalent to a theoretical plate" or more simply the plate height (H) which can be thought of as the mean downstream equilibration distance within the column. Mathematical analysis of the column process allows H to be expressed in terms of column parameters such as the linear flow velocity, particle diameter, retention ratio, and diffusion coefficients.

When flow variations are countered only by lateral diffusion the equilibration time is constant, and therefore the equilibration distance measured downstream (that is, the plate height) is proportional to the fluid velocity, u . If, on the other hand, the dispersion is countered only by the tortuous nature of the flow itself, fluid velocity will have no effect on H , because the equilibration distance is governed by the geometry of the column. In practice both counter-dispersion mechanisms are important and the dependence of H on u is a compromise between $H=\text{constant}$, and $H \propto u$. Over about two orders of magnitude of u , H is found to follow a fractional power of u :

$$H = Cu^n \text{ where } 0 < n < 1 \quad (2)$$

Typical experimental dependences are shown in Fig. 6 for some column packings used in HPLC. n may be as low as 0.25 and as high as 0.7, but, generally speaking, a low exponent is desirable. At much lower velocities than those commonly used in HPLC, longitudinal diffusion becomes important. The dispersion so produced increases with the time of residence in the column, and, at very low velocities, H rises again as shown by the broken lines in Fig. 6.

The non-equilibrium theory of dispersion⁸ just described explains peak broadening in terms of a balance between the dispersive effects of flow variations across the column, and the counteracting effects of the tortuous downstream flow and transverse diffusion. As both the diffusion time and the "reticulation" of velocities over the column cross section

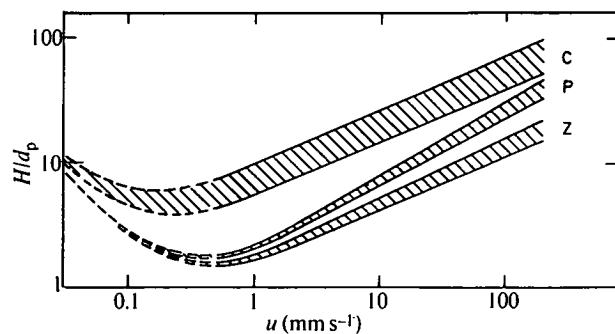


Fig. 6 Dependence of reduced plate height, H/d_p , on fluid velocity, u , for 'Zipax' (Z), 'Porasil' (P), and 'Chromosorb' (C). Lower limits of bands are for unretained solutes, upper limits for retained solutes giving maximum dispersion. Particle diameter 40 μm .

depend on particle diameter, d_p , one predicts that better resolution will be obtained with smaller particles. This is well authenticated and is clearly shown by the chromatograms in Fig. 7. Columns were of identical size, and the elution time was kept constant for particle diameters between 22 and 115 μm . One might well ask why even smaller particles (yielding still better resolution) have not been used. Apart from the unavailability of smaller particles, two problems arise when one tries to do this. First, smaller particles are more difficult to pack, and, because uniform packing is essential for high performance in chromatography, the expected improvement in performance does not always materialize if particular size is reduced too far.

Second, the driving pressure required for comparable elution speed increases as $1/d_p^2$, and the particle diameter does not have to be reduced much before the maximum pressure capability of most equipment is exceeded. If we assume that columns can be equally well packed whatever the particle size, it can be shown¹⁹ that the analysis times for the resolution shown in Fig. 7 with 22 μm particles can be greatly reduced if particle size and column length can be appropriately chosen (see Table 1). These figures are useful chiefly because they show how far we may still expect to improve the performance of existing equipment, and what technological problems are likely to be met during progress towards very fast liquid chromatography with elution times of seconds instead of minutes.

Although the theory suggests that the resolution shown in Fig. 7 for 22 μm particles could be obtained in only 4 s with a column 5 mm long containing 0.5 μm particles, the practical difficulties in achieving this would be insuperable,

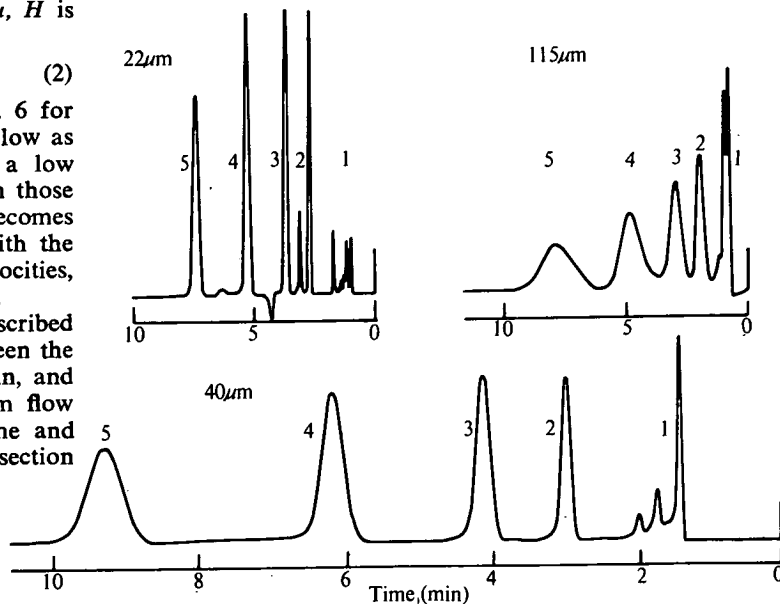


Fig. 7 Effect of particle size on resolution. Column length, 1 m; elution time for last peak, 7 to 10 min; stationary phase, BOP on 'Zipax'; mobile phase, cyclohexane; sample, about 10 μg . 1 = Unretained solute; 2 = 2,4-xylene; 3 = o-cresol; 4 = m-cresol; 5 = phenol.

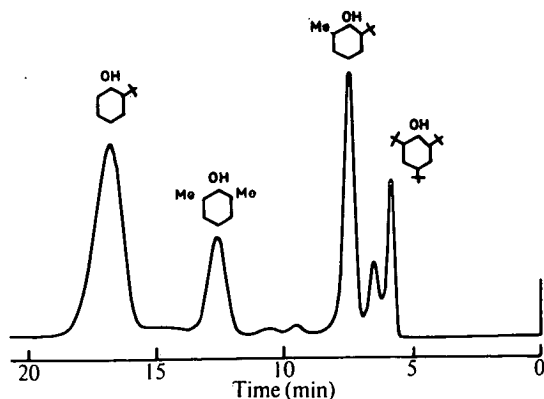


Fig. 8 Separation of antioxidants by adsorption chromatography on 'Corasil II'. Column length, 500 mm; particle size, 44 to 53 μ m; mobile phase, cyclohexane; —+, t-butyl group.

for the detection and injection devices would have to be so minute as to be beyond the reach of present day technology. The practical limit is likely to be reached with particles of 5 to 10 μ m. These would give high resolution analyses in 1 min or so with columns of reasonable length (100 to 500 mm). Detectors would not then have to be very much smaller than at present. An improvement of about ten-fold in performance should be possible in the quite near future.

Table 1 Calculated Elution Times for 'Zipax' of Different Particle Sizes using a Pressure Drop of 3,000 Pound Inch⁻²

Particle diameter (μ m) ψ	Column length (mm)	Analysis time (s)
0.2	7	40
0.25	3	5
0.5	5	4
1	14	7
2.5	50	15
5	140	30
10	370	50
25	1,400	110
50	3,900	220
100	10,300	400

The theory outlined in this article also sets out guidelines for the optimum configuration for a packing material; it clearly indicates that conditions should be chosen so that both counter-dispersion mechanisms are effective in reducing peak spreading. In the flowing parts of the mobile phase both the tortuous flow and molecular diffusion counter the dispersive tendency, but in the stagnant regions only molecular diffusion operates and this mechanism becomes increasingly ineffectual as the flow rate increases (see Fig. 5). In the ideal packing the stagnant regions are therefore minimized, and this explains why the gradients of the plate height curves for porous materials shown in Fig. 6 are greater than those for surface layered particles.

The curve in Fig. 6 for 'Chromosorb' (a well known support popular in gas chromatography) is interesting, for it exhibits a large plate height but a low gradient. Although 'Chromosorb' is a porous material, and so might be expected to give a steeper dependence like 'Porasil', it is very difficult to pack, and the chief cause of its poor performance is not particle porosity but the poor geometry of the interparticle space which probably leads to channelling and other undesirable flow characteristics.

The most sophisticated support at present available for HPLC is probably 'Zipax', which was invented by Kirkland¹². It consists of small glass spheres which are coated with microspheres of silica deposited from a silica sol. By suitable control of conditions, successive layers of silica are deposited and finally bound to the glass surface. The final result is a bead covered with a hard porous layer about

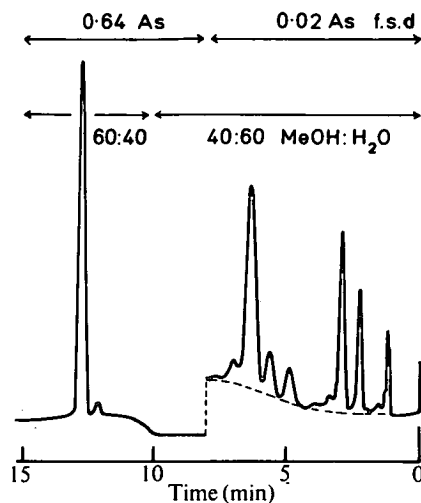


Fig. 9 Analysis of 'Trithion' on 'Permaphase'. Column length, 1 m; stationary phase, octadecyl silane chemically bonded to support.

1 μ m thick which can retain up to 1% by weight of stationary phase. An adsorptive material of similar configuration is Waters Associates' 'Corasil'²⁰ in which the bead is coated with a layer of silica gel with fine pores. This material is useful for separating substances which would normally be separated by thin layer chromatography. Fig. 8 shows the separation of some antioxidants on this material. An earlier approach to surface layered beads is that of Horvath, Preiss and Lipsky²¹, who coated solid glass beads with a thin layer of ion exchange resin. This material offered a significant improvement in the speed of analysis of nucleotides, but it has been overtaken in speed by 'Zipax' coated with ion exchange resin²².

An important technique in classical liquid adsorption chromatography was gradient elution or solvent programming. This technique has been applied with success in HPLC and is the analogue of temperature programming in gas chromatography. In gradient elution the composition of the solvent is changed either gradually or stepwise during the analysis of the sample; mixtures containing substances of a wide range of polarity can then be separated in a single analysis with good resolution at all points of the chromatogram²³. Until recently gradient elution was restricted to adsorption and ion exchange chromatography because it is essential that the stationary phase be insoluble in the mobile phase. Its range has now been greatly extended by the invention of partitioning materials in which the stationary phase is chemically bonded to the support²⁴. The use of such a material for a solvent programmed separation is shown in Fig. 9. The chromatogram of the commercial insecticide 'Trithion' shows one major component and several minor ones. As in gas chromatography, the identification of eluted fractions is an important part of a complete

Table 2 Formula of 'Trithion' and Masses of Possible Fragments

		$\begin{array}{c} \text{S} \\ \\ \text{C}_2\text{H}_5\text{O}-\text{P}-\text{S}-\text{CH}_2-\text{S}-\text{C}_6\text{H}_4-\text{Cl} \\ \\ \text{H}-\text{C}_2\text{H}_4\text{O} \end{array}$					
Cumulative mass number from left	45	153*	185	199*	231	307	342* 344*
Cumulative mass number from right	342* 344*	297* 299*	296* 298*	189* 191*	157* 159*	143* 145*	111 113
							35 37

* These masses are those actually recorded.

analysis. When using liquid chromatography this is readily achieved by mass spectrometry. The eluent fraction containing the appropriate peak is collected and most of the solvent evaporated. About 5 μ l. of the residue is then taken up on a quartz sinter attached to the direct injection probe of the mass spectrometer (in our case an AEI model MS 902). The remaining solvent is evaporated in air and the probe inserted into the instrument. Identification of the principal components from a single chromatographic run is then possible. In this way the chief peak in Fig. 9 was conclusively identified as 'Trithion'. The formula of the compound and the masses of possible fragments are shown in Table 2.

State of the Art and the Future

The most important features of the new form of liquid chromatography are its speed and versatility. Although HPLC has existed only for a few years a large number of different types of compound have been separated. Some idea of their range is given by Table 3 which lists a selection of applications. Most of them have been carried out on surface layered materials, and in all cases good resolution was obtained in less than 20 min and sometimes in as little as 1 or 2 min. Sample sizes were generally between 1 and 50 μ g. Clearly sample type is no limitation, and the fact that polymers can readily be separated by gel chromatography shows that, by contrast with gas chromatography, HPLC is not limited by molecular weight.

Table 3 Some Classes of Compounds Analysed by HPLC

Simple aromatic compounds:

Phenyl alcohols, alkyl substituted phenols, amines, pyridines and quinolines, nitro benzene and derivatives, benzoic acids, phthalic acids and derivatives, sulphur and selenium heterocyclic compounds, anthraquinone derivatives, polynuclear aromatic compounds.

Antioxidants:

Amines, t-butyl phenols, polyhydric phenols and hydroquinones.

Plasticizers:

Long chain phthalate esters.

Pesticides:

DDT, 'Aldrin', EPN, carbaryl, 'Abate', phosphorus insecticides.

Herbicides:

Phenoxyacetic acid derivatives, 'Tandex', and other urea herbicides.

Drugs, analgesics and antibiotics:

Aspirin, caffeine, sulphonamides, penicillins.

Biochemicals and natural products:

Steroids, nucleic acid bases, ribonucleosides, alkaloids.

Undoubtedly the principal areas where improvements will be made are in column packings and detectors. Detector technology is still limited, for the only commercial detectors which are widely used and reliable are the ultraviolet photometer and the RI monitor; the last of these is barely sensitive enough for other than major component analysis. It seems likely that all highly sensitive detectors will have to be selective, but then many applications will best be served by specific detectors. The most likely candidates for the immediate future are the fluorometer and the polarograph, both of which have sensitivities comparable with the ultraviolet photometer for those substances to which they respond, but transport detectors of novel design and with facilities for detection of specific elements look most attractive if the present mechanical problems can be solved.

In column technology the further development of chemically bonded stationary phases with a wide range of functionality will have an important influence on the progress of HPLC, and for the time being surface layered materials will predominate. As the particle size is reduced, however, one operates further down the plate height curve (Fig. 6), progressively nearer the minimum, and with 5 or 10 μ m particles much of the apparent advantage of the layered

supports disappears. Thus the future probably lies in the development of sophisticated porous materials based on silica or silica gel with particle diameters of 5 to 10 μ m.

A question that is often asked is whether the new form of liquid chromatography can be scaled to the preparative level. There seems no reason why this cannot be done. Indeed there is good evidence^{25,26} that very wide columns, in which the sample is injected centrally, can give higher efficiencies than conventional narrow columns. This is because lateral dispersion is slow and in a wide column a sample injected centrally may never reach the walls before it emerges from the column. This happens, for example, with a column of bore 10 mm and length 500 mm, filled with 50 μ m particles. Wall effects are absent, and, provided the packing is uniform, very high efficiency can be obtained. With existing 2 mm bore columns it is possible to elute 100 μ g without significant peak distortion. 2 cm bore columns can easily be envisaged, so charges of 10 mg can probably be separated without loss of resolution. By using the kind of repetitive injection systems common in preparative scale GC, the preparation of gram quantities of highly pure compounds by HPLC seems quite feasible.

Conclusions

High performance liquid chromatography is undoubtedly an analytical method for the future. In only three years it has demonstrated its power to analyse complex mixtures in fields such as drugs, pesticides, high molecular weight aromatics, plasticizers, antibiotics, organic chemicals generally, and biochemicals. It has all the advantages possessed by gas chromatography apart from price, but it does not require compounds to be volatilizable. Although its sensitivity is somewhat below that of GC, it is considerably more sensitive than GC was at such an early stage in its development, say, around 1956. The speeds which are currently being attained, although excellent, can probably be improved about ten-fold without any dramatic changes in technology, and such improvement may be confidently expected over the next few years. Within the same period automatic prep-scale liquid chromatographs should become available.

- ¹ Tswett, M., *Trav. Soc. Nat. Warsovie*, **14** (1903).
- ² Kuhn, R., and Lederer, E., *Naturwissenschaften*, **19**, 306 (1931); *Ber. Deutsch. Chem. Gerell.*, **64**, 1349 (1931).
- ³ Martin, A. J. P., and Synge, R. L. M., *Biochem. J.*, **35**, 1358 (1941).
- ⁴ Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224 (1944).
- ⁵ Kirchner, J. C., Miller, J. M., and Keller, G. J., *Anal. Chem.*, **23**, 420 (1951).
- ⁶ Phillips, C. G. S., *Discussions Faraday Soc.*, **7**, 241 (1949).
- ⁷ Martin, A. J. P., and James, A. T., *Biochem. J.*, **50**, 679 (1952).
- ⁸ Giddings, J. C., *Dynamics of Chromatography*, Part 1 (Marcel Dekker, New York, 1965).
- ⁹ Giddings, J. C., *Anal. Chem.*, **35**, 2215 (1963).
- ¹⁰ Hamilton, P. B., *Anal. Chem.*, **30**, 914 (1958).
- ¹¹ Snyder, L. R., *Principles of Adsorption Chromatography* (Dekker, New York, 1968).
- ¹² Kirkland, J. J., *J. Chromatog. Sci.*, **7**, 7 (1969).
- ¹³ *Modern Practice of Liquid Chromatography* (edit. by Kirkland, J. J.) (Wiley, New York, 1971).
- ¹⁴ Bombaugh, K. J., and Little, J. N., *J. Chromatog.*, **16**, 47 (1964).
- ¹⁵ Kirkland, J. J., *Anal. Chem.*, **40**, 391 (1968).
- ¹⁶ Koen, J. G., Huber, J. F. K., Poppe, H., and den Boef, G., *J. Chromatog. Sci.*, **8**, 192 (1970).
- ¹⁷ Hupe, K. P., and Bayer, E., *J. Gas Chromatog.*, **5**, 197 (1967).
- ¹⁸ James, A. T., Ravenhill, J. R., and Scott, R. P. W., *Gas Chromatography 1964*, 197 (Institute of Petroleum, London, 1965).
- ¹⁹ Knox, J. H., and Saleem, M., *J. Chromatog. Sci.*, **7**, 614 (1969).
- ²⁰ Waters Associates, *Anal. Chem.*, **42**, 18A (1970).
- ²¹ Horvath, C. G., Preiss, B. A., and Lipsky, S. R., *Anal. Chem.*, **39**, 1423 (1967).
- ²² Kirkland, J. J., *J. Chromatog. Sci.*, **8**, 72 (1970).
- ²³ Snyder, L. R., *J. Chromatog. Sci.*, **8**, 692 (1970).
- ²⁴ Kirkland, J. J., and De Stefano, J. J., *J. Chromatog. Sci.*, **8**, 309 (1970).
- ²⁵ Knox, J. H., and Parcher, J. F., *Anal. Chem.*, **41**, 1599 (1969).
- ²⁶ De Stefano, J. J., and Beachell, H. C., *J. Chromatog. Sci.*, **8**, 434 (1970).

The Performance of Packings in High Performance Liquid Chromatography (HPLC)

I. Porous and Surface Layered Supports

by Gordon J. Kennedy and John H. Knox, Department of Chemistry, University of Edinburgh, Scotland

Abstract

The performance of Porasil®, Corasil®, Corning Porous Glass (CPG), and Zipax® are compared for retained and unretained solutes by comparison of plots of the logarithm of reduced plate height against the logarithm of reduced velocity using 48 micron particles (275/325 mesh); and the type of information which can be obtained from such plots is discussed. Zipax gives the best performance for both retained and unretained solutes. The other three supports give similar plate height curves for retained solutes in both partition and adsorption modes, but Corasil gives the best results for unretained solutes. The higher plate heights and gradients of plots for the porous materials (Porasil and CPG) than for Zipax arise from the necessity of molecules to diffuse through stagnant mobile phase held within the pores of the supports. The higher plate height for Corasil than for Zipax for retained solutes is thought to indicate slow mass transfer within the fine pore structure of the silica gel surface layer.

The dependence of plate height upon velocity for the porous materials may be quantitatively interpreted in terms of Giddings' theory for diffusional mass transfer in such materials.

Introduction

The performance of column packings in LC may conveniently be evaluated (1,2) by comparing plots of the reduced plate height, $h = H/d_p$, against the reduced fluid velocity, $v = u d_p/D_m$, where H is the height

equivalent to a theoretical plate, d_p is the particle diameter, u the mean linear velocity of the mobile phase, that is the column length divided by the retention time of an unretained solute, and D_m is the diffusion coefficient of the solute in the mobile phase. This method of comparison is suggested by the theory of chromatography (3) which predicts that, for equally well packed columns of any material, common h versus v or $\log h$ versus $\log v$ curves should be obtained for all particle sizes and all mobile phases, including gases. In practice identical plots are not generally obtained with particles of diameter below 100 μm since smaller particles are more difficult to pack and more difficult to obtain in narrowly sized fractions (2,4,5). Thus the $\log h$ - $\log v$ plots tend to lie higher for smaller particles. However improvements are continually being made in the uniformity of supports both as regards size and shape and in packing methods, with the result that the gap between practice and theory is gradually closing. Logarithmic plots are preferable to linear plots for three reasons. First, wide ranges of velocity can be accommodated and "point" diameters can con-

1. Giddings, J. C., *J. Chromatog.* **13**, 301 (1964).
2. Knox, J. H., and Saleem, M., *J. Chromatog. Sci.* **7**, 745 (1969).
3. Giddings, J. C., "Dynamics of Chromatography Part 1," Marcel Dekker Inc., New York 1965.
4. Knox, J. H., *Anal. Chem.* **38**, 253 (1966).
5. Snyder, L. R., *J. Chromatog. Sci.* **7**, 352 (1969).

veniently be used to indicate the proportional accuracy of the data. Second, logarithmic plots are often nearly straight lines of fractional gradient (2,5,6) in contrast to the usual (h, ν) plots, and these gradients are characteristic of the supports used: surface layered materials give gradients around 0.3, porous materials around 0.6, and ion exchange resin beads (7) around 0.8. Third, at low velocities the logarithmic plots have well-defined common asymptotes with gradients of -1.0 which pass close to the origin.

According to theory (3), band dispersion arises in chromatography from only two sources. The first is axial molecular diffusion, and the second is non-equilibrium between parts of the column where different velocities prevail. Axial molecular diffusion gives the contribution to the plate height shown in Equation 1

$$H = (2\gamma D_m/u); \quad h = 2\gamma/\nu \quad \text{Eq. 1}$$

γ is called the obstructive factor for molecular diffusion and is about 0.6 for randomly packed spheres, and about 0.8 for diatomaceous earths (8,9). For highly porous materials like Porasil and Corning porous glass (CPG) γ is likely to be between 0.8 and 1.0.

Failure to attain perfect equilibrium arises firstly because there is a large scale velocity difference between the mobile and stationary phases, and secondly because there are wide variations of velocity within the mobile phase itself, part of which is streaming in the interparticle space, and part of which may be stagnant within the pores of a porous support. The dispersive effect of these flow variations is countered by transverse molecular diffusion, and, in the streaming part of the mobile phase, by the flow itself whose tortuous path independently carries molecules from slow to fast moving regions and vice versa.

A rigorous theory for the total contribution to H from non-equilibrium has not yet been developed, but the theoretical ideas of Giddings (3) have gone a long way towards this. H can be thought of (to a reasonable approximation) as compiled from contributions which arise from different equilibration mechanisms. Thus the contribution which arises from the balance of non-equilibrium in the stationary phase where the mechanism of equilibration is diffusion has the form of Equation 2

$$H = q \frac{k'}{(1+k')^2} \frac{d_f^2}{D_s} u; \quad h = q \frac{k'}{(1+k')^2} \frac{d_f^2}{D_s} \frac{D_m}{\nu} \quad \text{Eq. 2}$$

q is a configurational factor relating to the geometry of the stationary phase, k' is the column capacity ratio, d_f^2 is the mean square film thickness of the stationary phase, and D_s is the diffusion coefficient of the solute in the stationary phase. In a similar way the contribution from equilibration by diffusion in the stagnant part of the mobile phase (3,10) in a porous support is given by Equation 3

$$H = q' \left\{ 1 + \frac{\alpha k'}{(1+k')} \right\}^2 \frac{d_p^2}{D_m} u; \quad h = q' \left\{ 1 + \frac{\alpha k'}{(1+k')} \right\}^2 \nu \quad \text{Eq. 3}$$

where q' and α are configurational factors for the mobile phase. The contribution arising from non-equilibrium in the streaming part of the mobile phase is

much more complex because both diffusion and flow cooperate to reduce it. The two processes are said to be "coupled" (3,11). Although theoretical expressions have been advanced for the dispersion, none correctly reproduce experimental data (4), and we have, for the present, to resort to an empirical equation. A good fit to the experimental data over a wide range of velocity is given by Equation 4 (12).

$$h = \{ (1/a) + (1/c' \nu^{n'}) \}^{-1} \quad \text{with } n' < 1 \quad \text{Eq. 4}$$

For a range of ν covering less than two orders of magnitude Equation 4 is well approximated by Equation 5

$$h = c' \nu^{n'} \quad \text{with } n' < 1 \quad \text{Eq. 5}$$

This equation holds equally well for GC or LC (2) and n' is readily established by plotting $\log h$ against $\log \nu$ for unsorbed solutes on glass beads or sand. It lies between 0.25 and 0.35. The total reduced plate height is the sum of the contributions given in Equations 1 to 5 and may be written as:

$$\begin{aligned} h &= (2\gamma/\nu) + c' \nu^{n'} + q' \{ 1 + \alpha k' / (1+k') \}^2 \\ &\quad + c_s [k' / (1+k')]^2 \nu \\ &= (2\gamma/\nu) + A \nu^{n'} + C \nu \end{aligned} \quad \text{Eq. 6b}$$

Under the conditions most widely used in high speed LC the first term is negligible, and over a moderate range of reduced velocity, say one order of magnitude, the dependence of h upon ν given by Equation 6 may be expressed approximately by Equation 7.

$$h = d \nu^n \quad \text{with } n < 1 \quad \text{Eq. 7}$$

where n must now be equal to or larger than n' . Equation 7 is a reduced version of Snyder's equation $H = D u^n$ for the dependence of plate height upon velocity in LC (5). Generally n will fall in the range 0.35 to 0.7. However n should increase with reduced velocity and the $\log h$ - $\log \nu$ plots become steeper as ν rises. In contrast, according to Equation 6, the slope n' for unretained solutes on non-porous materials decreases with increase of ν (12-14).

As ν falls towards unity h falls to a minimum at a reduced velocity somewhere between 1 and 10. At lower reduced velocities h rises due to the growing influence of axial molecular diffusion (first term in Equation 6). This trend is well known in GC where it is the practice to work near the minimum in h ; how-

6. Waters, J. L., Little, J. N., and Horgan, D. F., *J. Chromatog. Sci.* 7, 293 (1969).
7. Hamilton, P. B., Bogue, D. C., and Anderson, R. A., *Anal. Chem.* 32, 1782 (1960).
8. Knox, J. H., and McLaren, L., *Anal. Chem.* 36, 1477 (1964).
9. Bennett, E. R., and Bolch, W. E., *Anal. Chem.* 43, 55 (1971).
10. Giddings, J. C., *Anal. Chem.* 34, 1186 (1962).
11. Giddings, J. C., *Anal. Chem.* 35, 1338 (1963).
12. Knox, J. H., and Parcher, J. F., *Anal. Chem.* 41, 1599 (1969).
13. Kaizuma, H., Myers, M. N., and Giddings, J. C., *J. Chromatog. Sci.* 8, 630 (1970).
14. Knox, J. H., and Saleem, M., *J. Chromatog. Sci.* 10, 80 (1972).

ever in LC for technical reasons it is normal to work at reduced velocities between 100 and 1000 and the low velocity rise in h is rarely seen.

Plots of $\log h$ against $\log v$ can reveal much about the dynamics of the equilibration processes in LC, and in particular we expect to be able to make the following inferences:

(1) The position of any $\log h$ - $\log v$ curve relative to the curve for a column containing large particles of the same material (say $d_p > 200 \mu\text{m}$) gives a good indication of the quality of the packing if unadsorbed solutes are used, and of how much improvement in performance may ultimately be expected from the material.

(2) The gradients of plots, n , at reduced velocities above about 20 (when axial diffusion may be ignored) should reflect the importance of equilibration by diffusion in the stationary phase or in the stagnant parts of the mobile phase. We may expect n to be about 0.3 for non-porous materials exhibiting rapid mass transfer and having no stagnant regions, while for ion exchange resin beads which exhibit slow mass transfer in the stationary phase n may approach unity. For porous particles n should be larger than for non porous supports and should rise with v .

(3) The dependence of h upon the column capacity ratio k' can reveal whether stationary or stagnant mobile phase processes are the more important in limiting equilibration. The plate height contribution given in Equation 2 for the stationary phase is zero for $k' = 0$ and $k' = \text{infinity}$ and passes through a maximum for $k' = 1$; the contribution given in Equation 3 for the stagnant part of the mobile phase is a minimum for $k' = 0$ and a maximum for $k' = \text{infinity}$. Thus any decrease in h as k' rises above unity indicates the importance of slow mass transfer in the stationary phase. For surface layered materials where the contributions to h from stagnant mobile phase will be minimal for unretained solutes, the dependence of h upon k' will give important information about the mechanism of equilibration in the stationary phase.

The purpose of this and subsequent papers is to explore some of these dependences and if possible to characterise the important equilibration mechanisms in the various supports available for high performance LC.

Experimental

The equipment was constructed in the laboratory and consisted of a high pressure piston pump (Orlita, Giessen, Model DMP 1515), a heavy walled 2.1 mm bore glass column, and UV detector (Varian Associates or Du Pont Model 410). Very low flow rates were generated either by gravity feed or by applying a controlled gas pressure to the eluent contained in a long narrow tube. Cyclohexane was used throughout as eluent and was degassed by reflux before passing through a 10 micron teflon sinter (2.5 cm diameter 3 mm thick) to the pump. Pressures were measured by Bourdon gauges (Negretti and Zambra; London) which also acted as pulse dampers and reduced the maximum pressure fluctuations at the column head to less than 2% of the inlet pressure.

Column connections were made of brass and were

attached to the columns by Araldite epoxy resin which was allowed to set at room temperature. Columns were of regular glass tubing which withstood pressures up to about 1500 p.s.i. (100 atm), and were operated at ambient temperature. Samples, normally 1 μl of 1% solutions, were injected through a 6 mm diameter septum with a 5 μl microsyringe. No problems were experienced injecting against inlet pressures below 1000 p.s.i. (70 atm) except that septum life was shorter the higher the operating pressure.

Columns were packed on a mechanical device which simultaneously rotated the column at about 180 r.p.m. and bounced it at about 100 times a minute. The height of the bounce was about 1 cm. Packings were added in a stream of air which passed first over the packing material contained in a small glass vessel and then into a funnel attached to the top column-fitting. This method ensured as far as possible that the particles of packing did not stick together while being added to the column. The column was struck with a light hard object during filling at the level of the top of the packing. The packing was not tamped. This procedure was adapted from that of Sie and van den Hoed (15) and is very similar to that found to be optimal by Kirkland (16). Filling time was about 15 min for a 50 cm column. The procedure gave plate height reproducibility from column to column of about 10% (see for example Figure 1).

Column packings were all 44-53 micron fractions sieved from batches received from the suppliers. Their physical properties are summarized in Table I. Microscopic examination showed them to be of good uniformity, both as regards shape and size. Coating of supports with stationary phase, β, β' -oxydipropionitrile (BOP), was carried out by the normal GC procedure by making a slurry of the packing with a solution of BOP in acetone, followed by evaporation of the excess acetone in a rotary evaporator under reduced pressure (water pump).

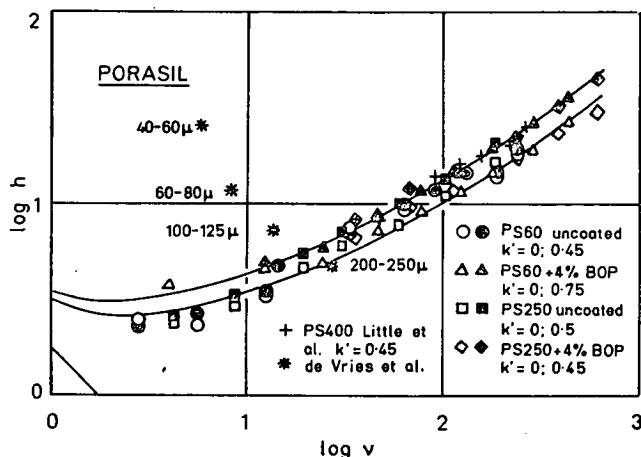


Figure 1. Reduced plate height—reduced velocity plots for retained and unretained solutes on Porasil PS60 and PS250 in both adsorption and partition modes. Little et al, (18) de Vries et al, (19).

15. Sie, S. T., and van den Hoed, N., *J. Chromatog. Sci.* 7, 257 (1969).

16. Kirkland, J. J., *J. Chromatog. Sci.* 10, 129 (1972).

For calculation of the reduced plate heights and reduced velocities, d_p was taken as the mean sieve opening, that is 48.5 μm . D_m was calculated by the Wilke Chang equation (3,17) for each solute. The values so obtained for solutes in cyclohexane were all close to $1.2 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, and this value was adopted as a common value for all solutes, any error being small compared to experimental errors. In comparing our data with those of others who used n-hexane, we have taken $D_m(\text{n-hexane}) = 3.2 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, n-hexane having about one third the viscosity of cyclohexane.

Plate heights were determined from the base width w and from the width of half height $w_{1/2}$, and are reported only for peaks of good symmetry with symmetry indices between 1.00 and 1.10. The symmetry index is defined by Equation 8.

$$\text{Symmetry index} = \frac{\text{Trailing peak half width, } w_t}{\text{Leading peak half width, } w_l} \quad \text{Eq. 8}$$

The leading and trailing half widths are obtained by drawing tangents to the points of inflection of a peak, and dropping a perpendicular from their intersections to the base line. The distance from the intersection of the perpendicular with the base line, to the intersection of one of the tangents with base line gives the half width.

Experimental Results

The experimental data are presented in Figures 1 to 7. All materials except Zipax can act either as adsorbents or as supports for stationary phase. Although Zipax has slight adsorptive properties it cannot be

used for adsorption chromatography because of its (intentionally) small adsorptive capacity. Porasil, Corning porous glass (CPG), and Corasil all have high surface areas (see Table I) and good adsorptive capacities. They give good peak shapes in adsorption chromatography. Coating with a polar stationary phase such as BOP greatly reduces their adsorptive capacity and enables them to be used for liquid-liquid chromatography. In the figures the k' values are quoted for the various samples used. Full details of the conditions employed are given in Table II*. Where the

*A referee has questioned whether chlorobenzene is truly unretained on the supports and adsorbents used in the work. We use the word "unretained" to mean "moving along the column at the same rate as solvent molecules." We have checked the retention of chlorobenzene by using pentane as a supposed "unretained" solute and observing its elution by the differential peak which it produces by virtue of changes in refractive index in the u-v detector. If pentane is assumed to have $k' = 0$, chlorobenzene has $k' \approx 0.1$. However the situation is not simple since the absolute retention volume of pentane depends upon the mobile phase used; with ethanol it is considerably lower than with cyclohexane, and so with the former pentane appears to be partially excluded. These observations spotlight the difficulty of defining what is meant by a genuinely unretained and un-excluded solute when adsorbents of the silica gel type are used. We are now carrying out experiments to elucidate the problem and hope to report shortly. In the meantime we take chlorobenzene to be unretained in the sense defined above. When a more reliable value for its k' becomes available the other values for k' listed in Table II can be corrected.

17. Wilke, C. R., and Chang, P., Am. Inst. Chem. Engr. J. 1, 264 (1955).

Table I. Physical Properties of Supports.

Name and supplier ^a	Description	Pore size Å	Pore volume cm ³ g ⁻¹	Surface area m ² g ⁻¹
Porasil PS60 (Waters)	Spherical particles; regular pore size silica-gel	< 100	0.85	350
Porasil PS250 (Waters)	Spherical particles; regular pore size silica-gel	100-200	0.85	200
Corning CPG75 (Corning)	Porous glass chips; silica; regular pore size distribution	70	0.45	300
Corning CPG240 (Corning)	Porous glass chips; silica; regular pore size distribution	230	0.82	70
Corasil I (Waters)	Fine pore silica-gel layered on glass spheres	probably < 50	0	7 (300) ^b
Corasil II (Waters)	As Corasil I but with double layer	probably < 50	0	14 (300) ^b
Zipax (du Pont)	5 layers of 200 nm silica spheres on larger glass spheres	1000	0	1.0 (15) ^c

^aSuppliers are Waters Associates, Framingham, MA 01701, Waters Associates (Instruments) Ltd., Stockport SX2 6PT, England. Corning Glass Work, Corning, New York 14830. Du Pont Company, Wilmington, Delaware 19898, or du Pont Co. (U.K.) Ltd., Hitchin, Herts SG4 0UR, England.

^bThe specific area of the silica gel layer is about 300 m²g⁻¹, the other figure refers to the material as a whole.

^cThe specific area of 200 nm silica spheres is 15 m²g⁻¹, the other figure refers to the material as a whole.

Table II. Details of Columns and Solutes.

Particle Size:	275/325 mesh or 44-53 μm diameter.		
Column Length:	50 cm.		
Column Temperature:	ambient.		
Eluent:	cyclohexane.		
Packing or Support	Mode ^a	Retained solute ^b and k'	
Porasil 60	Adsorption	acetophenone	0.45
	Partition (4% BOP)	benzaldehyde	0.75
Porasil 250	Adsorption	acetophenone	0.5
	Partition (4% BOP)	nitrobenzene	0.45
Corning CPG70	Adsorption	acetophenone	6.0
	Partition 4% BOP)	2,4-dimethyl 6-tert butyl phenol (topanol)	1.0
Corning CPG240	Adsorption	acetophenone	4.0
	Partition (4% BOP)	topanol	0.4
Corasil I	Adsorption	2-tertbutyl phenol	2.5
	Partition (1% BOP)	topanol	0.4
Corasil II	Adsorption	topanol	0.8
	Partition (1% BOP)	topanol	0.5
		nitrobenzene	0.6
		2,4-dinitrotoluene	3.2
		2-nitroaniline	11.5
Zipax	+1% BOP	3-phenyl propanol	0.4

^aIn the adsorption mode uncoated materials were used which had been activated by heating in vacuo to 120°C for 2 hours before packing. In the partition mode BOP was added in the percentages indicated by weight.

^bChlorobenzene has been assumed to be unretained in all cases, but see footnote.

same solute was used in both adsorption and partition modes it is seen that coating with BOP reduces k' , but whether adsorption is then completely eliminated has not been established.

Figure 1 shows data for retained and unretained solutes on Porasil PS60 and PS240. h is slightly higher for retained than unretained solutes but the difference is small (about 25% in h). Essentially identical results are obtained for adsorption and partition chromatography for both materials. The gradient n lies between 0.5 and 0.6 and there is some tendency for n to rise with v . The curves through the data have been drawn according to Equation 6b (see discussion) and show a reasonable fit between experiment and theory. The flattening of the plot at low values of v reflects the dispersive effects of axial diffusion. The low velocity asymptote, corresponding to the first term of Equation 6, is shown at the left hand lower corner of the figure.

Figure 1 shows data from four independently packed columns, two of which contain packing bearing 4% w/w stationary phase. The close agreement between the four sets of data shows the reproducibility of the packing method. Our data compare well with those of Little, Horgan and Bombaugh (18) for Porasil PS400 but are somewhat better than theirs for PS60. Also shown are some early data of de Vries *et al.* (19) who investigated the role of particle size with Spherosil (the French name for Porasil). They obtained increasingly poor results as the particle size was reduced, and only with large particles of 200-250 μm diameter did they obtain reduced plate heights as low as ours. The comparison shows that consid-

erable improvements have been made since 1967 in techniques of packing small particles and supports our contention that the reduced plate height-reduced velocity plot is to be preferred to the simple plate height-velocity plot in assessing how the performance of any column compares with the ideal; for example, that obtainable with optimally packed large glass beads. The use of reduced plots as an ultimate measure of performance does not, of course, imply that one can obtain better separations by using larger particles even though they normally give lower (h, v) plots. Smaller particles will nearly always give faster separations as pointed out by Snyder and others (20,21) until a certain practical limit is reached (22). The limit is set by problems of packing small particles, reduction in dead volumes, and pressure availability.

Figures 2 and 3 show data for Corning porous glass chips (CPG). This material is made by leaching two phase borosilicate glass (23) which has been sub-

- Little, J. N., Horgan, D. F., and Bombaugh, K. J., *J. Chromatog. Sci.* **8**, 625 (1970).
- de Vries, A. J., le Page, M., Beau, R., and Guillemin, C. L., *Anal. Chem.* **39**, 935 (1967).
- Snyder, L. R., "Gas Chromatography 1970," (R. Stock and E. Perry, eds.), Institute of Petroleum, London 1971, p. 81.
- Knox, J. H., and Saleem, M., *J. Chromatog. Sci.* **7**, 614 (1969).
- Done, J. N., Kennedy, G. J., and Knox, J. H., *Nature* **237** 77 (1972).
- Filbert, A. M., and Hair, M. L., *J. Chromatog. Sci.* **7**, 72 (1969).

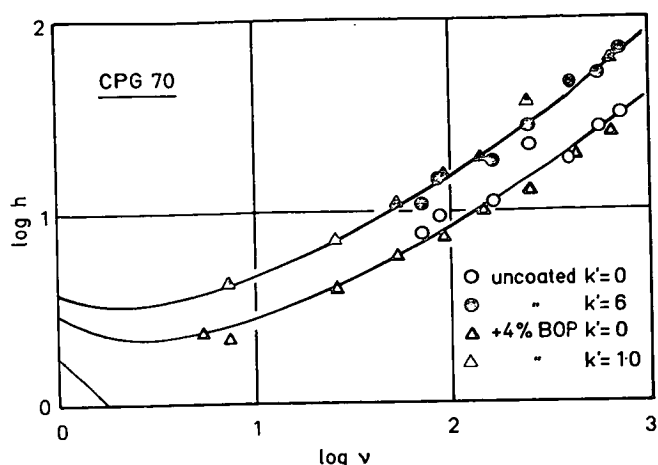


Figure 2. As for Figure 1 with Corning Porous Glass CPG70.

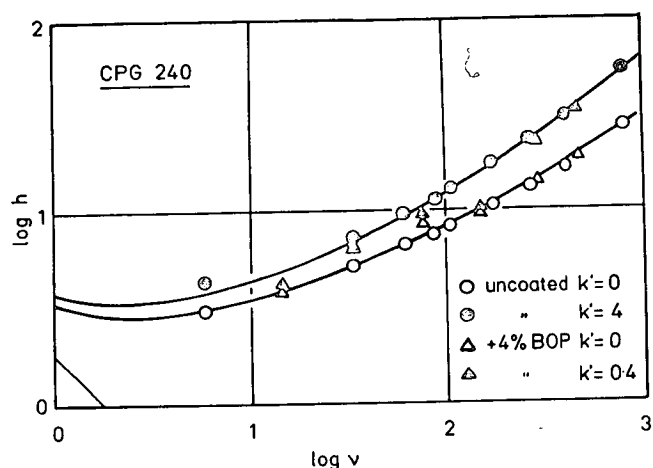


Figure 3. As for Figure 1 with Corning Porous Glass CPG240.

mitted to a controlled cooling procedure. The process gives a porous silica of very uniform pore size, and its physical structure is probably not unlike that of Porasil which is a spherical silica gel subjected to heat and hydrothermal treatment (24). As with Porasil there is little difference between the performance of CPG's with different pore sizes whether in adsorption or partition chromatography. The dependence on column capacity ratio, k' , is somewhat greater than that found with Porasil but this is because the plate heights for unretained solutes are lower, not because the plate heights for retained solutes are higher. Indeed Porasil and CPG perform almost identically with retained solutes. The gradients of the plots are again between 0.5 and 0.6, with n showing a distinct rise with increasing velocity. Contrary to expectation, the randomly shaped Corning chips packed as well if not better than the spherical Porasil particles.

Figures 4 and 5 show data for Corasil. Broadly speaking the plate heights are similar in both adsorption and partition modes as was found for Porasil and CPG, and there is no significant difference in performance between Corasil I and Corasil II, (Figure 4). What is most noticeable is the strong dependence of h upon k' . The gradient n for unretained solutes is about 0.35, a value typically found for impervious

materials, while that for moderately retained solutes is about 0.7. The curves for retained solutes are very similar to those on Porasil and CPG, but for unretained solutes they are much lower. Figure 5 shows plots for Corasil II using solutes with different k' values. The dependence upon k' is not very clear, and is at least partly a function of the nature of the solute. However h seems to rise quite sharply as k' increases from zero to reach a maximum for k' between 0.8 and 3. At higher values of k' , h may decrease again. Independent work employing a wider range of k' (25) confirms the present results and shows that slow mass transfer in the stationary as well as in the stagnant mobile phase is important in producing non-equilibrium in Corasil. The low plate height curve for unretained solutes shows that the cause of the relatively poor performance of Corasil with retained solutes is not to be found in its packing characteristics, but must be related to the structure of the silica gel surface layer.

Figure 6 gives the data for Zipax. For both re-

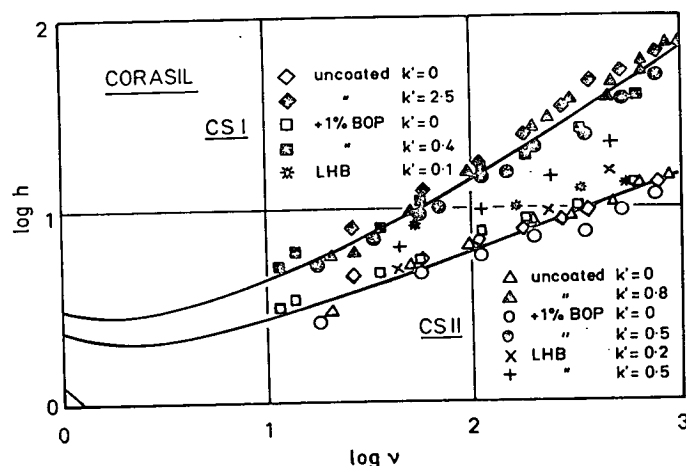


Figure 4. As for Figure 1 with Corasil I and Corasil II. LHB = Little et al. (18).

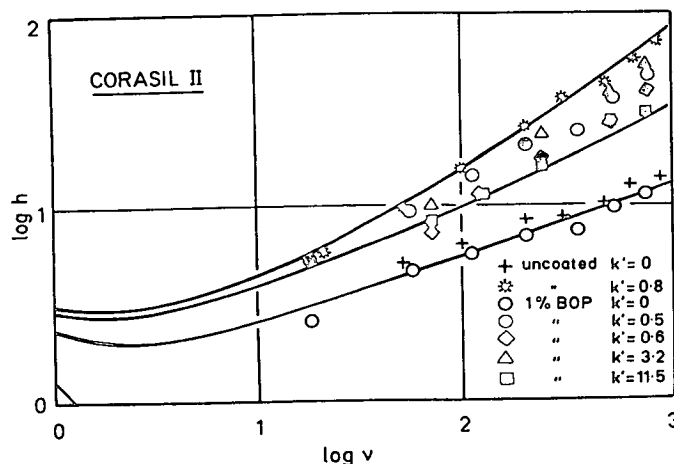


Figure 5. As for Figure 1 with Corasil II showing the effect of changing column capacity ratio.

24. Pechiney-St. Gobain, French Patent, 1,493,240 (1967).
25. Knox, J. H., and Done, J. N., unpublished data.

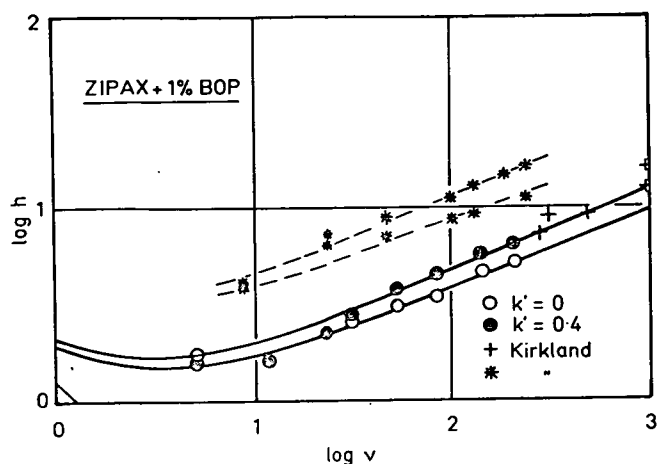


Figure 6. As for Figure 1 with Zipax. Data of Kirkland. (+16, *26).

tained and unretained solutes it gives lower plate heights than any of the other materials investigated. The gradient $n = 0.35$ is also lower than for any other material except Corasil using unretained solutes. Our data agree well with the recent data of Kirkland (16), being a slight improvement on his earlier data (26). The excellent performance of Zipax vindicates Kirkland's original contention (26) that surface layered particles should give near ideal performance in liquid chromatography, especially at high fluid velocities, and shows that the thin porous surface layer of Zipax offers little impediment to equilibration.

Discussion

The data of Figures 1 to 6 broadly confirm the ideas outlined in the introduction. Thus unadsorbed solutes show lower plate heights and lower values of n on surface layered materials than on porous materials indicating that equilibration by diffusion in the stagnant parts of the mobile phase is important in the latter. Although straight lines in accordance with Equation 7 may be drawn through the data within experimental error, a slightly better fit is obtained with curves in which the gradient rises with v as would be predicted on the basis of Equation 6 if h contained a term proportional to v . We have therefore drawn the full lines in Figures 1 to 6 according to Equation 6b where γ has been taken as 0.6 for the non-porous materials Zipax and Corasil, and 0.9 for Porasil and CPG. The values of A and C are listed in Table III.

Generally A is slightly higher for retained than unretained solutes being about 1.3 for $k' = 0$ and 1.9 for k' about unity. The A -term arises from the interparticle flow and is roughly the same for all supports except Zipax, for which it is noticeably lower. The weak dependence of A upon k' is generally in line with the predictions of Giddings (10) for the part of h controlled by molecular diffusion. The low value of A for Zipax suggests that we have succeeded in packing this material better than the other supports. Why this should be is not clear and requires further

study, but it is worth noting that the data obtained by Knox and Parcher (12) for 480 μm glass spheres gave a still lower value of $A = 0.4$. This seems to be about the lowest value which can be achieved by random packing of spheres; hopefully with further work it ought to be possible to attain similar values with small particle materials.

The values of C indicate the differing importances of non-convective mass transfer in the stationary phase and stagnant mobile phase. For unretained solutes C has a significant value only with porous materials. C is essentially zero for unretained solutes on the surface layered materials, and the small finite values shown in Table III may be attributed to errors in the assumed value of $n' = 0.33$ and to experimental imperfections. Except for Zipax C is considerably larger for retained than for unretained solutes, and this is particularly so for Corasil.

Table III. Experimental Parameters for Plate Height Equation.

$$\text{Equation } h = (2\gamma/v) + Av^{0.33} + Cv$$

Assumed values: γ for non porous materials = 0.6

γ for porous materials = 0.9

Material	Degree of Retention	A	C
Porasil PS60	Retained	1.72	5.0×10^{-2}
and PS250	Unretained	1.46	3.6×10^{-2}
Corning CPG240	Retained	1.71	4.3×10^{-2}
	Unretained	1.40	1.7×10^{-2}
Corning CPG70	Retained	1.88	6.0×10^{-2}
	Unretained	1.18	2.9×10^{-2}
Corasil	Retained ^a	2.04	5.9×10^{-2}
	Unretained	1.26	0.25×10^{-2}
Zipax	Retained	0.95	0.32×10^{-2}
	Unretained	0.77	0.30×10^{-2}
Glass beads 480 μm	Unretained ^b	0.37	0

^aValues of A and C are for substances giving highest plate heights and are dimensionless.

^bFrom Knox and Parcher (12), for 480 μm diameter glass beads. Particle diameter otherwise 48.5 μm .

The values of C may be compared with those predicted by the non-equilibrium theory of Giddings (3,10) who gives the value shown in Equation 9 for porous spheres.

$$h = Cv = \frac{\{(1-\phi) + \phi(k'/(1+k'))\}^2}{30(1-\phi)\gamma'}$$

Eq. 9

ϕ is the fraction of mobile phase in the interparticle space and is around 0.5 for most materials; γ' is the obstructive factor for diffusion in the stagnant mobile

term accounts for the contributions to k arising from the stationary phase and stagnant regions of the mobile phase. C depends on the nature of the support and the configuration of the stationary phase. The dependence of solute retention volumes on the difference in adsorption free energies between the solute and the eluent is examined for a number of solute-eluent pairs in adsorption chromatography on Corning CPG^R 75. The retention volumes of isotopically labelled samples of three eluents were determined and it is shown that, where the solute adsorption free energy is less than that of the eluent, the solute retention volume is less than that of an isotopically labelled mobile phase sample.