# Development of New Packing Materials <br> for High Performance Liquid Chromatography 

## by

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Thesis submitted for the degree of Doctor of Philosophy

## DECLARATION

This thesis is the original work of the author, unless otherwise stated, and has not been submitted previously for any other degree.

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During the course of this work, as well as attending departmental seminars 1 have also attended the following courses and meetings.

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## INTRODUCTION TO LIQUID CHROMATOGRAPHY

### 1.1. Historical Introduction

The fundamental concept of chromatography is straightforward. Different chemical substances are separated in a column packed with a particulate material which is often known as the stationary phase. A mobile phase or eluent is passed down the column and the substances dissolved in the eluent interact with the column packing. It is the differences in strength of this interaction that provides the basis for the chromatographic separation.

The origin of the technique dates back to the turn of the century. In 1903 Michael Tswett a Russian Botanist carried out work on the separation of plant chlorophylls(1) using a column packed with calcium carbonate. Tswett invented the name of chromatography to describe his new analytical process(2,3). It is now generally accepted that Tswett was the first who clearly understood the processes involved in chromatography(4).

Somewhat before this time several other workers had been involved in similar types of analysis. Runge(5) studied the separation of dyes in the latter half of the 19 th century, using a coarse form of paper chromatography. While Day a petroleum chemist carried out studies on the composition of crude petroleum(6) using packed columns.

After Tswett's death in 1919, interest in chromatography waned and during the next decade, barring the work of $\operatorname{Palmer}(7)$ on carotins in milk, there was no real use of the technique. Then in 1930 there was a revival of interest,
sparked off by the rediscovery of Tswett's techniques by Kuhn, Lederer, and Winterstein(8).

Up until this time chromatography was based on the adsorption forces between liquids and solids.

In 1941 Martin and Synge (9) produced their classical paper describing the separation of neutral amino acids as a result of the differing partition ratios of the solutes, between water (the stationary phase) held on an inert support, and chloroform (the mobile phase) an immiscible organic eluent. This paper laid the foundation of the second mode of chromatography; Liquid-Liquid Partition Chromatography (LLC). Martin and Synge introduced the concept of the theoretical plate, defining the height equivalent to a theoretical plate as - "the thickness of a layer such that the solution issuing from it is in equilibrium with the mean concentration of the solute in the non-mobile phase throughout the layer".

The work of Martin and Synge proved a great step forward in the understanding of chromatography, indeed they also saw that column efficiency could be improved with smaller particles and higher pressures. Furthermore the column should be operated at an optimum flowrate and that a lack of uniformity in flow would lead to a drop in column efficiency. Finally Martin and Synge realised that their theory could be applied to gases as well as liquids. Following this proposal, Martin and James(10) demonstrated the new technique Gas Liquid Chromatography in 1952. There then followed a decade of massive development in Gas Chromatography(GC). This development produced a technique that was fast, precise, quantitative, highly sensitive and suitable for automation.

Running parallel to this development was that of Thin Layer Chromatography (TLC), first carried out by Ismailov and Schraiber(11) and developed by Kirchner et al(12) in 1951. The improvement in plate preparation techniques largely due to Stahl(13) made the method of analysis much more effective, sensitive and reproducible.

This period also saw the first description of reversed phase chromatography by Howard and Martin(14), where the mobile phase is more polar than the stationary phase. Also around this time gradient elution was described by Alm, Williams and Tiselius(15). Here the eluent was changed in a stepwise manner to alter the interaction of the solute with the stationary phase.

As the importance of GC grew a large amount of work was done which laid down a comprehensive theory of chromatography. Major contributions were made by Van Deemterr(16), Golay(17), Giddings(18) and Knox(19), so that by the late 1960s GC had a firm theoretical base. However the theory of LC had progressed little since the work of Martin and Synge and analysts interested in separating compounds with high polarity, high molecular weight, or thermally unstable had to use classical LC. Typically the separations of proteins, surfactants, metabolites and dyes were restricted to the long labourious methods associated with gravity fed LC.

Giddings(18) was the first to clearly establish that the GC theory concerning the interaction of particle size, mobile phase velocity, pressure drop, and column efficiency could be applied to LC provided the correct scaling factors were used. The scaling factors arose from two factors. Firstly because liquids are some one hundred times more viscous than gases, the operating pressures have to be correspondingly higher. Secondly because the diffusion rates of liquids are some $10^{3}$ to $10^{4}$ times smaller than those of gases, the distance the
solute diffuses should be reduced according to the square root of this factor to give equivalent times of mass transfer; particles must then be 30 to 100 times smaller. Giddings improved the plate theory of Martin and Synge with theories based on random walk and non-equilibrium. The introduction of reduced parameters by Giddings(20) as a method of direct comparison between one column and the next, led to the prediction of optimum particle size for particular operating conditions(21). As a consequence of the smaller the columns used in modern LC, close attention had to be paid to sample introduction, ensuring that the sample was introduced as a narrow band.

The development of modern LC now became rapid. Between 1967 and 1969, Huber(22), Horvarth, Preiss and Lipsky(23) and Kirkland(24) described the first high performance chromatographic systems, designed to give analysis times comparable to those of GC.

At this time two other major improvements were made. The first improvement was the development of equipment, especially of detectors. This resulted in highly sensitive UV absorbance detectors, refractive index detectors(25), electrochemical detectors(26) and spectrofluorometric detectors(27). Development has continued in this field and it is now possible to interface the HPLC system directly with a mass spectrometer(28). Other new features of High Performance Liquid Chromatography (HPLC) equipment were the use of high pressure pumps, and GC-type injection devices. The development of detector, pumping and injection systems were responsible for bringing HPLC into the age of instrumental chemistry.

The second improvement was made in the production of packing materials. In classical LC which used a gravity feed (i.e. a very low pressure drop), packing materials were generally porous particles of $30-100 \mu \mathrm{~m}$ in diameter.

Kirkland(24) found that by using a "controlled surface porosity packing" performance could be improved. By making a material with a non-porous core and coating this in a thin porous layer the mass transfer problems arising from slow diffusion into and out of stagnent mobile phase held within the pores were virtually eliminated. Typically these packings were $30 \mu \mathrm{~m}$ in diameter, the porous coating being $1-2 \mu \mathrm{~m}$ thick. These packings were termed pellicular. Pellicular packings had much improved mass transfer properties, their loading capacity was very much less than that of fully porous silica gels due to the low surface area of the particles.

The next stage in the development of packings for HPLC was that of smaller fully porous particles of $5-10 \mu \mathrm{~m}$ in diameter(29). This reduction in particle size would in theory produce a good compromise between efficiency, pressure drop, analysis time and reproducibility of production. At the time it was believed that the packing of particles would prove a severe problem as the diameter decreased, especially using the existing dry-packing methods(30). However the development of slurry packing maethods largely solved this problem(31,32,33).

It now appears that small particles may actually be easier to pack using these methods. Small particles form a better slurry than the larger particles. A suspension of small particles is more stable than one of large particles, as the larger particles tend to sediment more rapidly during the packing procedure, producing a non-uniform particle bed.

Following development of the pellicular and microparticulate particles, came the production of bonded phases which have subsequently displaced liquid-liquid partition chromatography $(34,35,36)$. These bonded phases were developed because adsorption chromatography proved unsuitable for highly
polar molecules and LLC proved difficult to use for such solutes. The bonded phase was chemically bound onto the support surface. The first of these phases were produced by Halasz and Sebastian(34), but subsequently a wide variety of functional groups have been bonded onto silica gel. These include hydrophobic groups, polar groups, and ion-exchange groups. Many different production methods and applications have been developed.

One of the most recently developed HPLC modes is that of High Performance Size Exclusion Chromatography (HPSEC). HPSEC is the modern LC equivalent of Size Exclusion Chromatography(SEC) where the separation is due to differences in molecular size and the resulting interaction with the packing material. The first SEC column packing materials were the soft organic gels developed by Moore(37), and by Porath and Flodin(38). These gels were sensitive to pressure and therefore unsuitible for HPLC. Rigid microparticulates were subsequently developed by $\operatorname{Haller}(39)$ and by De Vries et al(40). Because of its importance in relation to this thesis the history, theory and prospects for High Performance Size Exclusion Chromatography (HPSEC) will be dealt with more fully in Chapter 2.

### 1.2. Different Modes of Liquid Chromatography

The different modes of modern HPLC all have essentially the same theoretical foundation, and they can very often be carried out using the same equipment. The different LC modes are variously classified according to, the nature and structure of the stationary phase and or according to differing interactions between the solutes and the phases.

### 1.2.1. Adsorption Chromatography (LSAC)

Adsorption Chromatography was the original technique used by $\operatorname{Tswett}(2,3)$. Nowadays the method is used for the separation of non-polar or moderately polar organic molecules. Molecules of high polarity tend to be strongly retained by typical oxide-based adsorbents and may even be irreversibly held onto the support, making chromatography very difficult. The basis for this form of chromatography lies in the relative partitioning of the solute between the hydroxylated adsorption sites on the packing and the non-polar (or weakly polar) mobile phase. Such separations are dependent on the adsorbent activity and control of this activity is important. Snyder (42) fias given a comprehensive treatment of this subject.

In modern LSAC, microparticulate silica gel is by far the most widely used adsorbent. The surface of this material carries various types of adsorption site, as illustrated in Figure 1.1, each with its own activity. This results in complex interactions between solute and support. By addition of deactivators, e:g. $\mathrm{H}_{2} \mathrm{O}$ to the silica gel a more uniform set of the sites can be achieved which leads to better chromatography.

### 1.2.2. Liquid-Liquid Partition Chromatography (LLC)

Liquid-Liquid Partition Chromatography was first developed by Martin and Synge (9). The different migration rates of solutes were based on their differing partition ratios for solutes between a moving and a stationary phase held on a porous inert support, the two phases being in thermodynamic equilibrium. It is possible to perform two types of LLC. In the first type, or "normal" type the stationary phase is polar and the mobile phase is non-polar. Such a system would be tri-ethylene glycol coated onto silica gel with a mobile phase of tri-ethylene glycol saturated hexane. This system could be used to separate solutes such as phenols and alcohols.

In the second type of partitioning system, termed "reversed phase", the stationary liquid is non-polar and the flowing eluent is polar. Reversed phase systems are more difficult to use as the forces responsible for binding the stationary liquid onto the support are now weak and the stationary phase is readily washed from the column. An example of a reversed phase system silanised
would be squalane coated onto $\uparrow$ silica gel and squalane saturated water/alcohol as the mobile phase.

As the two phases are in thermodynamic equilibrium only isocratic elution can be used effectively. If gradient elution were used the composition of the eluent and the thermodynamic equilibrium between the mobile and stationary phases would be difficult to maintain. The difficulty in covering a wide range of compounds coupled with the inherent instability of the non-polar stationary liquid led to development of chemically bonded stationary phases which have now almost completely taken over from LLC.

### 1.2.3. Bonded Phase Chromatography (BPC)

Bonded Phase chromatography has been made possible by modification of the surface characteristics of a packing material so that a wider variety of solute support interactions are possible. Here the character of the support can be altered by chemically bonding organic functional groups onto its surface. These bonded groups fall into three main catagories.

1. Hydrophobic groups e.g. $-\mathrm{C}_{18} \mathrm{H}_{37},-\mathrm{C}_{8} \mathrm{H}_{17},-\mathrm{C}_{2} \mathrm{H}_{5},-\mathrm{CH}_{3}$
2. Polar groups e.g. $-\mathrm{CH}_{2} \mathrm{CH}_{2} \mathrm{CH}_{2} \mathrm{NH}_{2},-\mathrm{CH}_{2} \mathrm{CH}_{2} \mathrm{CN}$
3. Ion exchange groups e.g. -sulphonic acids , -quaternary ammonium groups

These groups are normally bonded onto the silica gel surface by the formation of silicon to carbon bonds.


The bonding is stable to hydrolysis provided the pH is maintained between 2 and 8. At low pH the silicon to carbon bond holding the bonded phase to the gel surface is subject to hydrolysis, while at high pH the silica gel itself are subject to hydrolysis leading to dissolution of the silica gel. Reversed phase bonded materials (as shown above) are employed in the great majority of HPLC applications carried out today. The techniques of bonding are also used to improve the quality Size Exclusion Chromatography materials, but here their purpose is to reduce interactions between the solute and the support surface.

### 1.2.4. Ion Exchange Chromatography (IEC) and Ion Pair Chromatography (IPC)

Ion Exchange Chromatography (one of the oldest forms of LC) was used originally to separate ionic species such as lanthanide metals and later amino acids. Carbohydrates $(43,44)$ in the form of their borate complexes were also
separated by IEC where as outlined previously, adsorption chromatography was not suite for such separations, due to the high polarity of the solutes. These highly polar solutes were able to displace the polar deactivator (usually water) from the surface. This partial reactivation of some of the adsorption sites resulted in poor resolution and peak shape. The classic ion exchange chromatographic systems employed derivatives of styrene divinylbenzene crosslinked resin with acidic or basic groups, such as $-\mathrm{SO}_{3} \mathrm{H}$ and $-\mathrm{N}^{+} \mathrm{R}_{3}$ groups.

In IEC the separation is due to the solute ions displacing mobile counter ions associated with the fixed ionic sites on the support. For example:


In ion pair chromatography, the ion pairs are formed in dynamic equilibrium between the solute ions and ions of a pairing agent. In contrast to the situation in ion exchange chromatography the pairing agent in IPC is not fixed to the surface of the support. Although ion pairs are present in both the mobile and stationary phases(45), the partition coefficent of an ion pair is normally in favour of the stationary phase, whereas the partition coefficent of the unpaired ions is in favour of the mobile phase. The addition of the pairing agent enhances retention and provides great flexibility in manipulation of retention. In this type of separation the mobile phase is often an aqueous system while the stationary phase is usually a hydrophobic bonded silica gel.

### 1.2.5. Size Exclusion Chromatography (SEC)

In SEC the separation of the solutes is achieved as a result of differences in their molecular size. This technique is commonly used for separations of polymers or other large molecules, say those with molecular weight greater than 2000. Solutes are distributed between the flowing eluent and the stagnant


#### Abstract

eluent held within the pores of the support. In SEC it is important that there is no adsorption of the solutes by the packing material if selectivity is to be dependent purely on molecular size. For separations by SEC to occur, the dimensions of the pores of the packing material must be similar to those of the molecules to be separated. Large solutes are then eluted first from the column as they are partially or totally excluded from the pores while smaller molecules permeate the pores more fully and have longer retention times.


The range of molecules separated by SEC is large and recently it has been used for the separation of macromolecules with molecular weight up to $10^{7}$ ).

Resolution in SEC is obviously affected by the pore size, pore volume and pore size distribution of the packing, and the detailed theory of the method will be dealt with in Chapter 2. Part of the work undertaken in this project was geared to produce a material suitible for High Performance Size Exclusion Chromatography (HPSEC) of large molecules.

### 1.2.6. Other Modes of Chromatography

There are several other chromatographic modes closely related to LC, some of the more promising include:

1. Affinity Chromatography. This technique exploits the biological specificity of a protein-ligand interaction. The ligand is bound to an inert support. The proteins affinity for the ligand may be altered by changing the mobile phase or its pH . This technique has already been used in protein analysis (46).
2. Electro-osmotic Chromatography. Here the flow of eluent through the porous bed is as a result of an applied electric field rather than by means of a conventional pump. The flow velocity is related to the applied potential. This electro-osmotic flow has a flat rather than a parabolic flow profile. This can
result in a lower dispersion due to flow, and thus a lower plate height(47).
3. Supercritical Fluid Chromatography. In this form of chromatography where the eluent is a supercritical fluid improved efficency is attained by exploiting the low viscosity and high diffusion coefficients associated with such fluids. Mass tranfer contributions are minimised by higher diffusion coefficients associated with a fluid with a density between gas and liquid, and at a temperature above the critical temperature.

### 1.3. Theoretical Aspects of Liquid Chromatography

### 1.3.1. Introduction

The ability of chromatography to separate solutes is governed by a combination of thermodynamic and kinetic factors. Thermodynamic processes are responsible for the differential migration rates of the solutes which partition themselves between the flowing and stationary zones within the column. Kinetic factors determine the spreading of solute bands as they migrate along the column. Resolution of solutes is determined by both the thermodynamic and kinetic factors. Thus for the best resolution the system must be tuned to give the optimum combination of solute separation and band broadening. These thermodynamic and kinetic processes can be treated independently and will be discussed in this chapter.

### 1.3.2. Definitions

In order to elucidate the theory of chromatography a number of basic quantities must be defined. Figure 1.2 gives a representation of the various ways in which volumes within the column may be expressed. The column may be split according to zones or phases. The mobile zone is defined as the flowing eluent within the column, $V_{0}$. This volume, $V_{o}$, does not include stagnent eluent held within the pores of the support. The stationary zone is made up of stagnent eluent within the pores and eluent adsorbed onto the support. If a solute is unretained and has no partition into the stationary zone then it will be eluted in a time $t_{0}$ corresponding to an elution volume $\mathbf{V}_{\mathbf{o}}$ the volume of the mobile zone, where:

$$
\begin{equation*}
v_{o}=f_{v} \cdot t_{0} \tag{1.1}
\end{equation*}
$$

and $f_{v}$ is the volume flowrate of the eluent. In SEC a solute eluting at $v_{0}$ is said
to be totally excluded.

The mobile phase comprises the total eluent held within the column, both flowing and stagnent, and has a volume, $\mathbf{V}_{\mathbf{m}}$. The stationary phase comprises partitioning material other than the components of the eluent and has a volume, $V_{s}$. If a solute passes through the column without interaction with the stationary phase it is said, in retentive chromatography to be unretained and will elute in a time $t_{m}$ and with an elution volume equivalent to the volume of the mobile phase $\mathbf{V}_{\mathbf{m}}$ where:

$$
\begin{equation*}
V_{m}=f_{v} \cdot t_{m} \tag{1.2}
\end{equation*}
$$

In SEC such a solute is termed fully permeating, but in LC it is termed "unretained". The volume of eluent components, $\mathbf{V}_{\mathbf{p}}$ within the pores of the support is then:

$$
\begin{equation*}
V_{p}=V_{m}-V_{o} \tag{1.3}
\end{equation*}
$$

If the solute interacts with the stationary phase the solute will be retained and will elute in a time $t_{m}^{\prime}$ corresponding to an elution volume $V_{R}$ where:

$$
\begin{equation*}
V_{R}=f_{V} \cdot \mathbf{t}_{\mathbf{R}} \tag{1.4}
\end{equation*}
$$

In order to represent the degree of retention, a capacity ratio is used. The phase capacity ratio is given by:

$$
\begin{equation*}
k^{\prime}=\left(t_{R}-t_{m}\right) / t_{m}=\left(V_{R}-V_{m}\right) / V_{m} \tag{1.5}
\end{equation*}
$$

and the zone capacity factor by:

$$
\begin{equation*}
k^{\prime \prime}=\left(t_{R}-t_{0}\right) / t_{0}=\left(V_{R}-V_{0}\right) / V_{0} \tag{1.6}
\end{equation*}
$$

Retention may then be expressed as:

$$
\begin{align*}
& t_{R}=t_{m}\left(1+k^{\prime}\right)  \tag{1.7}\\
& V_{R}=v_{m}\left(1+k^{\prime}\right) \tag{1.8}
\end{align*}
$$

or,

$$
\begin{align*}
& t_{R}=t_{0}\left(1+k^{\prime \prime}\right)  \tag{1.9}\\
& V_{R}=t_{0}\left(1+k^{\prime \prime}\right) \tag{1.10}
\end{align*}
$$

The mean linear flow velocity of the mobile zone, $u_{\boldsymbol{\alpha}}$ is defined as:

$$
\begin{equation*}
u_{o}=L / t_{0} \tag{1.11}
\end{equation*}
$$

where $L$ is the column length. The mean linear flow velocity of the mobile phase is,

$$
\begin{equation*}
\mathrm{u}_{\mathrm{m}}=\mathrm{L} / \mathrm{t}_{\mathrm{m}} \tag{1.12}
\end{equation*}
$$

$U_{m}$ is generally called the linear flowrate of eluent. in retentive LC the velocity with which a solute moves along a column, $u_{\text {band }}$, is often expressed relative to the mobile phase velocity as the Retention ratio, R, where:

$$
\begin{equation*}
R=u_{\text {band }} / u_{m}=1 /\left(1+k^{\prime}\right) \tag{1.13}
\end{equation*}
$$

After injection a narrow solute band rapidly adopts a Gaussian profile where the width of the solute band, $\sigma_{z^{\prime}}$ is proportional to the square root of the distance migrated. The distance migrated along the column being given by,z.

$$
\begin{equation*}
\sigma_{z}^{2^{\prime}} \alpha z^{C} \tag{1.14}
\end{equation*}
$$

Thus for a column of length $L$,

$$
\begin{equation*}
\sigma_{z}^{2}=H . L \tag{1.15}
\end{equation*}
$$

H has the dimensions of a length and is called the "height equivalent to a theoretical plate" (plate height) following the work of Martin and Synge (9). Obviously the smaller the value of H the narrower the peak. For the purposes of measurement the base of the peak is often used, where the base width of a peak, $w_{z}$ is denoted by the intersection of the tangents at the points of inflection with the base. This can be seen in Figure 1.3. The base width is then equivalent to:

$$
\begin{equation*}
w_{z}=4 \sigma_{z} \tag{1.16}
\end{equation*}
$$

and

$$
\begin{equation*}
H=1 / 16 \cdot w_{z}^{2} / L=L / 16 \cdot\left(w_{t} / t_{R}\right)^{2} \tag{1.17}
\end{equation*}
$$

As a measure of column efficiency the number of theoretical plates to which the column is equivalent, $N$, provides a guide to the column's performance, the higher the number of theoretical plates the better the column.

$$
\begin{equation*}
N=L / H \tag{1.18}
\end{equation*}
$$

Equations (1.17) and (1.18) enable the efficiency to be expressed as:

$$
\begin{equation*}
N=16 .\left(L / w_{z}\right)^{2}=16 .\left(t_{R} / w_{t}\right)^{2}=16 .\left(V_{R} / w_{v}\right)^{2} \tag{1.19}
\end{equation*}
$$

The development of reduced parameters by Giddings(20) allowed a comparison between systems with different sizes and different solute/eluent characteristics. The reduced plate height, h , expressed as :

$$
\begin{equation*}
h=H / d_{p} \tag{1.20}
\end{equation*}
$$

gives the plate height in terms of particle diemeter, $d_{p}$. The reduced velocity, $v$,
measures of the flowrate over a particle relative to the diffusion rate across a particle.

$$
\begin{equation*}
v_{o}=u_{o} d_{p} / D_{m} \tag{1.21}
\end{equation*}
$$

where $D_{m}$ is the diffusion coefficient of the solute in the eluent.

### 1.3.3. Resolution

The goodness of separation of any two solutes by chromatography can be described quantitatively by the resolution, $\mathbf{R}_{\mathbf{s}^{\prime}}$ as shown in Firure 1.4. $\mathbf{R}_{\mathbf{s}}$ is defined by Equation (1.22).

$$
\begin{equation*}
R_{S}=\Delta z / w \tag{1.22}
\end{equation*}
$$

where $\Delta z$ is the separation of the peak maxima and $w$ is the mean peak width.

$$
\begin{gather*}
R_{s}=2 .\left(z_{2}-z_{1}\right) /\left(w_{1}+w_{2}\right)  \tag{1.23}\\
R_{S}=2 .\left(t_{R 1}-t_{R 2}\right) /\left(w_{t 1}+w_{t 2}\right)  \tag{1.24}\\
R_{S}=2 .\left(V_{R 1}-V_{R 2}\right) /\left(w_{v 1}+w_{v 2}\right) \tag{1.25}
\end{gather*}
$$

A value of $R_{S}=1.5$, will give near baseline resolution of two peaks. The separation $\Delta z$, is controlled thermodynamically and the mean width, w by kinetic processes. The resolution expression can be expanded to:

$$
\begin{equation*}
R_{S}=0.5 \cdot(\alpha-1) /(\alpha+1) \cdot k^{\prime} /\left(1+k^{\prime}\right) \cdot N^{1 / 2} \tag{1.26}
\end{equation*}
$$

where $\alpha=k_{2}^{\prime} / k_{1}^{\prime}$ and $k^{\prime}$ is the mean capacity ratio. From the above equation it is clear that for any separation, defined by specifing a value of $R_{s}$; three conditions must be met:

1. There must be a difference between $k_{1}$ and $k_{2}$ so that $\alpha \neq 1$
2. The solutes must retained so that $k^{\prime} \neq 0$
3. There must be a adequate number of theoretical plates to achieve the separation.

Conditions 1 and 2 are thermodynamic in nature while condition 3 is kinetic. Significant improvement in resolution is achieved more easily by altering the $\alpha$ - value than by alterating $N$. However adequate $N$ is a necessary starting point for any attempt to resolve a pair of closely related solutes.

### 1.3.4. Thermodynamics

The column divides into two zones, the mobile zone, containing the fluid outwith the particles and the stationary zone. The stationary zone includes the support phase and the stagnent eluent held within the pores of the support and any stationary phase. As a result of the rapid partition between the two zones the separation is achieved. As already seen the relative speed at which a solute band moves down the column is given by

$$
u_{\text {band }} / u_{0}=\text { fraction of molecules in the mobile zone }
$$

where $u_{0}$ is the mobile zone velocity. In terms of the relative quantities of solute in each phase,

$$
\begin{gather*}
u_{\text {band }} / u_{0}=q_{s z} /\left(q_{s z}+q_{m z}\right)=v_{0} / v_{R}  \tag{1.27}\\
=1 / 1+k^{\prime \prime}
\end{gather*}
$$

where $q_{s z}$ is the amount of solute in the stationary zone and $q_{m z}$ is the amount of solute in the mobile phase. The zone capacity ratio is then $k^{\prime \prime}=q_{s z} / q_{m z}$. As this is a near equilibrium process we may write

$$
\begin{equation*}
k^{\prime \prime}=c_{s z} \cdot V_{s z} / c_{m z} \cdot V_{m z} \tag{1.28}
\end{equation*}
$$

where $c_{s z}$ and $c_{m z}$ are the concentrations of solutes in the stationary and mobile zones. $V_{s z}$ and $V_{m z}$ are the volumes of the respective zones. The themodynamic distribution coefficient between the zones can be written as:

$$
\begin{gather*}
\mathrm{K}^{\prime \prime}=\mathrm{c}_{\mathrm{sz}} / \mathrm{c}_{\mathrm{mz}}  \tag{1.29}\\
\mathrm{k}^{\prime \prime}=\mathrm{K}^{\prime \prime} \cdot \mathrm{V}_{\mathrm{sz}} / \mathrm{V}_{\mathrm{mz}} \tag{1.30}
\end{gather*}
$$

The volume of the mobile zone is the volume of the flowing eluent within the column $V_{0}$. In pure SEC the separation mechanism does not rely on interaction between the solutes and the stationary phase. The separation is achieved as a result of partition between the flowing eluent, $\left(V_{0}\right)$, and the eluent held within the pores, $\left(V_{p}\right)$. (If we were to decribe SEC in terms of retentive LC then the pore volume could be regarded as "the stationary phase").

$$
\begin{align*}
& k^{\prime \prime}=K^{\prime \prime} \cdot V_{P} / V_{0}  \tag{1.31}\\
& V_{R}=V_{0}\left(1+k^{\prime \prime}\right)  \tag{1.32}\\
& V_{R}=V_{0}+K^{\prime \prime} \cdot V_{P} \tag{1.33}
\end{align*}
$$

This is the fundamental equation describing retention and is directly applicable to Size Exclusion Chromatography. $K^{\prime \prime}$ can take values only within the range 0 to 1 in SEC. In retentive chromatography unretained solutes are defined as those which may enter the stagnant mobile phase within the pores of the support. Their relative velocity along the column is then by convention defined relative to the mean eluent velocity in the mobile phase, that is:

$$
\begin{equation*}
u_{\text {band }} / u=q_{s} /\left(q_{s}+q_{m}\right)=v_{m} / v_{R} \tag{1.34}
\end{equation*}
$$

where $u$ is the mobile phase velocity, and is the same as $u_{m}$ of equation (1.12).

$$
\begin{gather*}
u_{\text {band }} / u=1 /\left(1+k^{\prime}\right) \quad k^{\prime}=q_{s} / q_{m}  \tag{1.35}\\
k^{\prime}=K^{\prime} \cdot v_{s} / v_{m} \tag{1.36}
\end{gather*}
$$

here $K^{\prime}$ is the equilibrium distribution between the mobile and stationary phases.

$$
\begin{align*}
& V_{R}=V_{m}\left(1+k^{\prime}\right)  \tag{1.37}\\
& V_{R}=V_{m}+K^{\prime} V_{s} \tag{1.38}
\end{align*}
$$

$\mathrm{K}^{\prime \prime}$ and $\mathrm{K}^{\prime}$ are related to the standard Free Energy change for transfer of solute from the mobile zone/phase to the stationary zone/phase and given by the Gibbs Free Energy expression. Then $\Delta G^{\boldsymbol{o}}$ is the standard free energy difference between the mobile and stationary zones. And for the transfer between zones we have

$$
\begin{equation*}
\Delta \mathrm{G}^{\theta}=- \text { R.T. } \mathrm{L}^{\prime} \mathrm{K}^{\prime \prime} \tag{1.39}
\end{equation*}
$$

and between phases

$$
\begin{equation*}
\Delta G^{\bullet}=- \text { R.T.LIn } K^{\prime} \tag{1.40}
\end{equation*}
$$

since

$$
\begin{equation*}
\Delta G^{\theta}=\Delta H^{\ominus}-T \Delta S^{\theta} \tag{1.41}
\end{equation*}
$$

we obtain

$$
\begin{align*}
& \left.\operatorname{Ln} K^{\prime}=\operatorname{Ln}\left(V_{s} N_{m}\right) \cdot \Delta S^{\ominus} / R V^{-}\left(\Delta H^{\infty} / R T\right)\right]  \tag{1.42}\\
& \left.\operatorname{Ln} K^{\prime \prime}=\operatorname{Ln}\left(V_{p} N_{0}\right) \cdot \Delta S^{\oplus} / R \quad \cdots\left(\Delta H^{\oplus} / R T\right)\right] \tag{1.43}
\end{align*}
$$

It can now be seen that there are both enthalpy and entropy contributions involved. In retentive chromatography $\Delta H^{\ominus}$ is dominant over $\Delta S^{\ominus}, \Delta H^{\ominus}$ is negative and $\Delta S^{\theta}$ is near zero. In size exclusion chromatography $\Delta H^{\top}$ is made near zero and thus $\Delta S^{\ominus}$ determines the separation. In SEC $\triangle S^{\ominus}$ is negative due to a restriction of the available molecular configurations within the pores of the support when compared to those available in the bulk eluent.

Exclusion can however arise from a positive $\Delta H^{\theta}$ between the stationary and mobile zones, and conversely polymers partially excluded by size, can be retained by having a finite but negative $\Delta H^{\ominus}$ for transfer from mobile to stationary zones.

There is often a question as to whether the capacity ratio should be referred to $\mathrm{V}_{0}$ or $\mathrm{V}_{\mathrm{m}}$. There is no correct answer since at the molecular level and at the level of subdivision in porous supports used in HPLC phase boundaries are not sufficiently sharp for the phase volumes to be precisely defined. However where the separation depends primarily on differences in $\Delta H$ it is better to use $V_{m}$ than $V_{o}$; but where the entropy change $\Delta S$ dominates it is better to use $V_{0}$.

### 1.3.5. Kinetics

Kinetic factors responsible for solute band broadening are illustrated in Figure 1.5. For the best resolution a minimum band width is desired within the time available. While Martin and Synge(9) realised that there was an optimum flowrate for separation, Van Deemter et al (16) were the first to separate the three main contibutions to band broadening. The processes involved were

- dispersion due to axial molecular diffusion
- dispersion due to tortuous flow
- dispersion due to slow mass transfer between mobile and stationary zones and within each zone.

These three dispersion processes are independent and the total peak variance can be expressed as,

$$
\sigma_{\text {TOTAL }}^{2}=\sigma_{1}^{2}+\sigma_{2}^{2}+\sigma_{3}^{2}
$$

This implies that the contributions to plate height could be considered to be independent.

$$
\begin{equation*}
\mathrm{H}_{\text {TOTAL }}=\text { H }_{\text {DIFFUSION }}+\mathrm{H}_{\text {FLOW }}+\mathrm{H}_{\text {SLOW }} \text { MASS TRANSFER } \tag{1.44}
\end{equation*}
$$

Van Deemter et al showed that the dispersion effect of axial diffusion would be smallest at high eluent velocities, that the effect of tortuous flow should be independent of flowrate, and that the effect of slow mass transfer would be larger at higher velocities. A compromise in eluent velocity was therefore required to give the best efficiency. This deduction had a profound influence on the subsequent development of gas and later liquid chromatography.

### 1.3.6. Band Broadening due to Axial Diffusion

As a solute band moves down a column, we have already seen that it's profile spreads out adopting a near Gaussian shape as described in equation(1.15). When band reaches the end of a column of length $L$, Hen:

$$
\sigma_{L}{ }^{2}=H . L
$$

The dispersion of a solute band due to diffusion whether stationary or moving is given by the Einstein Diffusion Equation.

$$
\begin{equation*}
\sigma_{L}{ }^{2}=2 D_{e f f} t \tag{1.45}
\end{equation*}
$$

where $t$ is the total time overwhich the dispersion is taking place and $D_{\text {eff }}$ is the effective diffusion coefficient. $D_{\text {eff }}$ is related to the solute diffusion coefficient thus,

$$
\begin{equation*}
D_{e f f}=\gamma^{\prime} D_{m} \tag{1.46}
\end{equation*}
$$

where $\gamma^{\prime}$ is a geometrical constant which allows for obstruction to diffusion by the packing material. The period over which dispersion takes place is given by the retention time,$t_{R}$. The difference in diffusion rates of the solute in the mobile and stationary phase will also affect the band width. Since

$$
\begin{equation*}
t_{R}=t_{m}\left(1+k^{\prime}\right) \tag{1.47}
\end{equation*}
$$

we obtain

$$
\begin{gather*}
\sigma_{z}^{2}=2 \gamma^{\prime} D_{m}\left(1+k^{\prime}\right) t_{m}  \tag{1.48}\\
\sigma_{z}^{2}=2 \gamma^{\prime} D_{m}\left(1+k^{\prime}\right) L / u \tag{1.49}
\end{gather*}
$$

Thus the contribution to reduced plate height is

$$
\begin{equation*}
\text { HIFF }=2 \gamma^{\prime} D_{m}\left(1+k^{\prime}\right) L / u \cdot\left(1 / L \cdot d_{p}\right) \tag{1.50}
\end{equation*}
$$

From equation 1.21

$$
\begin{gather*}
v=u \cdot d_{p} / D_{m}  \tag{1.51}\\
H_{\text {DIFF }}=2 \gamma^{\prime}\left(1+k^{\prime}\right) / v=B / v \tag{1.52}
\end{gather*}
$$

where $B$ is a constant for the solute in question.

### 1.3.7. Band Broadening due to Tortuous Flow

Due to the random velocity changes within the column both parallel and perpendicular to the direction of flow, there is a complex matrix of variations in axial velocity across and along the bed. This results in a solute dispersion.

According to Giddings there are two processes that act to oppose this dispersion. Firstly random velocity changes along any flow line mean that a solute molecule will sample most of the possible velocities within a short space of time. Secondly transfer from one flow line to another by transverse diffusion will also result in the solute experiencing different velocities. The theory of this interaction was developed by Giddings and the term "coupling" was used to cover this. Experiments on glass bead columns (50) show that this dispersion exhibits a positive dependance on eluent velocity, rather than the velocity independent dependance supposed by Van Deemter.

At low flowrates the processes opposing dispersion are enhanced while at high flowrates the variation in streamline velocities is enlarged therefore dispersion is increased. Theoretical treatment of these factors has not yet provided an exact expression however experimental work $(50,51)$ indicates that the dispersion due to flow irregularities can be approximated by:

$$
\begin{equation*}
\mathrm{h}_{\text {FLOW }}=A \nu^{0.33} \tag{1.53}
\end{equation*}
$$

This modified expression provided a better correlation to experimental data than the van Deemter expression where;

$$
\begin{equation*}
h_{\text {FLOW }}=A \tag{1.54}
\end{equation*}
$$

### 1.3.8. Band Broadening due to Slow Mass Transfer

This contribution to band dispersion shows a positive dependance on eluent velocity and arises from slow equilibration between the stationary and mobile zones. When a solute molecule is in the stationary zone it may be left behind as the remaining solute band in the mobile zone passes by. For a solute molecule that passes over a particle the time taken to diffuse into and out of the particle while the solute band continues to move down the column is dependent on the distance the solute has to diffuse, which in turn is dependent on the particle size and the diffusion coefficient. The Giddings coupling theory provided an expression for the plate height contribution that arose from this slow equilibration.

$$
\begin{equation*}
H_{\text {MASS TRANSFER }}=c, k^{\prime \prime} /\left(1+\mathrm{k}^{\prime \prime}\right)^{2} \cdot \mathrm{D}_{\mathrm{m}} / \mathrm{D}_{\mathrm{sm}} \cdot \mathrm{u} \tag{1.55}
\end{equation*}
$$

where $D_{s m}$ is the diffusion coefficient in the stationary zone, $D_{m}$ isthe diffusion coefficient in the mobile zone. The value of $D_{s m}$ is diffucult to evaluate as the composition of the stationary zone is not necassarily constant(49). For spherical particles the Giddings expression reduces to

$$
\begin{equation*}
h=1 / 30\left(\bar{k}^{\prime \prime \prime} /\left(1+k^{\prime \prime}\right)^{2}\right) v \tag{1.56}
\end{equation*}
$$

For practical purposes the expression may be given as

$$
\begin{equation*}
h=C v \tag{1.57}
\end{equation*}
$$

The complete expression for the reduced plate height is then:

$$
\begin{equation*}
h=B / v+A v^{0.33}+C v \tag{1.58}
\end{equation*}
$$

At high velocity $\mathrm{A}^{\prime}$ due to mass transfer and flow are a maximum while at low velocity the dispersion due to axial diffusion is a maximum. The expression
obviously exhibits a minimum as can be seen in Figure 1.6. Since Equation (1.58) is dimensionless identical $h$, $v$ plots should be obtained for a wide variety of LC systems with differing $d_{p}$ and with solutes having differing $D_{m}$ values. This is indeed demonstrated by the extensive literature reporting $h, v$ data. Typical values for A, B, and C are also shown.

### 1.4. Optimum Dimensions for Packed Columns

The pressure drop across a packed bed is given by the simplified form of the Kozeny-Carmen equation(52)

$$
\begin{align*}
& \Delta P=\phi \eta \lambda^{2} / t_{m}  \tag{1.59}\\
& t_{m}=\phi \eta \lambda^{2} / \Delta P  \tag{1.60}\\
& t_{m}=N^{2} h^{2} \phi \eta / \Delta P \tag{1.61}
\end{align*}
$$

where $\phi$ is the column resistance factor, related to the porosity of the bed and $\eta$ is the solvent viscosity. Thus for a given pressure drop in a particular column the minimum value of $t_{m}$ will occur when the reduced plate height is a minimum, which in turn occurs at the optimum reduced velocity.

$$
\begin{align*}
v_{\text {opt }} & =u_{m} d_{p} / D_{m}  \tag{1.62}\\
= & \left(L / t_{m}\right)\left(d_{p} / D_{m}\right)
\end{align*}
$$

The column length $L$, may be expressed as the reduced length $\lambda$, where $\lambda$ is defined as:

$$
\begin{equation*}
\lambda=L / d_{p} \tag{1.63}
\end{equation*}
$$

Equation ((1.62)) is then

$$
\begin{equation*}
v_{\mathrm{opt}}=\lambda d_{p}^{2} / t_{m} D_{m} \tag{1.64}
\end{equation*}
$$

Substituting for $\mathrm{t}_{\mathrm{m}}$;

$$
\begin{equation*}
v_{\mathrm{opt}}=\lambda d_{p}^{2} / D_{m} \cdot\left(\Delta P / \lambda^{2} \phi \eta\right) \tag{1.65}
\end{equation*}
$$

The particle diameter under optimum conditions is then

$$
\begin{equation*}
d_{p}=\left(\nu_{\mathrm{opt}} \mathrm{D}_{\mathrm{m}} \phi \eta \lambda / \Delta P\right)^{1 / 2} \tag{1.66}
\end{equation*}
$$

If we replace $\lambda$ by $N . h$ then

$$
\begin{equation*}
d_{p}=\left(\nu_{\mathrm{opt}} D_{\mathrm{m}} \phi \eta N h / \Delta P\right)^{1 / 2} \tag{1.67}
\end{equation*}
$$

In retentive HPLC where typically solutes are of low molecular weight, the optimum particle size under the following working conditions

$$
\begin{aligned}
& h=2 \\
& N=10,000 \\
& D_{m}=10^{-9} \mathrm{~m}^{2} \mathrm{~s}^{-1} \\
& \phi=1000 \\
& \eta=10^{-3} \mathrm{Nsm}^{-2} \\
& \Delta \mathrm{P}=10^{7} \mathrm{Nm}^{-2} \\
& v_{\text {opt }}=5
\end{aligned}
$$

would give an optimum particle diameter of about $3.2 \mu \mathrm{~m}$.

However in HPSEC the solutes are much larger and typically may have a molecular weight of 100,000 . The value of the Diffusion Coefficient is now much smaller and is a function of the molecular weight. The diffusion coefficient may be estimated from the formula(53):

$$
\begin{equation*}
D_{m}=\left(R T / 6 \pi \eta_{o} N_{o}\right) \cdot\left(10 \pi N_{o} / 3 K\right)^{1 / 3} \cdot M^{-(1+a) / 3} \tag{1.68}
\end{equation*}
$$

where $N_{0}$ is Avagadro's number, $C^{K}, R$ and $T$ are constants and $M$ is the molecular weight. This expression simplifies so that

$$
\begin{equation*}
D_{m} \propto M^{1 / 2} \tag{1.69}
\end{equation*}
$$

In a typical HPSEC analysis the diffusion coefficient of macromolecular solute may be a hundred times smaller than in the retentive HPLC mode. This smaller diffusion coefficient results in the optimum particle size being around $0.3 \mu \mathrm{~m}$.

At the same time as the diffusion coefficient and optimum particle size decrease, the pore size required to perform the analysis will increase according to the expression(54):

$$
\begin{equation*}
\text { Solute Diameter }=0.246 \times \mathrm{M}^{\mathrm{a}} \AA \tag{1.70}
\end{equation*}
$$

where $\mathbf{a}=0.588$ for polystyrenes.

The production of packing materials able to withstand the pressures used in HPSEC, with pores sizes of the same magnitude as the particle, is not possible. Therefore a comprimise has to be reached between particle size and pore size when considering the "optimum" dimensions of HPSEC packing materials.

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Figure illustrates how the different volumes within the column may be divided according to zones or phases.


- $\mathrm{V}_{\text {support }}$ - is the volume of the solid support.
- $V_{s}$ - is the volume of the stationary phase.
- $V_{p}$ - is the volume of the pores.
$-V_{o}$ - is the volume of flowing eluent, the mobile zone.
- $V_{m}$ - is the volume of the mobile phase.
- $V_{s z}$ - is the volume of the stationary zone.

FIGURE 1.3

The figure defines the various parameters describing retention and peak width in terms of elapsed time and elution volume.


Elution Volume

FIGURE 1.4

The parameters required to describe the resolution of a column are illustrated below.


FIGURE 1.5

The major mechanisms responsible for the dispersion of solute bands in chromatography are illustrated below:

Two adjacent solute molecules are separated by the dispersion forces such that after a period of time they are separated by a distance, $d$.

1. shows the dispersion as a result of axial diffusion.
2. shows the dispersion due to tortuous flow through the column.
3. shows the dispersion as a result of slow equilibration between the stationary and mobile zones.
(1)

(2)


The equation describing the dispersion of the solute bands

$$
h=B / v+A v^{1 / 3}+C v
$$

may be plotted as follows:


## CHAPTER 2

## SIZE EXCLUSION CHROMATOGRAPHY

Size Exclusion Chromatography(SEC) differs from other modes of chromatography in that the separation process is based on entropic differences rather than enthalpic differences for transfer of solutes between the mobile and the stationary zones. In SEC it is the relative dimensions of the solute and pores within the packing which are responsible for the separation obtained (1).

### 2.1. History

The first use of size exclusion in chromatography was in 1926 when McBain reported zeolites(2) acting as differential molecular sieves. In 1930 Friedman(3) showed that transport of urea and glycerine through agar gels was dependent on the pore size of the gel. Polson(4) showed that the penetration of proteins into agar gels was dependent on the proteins size and the gel concentration and thus the pore size. The development of SEC proper began in 1959 with a technique introduced by Porath and Flodin(5), for the separation of biopolymers. Porath and Flodin studied several packing materials including cellulose, starch and polyvinyl alcohol. However they found cross-linked dextrans to be the most useful(6). These materials were made by the emulsification of dextrans in an organic medium, followed by treatment of the emulsion with epichlorohydrin(5). This resulted in glycerol cross-links being formed between the dextran chains. By alteration of the degree of cross-linking the porosity and pore size of the dextran could be controlled. These cross-linked dextrans were marketed under the name, Sephadex.

Although these gels had poor mechanical stability and could only be used with low flowrates, they still represented a major advance. The techniques allowed
biochemists to separate biopolymers in an aqueous eluent. The packing materials are still widely used no alternative is as yet availible. The method became known as Gel Filtration Chromatography (GFC) and was applicable to separations in aqueous media only.

During the next few vears other polymer packing materials were studied including, agarose gels(7), polyacrylamides(8), polymethacrylates and cellulose. The first hydrophobic packings were repórted by Vaughan(9). These were produced by cross-linking polystyrene with divinyl-benzene. The extent of cross-linking could be altered by changing the relative amounts of organic dilutants within the reaction mix. In 1964 Moore(10) made a systematic study of the effect of these modifiers on the resultant polymer. He noted that permeability and rigidity were related to the concentration of the dilutant used. Polystyrene gels based on this study were commercially availible under the name Styragel. Moore also described how the technique could be used for the rapid determination of the molecular weight distribution of polymers. The technique when used with organic eluents became known as Gel Permeation Chromatography (GPC) (11).

However separations were slow and cumbersome, perhaps requiring many metres of column because of the low pressure stability of the porous supports (less than 250psi). Application of the higher pressures required for increased flowrate resulted in compression of the porous matrix.

During the 1960's as chromatographic theory developed, SEC speed and resolution also improved. The development of microparticulate and rigid supports with a high mechanical stability was next improvement in SEC. Haller(12) described the first rigid packing material. This was a porous glass powder of regular pore size made by heat treatment followed by leaching of sintered alkali borosilicate glass. This material was produced in a number of different pore sizes ranging from
$7-300 \mathrm{~nm}(13)$. It was sold under the name of Corning Porous Glass.

De Vries et al (14) developed the first porous silica packing for SEC in 1966. The inert nature of the silica gel together with its rigidity at high back pressure were obvious advantages over the organics gels with regard to the speed and reproducibility of separation achieved. These SEC silica gels were marketed as Porasil.

Further understanding of the retention mechanism, solute structure and the effect of packing methods have lead to the development of a wide range of packing materials(15) being available on the market. With the development of chromatography in biochemistry and medicine the preparation of supports giving high resolution good reproducibility and high biological recovery has led to the further development of $\operatorname{HPSEC}(16)$. In this field the development of wide pore packing materials is now of interest(17).

### 2.2. Theoretical Aspects of SEC

### 2.2.1. Introduction and Principles

In all forms of LC other than SEC the separation is dependant on a retentive interaction of the solute with the packing surface. This results in the components being eluted after $V_{m}$. SEC relies on no such interactions. Indeed great care must be taken to avoid them. Species that are too large to enter the pores of the packing elute in a volume $V_{0}$, the interstitial column volume. As the solute size decreases,solute molecules can access more of the pore volume. This results in a higher elution volume. If a solute is sufficiently small to penetrate all the pores of the packing the solute will elute in a volume $V_{m}$ where
$V_{f}$ is the column pore volume. $V_{m}=V_{o}+V_{p}$

If the solute is eluted between the two extremes then

$$
\begin{equation*}
V_{R}=V_{o}+K \cdot V_{p} \tag{2.2}
\end{equation*}
$$

where $K$ represents the fraction of pore volume into which the solute can gain entry. For true SEC the range of values of $K$ lies between 0 and $1 . K$ is variously called the Distribution Coefficient, the Exclusion Coefficient or the Permeation Coefficient. The first of these will be used in further discussions. $K$ of Equation (2.2) is equivalent to $K^{\prime \prime}$ of Chapter 1 equation (1.39).

If we apply traditional LC terminology to describe elution then:

$$
\begin{equation*}
v_{R}=f_{v} \cdot t_{R} \tag{2.3}
\end{equation*}
$$

and

$$
\begin{equation*}
k^{\prime}=\left(V_{R}-V_{m}\right) / V_{m} \tag{2.4}
\end{equation*}
$$

However as $V_{R}<V_{m}$ at all times, this implies that $k^{\prime}$ is negative. The zone capacity factor however is always positive

$$
\begin{equation*}
k^{\prime \prime}=\left(V_{R}-V_{0}\right) / V_{0} \tag{2.5}
\end{equation*}
$$

In order to avoid describing relative retention in terms of negatives, $k^{\prime \prime}$ is the more useful term in. SEC.

### 2.2.2. Representation of Elution Data

The normal way of representing SEC data is as a semi-logarithmic plot where a molecular size parameter is plotted against the degree of retention. For molecular size, Log (Molecular Weight) or Log (Molecular Radius) are commonly used. The degree of retention may be expressed as $V_{R}$, the retention volume or as $K$, the distribution coefficient. Figure 2.1 shows a typical curve, taken from reference 18.

### 2.2.3. Resolution

The number of peaks that may be resolved by SEC is relatively low compared to the number resolvable by other modes of chromatography(19). This is because of the limit placed upon the method by the accessible pore volume. As there is no interaction with the surface the physical limits of retention are $V_{o}$ and $V_{m}$ while in other forms of chromatography the retention volume may be many times larger than $V_{m}$. The peak capacityjsdefined as the number of peaks which can be fitted in between the first and last elution peaks with all peaks having resolution $R_{s}$. Clearly the number of peaks that can be separated will be proportional to the resolution required between the solutes. According to Giddings (19):

$$
\begin{gather*}
n \propto R_{s}  \tag{2.6}\\
n=1+\text { Constant } \cdot / N \cdot \ln \left(V_{R M A X} / V_{R \text { MIN }}\right) \tag{2.7}
\end{gather*}
$$

The value of the constant will depend on the resolution required. The peak capacity is controlled by the difference in the elution volumes of the first and last peaks.

The volume of the pores $V_{p}$ is dependent on the particle porosity, $\phi$, and typically in LC:

- $V_{o}$ may occupy $40 \%$ of the column volume
- $V_{p}$ may occupy $30 \%$ of the column volume
- $V_{s}$ may occupy $30 \%$ of the column Volume

In retentive LC the peak capacity is large as the maximum elution volume is not limited by the volume of the pores. The difference between the elution volume of the first and last peaks is large.
$V_{\text {LAST PEAK }} / V_{\text {FIRST PEAK }}=10$

However in Modern SEC the value of $\Delta \ln \frac{V_{\operatorname{An}}}{V_{\text {HiN }}}$ is much smaller, and it is dependent on the ratio of pore volume to the volume outside the pores.

$$
\begin{equation*}
V_{\text {max retention }} / V_{\text {min retention }}=V_{0}+V_{p} / V_{0}=2 \tag{2.8}
\end{equation*}
$$

In SEC the minimum retention volume corresponds to $V_{0}$ while the maximum retention volume is $\left(V_{o}+V_{p}\right)$. The only ways of increasing this difference are to either decrease $V_{0}$ or increase $V_{p}$. A decrease in $V_{0}$ will result in lower bed porosity and lower flowrates. This approach is typical of the swollen gels used for SEC. The other alternative is to increase the pore volume, however this results in the production of weak particles. This project deals in part with the development of strong higher pore volume silica gels for HPSEC.

### 2.3. Retention

In Retentive LC it is widely agreed that thermodynamic effects form the basis for retention. In SEC there has been widespread argument and several theories have developed to explain the partial exclusion. The basis of these will be outlined in this section.

### 2.3.1. Retention as a Result of Restricted Diffusion

This theory describes the separation of solutes as being dependant on their rate of diffusion into and out of the pores of the support(20,21). The smaller molecules are able to diffuse through the porous support more easily than the large ones due to their higher diffusion coefficients. Since the large solutes have smaller diffusion coefficients they are presumed to permeate less far into the pores of the packing and thus are less retained. This model results in what is effectively two processes combining. Firstly exclusion due to the molecular size, and secondly separation due to slow diffusion.

Ackers(20) produced a expression where the retention was given by

$$
\begin{equation*}
K=(1-r / R)^{2} \cdot\left(1-2.104 r / R+2.09(r / R)^{3}-0.95(r / R)^{5}\right) \tag{2.9}
\end{equation*}
$$

where, $r$ is the solute radius and $R$ is the pore radius.

However for this expression to be related to the diffusion rate the degree of permeation should be linked to the solvent velocity through the column. In the above expression there is no mention of solvent velocity, and the expression relies purely on geometry. Yau and Malone(21) produced a model relating the retention of the solute to its diffusion rate. The diffusion rate of the solute is related to the frictional forces as well as molecular size. This expression did contain variables relating to eluent flowrate.

$$
\begin{gather*}
V_{R}=V_{0}+V_{A} \cdot\left(k /\left(\pi u M^{b}\right)^{1 / 2} \cdot\left(1-\exp \left(-u M^{b} / k^{2}\right)\right)\right. \\
x \quad \operatorname{erfc}\left(u M^{b}\right)^{1 / 2} / k \tag{2.10}
\end{gather*}
$$

where $u$ is the eluent velocity, $M$ is the molecular weight, $V_{o}$ is the mobile zone volume, $\mathrm{V}_{\mathrm{A}}$ is the pore volume and k and b are constants.

This expression gave fairly good agreement with experimental plots as can be seen from Figure 2.2. These models were later proven unsuitable as it was found that the retention volume was effectively independant of flowrate. Further experimentation in this direction used the now generally accepted steric exclusion model with the differing diffusion rates being used as a secondary mechanism to explain speculated flowrate dependance. It has since been shown that the flowrate has no influence on SEC retention. It is the skewness of peaks at high flowrate that has given the impression of some kind of flowrate dependance.

### 2.3.2. Flow Models

Separation by flow (SBF) was a model developed by Marizo and Guillman(22,23) based on the postulate that larger solutes were unable to gain aress to the whole surface and therefore were forced into the faster flowing eluent channels. In such a system solvent viscosity and the solute to wall exclusion in the flow channets would affect the retention. The use of non-porous packing to examine these effects showed that there was no retention. This indicates that it was the pores that were responsible for the separation(24). These flow models have been reviewed by Casassa(25).

### 2.3.3. Thermodynamic Equilibrium Models

Carmichael(26) proposed a model in which the number of times a solute penetrates the pores of the packing will depend on its radius of gyration. This radius constantly fluctuates and therefore the solute may be held within a pore as a result of this fluctuation. This was the basis of retention and the broadening of peaks could also be explained by this model.

It is now generally accepted that the SEC mechanism can be formulated purely on the basis of the thermodynamic equilibrium of the solute partitioning itself between the the volume inside and the volume outside the pores(1). As explained in equation (2.2) the retention of a solute is

$$
\begin{equation*}
V_{R}=V_{0}+K \cdot V_{p} \tag{2.11}
\end{equation*}
$$

where $K$ is the equilibrium thermodynamic distribution coefficient between the mobile and stationary zones.

$$
\begin{equation*}
\mathrm{K}=\mathrm{c}_{\mathrm{sz}} / \mathrm{c}_{\mathrm{mz}} \tag{2.12}
\end{equation*}
$$

In terms of the free energy change $\Delta G^{\circ}$, for solute transfer from the mobile to
stationary phase:

$$
\begin{equation*}
\Delta G^{\theta}=-R T \ln K \tag{2.13}
\end{equation*}
$$

Whence:

$$
\begin{equation*}
K=e^{-\Delta \hat{H} / R T+\Delta \widehat{S T R}} \tag{2.14}
\end{equation*}
$$

where $\Delta H^{\theta}$ is the enthalpy change involved in solute transfer from mobile to stationary phase, and $\Delta S^{\ominus}$ is the standard entropy change. For other modes of LC as mentioned earlier the interaction between solute and surface of the packing dominates. i.e. $\Delta H^{\ominus} / T \gg \Delta S^{\ominus}$ and we may write

$$
\begin{equation*}
K=e^{-\Delta \widehat{H} / R T} \tag{2.15}
\end{equation*}
$$

The process is enthalpy controlled. However in SEC the process is entropy controlled(27) and $\Delta H=0$ thus

$$
\begin{equation*}
K=e^{\Delta \tilde{\mathrm{S} / R}} \tag{2.16}
\end{equation*}
$$

This expression has been demonstrated by Casassa and Tagami(28). For small molecules able to penetrate all the pores of the support there is little change in the comformational entropy, thus $\Delta S=0$ and $K=1$ For large solutes there is a large restriction on the conformational entropy and so $\Delta S<0$ and $K<1$.

In retentive LC there is a direct temperature dependance in the equilibrium expression. In SEC there is no direct temperature dependance in the equilibrium expression and therefore retention in SEC is independant of temperature. The effect of temperature in SEC is seen only in alteration of solute configuration which would in turn alter the entropy of the system. For non-rigid packing materials temperature may affect the pore structure and thus alter retention, but if the structure of the support matrix is fixed there should be no change in retention
with temperature. Temperature does of course affect the peak width but this is common to all LC. Band broadening will be discussed later.

Further evidence for the retention being based on steric exclusion is given by the independance of retention volume and flowrate. The thermodynamic mechanism is the basis for various SEC theories regarding retention. If there is no interaction with the packing surface then variation in the SEC calibration curve arise from variation in solute conformation, pore volume of the column, pore size of the packing, pore shape and pore size distribution. The effect of these variables is well documented $(1,29)$.

A final and conclusive argument for' the thermodynamic basis of SEC came from Giddings' non-equilibrium theory $(30,31)$ which requires that for narrow peaks the distribution of the solute between mobile and stationary zones must be very close to equilibrium. Significantly slow equilibration will necessarily give very wide peaks and a large and readily measurable $C$ term in the plate height equation. There is no evidence of unduly wide peaks in SEC for near monodisperse solutes.

### 2.4. Calibration Curves

The shape of Size Exclusion Calibration Calibration (SECC) curves shows a dependence on

- solute conformation
- pore configuration

In order to make a theoretical study of the effect of solute shape on the elution volume the shape size and distribution of the pores of the packing material must be assumed.

The simplest realistic model comprises a network of pores of infinite length
and equal diameter. Once the pore structure is assumed an evaluation of solute shape effects may be carried out. The reverse method is also possible, where the solute shape is assumed and the pore structure examined.

### 2.4.1. Variation of Solute Conformation

Hard Spheres - This model has a great advantage in its inherent simplicity as can be seen in Figure 2.3A. The thermodynamic process can be considered as the restriction of spatial freedom. The centre of mass of the solute is completely free to move within the dashed circle but it may not approach the pore wall any closer than that dashed circle. This in effect means that the solute is excluded from that region.

$$
\begin{equation*}
K=V_{R}-V_{0} / V_{p}=V_{a c c} / V_{p} \tag{2.17}
\end{equation*}
$$

where $V_{p}$ is the total pore volume and $V_{a c c}$ is the pore volume to which the solute may gain access.

$$
\begin{gather*}
V_{p}=\pi R^{2} L  \tag{2.18}\\
V_{a c c}=(R-r)^{2} \pi L  \tag{2.19}\\
K=(R-r)^{2} / R^{2}  \tag{2.20}\\
K=(1-r / R)^{2} \tag{2.21}
\end{gather*}
$$

where $L$ is the length of pore, $R$ is the pore radius and $r$ is the solute radius.

Rigid Rods - In Figure 2.3B. the case where the solute is rod shaped is examined. Here when the rod of length $2 \mathbf{a}$ is at a distance $>$ a from the pore wall the solute will have complete spatial freedom. However its centre of mass is limited to the zone $(R-a)^{2} \pi L$. In this model the solute may adopt a second configuration(ii), here the solute can move closer to the wall but the possible
configurations are now limited. As the configuration changes from (i) to (ii) the number of spacial positons of the solute decreases. So that as explained by Casassa(25) there is a region $x$ defined as the distance $x$ of the centre of mass of the rod from the wall such that

$$
\begin{equation*}
a>x>\left(\left(R^{2}+a^{2}\right)^{1 / 2}-R\right) \tag{2.22}
\end{equation*}
$$

in which the solute will be able to adopt limited configurations. Furthermore the molecule may be perpendicular to the plane of the circular cross-section shown in Figure 2.3B., and thus would be able to enter the hitherto restricted volume. Giddings(32) experimented with different shaped pores and rods and developed an expression for the general exclusion coefficient of both infinitely thin rods and capsule shaped molecules within the pores. These functions are much more complex than the spherical solute equations, as the orientation of the solute is now important. Giddings used statistical models to describe the exclusion effects, and suggested that the distribution coefficient could be represented by

$$
\begin{equation*}
K=\int e^{-u(q) / k T} d q / \int d q \tag{2.23}
\end{equation*}
$$

where $q$ represents the co-ordinates relating to the solute position within the pore. $\mathbf{u}(\mathbf{q})$ is the energy associated with the geometrical comformation of the solute, defined by its co-ordinates, $q$.

The simplest rod shapes produce very complex expressions. When these molecular shapes are compared to other types, the rods have a less well defined exclusion limit. That is the point at which $K=0$ is approached much more gently in these curves. This can be seen in Figure 2.4 The gradual approach is due to the rod like molecule entering the pores end on rather than as shown in Figure.2.3B.

Random Coils - This is illustrated in Figure 2.3C. where it is now clear that at
any particular time any part of the solute molecule may be located in any part of the pore. However the walls of the pore do influence the conformational freedom of the solute. The distribution coefficient in this case is given by:

$$
\begin{equation*}
K=4 \Sigma B_{m}^{-2} \cdot \exp \left(\left(-\beta_{m} \cdot r_{g} / R\right)^{2}\right) \tag{2.24}
\end{equation*}
$$

where $B_{m}$ is the $m$ th root of the Bessel function of the first kind, of order zero. The difference between the $K$ values calculated for hard spheres and random coils is however small. The difference is sufficiently small that for all practical purposes random-coil molecules can be treated as rigid spheres

## Effect of solute conformation on the Calibration Curve

The relationship between molecular size and molecular weight depends upon the molecular conformation. Thus for a hard sphere the effective radius $r$ used to calculate the curves is simplifed to relate to the molecular weight by equation (2.25) for hard spheres:

$$
\begin{equation*}
r \propto M^{1 / 3} \tag{2.25}
\end{equation*}
$$

and for rigid rods by equation (2.26):

$$
\begin{equation*}
\mathrm{L} \propto \mathrm{M} \tag{2.26}
\end{equation*}
$$

For random coils the effective radius is related to the radius of gyration $\mathbf{r}_{\mathbf{g}}$ by the equation (2.27):

$$
\begin{equation*}
r=0.886 r_{g} \tag{2.27}
\end{equation*}
$$

and to the molecular weight through equation (2.28):

$$
\begin{equation*}
r_{g}=a M^{b} \tag{2.28}
\end{equation*}
$$

Here $a$ and $b$ are constants and $a$ :e different for different polymer solvent systems(28). which are obtained from the Mark-Houwink expression relating molecular weight to intrinsic viscosity. For example a system of polystyrene solutes in tetrahydrofuran would give

$$
\begin{equation*}
r_{g}=0.137 \mathrm{M}^{0.588} \AA \tag{2.29}
\end{equation*}
$$

and the effective radius of the polystyrene

$$
\begin{equation*}
r=0.123 \mathrm{M}^{0.588} \AA \tag{2.30}
\end{equation*}
$$

The solute conformation clearly affects the $K$ value obtained for a particular molecular weight . Molecules with compact structures will tend to elute later than extended structures.

### 2.4.2. Variation of Pore Structure

## Pore Size

For the purposes of a theoretical model, if we consider the support as containing only one pore size then as expected variation in pore size will result in a different molecular weight range being separated. Altering the pore size will merely shift the separation to a higher or lower molecular weight separation. Figure 2.5 indicates the predicted Size Exclusion Calibration Curve (SECC) obtained with differing pore sizes. It is very important to note that to separate solutes of differing molecular weights it is not necessary to have a range of pore sizes, provided the molecular weights do not differ by too much. If a wider range of molecular weight separation is required then this can be achieved by widening the pore size distribution. The resolution between closely related solutes will however be sacrificed. $\qquad$


## Pore Shape

Model calculations(19) show that differing pore shape and solute geometry have only a slight effect on predicted SECC. In determination of calibration curves the effect of pore shape has been studied in great detail by Giddings et al.(25), van Kreveld(34) and Knox and Scott(35). The subject has also been well reviewed(1,29). The expressions are examined below. In the expressions the solute is regarded as a sphere of effective radius $r$.

### 2.4.2.1. Fixed Pore Models

These models describe the shape of the pore and it is assumed that only one pore size is present.

Pores considered as infinite flat plates(25)

$$
\begin{equation*}
K=(1-r / R) \tag{2.31}
\end{equation*}
$$

Cylindrical Pores(25)

$$
\begin{equation*}
K=(1-r / R)^{2} \tag{2.32}
\end{equation*}
$$

Spherical Pores(35)

$$
\begin{equation*}
K=(1-r / R)^{3} \tag{2.33}
\end{equation*}
$$

Rectangular Pores(30)

$$
\begin{equation*}
K=\left(1-(r / R) \cdot\left(1 / 1+P^{\prime}\right)\right) \cdot\left(1-(r / R) \cdot\left(P^{\prime} / 1+P^{\prime}\right)\right) \tag{2.34}
\end{equation*}
$$

where $R$ is the radius of the pore obtained from

$$
\begin{equation*}
\mathrm{R}=2 x \text { (Pore.Vol)/(Surface.Area) } \tag{2.35}
\end{equation*}
$$

and $P^{\prime}$ is the ratio of the long to short side of the rectangle.

Conical Pores(33)

$$
\begin{equation*}
K=(1-r / R)^{2}(1-r / L \tan \theta) \tag{2.36}
\end{equation*}
$$

As the pore models become more intricate the expressions describing them become more complex. For example in a model describing the pores as inverse cylinders(34) where the pores are represented by the voids between touching spheres. Expressions have been derived for different co-ordination numbers. Figure 2.6 indicates the different geometries that may be obtained. The value of $K$ for a system of four touching cylinders is given by:

$$
\begin{equation*}
K=1-\left(2 \rho-\rho^{2}\right)^{1 / 2}-(1-\rho)^{2} \cdot(\pi / 4-\theta) \tag{2.37}
\end{equation*}
$$

Where $\rho=r / R$ and $\theta=\cos ^{-1}(1 / 1+\rho)$ The alteration made in the predicted SEC calibration curves by using these models is slight. Figure 2.7

### 2.4.2.2. Random Pore Models

The pore models described have been of regular geometry and uniform in size of pores. In an attempt to describe the porous network more realistically, random pore models were devised $(25,34,35)$. Giddings(25) calculated the calibration curve using a model where the pores were considered as randomly arranged planes. The distribution coefficient was given by

$$
\begin{equation*}
K=e^{-2 r / a} \tag{2.38}
\end{equation*}
$$

where $a$ is the effective mean pore radius, calculated from the pore volume and surface area.

A network of randomly overlapping spheres was considered by Van Kreveld and Van den Hoed(34). The value of $K$ was then given by

$$
\begin{equation*}
K=\exp \left(-4 / 3 \pi N(R+r)^{3}\right) / \exp \left(-4 / 3 \pi N R^{3}\right) \tag{2.39}
\end{equation*}
$$

The random geometry described above was also dealt with by Knox and Scott(35) who simulated a bed of touching randomly packed porous spheres. The size distribution of the spheres was taken to be Gaussian. They found that this model and that of Van Kreveld gave reasonable agreement to experimental data. The modelled curves and the experimental data did however differ markedly at large pore/high molecular weight end of the SECC. The experimental curves showed a more gradual transition towards total exclusion than the modelled curves predicted. In order to mimic exclusion material it was important that the model had a similar porosity to the actual material. Also it was necessary to have a range of pore sizes. Knox and Scott found that the best fit to experimental data was obtained using a model of cylindrical pores having a distribution corresponding to that found by mercury porosimetry.

### 2.5. Size Exclusion Chromatography Peak Broadening

In SEC the broadening in peaks has two major sources, the first, common to all forms of LC, arising from Kinetic factors. This is associated with eluent flowrate, diffusion rate, particle size etc.. The second source of broadening is the polydispersity of the solute which results in molecules of the sample having a cange of elution volumes.

For a chromatographic peak the peak variance determitoned by these effects are independent. If we assume a Gaussian peak then the total variance is given by the sum of the components.

$$
\begin{equation*}
\sigma_{v \text { total }}^{2}=\sigma_{v \text { kinetics }}^{2}+\sigma_{v \text { polydispersity }}^{2} \tag{2.40}
\end{equation*}
$$

The two contributions can be treated separately, and in Chapter 1 the kinetics of band spreading have been discussed in relation to general chromatographic theory.

### 2.5.1. Polydispersity

The fact that even the most highly refined polymer standards give relatively broad peaks in SEC, led to the suspicion that this form of chromatography was inherently less efficient than retentive LC. This in fact is not the case. Knox and McLennan(36) have shown that a major portion of the observed band broadening arises not from poor chromatography but from the distribution of the molecular weights that are associated with a polymer standard. This distribution solute sizes is represented by the polydispersity, $\mathbf{P}$, where

$$
\begin{equation*}
P=M_{w} / M_{n} \tag{2.41}
\end{equation*}
$$

$M_{w}$ is the weight average molecular mass and $M_{n}$ is the number average molecular mass of the polymer. Even when $\mathrm{P}=1.01$ the drop in apparent column efficiency is large. For example if a monodisperse solute gave say 10,000 theoretical plates, a polydisperse solute ( $\mathrm{P}=1.01$ ) would give only 3800 plates(18).

The apparently large drop in efficiency is due to the range of molecular weights that are often present in polymer solutes. Knox and McLennan showed that for a Gaussian molecular weight distribution the polydispersity was related to the standard deviation as follows:

$$
\begin{equation*}
/\left(\sigma_{m} / M_{n}=(P-1)^{1 / 2}\right. \tag{2.42}
\end{equation*}
$$

According to the above equation even small values of polydispersity will correspond to a wide molecular size distribution in the sample. The wide peak arise as the solute is infact a number of solutes of differing size, each of these solutes may penetrate the pores to a different extent and thus differing distribution coefficients are obtained from Equation (2.2), as

$$
\begin{equation*}
k^{\prime \prime}=K \cdot V_{p} / V_{o} \tag{2.43}
\end{equation*}
$$

Knox and Mclennan(37) produced a statistical treatment of this dispersion and showed that the variance introduced into a peak by its polydispersity was

$$
\begin{equation*}
\sigma_{v}^{2}{ }_{v} \text { polydispersity) }=S^{2}(P-1)(1+\alpha) \tag{2.44}
\end{equation*}
$$

where $S$ is the reciprical of the gradient of the linear part of the SEC calibration curve. The S term gives a measure of the selectivity of the column. The $\alpha$ is a correction term and is a weak function of the polydispersity.

The total column variance due to the column packing is now given by

$$
\begin{gather*}
\sigma_{\text {TOTAL }}^{2}=\sigma_{\text {POLYDISPERSITY }}^{2}+\sigma_{\text {KINETICS }}^{2}  \tag{2.45}\\
\sigma_{\text {TOTAL }}^{2}=S^{2}(P-1)(1+\alpha)+H V_{R}^{2} / L \tag{2.46}
\end{gather*}
$$

the apparent plate height, $\mathrm{H}_{\text {app }}$

$$
\begin{gather*}
H_{\mathrm{app}}=\mathrm{L} \cdot\left(\sigma_{\text {TOTAL }}^{2} N_{\mathrm{R}}^{2}\right)  \tag{2.4}\\
H_{\mathrm{app}}=\mathrm{L} . \mathrm{S}^{2}(\mathrm{P}-1)(1+\alpha) N_{\mathrm{R}}^{2}+\mathrm{H} \tag{2.48}
\end{gather*}
$$

The true plate height is then

$$
\begin{equation*}
H=H_{\mathrm{app}}-L(P-1)(1+\alpha)\left(S / N_{R}\right)^{2} \tag{2.49}
\end{equation*}
$$

The effects of, spreading due to kinetics and polydispersity can be separated by plotting $H_{\text {app }}$ against the column length, L .

### 2.5.2. Non-ideal SEC Effects

Variation in elution volume may be observed when interactions other than those associated with normal steric exclusion take place. These interactions are varied and their effect often dependent on the solute.

Adsorption Effects - If a solute is able to adsorb onto the surface of the
packing it will exhibit a larger retention volume. Such adsorptions are common if there are free sites available on the surface of the support. The removal of these sites would restore the SEC character of the packing. This is done by deactivation of the surface groups. Often an organic group may be bonded onto the surface of silica gel to remove free silanol groups (the bonded groups may be $-\mathrm{C}_{4} \mathrm{H}_{9}$ or $-\mathrm{C}_{8} \mathrm{H}_{17}$, using silane chemistry).

Hydrophobic interactions may be removed by addition of an organic solvent or by reducing the ionic strength of the eluent and thus reducing the affinity of the solute for the support.

For high molecular weights adsorption can be extreme due to the large number of possible interactions that would be associated with say a complete protein. Combating and making use of the interactions will be discussed in Chapter 3.

Ion Effects - When the solutes can exist in a charged (ionised) form, there are different types of possible interactions that may take place.

- ion exchange effects where the packing may exchange its surface groups. For example, if silica gel is used the silanol group may exhibit some cationic exchange behaviour.
- Donnan potential effects, results only in the case of ionised solutes. They arise due to an interaction between the solute and the potential gradient associated with the particles in the packing material. Figure 2.8 illustrates the potential that may exist within the packing material. The effect implies that solutes of one charge will be more highly retained? In the case of oppositely charged solutes, ion exclusion effects occur and solutes suffer electrostatic repulsion.

Viscosity Effects - These tend to arise if the injected sample is too concentrated. At high sample concentration the viscosity of the sample can be significantly greater than that of the mobile phase viscosity. As a result the eluent tends to push through the sample rather than carry it along the column. The outcome is a distorted peak and a higher retention volume.

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FIGURE 2.1

Figure illustrates a typical Size Exclusion Chromatography Calibration Curve. The retention volume $\left(V_{R}\right)$, or the distribution coefficent $(K)$, is plotted against the log of solute molecular size.


The SECC Curve obtained using the theoretical model of restricted diffusion of Yau and Malone is compared against experimental data.


Taken from reference 21

Within a cylindrical pore the volume accessible to a solute depends on the solute shape. Below three different solute shapes are shown:

- a) hard sphere of radius, r.
- b) rod of length L - two extremes of orientation.
- c) random flight chain - restricted in allowable conformations.


FIGURE 2.4

The theoretical dependance for the elution of rod like solutes on pore structure is compared against the curves for spherical solutes.


Divtribution coefficient $\boldsymbol{k}$. for thin rods of length $L_{\text {: }}$.
A. for various pores versus the dimensionless parameter $l$. a .


- Distribution copfticient $A$. for spherical molecules of radius $r$.


FIGURE 2.5

Illustration of how the SECC Curve is displaced with differing pore sizes.


PORE $\mathrm{A}>$ PORE $\mathrm{B}>$ PORE $C$

A number of different pore shapes have been outlined.

## CYLINDER

$$
K=(1-\Gamma / R)^{2}
$$

$R=$ pore radius

$r=$ solute radius

## INVERSE

## 4-CYUNDER

$$
\begin{gathered}
K=\frac{\left.1-\sqrt{(2} p-p^{2}\right)-(1-p)^{2}(\pi / 4-\theta)}{(1-\pi / 4)} \\
p=r / R \quad \theta=\cos ^{-1}(1 /(1+p))
\end{gathered}
$$



RANDOM

## SPHERE

$$
K=\frac{\operatorname{expl}\left[-4 / 3 \pi N(r+R)^{3}\right]}{\exp [-4 / 3 \pi N R\}^{3}}
$$

$N=\begin{gathered}\text { No. of spheres per unit } \\ \text { volume }\end{gathered}$



FIGURE 2.8

The formation of a Donnan Potential as illustrated below, may be responsible for some non-ideal exclusion effects.


## CHAPTER 3

## ASPECTS OF LARGE MOLECULE CHROMATOGRAPHY

### 3.1. Introduction


#### Abstract

The development of High Performance Liquid Chromatography (HPLC) has led to a well understood variety of techniques primarily suited to the separation of small molecules with molecular weights typically less then 2000. Larger molecular weight, samples have in the past been considered unsuitable for HPLC. This was because the HPLC systems available gave poor resolution and poor recovery of these large biologically active and often polyfunctional solutes. Furthermore the biological activity of these molecules was often lost during chromatography. However the demand for an HPLC support suitable for the chromatography of proteins and other large biopolymers in the biochemical, biological and biotechnological industries has led to renewed interest in packing material development for both analytical and preparative HPLC (1).


Until recently, chromatography of biopolymers and proteins was performed with gravity fed LC, which used semi-rigid gels which in turn tended to swell in organic solvents. These gels gave low efficiencies and were generally unsuitable for high solvent flow. Typically an analysis using such a system may last at least 24 hours.

The research into HPLC packing materials for biopolymer separation is now being directed towards the production of a rigid porous support which should contain uniformally sized particles that are mechanically stable and fully porous. In a recent publication (2) Regnier described the demands on this new packing
material.

- "A superior support for the separation of biological macromolecules must be available in a variety of particle and pore sizes and surface areas, be of a narrow PSD and separate in only one mode at a time and be mechanically stable to 100 bar."

In this chapter a general study has been made of the different modes of HPLC used for large molecule chromatography and the packing material design that gives the most useful resolution for that particular mode. Large molecules can be chromatographed in three main modes.

\author{

1. Ion Exchange Chromatography <br> 2. Bonded Phase Chromatography <br> 3. Size Exclusion Chromatography
}

### 3.2. Ion Exchange Chromatography

Ion Exchange Chromatography has been used for many years to separate biopolymers in classical LC. This mode of chromatography is based on the interaction between the solute molecule and a charged liquid held on the packing material. These liquids may be positive or negatively charged depending on the requirements of the separation. The elution of bound proteins is usually achieved with either an ionic strength or pH gradient. In High Performance Ion Exchange Chromatography, the packing materials are usually either silica based bonded supports (3), or organic materials (4). As with other HPLC modes the packing material stability is essential and thus for this reason silica gel is a good choice as a base material.

The separation in HPIEC relies on interaction between the solute and the
packing material surface. Thus it is essential that the solute molecules must have unrestricted access to this surface for the best chromatographic results. As around $95 \%$ of the particle surface in conventional HPIEC materials is held within the pores, HPIEC of biopolymers whose size limits access to the pores is correspondingly poor. Therefore in order to allow solutes free access to the packing surface and maintain the column loading capacity of the silica gel wide pore silica gel packings are required(5).

As the solute size increases, the pore size must also increase and thus pores of diameter 250-350A have been shown to be suitable for protein chromatography where the solute molecular weight is around 50,000 . The maximum resolution of any solute pair will be achieved when the support diameter is matched with solute size to provide the most advantageous conditions of solute diffusion. Finally the surface area of the packing must be maintained at a sufficiently high value, to retain the packing material loading capacity.

### 3.3. Bonded Phase Chromatography

In the main, bonded phase chromatography of proteins is carried out using hydrophobic phases (e.g. alkyl bonded groups) when the technique is known as reverse phase chromatography. Separations have been achieved on conventional bonded packing materials (6) but as with HPIEC wider pore packing materials tend to give better results (7-9). The most common packings available, are silica gel based packing.

The character of the bonded phase is easily altered by the different groups that may be chemically attached to the silica gel. Polar groups have successfully been used by Kiselev et al (10) for the separation of viruses in aqueous media. Non-polar bonded phases have been used more frequently, and detailed studies of
the effect of the different bonded groups have been made. In contrast to the reverse phase chromatography of small molecules where octyl and octadecyl phases are common, the best recovery and resolution of proteins and biopolymers is often obtained with butyl and propyl bonded groups (11).

As in HPIEC few proteins may be chromatographed isocractically with RP HPLC; the peaks tend to be broad and the difference between total retention and non-retention is very small (12), and gradient elution is very common. The retention of the solutes can be varied by altering the mobile phase pH , or salt concentration or organic content. In the past one of the major problems with this type of HPLC has been the denaturing or unfolding of the solute molecule during chromatography which results in loss of biological activity, and in cases where biological activity and efficiency are important, this is obviously a problem. As a result of this problem a large amount of work on the development of High Performance Hydrophobic Interactive Chromatography (HP-HIC) has been done. This is a specialised form of bonded reverse phase chromatography where the biological activity of the solute molecules is maintained (13).

### 3.4. Size Exclusion Chromatography

Size Exclusion Chromatography (SEC) is the simplest of the various chromatographic techniques, there is no interaction between the packing surface and the solute molecules. The solutes are eluted through a neutral hydrophobic bed and are separate according to their hydrodynamic volume which is usually proportional to the solute molecular weight. The technique has been widely used in protein purification where the absence of adsorptive interaction with the packing surface is a prerequisite.

The fractionation limits of SEC packing materials are largely dependent on pore
size and a detail account of the separation mechanism has been given in Chapter 2. Early SEC supports provided limited resolution and poor recovery but recent developments in packing material efficiency, permeability and surface properties of mostly silica based materials have enabled SEC to be used in modern LC where the technique has been termed High Performance Size Exclusion Chromatography (HPSEC) (14). These improved materials offer a comparable performance to soft gels with as much as a 100 fold reduction in separation time. The demand on HPSEC from the application field in terms of resolution, molecular weight accuracy and recovery imply that tailor made packing materials with specific chemical and physical properties should be developed.

In general the resolution obtained from HPSEC depends on the solute being separated, the particle size, the pore size, the pore size distributon and the mechanical stability of the packing material. The packing material must be strong and this has been the advantage of the silica based packing materials in other HPLC modes. The case for using silica based packing materials for HPSEC, makes the control of the physical properties described above more important. Another important part of HPSEC packing material design(14), is the control of unwanted solute adsorption by surface silanol groups.

HPSEC may be subdivided into ideal HPSEC where only the pore structure is responsible for separation and non-ideal HPSEC where eluent conditions may be manipulated to give improved selectivity by introducing further interactions.

In ideal HPSEC the maximum resolution is obtained by minimising the PSD and maximising the pore volume of the packing material (15). Wider solute size ranges are often covered by using several packing materials with differing PSD. Regnier has demonstrated the improved efficiency attainable using higher pore volume packing materials (16) and this is an obvious area in which silica gel based
materials may be improved.

In the situation of non-ideal SEC the eluent composition is used to change the selectivity of the chromatographic system (17). During a typical analysis the interaction between proteins and the packing material can be varied by careful control of eluent ionic strength or the pH of the mobile phase. Several studies have been made showing how protein elution differs with mobile phase variation and also with the protein type that is being chromatographed (18 and 19).

### 3.5. Summary of Packing Material Requirements

For large molecule chromatography the packing material design depends on firstly the mode of HPLC in which it will be used and also on solute that will be separated, There are several general requirements.

1. The packings should be available in a range of sizes from $3-10 \mu \mathrm{~m}$ in diameter for analytical work and $10-20 \mu \mathrm{~m}$ in diameter for preparative work.
2. The packings must be mechanical stable to allow high solvent throughput and must not breakdown during usage. This in practice means the materials must be stable to at least 4000 psi although many HPLC packing materials are slurry packed at pressure up to $10,000 \mathrm{psi}$.
3. The packings should have wide pores that will allow greater solute diffu'sion within the pores and also it will allow greater access of the solute to the particle surface. The pore sizes in which the packings should be made are inthe range of 100 up to 800 Angstroms in diameter depending on the application.
4. For HPSEC the production of packing materials with a narrow PSD is required to give the best resolution.
5. A high pore volume material should be made as this will give better resolution in HPSEC and will allow a greater loading capacity in the relative HPLC modes.
6. The packing materials should be of good chemical stability, while silica gel has been suitable in the past it may become important to carry out separations at higher $\mathrm{pH}(\mathrm{pH}>8)$ and in this case either the silica surface must be protected or alternative materials should be used.
7. The packing surface should be as chemically homogeneous as possible so that bonding of specific substrates to the gel surface to alter its properties may be easily achieved. It is also necessary to make a packing material that has controllable adsorptive properties so that solute recovery is maintained at the highest possible level.

The work reported in this thesis concerns the production and development of a high pore volume silica gel with wide pores and a good mechanical stability. The combination of these properties should make such a material useful for HPLC of macromolecules.

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## SILICA GEL FOR HPLC AND HPSEC

### 4.1. Introduction

In HPLC pressurised flow results in faster separations being obtained. However this improvement is limited by the extent of solute dispersion on passage along the column bed. For best efficiency it is important to use:

- a homogeneous bed - giving uniform flow across the column
- a bed in which the solute diffusion distances are minimised
- strong particles which are able to withstand the pressures used (i.e. $>4000 \mathrm{psi}$ )

Silica gel has over the last fifteen years become the most common packing material used in HPLC. As the demands of HPLC have multiplied, methods of production of the different silica gels with specific chemical and physical properties have developed.

As explained in Chapter 1 the first silica gels used in modern LC were large fully porous particles some $40 \mu \mathrm{~m}$ in diameter. These were followed by the production of pellicular particles with a porous coating. The materials in use today are fully porous particles of $10 \mu \mathrm{~m}, 5 \mu \mathrm{~m}$ and $3 \mu \mathrm{~m}$ in diameter. The particle shape for most commercial gels is spherical although some irregulars are still available.

In design of a chromatographic silica gels several characteristics must be given careful consideration. These characteristics are important to the chromatographic performance achieved. They include Particle Shape, Particle Size, Particle Size Distribution, Pore Shape, Pore Size, Pore Size Distribution, Pore Volume and Specific Surface Area.

For most modes of HPLC the separation achieved is dependent on the interaction between the solute and the stationary phase held on the support surface. For such techniques the surface area of the packing material as well as the pore size will determine the loading capacity and retention. Typically silica gels used for adsorption chromatography would have surface areas of around $100-400 \mathrm{~m}^{2} \mathrm{~g}^{-1}$ with a pore volume of $0.4-0.8 \mathrm{ccg}^{-1}$ and a mean pore size of 60-200 $\AA$. For small molecules the surface structure is much more important than the pore structure. However for the separation of larger molecules by retentive HPLC the pore characteristics become crucial and therefore have to be considered in relation to the gel production. A compromise must be made between pore size, surface area, particle porosity and gel strength.

For HPSEC the retention mechanism is different from that in retentive LC, as ideally the stationary phase is made up of eluent within the pore volume alone. Here the retention of solutes is determined by pore size and pore size distribution. The work undertaken concerned the production of silica gels specifically for use in HPSEC and accordingly the following discussion will be geared towards the factors involved in such production.

The object was to produce a silica gel of higher pore volume than commonly used in retentive LC. These gels of higher pore volume and wider pore size would give better chromatography of very large molecules. As explained in. Chapter 2 the resolution in HPSEC is determined by the structural
properties of the pores in the packing material. The pore size, pore volume, pore size distibution and particle porosity all affect the chromatographic ability of the packing material. The peak capacity in HPSEC is given by

$$
\begin{equation*}
n=1+0.25 / N \ln \left(V_{R \operatorname{MAX}} / V_{R \operatorname{MIN}}\right) \tag{4.1}
\end{equation*}
$$

The value of $V_{R \text { MIN }}$ is the volume of the interparticle space within the column, while $V_{R}$ MAX is the volume of both the interparticle space and the pores within the column.

Silica gel can be formed and fabricated with a number of different physical characteristics depending on the starting material and reaction conditions during production. The porous structure created is controlled by:

1. Type of chemical reaction used to form the gel
2. Experimental conditions

In aqueous solution silica may exist in several forms, as soluble silicates or if sufficiently dilute as a solution of monosilicic acid, $\mathrm{Si}(\mathrm{OH})_{4}$. Alternatively silica may be present as a silica sol, a colloidal dispersion of amorphous silica.

These sources of silica are made use of in the production of the different forms of silica gel. The gels may be catagorised as:

1. Hydrogel - a gelatinous, semi-rigid network of linked colloidal particles where the aqueous media is still present.
2. Xerogel - the rigid network obtained by the drying of the hydrogel
3. Aerogel - where the network is obtained without the loss of pore volume normally associated with xerogel production.

In all these systems the solid silica is dispersed in either a liquid or a gaseous phase. As to their use in Chromatography, it is the xerogels that are most important due to the ease of production of a mesoporous material.

### 4.2. Silica Gel Structure

Silica gels consist of a porous structure where the framework comprises dense amorphous silica(1). In amorphous silica each silicon atom has tetrahedral co-ordination with oxygen throughout the internal structure. Within the internal structure the gel is held together by siloxane bonds.


These siloxane bonds hold the primary colloidal units of the gel together. Figure 4.1 illustrates the particle structure. At any surface either within the particle or on the external surface of the gel the tetrahedral co-ordination is maintained by the presence of hydroxyl groups(2). These groups are of two major types:

Mono hydroxy $\quad \mathrm{Si}-\mathrm{O}-\mathrm{H}$

Di hydroxy $\quad \mathrm{Si}-(\mathrm{O}-\mathrm{H})_{2}$

On the surface the hydroxyl groups may be

- Free Hydroxyl groups
- Vicinal Hydroxyl groups, that may exhibit Hydrogen bonding.
- Hydroxyl groups with adsorbed water molecules

The highly disordered nature of the gel surface means that all these different groups will be present in any one sample.

### 4.2.1. Solubility of Silica Gel

The solubility of silica gel in aqueous media is a function of three main variables.

## Solubility $=\mathrm{f}($ Temp, pH, Particle Size )

The solubility of amorphous silica gel increases with temperature.

$$
\begin{equation*}
d \ln S / d T=\Delta H / R T^{2} \tag{4.2}
\end{equation*}
$$

The pH affects solubility in that at high pH the hydrolysis of silica gel to soluble species is favoured.

$$
\begin{gathered}
\mathrm{SiO}_{2}+2 \mathrm{H}_{2} \mathrm{O}=\mathrm{Si}(\mathrm{OH})_{4} \\
\mathrm{SiO}_{2}+\mathrm{H}_{2} \mathrm{O}+\mathrm{OH}^{-}=\mathrm{Si}(\mathrm{OH})_{3} \mathrm{O}^{-}
\end{gathered}
$$

The variation in solubility is slight from $\mathrm{pH} 1-9$, but above pH 9 increased solubility is observed due to the formation of silicate ions.

$$
\mathrm{Si}(\mathrm{OH})_{4}+\mathrm{OH}^{-}=\mathrm{Si}(\mathrm{OH})_{5}^{-}
$$

The variation of solubility with pH is shown in Figure 4.2

The third major factor in the solubility of silica gel is the particle size. For small amorphous silica particles variations in solubility are more pronounced. These variations have been shown to be related to the radius of the particles. Alexander(3) produced data showing that for a particular silica species the
solubility increased with decreasing particle size. Charles(4) found that the rates of dissolution of porous silica glass was related to the high local solubility of the silica surface. This high solubility was due to the small highly convex radius of curvature. Figure 4.3 illustrates the variation of solubility with particle size and radius of curvature. The solubility expression is given by:

$$
\begin{equation*}
\ln \left(S_{r} / S_{i}\right)=(2 E V / r R T) \tag{4.3}
\end{equation*}
$$

where, $S_{r}$, is the solubility of a particle radius $r, S_{i}$, is the solubility of a flat surface, $E$ is the interfacial surface energy, $V$ is the molar volume, $R$ is the gas constant and $T$ is the temperature.

### 4.2.2. Solution/Deposition in Silica Gel

The variation of solubility with particle size has pronounced effects in solution/deposition processes associated with the ageing of silica gels. Figure 4.4 illustrates the typical make up of a silica gel particle and the structural change associated with solution/deposition is also shown.

In silica gel both convex and concave radii are present. As such the least soluble surface, that surrounded by the most siloxane bonds, isassociated with the areas of contact between particles. These areas of contact or "necks" have the lowest solubility and the solubility of other surfaces relative to these "necks" may be expressed as:

$$
\begin{equation*}
\ln \left(S_{r} / S_{r^{*}}\right)=2 E V / r R T \tag{4.4}
\end{equation*}
$$

where $S_{r^{*}}$ is the solubility of the most highly convex surface. All other solubilities within the gel will lie between the solubility of the most convex area and the solubility of the most concave area.

An approximation to the rate of the silica gel dissolution may be given as

$$
\begin{equation*}
R_{S O L}=\exp \left(-E_{a} / R T\right) \cdot A_{r} \cdot\left(S_{r}-S_{r^{*}} / S_{r} \dot{*}\right) \tag{4.5}
\end{equation*}
$$

where $R_{S O L}$ is the rate of dissolution of silica of radius $r$. $E_{a}$ is the activation energy. $R$ is the gas constant and $T$ is the temperature. $S_{r}$ is the solubility of particle radius $r$ and $S_{r}$ : is the solubility of the least soluble area of the gel. $A_{r}$ is the area of particles radius $r$.

The rate of dissolution of particles of a specific size is dependant on the surface area of the particles, their solubility and the temperature of reaction. Once in solution the particles may rapidly redeposit on the surface of undissolved silica gel. The most favourable surfaces are those of low solubility.

A fourth factor in the solubility is the effect of electrolyte within the network, in particular metal ions. Iler(2) has shown that the presence of aluminium ions will increase the solubility of colloidal silica. Other ions like $\mathrm{Na}^{+}$ and $\mathrm{Fe}^{2+}$ have also been shown to have an effect on the solubility.

### 4.3. Production of Silica Gels

As mentioned earlier the production of the gel depends on, among other variables, the starting material used to form the gel. The production of silica gels is generally achieved from one of three main routes:

- The acidification of sodium silicate solution
- The gelling of colloidal silicas
- Hydrolysis and polycondensation of silicon compounds such as $\mathrm{SiCl}_{4}$ or $\mathrm{Si}(\mathrm{OEt})_{4}$

These produce hydrogels, which may then be dried to give a xerogel. The structure of the xerogel is a function of the strength of the material. By
reinforcement treatments of these gels the the strength and structures may be altered.

### 4.3.1. Silica Gel from Silicate Solution

### 4.3.1.1. Acidification

In solution silicates exist in equilibrium with monosilicic acid $\operatorname{Si}(\mathrm{OH})_{4}$.

$$
\begin{gathered}
\mathrm{Si}(\mathrm{OH})_{5}^{-}=\mathrm{Si}(\mathrm{OH})_{4}+\mathrm{OH}^{-} \\
\mathrm{SiO}_{2}+2 \mathrm{H}_{2} \mathrm{O}=\mathrm{Si}(\mathrm{OH})_{4} \\
2 \mathrm{HSiO}_{3}^{-}=\mathrm{Si}_{2} \mathrm{O}_{5}^{2-}+\mathrm{H}_{2} \mathrm{O}
\end{gathered}
$$

Monosilicic acid is sparingly soluble in water and if its concentration rises above $100-200 \mathrm{ppm}$ then polymerisation may occur. This polymerisation may continue to form either a gel of discrete particles or an extensive 3D network throughout the solution. Carmen(5) first outlined the stages in the polymerisation to be

1. Polymerisation to form primary colloidal particles
2. Growth of colloidalparticles
3. Linking of colloidal particles forming a network

The individual stages of the polymerisation have been dealt with in detail by $\operatorname{ller}(2)$ First the silicate ions are neutralised by addition of acid to form neutral $\mathrm{Si}(\mathrm{OH})_{4}$. In the next stage the monosilicic acid polymerises to form polysilicic acids of relatively low molecular weight, by the expulsion of water.

$$
\begin{gathered}
\mathrm{Si}(\mathrm{OH})_{4}+\mathrm{Si}(\mathrm{OH})_{4}=(\mathrm{HO})_{3} \mathrm{Si}-\mathrm{O}-\mathrm{Si}(\mathrm{OH})_{3}+\mathrm{H}_{2} \mathrm{O} \\
(\mathrm{HO})_{3} \mathrm{Si}-\mathrm{O}-\mathrm{Si}(\mathrm{OH})_{3}+\mathrm{Si}(\mathrm{OH})_{4}= \\
(\mathrm{HO})_{3} \mathrm{Si}-\mathrm{O}-\mathrm{Si}(\mathrm{OH})_{2}-\mathrm{O}-\mathrm{Si}(\mathrm{OH})_{3}+\mathrm{H}_{2} \mathrm{O}
\end{gathered}
$$

After this inital polymerisation there are three possible outcomes. Firstly gel formation can occur throughout the medium, secondly particle growth can occur resulting in the formation of colloidal sol and thirdly both mechanisms may occur giving a gel network of larger primary particles. The rate at which gelling occurs is a function of several variables.

$$
\text { Gelation Rate }=f(\text { Temp,pH,Silica Conc'n,Electrolyte })
$$

### 4.3.1.2. Temperature

A higher temperature will tend to favour particle growth due to the increased solubility and larger number of particle collisions.

### 4.3.1.3. Silica Concentration

Increased silicate concentration will result in faster gelling as more silicic acid would be present in the solution thus the linking between particles becomes more likely. Faster gelling would also occur the smaller the initial colloidal particles.

### 4.3.1.4. Electrolyte Concentration

The concentration of electrolyte affects the electrical potential of the solute. electrical
The electrolyte will tend to decrease the/double layer thickness round the dispersed silica particles. This allows closer approach of the particles and thus speeds up their aggregation.

### 4.3.1.5. The Effect of pH

Figure 4.5 shows the time taken for gel formation from sodium slicate varies with pH . At low $\mathrm{pH}(\mathrm{pH}<2)$ the condensation reaction reaction is catalysed by the $\mathrm{H}^{+}$ion. This results in slow particle growth and small colloidal particles up to 1 nm forming. As the pH increases to $\mathrm{pH} 4-5$ there is less $\mathrm{H}^{+}$in the system. The isoelectric point of silica lies in this region and it is here that gelation occurs at its most rapid. The gel network spreads quickly through the solution. In the presence of lower hydrogen ion concentration, i.e. higher pH the particles take on a negative charge, resulting in repulsion between them. By pH 6 the gelation rate/rapidly decreased and by pH 9 the gelation rate is negligible. At pH 9 the rate has slowed so much that a stable colloidal sol containing silica gel particles up to say 50 nm in diameter can be formed.

Thus for the production of hydrogels from silicate solutions pH control is essential. At low pH the colloidal nuclei will grow only slightly before gelling, giving high surface area gel. Whereas at high pH much larger particles are formed before gelation. A gel formed from these larger particles will tend to have a lower surface area.

### 4.3.2. Silica Gel from Colloidal Silica

Colloidal silica sols are dispersions of amorphous silica. The dispersion contains discrete particles. As discussed in the previous section the colloidal silica can be produced by the growth of particles when silicate solutions are acidified. Techniques in production of these sols have advanced from sols stable with $10 \%$ silica in the 1930 's to sols with up to $50 \%$ silica by weight(6).

The method of producing the highly concentrated sols was first developed by Bechtold and Snyder(6). A dilute solution of sodium silicate was passed through a cation exchanger to produce a solution of monosilicic acid. Alkali
was added to stabilise the solution, at this stage the sol would be about $3.5 \%$ silica. The next stage in the production involved heating a small portion of the sol to encourage particle growth. Further silicic acid was then slowly added to the mix, whereupon the silicic acid was deposited onto the surface of the particles. Using this method sol particles can be formed and stabilised, giving particles 10-130 nm in diameter. Water must be evaporated, otherwise the colloidal sol will always give $3.5 \%$ silica. In this way the sol is concentrated.

The colloidal sol may now contain perhaps $30 \%$ or as much as $50 \%$ silica by weight, and is stabilised by high pH pH 9-10. The stabilisation is brought about by the addition of alkalis such as NaOH or $\mathrm{NH}_{4} \mathrm{OH}$. In order to gel the sol the pH must be dropped in a manner similar to the acidification of silicate solution. With a lower pH the interparticle repulsive forces are decreased and gelation is encouraged.

The characteristics of a gel produced in this manner are dependent on

- The primary particle size of the colloidal particle. Smaller particles give a higher surface area. For example a 5 nm silica sphere will give an area of about $550 \mathrm{~m}^{2} \mathrm{~g}^{-1}$, and a 50 nm sphere an area of $55 \mathrm{~m}^{2} \mathrm{~g}^{-1}$.
- The concentration of the primary particles: A higher concentration will resuit in a more compact structure and a lower porosity.
- The effects of pH , temperature, and electrolyte on gelation rate as discussed previously: The major effects being in the degree of interparticle bonding.


### 4.3.3. Silica Gel from Hydrolysis and Condensation of Silicon Compounds

Many studies have been carried out on the production of silica gel by the hydrolysis of silicon compounds. In particular these studies have used Silicon tetrachloride or Tetralkyl silicates. The methods of Stober et al(7) and Unger(8) are able to produce colloidal spheres of uniform size. The size of the particle produced depends on the type of alkyl silicate used. Stober et al have produced particles up to $1 \mu \mathrm{~m}$ in diameter by using low alkyl silicates $\left(\mathrm{Si}(\mathrm{OEt})_{4}\right.$ or $\left.\mathrm{Si}(\mathrm{OMe})_{4}\right)$.

The process takes place in several stages, the first is a hydrolysis of the tetra ethyl silicates in an alcoholic solution with ammonia as a catalyst. This hydrolysis results in the formation of polyethoxysilanes: eg.

$$
2 \mathrm{Si}(\mathrm{OEt})_{4}=(\mathrm{EtO})_{3} \mathrm{Si}-\mathrm{O}-\mathrm{Si}(\mathrm{OEt})_{3}+\mathrm{H}_{2} \mathrm{O} \longrightarrow
$$



Continued hydrolysis results in complete replacement of the alkoxy groups by hydroxyl groups giving a silica gel with a very high degree of fine channels.

## Here the factors that control the structure of the final gel are

- The molecular weight of the polyethoxysilane. The larger the polymer unit the more open the structure.
- The type of catalyst used and their concentration affect the rate of chain linkage.
- The relative proportions of the non-solvent mix from which the gel has been precipitated. By alteration of the conditions particles of a specific size will be deposited from solution.
- The reaction temperature will also affect the rate at which particles are grown and deposited from solution.


### 4.4. Gel Strengthening

The strengthening of the gel structure can be carried out in several ways. Figure 4.6 outlines several of the methods that can be used to strengthen the gel. The experimental use of some of these methods will be discussed in the next chapter.

### 4.4.1. Gel Strengthening from the Hydrogel

The strengthening of the gel can be carried out in two ways:

[^1]2. Solution / deposition processes which make significant changes to the surface area and pore size of the gel. This type of process is often termed "aging" of the gel. These processes make use of the differences in solubility between the individual particles and within the particles themselves. The areas around points of contact of individual particles being less soluble. This has been explained previously. The most common form of this type of process is "hydrothermal treatment" where the treatment is carried out by immersion of the gel in water at an elevated temperature and pressure. The term is used when referring to treatment above $100^{\circ} \mathrm{C}$ usually in an autoclave. Hydrothermal treatment may however refer to reactions carried out at room temperature as well as those at elevated temperature.

### 4.4.2. Drying Silica Gel

In all of the methods described the gel produced is a hydrogel with liquid, namely water held within the pores of the material. To make a gel for HPLC this liquid must be removed and the gel structure finalised. This removal of liquid can often cause changes in the gel structure.

The removal of liquid from the pores subjects the gel structure to enormous compression forces, arising from surface tension effects, that tend to pull the pore walls together. The compression forces associated with the removal of water are given by; where $\theta$ is the contact angle and $\gamma$ is the surface tension

$$
\begin{equation*}
F_{c}=2 \pi r \gamma \cos \theta \tag{4.6}
\end{equation*}
$$

where $F_{c}$ is the force on the walls due to the evaporation. This force results in
the particle being subjected to extremely large pressures where the resultant pressure is:

$$
\begin{gather*}
P=F_{c} / A=2 \pi r \gamma \cos \theta / \pi r^{2}  \tag{4.7}\\
P=2 \gamma \cos \theta / r \tag{4.8}
\end{gather*}
$$

For typical silica gels, assuming a zero contact angle the resulting pressures for various pore sizes are listed below:

| Pore Diameter <br> $(\mathrm{nm})$ | Pressure <br> $\left(\mathrm{Nm}^{-2}\right)$ |
| :---: | :---: |
|  |  |
| 10 | $29.6 \times 10^{\ddagger 6}$ |
| 20 | $14.8 \times 10^{+6}$ |
| 50 | $5.96 \times 10^{\ddagger 6}$ |
| 100 | $2.98 \times 10^{\frac{46}{4}}$ |

The result of this that some particle deformation takes place on drying.

This compression will obviously increase the packing density of the gel. The compression force arises due to the surface tension of the liquid held within the pores. Figure 4.7 indicates the effect of liquid being evaporated from the pores of the gel. How the gel structure behaves on drying is dependent on the initial hydrogel gel structure and the degree of coalescence of the primary colloidal particles in the gel before drying.

Foster(9) described the surface tension forces as being related to pore size. The surface tension forces acting on the pore walls are inversely related to the width of the pore. Thus high area gels riddled with fine channels will suffer a much larger degree of shrinkage than wide pore materials upon drying.

Gels made from fine colloidal silica particles (Diameter around $2-5 \mathrm{~nm}$ ) may experience very large forces. On the other hand the compression experienced
by gels made from $10-20 \mathrm{~nm}$ particles will be much reduced. The result of this effect is that gels made from larger particles, dry with a more open structure but they are also much more fragile.

The stages in drying have been outlined by ller

- Solidification of gel network - with liquid still remaining within the pores.
- Strengthening of the particle by increased coalescence at points of contact, through aging.
- Shrinkage of the particle as a whole as water evaporates giving a particle of a minimum radius determined by the compressive forces.
- Evaporation of the remaining water from within the pore and the development of stress within the structure.
- Possible fracture of the silica particles, if large

Several ways of reducing the shrinkage of silica gels have been developed.

1. Strengthening of the gel by reinforcement prior to drying
2. Reducing the surface tension of the liquid being removed from the pores
3. Enlarge the pores and increasing coalescence by aging the gel (eg. Hydrothermal Treatment)
4. Dry the gel by heating at a temperature above the critical point of the liquid held within the pores. In this way there is
no liquid-vapour interface, and thus no surface tension forces.

### 4.4.3. Shaping the Gel Particles

In HPLC it is generally thought desirable that the packing materials be spherical in nature. Several techniques have been used to produce the particles.

Emulsification - The colloidal silica or sodium silicate is emulsifed in an immiscible liquid while the gelling takes place.

Spraying - Small droplets are sprayed into a hot air drier that gels the particle before it sediments.(10)

Coacervation - where colloidal silica combines with a water soluble organic compound that deposits from solution and solidifies.

### 4.4.4. Strength of Silica Gels

The strength of a silica gel is related to the primary colloidal particle size and to the extent of interparticle bonding.

The bonding between colloidal particles has already been described as being by condensation of silanol groups forming siloxane bonds. If this bonding is only slight then particles of high porosity and low mechanical stability are formed. These gels are indicated in Figure 4.8 which shows the situation as increased coalescence between the particles occurs. The extent of siloxane bonding at the particle joints or "necks" increases the strength of the gel. This results in a stronger, denser and less flexible structure.

The determination of the extent of interparticle bonding has been carried
out by electron microscopy and partial dissolution methods. While the determination of mechanical strength of particles has been studied by Meissner et $a l(11)$ and has been found to be inversely related to the particle diameter and porosity.

### 4.5. Modification of Silica Gel structure

It has been possible to strengthen silica gels by chemical and thermal treatments. The two techniques used are "sintering" and "hydrothermal treatment". The surface area, pore volume and gel structure can be altered by sintering in an inert atmosphere (air, vacuum) at high temperature, 600-1000 ${ }^{\circ} \mathrm{C}$. This results in a decrease in surface area and pore volume. A similar effect can be obtained by heating the gel in a current of steam. For this type of treatment a lower temperature is required to give structural changes comparible to sintering. At still lower temperature $100-250^{\circ} \mathrm{C}$ treatment in an autoclave with liquid water results in a dramatic decrease in surface area without loss of pore volume. Above $250^{\circ} \mathrm{C}$ pore volume as well as surface area is lost.

### 4.5.1. Sintering of Silica Gel

Sintering of silica gel has been carried out by heating the gel in a vacuum(12), in air(13), or in a current of steam(14) for a set period of time. The behaviour of silica gel under the action of heat has been thoroughly studied.

As the temperature rises the surface water molecules are removed from the silica gel, such that at a temperature of $100-140^{\circ} \mathrm{C}$ all are lost. Continued treatment up to $300^{\circ} \mathrm{C}$ will remove any physically adsorbed water held within the gel pores. Over the range $300-500^{\circ} \mathrm{C}$ reversible condensation occurs between the vicinal $O H$ groups on the gel surface.


Above a temperature of $500^{\circ} \mathrm{C}$, depending on structure of the gel, condensation of silanol groups is no longer reversible. Surface migration within the silica gel results in condensation across the pores and narrow cavities within the gel. This condensation results in stronger bonding between the particles. As the temperature increases the surface mobility increases and a rapid loss of surface area and pore volume occurs. As the pores contract the silica gel shrinks correspondingly.

For gels of relatively uniform pore structure the sintering process occurs over a relatively narrow range of temperatures. Furthermore a macroporous material will tend to be more resistant to sintering than a gel containing micropores. Thus gels made from smaller primary particles will sinter more strongly and rapidly than a gel made from large particles.

The presence of impurities within the gel structure alters the sintering mechanism. These impurities are the oxides of $\mathrm{Na}, \mathrm{Fe}, \mathrm{Ca}$, and Al . These impurities tend to catalyse the sintering process. The rate of sintering varies within the gel, it has been suggested that this is due to the geometrical and chemical heterogeneity of the silica gel structure(15).

### 4.5.2. Hydrothermal Sintering

In the presence of steam "hydrothermal sintering"(16-19) occurs. The effects associated with sintering are seen but at lower temperatures. The change in structure associated with bulk mass transfer within the silica gel is catalysed by the steam. The $\mathrm{Si}-\mathrm{O}-\mathrm{Si}$ is hydrolysed by adsorbed water which decreases the activation energy of the surface diffusion process that is responsible for the gel strengthening. Table 4.1 indicates the effect of steam treatment in comparison to that of sintering.

### 4.5.3. Hydrothermal Treatment

Hydrothermal treatment(HTT) can be applied to both xerogels (where the structural skeleton is fixed) and to hydrogels that have never been dried. This type of treatment has a different mechanism from the sintering processes in that a solution deposition mechanism is responsible for the structural changes. The term usually refers to treatment of a gel in an aqueous medium at temperatures above $100^{\circ} \mathrm{C}$. The treatment is usually carried out in an autoclave.

The mechanism is a solution deposition process where the most soluble parts of the gel (small particles with a high positive radius of curvature) are preferentially dissolved. These particles are then reprecipitated on the surface of the least soluble parts of the silica gel. The least soluble area in a gel is the area of negative curvature associated with the points of contact between spheres. It is in this way that the gel strength is increased.

In the production of a chromatographic packing material several variables within the hydrothermal treatment have been considered and studied:

- The structural properties of the product
- The effect of temperature
- The effect of duration of treatment
- The pH of the system
- The effect of impurities
- The relative amounts of reagents.

The effect of HTT on silica gel pore structure has been studied by many workers. Kiselev et al(19,20) worked on several of the variables.

### 4.5.3.1. Structural Microporosity

It has been found that during hydrothermal treatment there can occur the formation of micropores $(21,22)$. which are very fine and only avallable for very small molecules (e.g. $\mathrm{H}_{2} \mathrm{O}$ ). A dependance was noticed between this microporosity and the conditions used for the hydrothermal treatment. As the temperature and pressurewere increased then the degree of microporosity also initially increased (this increase was most apparent between 130 and $150^{\circ} \mathrm{C}$ and also during the early stages of treatment). The microporosity then decreased and almost disappeared as the treatment continued to high temperatures.

The formation of micropores also seems favoured by the silicas that initially have a structure of small tightly packed spheres. The formation of micropores in the early stages of HTT is due to the overgrowth of the pore openings within the structure. Any deposited silica at these pores entrances is still highly reactive (soluble) and will be dissolved and reprecipitated on less reactive sites.

### 4.5.3.2. Temperature Effects

The temperature of hydrothermal treatment has been found to be the major variable in this form of modification $(20,23)$. As the temperature rises there is a increased widening of pores corresponding to the higher solubility of silica gel. This process is favoured up to around $250^{\circ} \mathrm{C}$ when the major changes are those associated with solution/deposition. That is a decrease in surface area without decrease in pore volume.

Kiselev et al(23) have studied the structural changes in silica gel at higher temperatures. Above $180^{\circ} \mathrm{C}$ the spherical nature of the primary particles decreases and a more spongy structure is formed.

If the silica gel undergoes HTT above $250^{\circ} \mathrm{C}$ pore volume is also lost from the structure. The growth of a spongy structure continues and above $300^{\circ} \mathrm{C}$ the formation of a secondary structure and a crystalline phase has been reported(23). Table 4.2 illustrates the effect of variation in treatment temperature.

### 4.5.3.3. Duration of Treatment

It has been shown that a longer treatment time leads to an increase in the diameter of the pores and also to an increase in the uniformity of the structure obtained(1,22). The changes in pore structure are greatest in the first few hours of treatment due to the presence of a larger number of small particles and the high silica concentration. The higher the solubility of the silica gel particles the greater the pore growth and at constant temperature the solubility is determined primarily by particle size. Thus in the first few hours of treatment where dissolution of silica gel is easiest the change in the structure occurs most rapidly.

Accompanying the widening of pores, as discussed earlier is the formation of micropores. If treatment is continued for say $15-20$ hours these micropores may be eliminated.

Kiselev(22) has shown that the prolonged treatment of silica gel will not significantly alter the pore size after five hours in the autoclave. Table 4.3 indicates the structural changes achieved. However prolonged treatment does result in a more uniform pore structure. This results from repeoted dissolution and reprecipitation of the smaller particles within the gel.

### 4.5.3.4. The Effect of Impurities

The purest silica gels are those made from the polycondensation of silicon compounds such as $\mathrm{SiCl}_{4}$ or $\mathrm{Si}(\mathrm{OEt})_{4}$. The silica gels made from commercial silica sols have as their main impurities the oxides of sodium, aluminium, iron and calcium $(19,20,23)$.

The metallic impurities may be removed by simple washing with acid, both HCl and $\mathrm{HNO}_{3}$ have been used. It has been shown that after washing (20) there is a considerable increase in the resistance of the gel to hydrothermal treatment. This is because the the solubility of the gel has trincreased. The heterogeneity introduced by impurities raises the solubility of the gel. Table 4.4 shows the effect of impurities during HTT.

### 4.5.3.5. The Effect of pH

The textural characteristics of silica gel can be altered by soaking the gel in ammonium hydroxide solution(24-26). As a result of the higher pH the solubility of the gel increases and the solution deposition process is enhanced. hydrothermal sintering at high pH has also been demonstrated by Le Page(27) as a method of increasing pore size.

Girgis(24) carried out extensive studies on the effect of pH and temperature of ammonical treatment. The improved solubility of silica gel at high pH led to an increase in the reaction rate. Table 4.5 indicates nature of the structural changes obtained.

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FIGURE 4.1
Diagram illustrates a cross-section through a silica gel particle made made from colloidal spheres.

## External Internal Surface <br> Siloxane Bonds <br> link the primary particles <br> Primary Colloidal Particle

Figure illustrates how the solubility of silica varies with pH


Sulubility of amorphous siliea versus pll: O. Nexander, $25^{\circ} \mathrm{C}$; Cherkinskii and $K n y a z^{\circ} k$ ova (160) $19^{\circ} \mathrm{C}$ :

Taken from reference 1

## FIGURE 4.3

Figure illustrates how the solubility of silica varies with particle size.


Variation in solubility of silica with radius of curvature of surface. The positive radii of curvature are shown in cross-section as particles and projections from a silica surface Negative'radii are shown as depressions or holes in the silica surface, and in the crevice between two particles.

FIGURE 4.4
An illustration of the change brought about in the silica gel structure by Hydrothermal Treatment.

## Before Treatment



After Treatment


The variation in the rate of Gelling with pH .



FIGURE 4.7

Evaporation of the liquid associated with the silica hydrogel leads to massive compression fores being exerted on the gel particles, resulting in a shrinkage of the particle.

(a)
(b)

s
(c)

(ل)

(e)


Evapurating film of silica sol to gel and drying: schematic cross-section. (a) sol: (b) coneentrited sol-beginning of aggregation; (c) gel compressed by surface tension; (d) fracturing of get by shrinkage: (e) dried loose pol fragments. W. water surfact; S. solid substrate.

The difference between high and low pore volume silica gel.

Low Pore Volume


Comparison of the effect of Hydrothermal Sintering and Sintering on two different experimental silica gels.


Data taken from Reference 20.

TABLE 4.2
Table showing the effect of Hydrothermal Treatment Temperature

These samples were treated in an autoclave with water for 6 hours.

| Temperature <br> $\left({ }^{\circ} \mathrm{C}\right)$ | Surface <br> Area <br> $\left(\mathrm{m}^{2} \mathrm{~g}^{-1}\right)$ | Pore <br> Volume <br> $\left(\mathrm{cm}^{3} \mathrm{~g}^{-1}\right)$ |
| :---: | :---: | :---: |
| Sample 1 |  |  |
| 20 | 772 | 0.36 |
| 100 | 571 | 0.36 |
| 150 | 245 | 0.36 |
| 250 | 47 | 0.36 |
|  |  | 720 |
| 100 | 545 | 0.37 |
| 150 | 259 | 0.39 |

Data taken from:
V.M.Chertov and V.V.Tsyrina: Kolloid. Zh., 47, 922, (1985)

Table showing the effect of Hydrothermal Treatment

## Duration on Silica Gel

| Temperature <br> $\left({ }^{\circ} \mathrm{C}\right)$ | Duration <br> (hours) | Surface <br> Area <br> $\mathrm{m}^{2} \mathrm{~g}^{-1}$ | Pore <br> Volume <br> $\mathrm{cm}^{3} \mathrm{~g}^{-1}$ | Pore <br> Diameter <br> A |
| :---: | :---: | :---: | :---: | :---: |
| - | - | 330 | 1.07 | 105 |
| 250 | 5 | 63 | 1.09 | 680 |
| 250 | 10 | 51 | 1.06 | 885 |
| 250 | 15 | 48 | 1.15 | 880 |
| 250 | 20 | 38 | 1.06 | 885 |

Data taken from Reference 23.

Table showing the Effect of Impurities in

Hydrothermal Treatment of Silica Gel

| Temperature <br> $\left({ }^{\circ} \mathrm{C}\right)$ | Duration <br> (hours) | Surface <br> Area <br> $\mathrm{m}^{2} \mathrm{~g}^{-1}$ | Pore <br> Volume <br> $\mathrm{cm}^{3} \mathrm{~g}^{-1}$ | Pore <br> Diameter <br> A |  |
| :--- | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |
| Silica Gel MSK | - | - | 286 | 1.01 | 140 |
| Impurity removed | 140 | 4 | 214 | 1.02 | 190 |
| Impurity present | 140 | 4 | 100 | 1.02 | 410 |
| Silica Gel ShSK | - | - | 300 | 0.92 | 120 |
| Impurity removed | 140 | 4 | 285 | 0.87 | 120 |
| Impurity present | 140 | 4 | 75 | 0.92 | 490 |

Data taken from Reference 21.

Table showing the effect of

Hydrothermal Treatment

| Temperature <br> $\left({ }^{\circ} \mathrm{C}\right)$ | Duration <br> (hours) | pH | Surface <br> Area <br> $\mathrm{m}^{2} \mathrm{~g}^{-1}$ |
| :---: | :---: | :---: | :---: |
| 140 | 3 | 9 |  |
| 140 | 3 | 10 | 300 |
| 140 | 3 | 11 | 210 |
| 140 | 3 | 12 | 110 |

Data taken from Reference 23.

## CHAPTER 5

## EOUIPMENT AND EXPERIMENTAL PROCEDURE

The major experimental sections were carried out using the following equipment.

### 5.1. Surface Area Determination

The surface area of porous silica gel was measured using gas adsorption techniques. The apparatus used was a home assembled gas line designed to measure the surface area using the B.E.T. Method(1). In Figures 5.1 and 5.2 the two versions of the apparatus used are illustrated. The difference between the two instruments will be outlined in Section 5.1.2.

### 5.1.1. The B.E.T. Method

The quantity of gas adsorbed on the surface was measured as a function of the gas phase pressure at constant temperature. From the isotherm produced it is possible to calculate the amount of gas required to cover the surface with a monomolecular layer. This value was then used to calculate the surface area.

For the purposes of this project the surface area was determined using nitrogen gas as the adsorbate. The B.E.T. equation describing the isotherm is given by

$$
\begin{equation*}
P /\left(P_{o}-P\right) V_{a d s}=1 N_{m} C(T)+(C(T)-1) P / N_{m} C(T) P_{o} \tag{5.1}
\end{equation*}
$$

where

- $P$ is the equilibrium adsorbate gas pressure at sample temperature.
- $V_{\text {ads }}$ is the volume of gas adsorbed at pressure, $P$, and at a temperature, $T$, converted to STP.
- $P_{o}$ is the vapour pressure of adsorbate at sample temperature.
- $V_{m}$ is the volume of gas corresponding to monolayer coverage.
- $C(T)$ is a constant relating to the adsorption of nitrogen on the material under test. $C(T) \propto \exp \left(\Delta H_{a d s}-\Delta H_{\mathrm{liq}}\right) / R T$; $\Delta H_{\text {ads }}$ is the heat of adsorption of the first monolayer. $\Delta H_{\text {lia }}$ is the heat of adsorption of subsequent layers, which is assumed to be equal to the heat of liquifaction.

A plot of $P / N_{\text {ads }}\left(P_{o}-P\right)$ against $P / P_{o}$ will give a straight line where

$$
\begin{align*}
& \text { The Gradient }=(C(T)-1) /\left(V_{m} C(T)\right)  \tag{5.2}\\
& \text { The Intercept }=1 / N_{m} C(T) \tag{5.3}
\end{align*}
$$

Thus

$$
\begin{equation*}
V_{m}=1 / \text { (Gradient + Intercept) } \tag{5.4}
\end{equation*}
$$

The surface area was then related to the volume of the monolayer by

$$
\begin{equation*}
\text { AREA }=\text { CONSTANT } \times V_{m} \tag{5.5}
\end{equation*}
$$

For nitrogen the value of the constant based upon a molecular area for nitrogen of $16.2 \times 10^{20} \mathrm{~m}^{-2}$, is $4.35 \mathrm{~g}^{-1} \mathrm{~m}^{-1}$.

### 5.1.2. Description of Glass Line

The home assembled : glass line used for surface area determination was first assembled as illustrated in Figure 5.1. An evacuation system comprising a rotary oil pump and a mercury diffusion pump were used to obtain sufficient vacuum in the line. The apparatus was designedso that gas could be introduced into known volumes (the calibration of which will be dealt with in Section 5.1.3), at a known pressure. This pressure was displayed on the pressure gauge. The pressure gauge was made by Thommon, Waidenburg, FRG. The Pirani gauge supplied by Edwards Ltd., Surrey, UK., was used to detect the presence of gas in the line. Normally the system was said to be fully evacuated when this gauge read $0.01-0.001$ torr. A pressure transducer, model $\mathrm{BHL}-4050$, supplied by CEC Instruments Ltd., Basingstoke, Hampshire, UK., detected the pressure in the sample volume $\left[\left(V_{s}+V_{0}\right)\right.$ or $\left.\left(V_{c}+V_{o}\right)\right]$. The signal from the tranducer was sent to a Sevoscribe 1 S recorder, supplied by Belmont Instruments, Glasgow, UK.

An improved version of the surface area line was made and is illustrated in Figure 5.2 The improvement was thought necessary, as it was suspected that the vapour pressure of the liquid nitrogen used during the determination, was significantly different from the $P_{o}$ value of 760 mmHg used in the early experiments. A nitrogen thermometer was introduced. The mercury manometer (M2) was added to measure the vapour pressure of the nitrogen at the temperature of the liquid nitrogen that surrounded the sample bulbs during use.

### 5.1.3. Calibration of Dosing and Sample Volumes

## Dose Volume

In Figures 5.1 and 5.2 the volumes between certain taps are indicated as $D_{1}, D_{2}$, $D_{3}$, and $D_{4}$; these were the dosing volumes. It was necessary to determine the volume of these sections of the line accurately so that the amount of gas contained in any one on these sections, and hence the amount of gas let into the sample chamber could be calculated.

For calibration, the first stage was to obtain a value for $V_{c}$, a detachable bulb. This was determined by filling the bulb with mercury and weighing. The density of mercury was assumed as $13.594 \mathrm{~g} \mathrm{~cm}^{-3}$ and $V_{c}$ was easily found. The bulb $V_{c}$ was then attached to the apparatus.

Three possible methods were used to calculate the dosing volumes. $V_{c}$ was attached at point " A ".

Method A

1) The line was filled with gas at a known pressure.
2) The pressure measured by the transducer was recorded.
3) The line bar $V_{c}$ was evacuated.
4) Tap $T_{c}$ and Taps 6,78 were all opened in turn and at each stage the pressure measured by the transducer recorded.

Then using:

$$
\begin{gather*}
P_{1} V_{c}=\left(V_{c}+V_{o}\right) P_{2}  \tag{5.6}\\
P_{2}\left(V_{c}+V_{o}\right)=\left(V_{c}+V_{o}+D_{2}\right) P_{3} \quad \text { etc } \tag{5.7}
\end{gather*}
$$

and repeating the operation the doze volumes were all calculable.

## Method B

1) The doze volume, $D_{2}$, was filled with gas at a known pressure.
2) The remainder of the system was evacuated.
3) Open Tap "T2" and record the equilibrium pressure.

The procedure was repeated for the other doze volumes and the calculation was carried out as before in Equations (5.6) and (5.7).

## Method C

1) Fill $V_{c}$ at a known pressure
2) Open tap into dozer
3) Record pressure
4) Evacute dozer and repeat.

This method enabled a series of measurements to be made without refilling the volume $\mathrm{V}_{\mathrm{c}}$. The calculation method was as before.

Using these methods values were determined for the dozing volumes used in surface area determination.

## Sample Volumes

The calculation of the sample tube volumes were made by replacing $V_{c}$ with each sample tube in turn. The sample tube once attached was surrounded with dewar containing liquid nitrogen. During calibration the level of the liquid nitrogen was kept constant. The neck of the sample tubes was reduced to minimise the variation in equilibrium pressure caused by any shift in the nitrogen level. The liquid nitrogen was required, because during measurement of surface area the sample would be surrounded in the same way. Once the sample tube was set up the sample volume was calculated using the methods described previously.

### 5.1.4. Determination of the Vapour Pressure of Liquid Nitrogen

At room temperature taps " T 4 " and T 5 " of Figure 5.2 were opened and nitrogen allowed into bulb $B$, until the pressure recorded by manometer 2 was significantly greater than atmospheric pressure (i.e. $850-900 \mathrm{mmHg}$ ). Tap "T4" was closed and a dewar containing liquid nitrogen placed round the tube $V_{T}$.

As the temperature in $V_{T}$, drops to the temperature of the liquid nitrogen; nitrogen in the closed volume condenses and the pressure recorded by manometer 2 drops. After a period of time ( -25 mins ), the equilibrium between the condensed nitrogen and the nitrogen vapour is reached and the pressures stabilises. This is the vapour pressure of nitrogen at the temperature of the nitrogen in the dewar. This value was then used as the $P_{o}$ value for the B.E.T. determination of equation 5.1 .

Using the Clausius Clapeyron equation the vapour pressure at a given temperature is found from

$$
\begin{equation*}
d(\ln P) / d T=\Delta H / R T^{2} \tag{5.8}
\end{equation*}
$$

a plot of $\ln P$ aginst $1 / T$ was drawn using literature data, as illustrated in Figure 5.3. The values obtained experimentally for the vapour pressure of nitrogen at the sample temperature over the duration of the thermometer's use fell in the range indicated by the broken lines. This variation in the value of $P_{o}$ was infact not significant in the calculation, however once fitted the thermometer was used for every determination and the value of $P_{o}$ used in calculations.

### 5.1.5. Experimental measurement of Surface Area

A known weight ( $0.1-0.15 \mathrm{~g}$ ) of the silica gel to be examined was introduced into a calibrated sample volume. Non-porous glass beads and glass wool were then placed on top of the gel sample to retain the sample while under vacuum. the sample was then attached to the surface area line by a swaglock fitting at outlined in Figure 5.4. A home made tube furnace of dimensions $3 \times 25 \mathrm{~cm}$ was placed round the sample volume and the temperature in the furnace was raised to $300^{\circ} \mathrm{C}$. The temperature in the furnace/ by means of a variac. At this temperature any adsorbed species on the silica gel were desorbed and pumped from the line. This furnace was left in position until all adsorbed species had been removed ( $30 \mathrm{mins}-8$ hours). In order to check that all adsorbed specief were removed, tap " T 2 " was closed for a few minutes and then reopened. If no increase in pressure was recorded by the Pirani gauge it was assumed that no desorption had taken place and there were insufficient adsorbed species present in the silica gel to affect the experimental value of the surface area.

The tap "T2" was now shut and the furnace removed and replaced by liquid nitrogen containing dewar. The level of the nitrogen was kept constant during the determination.

Measurement of the surface area was then carried out by the introduction of known amounts of gas through tap "T6" or "T9". Before starting the measurement the entire system was evacuated, then with taps "T2, T6, T9 and T4" all shut, gaswas introduced into the remainder of the system up to a known pressure of between 900 and 1000 mmHg on the pressure gauge. This pressure was the dozer pressure ( $\mathrm{P}_{\mathrm{D}}$ ).

A known amount of gas was then introduced into the previously evacuated section of the apparatus. The amount of gas introduced was known as the
pressure of the gas in the line was $P_{D}$ and the volume of each section of the line had been previously calculated (Section 5.1.3).

In Figure 5.5 a typical recorder trace for a sample during the measurement of surface area is shown. On introduction of the gas, the pressure transducer records a dramatic rise in gas pressure. This is followed by a period of equilibration as the gas within the sample volume is adsorbed onto the gel surface. This is quite clearly seen as a gradual decrease in pressure in the sample volume, as shown in Figure 5.5. Once the trace has become horizontal the system is assumed to have reached its equilibrium and the equilibrium pressure is recorded.

This introduction of gas and subsequent equilibration are repeated several times until the pressure in the sample volume has reached about $0.25 \mathrm{P}_{\mathrm{o}}$. From the values of the equilibrium pressure, the value of the amount of gas adsorbed in each doze can be calculated. This calculation is illustrated in Table 5.1. Once the volume of gas adsorbed has been calculated it is then converted to give the amount of gas adsorbed at STP ( $V_{\text {ads }}$ ). In this way data for a plot of $P /\left(P_{o}-P\right) V_{\text {ads }}$ against $P / p_{o}$, is built up.

The data was then plotted and using values obtained from the plot in Equations (5.2) - (5.4) the surface area was calculated. Initially the calculation was carried out manually, but it was later transfered first to a Fortran program run on the EMAS mainframe computer and then to a BBC microcomputer running Basic. The programs used are shown in Appendix 1. In Figures 5.6 and 5.7 typical isotherms and B.E.T. plots obtained for silica gel samples are illustrated.


#### Abstract

5.2. Measurement of Pore Volume and PSD

Four different methods were employed in the course of the project. The subject of PSD determination will be dealt with in greater detail in a later chapter. The methods used were


- Water method
- Low Pressure Mercury Porosimetry
- High Pressure Mercury Porosimetry
- Chromatographic methods


### 5.2.1. Water Method

The determination of pore volume was made by observing the amount of water required to fill the pores of the gel under test.

The procedure involved titrating a known mass of silica gel with water. Initially the silica gel was free flowing, but as more water is added the pores fill up and the end point was reached when the silica gel became "wet" and the particles would stick to each other and the walls of the beaker. At this point:

Specific Pore Vol. = Vol. of water added/Mass of Silica Gel

Typically the measurement wascarried out with around $2-5 \mathrm{~g}$ of material, as this ensured a reasonable titre. The method gave values that were very subjective in nature, as the value obtained for the pore volume relies on determining when the gel is just "wet". This method was not suitible for reproducible measurements and after initial trials was not used.

### 5.2.2. Low Pressure Mercury Porosimetry

## Apparatus

This was a simple thick walled 1 ml pipette calibrated in 0.1 ml steps and able to withstand pressures of up to at least 20 bar. The shape of the pipette was changed and it was sealed at one end. The glass tube was encased in a steel frame, and via the frame the tube was connected to a solvent delivery pump capable of producing a solvent pressure in the tube of 500 psi. Figure 5.8 illustrates the apparatus filled with sample and ready for use.

## Calibration

The glass tube was weighed and known weights of mercury were added up to each calibration mark on the pipette. The level of the mercury was checked with a travelling microscope. Once the level of the mark and the mercury coincided the volume of the tube between calibrated marks was calculated from the weight and density of mercury. In Figure 5.9 a typical set of calibration data is shown.

## Measurement

A known weight of silica gel was placed in the glass tube, on top of this was placed a known weight of glass wool. the purpose of the wool was to retain the gel while the tube was connected to a vacuum line. The tube was connected to a specially designed $T$-piece, that enabled the tube to be evacuated on a vacuum line and then while still under vacuum mercury could be poured into the tube. The $T$-piece used is illustrated in Figure 5.10 Once the tube was evacuated and the mercury added the tube was reweighed to determine the weight of mercury in the tube.

As a result of the evacuation, at this stage the volume inside the tube was made up of:

Silica Gel<br>Glass Wool<br>Mercury<br>Pores within the gel particles<br>Pores between the gel particles

The tube was connected to the pump as previously described and on application of pressure, mercury was forced into the large spaces associated with the interparticle space but not into the pores themselves. The mercury was not able to enter the pores as it is a non-wetting liquid such that the pressure required to push mercury into the pore is given by:

$$
\begin{equation*}
\Delta P=2 \cdot \gamma \cdot \cos \theta / r \tag{5.9}
\end{equation*}
$$

- $\Delta \mathrm{P}$ is the applied pressure
- $\gamma$ is the surface tension of mercury
- $r$ is the pore radius
- $\theta$ is the contact angle of mercury on the porous silica gel

Figure 5.11 shows the minimum pore size entered by mercury at differing applied pressures. Quite clearly the effect of mercury entering the pores will not be significant for normal chromatographic gels or even wide pore gels where the pores were between 300 and 1000 A in diameter. The final pore volume was measured at a pressure of 500 psi , at this pressure the smallest pores that the mercury could enter are 4300 A in diameter. During the application of pressue the level of mercury in the tube is measured relative to the nearest calibrated mark. At this point the highest alibrated mark below the mercury is also noted. Now as the mercury has been forced into all the
interparticle space the volume up to the top of the mercury is comprised of:

```
Silica Gel
Glass Wool
Mercury
Pores within the particles.
```

The mercury volume, glass volume and silica volume are all calculable from their weights and densities. A typical calculation is shown below:

Calculation of Pore Volume for a sample of silca gel.

Mass of Gel in tube $=0.1007 \mathrm{~g}$
Mass of glass in tube $=0.0615 \mathrm{~g}$
Mass of Mercury in tube $=7.4594 \mathrm{~g}$
Pressure Applied $=500 \mathrm{psi}$
Volume up to calibrated mark $=0.6684 \mathrm{ml}$
Height of mercury above mark $=1.65 \mathrm{~cm}$
Height difference between marks either side of Mercury=a=1.73cm
Volume difference between marks either side of Mercury $=b=0.095 \mathrm{ml}$
Extra Vol. = b x (1.65/a)
Total Vol. in Tube $=0.6684+[b x(1.65 / a)]$

Volume of Silica Gel $=\mathrm{s}=0.1007 /$ Density of Silica $\left(2.2 \mathrm{gcm}^{3}\right)$
Volume of Glass $=\mathrm{g}=0.0615 /$ Density of Glass $\left(2.2 \mathrm{gcm}^{3}\right)$
Volume of Mercury $=m=7.4594 /$ Density of Mercury ( $13.594 \mathrm{gcm}^{3}$ )

Volume of Pores $=$ Total Vol. $-s-g-m=0.0724 m l$

The pore volume is then:
Pore Volume $=$ Volume of Pores $/$ Mass of $\mathrm{Gel}=0.72 \mathrm{~cm}^{3} \mathrm{~g}^{-1}$

The effect of increased applied pressure on the calculated pore volume is shown in Figure 5.12 As can be seen from the diagram the effect would only be important if the material had a wide Pore Size Distribution, PSD, and a mean
pore diameter greater than 4500A.

### 5.2.3. High pressure Mercury Porosimetry

High pressure mercury porosimetry was carried out by Shandon Southern Products, Runcorn U.K. using a Micromeritics Pore Sizer, Model 9305. This technique used the same theory as the previous mercury method, however on this occasion the mercury was forced into the pores. Assuming that the contactrangle of mercury on silica gel was $140^{\circ}$ this instrument which was capable of delivering 30,000 psi, could enter pores as small as $30 \AA$ at that pressure. These measurements were only carried out when the PSD was required as well as the pore volume.

### 5.2.4. Chromatographic Methods

Using the Knox and Scott(2) method the pore volume and PSD of gels was determined from their Size Exclusion Calibration Curves (SECC). The calibration curves were obtained using highly refined polystyrene standards that had low polydispersity, typically less than 1.1. These standards were obtained: from Polymer Laboratories Ltd., Churchstretton. U.K. The complete list of standards used is given in Figure 5.13 This method was used for the determination of PSD rather than pore volume alone and will be discussed in Chapter. 7.

### 5.2.5. Chromatographic System

HPSEC was carried out on home assembled equipment. An Altex 110A, high pressure pump or Shandon HPLC pump was used. Detection was with a Cecil UV photometer, a Shandon UV photometer or a Spectroflow UV photometer. The eluent used was methylene chloride of HPLC grade, supplied by Rathburn Chemicals, Walkerburn. U.K. Column preparation for any test material was by slurry packing, using a Shandon Packer made by Shandon Southern Products Ltd. The gel was suspended in methanol and then packed into Shandon Type
columns with an applied pressure of 3000 psi. The columns used varied in length from 100 mm to 250 mm with a internal diameter of 4.6 to 6.4 mm .

### 5.3. Pressure Testing

All pressure tests were performed in Shandon type columns, $100 \times 4.6 \mathrm{~mm}$ ID. The test solvent was iso-propyl alcohol. The pump used was a Shandon HPLC packing pump, for tests below 8000 psi. For pressures greater than 8000 psi a Haskel pưmp pàmade by Haskel Engineering and Supply Company, California USA, was used. Work requiring pressures greater than 8000psi was carried out at Shandon Southern Products Ltd., Runcorn, UK. The method used to determine the mechanical strength will be discussed in Chapter 6.

### 5.4. Autoclave Treatments

The autoclave treatments were carried out in three different systems during the course of the project.

- Small scale testing was carried out in stainless steel tubes
$250 \mathrm{~mm} \times 10 \mathrm{~mm}$ ID sealed with Swaglock end fittings. A
maximum of 20 ml was placed in these tubes. The heating of
the tubes was carried out by suspending the steel tube in a
mounted furnace.
- Larger treatments were carried out in a 1 litre stirred autoclave made by Baskerville and Lindsay, Manchester, England. this system was able to accommodate up to 700 ml of reaction mix.
- For large scale treatments a 2 litre rocking autoclave was used. This was supplied by C.W.Cook and Sons. Ltd.,


#### Abstract

Birmingham, England. Here up to 1750 ml were placed in the autoclave at any one time. The heating and cooling profile for this autoclave were recorded and are shown in Figure 5.14


An important aspect regarding the autoclave treatment of these silica gels ,which became apparent with large batch treatments, was the expansion of water at elevated temperature. In Figure 5.15 the density of water at temperatures above $100^{\circ} \mathrm{C}$ is shown. Quite clearly when carrying out treatment in the autoclave, significant allowances must be made for the expansion of water.

### 5.5. Furnace Treatments

For sintering treatments two systems were used. Small test batches (up to 3 g ) were heated in a Carbolite Tube Furnace, Sheffield, England. The heating area in the tube furnace was approximately 100 mm in length and 25 mm in diameter. Here the temperature that could be obtained was up to $1400^{\circ} \mathrm{C}$.

For larger samples a muffle furnace supplied by Gallemkamp, U.K. was used. Here the furnace dimensions were $400 \times 120 \times 150 \mathrm{~mm}$. Here a temperature of $900^{\circ} \mathrm{C}$ could be obtained.

### 5.6. Materials

Production of Silica Gels

All the silica gels produced in Edinburgh during this work were made from
colloidal sols. Experimental batches $\mathrm{H} .1-->\mathrm{H} .12$ and experimental batches MB1 --> MB5 were made from Ludox HS40. The experimental batch SX30 was made from Monsanto $S \times 30$ sol. The properties of these sols are described in detail in Chapter.6. The small gel particles were made by dispersing the sol in petroleum ether. The dispersion was achieved using a high speed rotary mixer. The mixer used was a Silverson L2R stirrer, made by Silverson Machines Ltd, Chesham, Buckinghamshire, UK..

## PSD Analysis of Silica Gels

The silica gels examined in Chapter 8 were a mixture of commercially available and experimental gels. The microcomputer used in the PSD determinations was a BBC Model B made by Acorn Computers Ltd., Cambridge, UK. The more powerful optimisation was made using the Edinburgh Multi-Access System at the Edinburgh Regional Computing Centre.

Experimental Gels

Silica Gel samples PR183, PR179, SSP501 and WP1004 were obtained from Shandon Southern Products Ltd. The series of silica gels 732HK2a-->732HK6a were obtained from Prof. K.K.Unger (Mainz,FRG)) Silica gels HR-WPS-2, MB5S and MB5U were produced in Edinburgh.

Commercial Gels

Hypersil was supplied by Shandon Southern Products Ltd. (Runcorn, Cheshire. UK), the Lichrosphere Si1000 was supplied by E.Merck, Darmstadt, FRG. The Zorbax PSM materials were supplied by DuPont de Nemours, NL. and the Vydac material was obtained from Varian Associates, Surrey, UK.
5.7. References

1. S.J.Gregg and K.S.W.Sing "Adsorption Surface Area and Porosity" Acad Press, London (1967)
2. J.H.Knox and H.P.Scott J. Chromatog., 316,311, (1984)


The Apparatus used for the determination of Surface Area by the B.E.T. Method


Figure showing the variation of vapour presure of nitrogen with temperature The dashed lines indicate the extremes of measured vapour pressure.

## Plot of Log Vapour Pressure against inverse of Temperature for Nitrogen



Diagram showing the packing and connection of the sample tube used on the Surface Area apparatus shown in Figures 5.1 and 5.2.


The trace shown below is typical of the output received from the recorder connected to the pressure transducer of the Surface Area Apparatus.

After the initial rise in pressure associated with the introduction of a gas dose, the pressure drops; once the pressure had stabilised the value was recored.

FIGURE 5.5


FIGURE 5.6

Diagram shows three typical B.E.T. plots obtained form the data collected during Surface Area analysis.

The gradient and intercept of these plots are used to calculate the surface area.
b.E.t. SURFACE ARER PLOT
b.E.t. SURFACE ARER PLOT


RELATIVE PRESSURE $100 \mathrm{P} / \mathrm{PO}$


RELATIVE PRESSURE $100 \mathrm{P} / \mathrm{PO}$
B.E.T. SURFACE AREA PLOT


FIGURE 5.7

Diagram shows three typical isotherms plotted from the data collected during Surface Area analysis.
B.E.T. ADSORPTION ISOTHERM

B.E.T. ADSORPTION ISOTHERM

B.E.T. ROSORPTION ISOTHERM


RELRTIVE PRESSURE $1100 \mathrm{P}, 201$

Calculation method as used for Surface Area Determination


FIGURE 5.8
The Apparatus used the determination of Particle Pore Volume, using Low Pressure Mercury Porosimetry.


Typical Calibration data for Low Pressure Mercury Porosimetry
Calibration
Mark on
Glass Tube
Volume up to
Calibration
Mark
$\left(\mathrm{cm}^{3}\right)$

Height Difference
between
Calibration Mark (cm)


Diagram illustrates the specially designed $T$-piece used to enable mercury to be poured onto the sample while under vacuum.


FIGURE 5.11

Schemmatic representation of a non-wetting liguid entering a pore.


Mercury can be forced into pores by the application of pressure. Below the pressures required to enter pores of specific sizes are listed.

| Contact Angle $140^{\circ}$ |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Pressure (psi) | . 100 | 300 | . 500 | 1000 | 2000 | 4000 | 5000 | 10000 |
| Pore Radius ( ${ }_{\text {( }}$ ) | 10650 | 3560 | 2135 | 1067 | 533 | 267 | 214 | 107 |

FIGURE 5.12
In this Figure the oberved pore volume is plotted against the pressure used in the Low Pressure Porosimetry. There is little observed change in pore volume above an applied pressure of $200-300$ psi.


FIGURE 5.13

Manufacturers' quoted values for the polydispersity of the polystyrene standards used in obtaining experimental SECC Curves.

| Molecular Weight | Polydispersity |
| :---: | :---: |
| $15,000,000$ |  |
| $8,000,000$ | 1.30 |
| $4,250,000$ | 1.06 |
| $1,650,000$ | 1.07 |
| $1,400,000$ | 1.07 |
| $1,150,000$ | 1.05 |
| 770,000 | 1.09 |
| 675,000 | 1.07 |
| 50,000 | 1.07 |
| 180,000 | 1.08 |
| 115,000 | 1.06 |
| 76,000 | 1.06 |
| 51,500 | 1.06 |
| 39,000 | 1.07 |
| 34,500 | 1.06 |
| 20,500 | 1.07 |
| 17,000 | 1.07 |
| 11,800 | 1.08 |
| 7,600 | 1.06 |
| 4,250 | 1.06 |
| 3,600 | 1.05 |
| 2,200 | 1.05 |
| 730 | 1.06 |
| 480 | 1.10 |
|  | 1.15 |

Experimental Measurements of the heating and cooling profile for the Cook Rocking Autoclave filled with water.


FIGURE 5.15

The Density of Water at Elevated Temperature.


## CHAPTER 6

## WIDE PORE SILICA GEL PRODUCTION

### 6.1. Introduction

As has already been seen the use of high pore volume silica gels results in improved chromatography of large molecules. In retentive chromatography the wider pores allow better access to the stationary phase, as a result of this is separating the nower of the column is improved. In non-retentive chromatography the resolution is improved by increasing the ratio of pore volume to interparticle volume. High pore volume materials improve this ratio, thus the potential resolution of the silica gel is enhanced.

The production of wide pore high pore volume silica gel from colloidal sols can be achieved by:

- Preparation of a high pore volume xerogel
- Modification of a high volume xerogel.

The high pore volume silica gels tend to have a poor mechanical strength and thus breakdown when used in HPLC. This weakness is due to the fact that the bonding between the primary particles of the gel is insufficient to resist the forces exerted on the gel as a result of the flow of eluent through the column.

The initial experimental study undertaken was concerned with the effect of gel reinforcement techniques (HTT and Sintering) on commercial silica gels. In this study Hypersil was examined and the effect of treatment was recorded by measurement of the surface area and pore volume of the gel as described in

Chapter 5. This part of the project dealt with the hydrothermal modification of low pore volume silica gel. Variation in the hydrothermal treatment given to the gel enabled a picture to be built up of how the gel could best be modified.

### 6.2. The Effect of Hydrothermal Treatment

Here small silica gel samples were autoclaved using sealed tubes as described in Chapter 5. A study was made of the effect of temperature, duration, and pH of treatment. A brief look at different silica gels and different reaction media Were also carried out.

### 6.2.1. Preliminary Modifications

The treatments carried out were designed to indicate the limits of the modifications attainable with HTT. Treatments were carried out in alkaline solution of pH 11 . The results listed in Table 6.1 and displayed in Figure 6.1 indicate that significant modification of the gel was possible with hydrothermal treatment.

From these results it is seen that the rate at which structural change occurs decreases with time. That is the fractional change of structural properties outlined by the log plot of surface area against time tends to level off. The extent of modification is determined more by the temperature of treatment than by the time as seen in Figure 6.1. The higher temperature gives a greater decrease in surface area, but the decline in rate with time is again seen. This suggested that to achieve a particular surface area the choice of the appropriate temperature will be the most important factor.

### 6.2.2. Variation of Reaction Duration

The preliminary modification experiment as mentioned above had indicated that a longer treatment time did not significantly change the structure after the
first few hours. Experiments with a longer duration confirmed this, as shown in Figure 6.2. These results would indicate that very long treatment is not necessary to produce structural change. However as mentioned in Chapter 4. Kiselev et al(1) carried out work showing that longer treatment although not significantly enlarging the pores did provide a more uniform Pore Size Distribution(PSD). In Figure 6.3 the high pressure mercury porosimetry data for a hydrothermally treated gel shows how observed pore size has increased with treatment. Further treatments were carried out for a standard duration of 18 hours. In this way any microporosity arising in the early stages was hopefully eliminated.

### 6.2.3. Variation of Autoclave Temperature

The preliminary experiments had indicated that temperature controlled the final structure attained during HTT. A more comprehensive study of the effect of temperature was made. The surface area modified gels are plotted against the temperature of treatment in Figure 6.4. A $\log$ plot of surface area against temperature is also shown in Figure 6.4. In both plots the variation in surface area is linear at intermediate temperatures. A steady drop in surface area was noted as the temperature rose while the pore volume remained relatively constant, up to $220^{\circ} \mathrm{C}$. Above this temperature the structure collapsed with significant loss of pore volume.

### 6.3. Variation of Reagent used for Treatment and pH

As explained in Chapter 4, the hydrothermal reaction is enhanced by the use of elevated pH . In Figure 6.6 the difference in modifications obtained at different pH by Girgis(2) is illustrated. These results indicated that elevated pH should be used to obtain the most rapid structural change in HTT.

Various different media for the modification were tried. Table 6.2 shows that
there was little difference in the effect of the different alkalis used. Although it should be noted that with all the alkaline solutions the effect was greater than treatment with water alone. Standardizing the pH at a value in the range $9-12$ is therefore desirable to obtain reproducible conditions. Accordingly it was decided to use $2.5 \% \mathrm{v} / \mathrm{v}$ ammonia solution for further modifications, as this reaction media allowed the elevation of pH without the introduction of metal counter ions. This particular solution has pH 11 .

### 6.3.1. Variation of Reactant Gel

A number of different silica gels were studied under HTT conditions. In Table 6.3 the properties of various starting materials for the production of these gels are outlined. From the results it can be seen that all the gels were significantly altered by HTT. Furthermore it is clear that the gels made from polymerisation of $\mathrm{Si}(\mathrm{OEt})_{4}$ or sodium silicate rather than from colloidal silica are much more susceptible to HTT . This is due to the fact that these materials are made up of much smaller units that obviously give the gel a higher surface area, which in turn makes the solution/deposition process in HTT much more effective in reducing the surface area. The fractional decrease in surface area is much larger in the other materials. The "Mihm" silica made by Laird(3) from the polymerisation of $\mathrm{Si}(\mathrm{OEt})_{4}$, the Partisil and Merckosorb made from sodium silicate solution all show poor resistance to HTT. The Hypersil material made from colloidal silica with larger primary particle size is less easily altered and the fractional decrease in surface area is much less than the other materials. This lower susceptibility of the Hypersil material would imply that the use of higher pH and higher temperature would be required to give comparable changes.

### 6.4. The Sintering of Silica Gels

The main aims of sintering the silica gels were to produce a gel with wide pores and also to strengthen the structure. This method of pore alteration could be seen as an alternative to HTT or could be used in conjunction with HTT. As discussed in Chapter 4 the structure of the silica gel could be altered by heating in air to high temperatures. The loss of water from the surface of the silica gel is followed, as the temperature is increased, by the condensation between the silanol groups, to form siloxane bonds. On further heating continued condensation occurs, particularly in the narrow pores and the structure as a whole tends to shrink.

The effect of sintering was studied on a number of silica gels. The samples studied had typical properties characteristic of the sources from which the gels had been made. In Table 6.4 the properties and sources of the various gels are outlined. Structural change due to sintering has been associated with the pore size distribution of the material. The range of temperature over which sintering of the structure occurs is dependant on the range of pore sizes present. Materials with a wide pore size distribution will be subject to sintering over a wider range of temperatures. These experiments examined the extent of sintering and the range over which the sintering occured.

This examination of the effect of sintering was undertaken.in conjunction with Dr Hassan Ceylan. The work carried out by Dr Ceylan has not been previously reported and is of direct relevance to the picture of the behaviour of silica gels during their sintering in air. Dr Ceylan carried out the initial experiments on silica gels made from colloidal silica sols.

A series of small silica gel samples were sintered by the methods described in Chapter 5. These gels were treated at a number of temperatures. The
sintering was carried out for 16 hours in air.


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The results of these experiments are seen in Figures 6.7 and 6.8. To obtain a more general picture of the sintering effect a number of other gels were examined. The properties of these gels are also listed in Table 6.4. In


 Figures 6.9-6.12 the structural changes are also illustrated.Evidently, all the gels are subject to sintering in air. The structural changes appear to begin around $500^{\circ} \mathrm{C}$ when pore volume is lost from the material. For materials of higher pore volume higher temperature must be applied in order to collapse the structure completely. Surface area is also lost from the materials over the same temperature range. This is to be expected as the formation of siloxane bonds across micropores will effectively remove the surface. Again the high surface area materials made from tetra-alkoxy silicates will require a higher temperature for complete collapse. Under sintering the gels made from colloidal silica behaved similarly to those produced by polymerisation. The modification of either structure could be achieved by sintering in air at temperatures between $600^{\circ}$ and $900^{\circ} \mathrm{C}$. The exact temperature to be used would be dependant on the initial structural characteristics. The impurify? content, and in particular the sodium content of the gel being sintered makes a significant contribution to the effect of sintering. The gel is more susceptible to sintering if it contains large amounts of sodium. This is clearly seen in the production of Zorbax silica gel(4).

### 6.5. Discussion of Results

The various treatments throughtout the project gave a broad outline of the behaviour of silica gel under different hydrothermal and sintering conditions. It should be noted that since this work has been undertaken similar studies have been reported (5). The primary aim of the above work in producing wide pore
gel was achieved. A reaction temperature of only $200^{\circ} \mathrm{C}$ in HTT being sufficient to give a very significant change. The temperature of reaction was the overiding factor in determining, the extent of modification. For example hydrothermal treatment at $200^{\circ} \mathrm{C}$ may continue to widen the pores up to six hours into the reaction. but significant alteration after this stage does not occur. However a slight rise in temperature enables wider pores to be obtained relatively easily. These results are in good agreement with the mechanism theory of Girgis(2) which infers that the effects of hydrothermal treatment are more pronounced at high dissolved silica concentrations. These high dissolved silica concentrations exist during the early stages of treatment and are more pronounced at elevated temperature. At the start of hydrothermal treatment, the range of primary particle surfaces available to be dissolved is at its greatest. The number of smaller more soluble particles is at its highest. As the treatment proceeds these small particles are removed from the silica gel and it becomes increasingly difficult to dissolve further silica from the larger structure that is formed.

The extent of the modifications that can be obtained can be seen in electronmicrographs of silica gel samples. In Plate. 1 and Plate. 2 Hypersil a typical narrow pore material is shown. In Plates.3-6 gels produced by the hydrothermal modification of silica gels clearly show a structure with extensively widened pores, while the basic skeletal structure is maintained.

The very high hydrothermal treatment temperature of $280^{\circ} \mathrm{C}$ produced a gel of poor uniformity as seen in Plates 1 - 6. This lack of uniformity may in part be due to uneven treatment. In order to ensure even gel treatment agitation should be employed throughout the treatment. Kiselev(1) has reported an increase in pore uniformity by the use of longer treatment times.

The use of ammonia pH 11 catalvsed the reaction and also provided buffering of the solution. When carried out in water the pH of the solution became dependant on the silica gel, a property that may vary with different production methods.

From the above results of hydrothermal treatment, the production of a suitably wide pored chromatographic material could be achieved noting that:

- Temperature control is the overiding factor in deciding what pore size can be attained.
- pH control will decrease the effect of impurities within the system. The pH primarily alters the rate of reaction and its control should lead to less batch to batch variation.
- More uniformly porous materials can be obtained using long treatment times (greater than 12 hours) at moderate temperatures, i.e. below $250^{\circ} \mathrm{C}$

The effect of sintering has been seen to be different from HTT. Pore volume(PV) and surface area(SA) are not independent during treatment. Both properties fall over a range of temperature controlled by the initial characteristics of the gel.

Prior to the drop in SA and PV most samples tend to exhibit a slight increase in SA and PV. This tended to occur at around $500^{\circ} \mathrm{C}$. This increase may be attributed to an opening of some previously closed pores as a result of surface migration of silica species. The apparent rise in surface area, could perhaps have resulted from a change in the surface chemistry of the silica gel,
producing slight changes in the adsorption forces and hence altering the area occupied by the nitrogen molecule.

It has previously been suggested (6) that the range of temperature over which sintering occurs is related to the uniformity of the pore structure. From these experiments this hypothesis has been borne out. Silica gels 732HK2a and 732HK6a shown in Figures 6.9 and 6.10 have a wide range of pore sizes as a result of their production method and the structural changes have occured from $500^{\circ} \mathrm{C}$ to $1000^{\circ} \mathrm{C}$. Hydrothermally treated silica gels with a narrow range of pore sizes tend to sinter between 700 and $900^{\circ} \mathrm{C}$, as shown in Figures 6.7 and 6.8 and Figures 6.11 and 6.12.

The sintering of silica gel is also dependant upon the impurity content of the silica species. For example silica gels made from pure silica containing no metal ions, and in particular sodium will not sinter below $1000^{\circ} \mathrm{C}$. However materials made from commercial sols stabilised by alkali, and in particular NaOH tend to sinter at lower temperatures, perhaps below $900^{\circ} \mathrm{C}$. This is most likely to be due to the metal ion interrupting the silica structure making it easier for the silica species to break the bonds holding them to the surface. occurs
Once thesébonds are broken rapid condensation/forming a more stable siloxane linkage. The gels made from colloidal sols, had all been stabilised by NaOH and all had sintered at a lower temperature than the purer silica gels.

### 6.6. Production of Wide Pore Silica Gel Packing Materials

Batches of silica gel were produced from colloidal silica sol. Within the experimental production several stages were examined.

1. Shaping of gel particles - spherical particles of specific sizes were to be produced.
2. Gelling time of particles
3. Washing of the gel was examined, in order to preserve the pore structure.
4. Drying of the gel.
5. Gel reinforcement techniques.

### 6.6.1. Shaping of the gel particles

Spherical porous particles, initially of sizes around $20 \mu \mathrm{~m}$ were produced. These were made using commercial sols of two major types, as described in Table 6.5. The particles were shaped by a process involving the even dispersion of the sol in an organic medium. This was carried out using thehigh speed mixer described in Chapter 5 . The emulsion formed had to be sufficiently stable to allow the non-porous primary colloidal particles within each dispersed droplet enough time to join together and harden as a discrete porous particle

Within the emulsification process several variables altered this process of discrete particle formation. These variables included:

- Speed of Stirring.
- Mesh Size through which the particles were forced.
- Viscosity of organic dispersant.
- Concentration of stabilising agent.

A study of the effect of these variables was undertaken using water rather than silica sols. An examination was made of the effect of the stirrer speed on particles size produced by the emulsifier. The mixing procedure for all experiments together with the results are outlined in Table 6.6. By increasing the stirrer speed it was possible to decrease the particle size formed. A study of the mesh size through which the emulsion was forced showed that this had no significant effect on the final particle size obtained. By lowering the viscosity of the organic dispersant larger particles as well as a larger size range were produced. Finally the concentration of the stabilising agent was seen to have a critical value, in that below a concentration of $1 \%$ in the petroleum spirit, the emulsion was unstable and rapidly separated into two layers. For the production of colloidal silica/pet. spirit emulsions it was necessary to stir the mixture much faster to obtain similar particle sizes to the above experiments. This was required because the silica sols were much more viscous than the water and therefore required more force to obtain an even dispersion of the required size. The stabilising agent used was Span 80, a non-ionic surfactant, that forms an adsorbed film round the dispersed particles thus inhibiting coalescence and coagulation.

### 6.6.2. Preparation and Gelling of Sol Particles

As described the dispersion had to be stable for a sufficient length of time to ensure hardening as individual particles. In Chapter 4, the gelling process was shown to be dependant on pH , temperature, particle size, and
concentration. Figure 6.13 shows how the gelling rate of the commercial sols used varies with pH.

The general procedure was as follows:

The sol was warmed on a water bath to $50^{\circ} \mathrm{C}$. At the same time the organic dispersant, petroleum ether (b.p. $100-120^{\circ} \mathrm{C}$ ), was also warmed to $50^{\circ} \mathrm{C}$. The sol was then acidified to lower the pH to 5.5 and thus encourage hardening. The sol and petroleum ether were then stirred at high speed for a set period of time at a specific speed. The emulsion formed was allowed to stand on the water bath for up to 2 hours at $50^{\circ} \mathrm{C}$. The conditions used for specific samples are indicated in Table 6.7.

The hydrogel particles so formed were highly porous and fragile. For example a sol containing $40 \% \mathrm{w} / \mathrm{v}$ silica, where the primary particles were of diameter 13-14 nm, on gelling and assuming no loss of associated water, would have a pore volume of around $2 \mathrm{ml} \mathrm{g}^{-1}$. They would be subject to contraction and/or fracture on drying.

### 6.6.3. Washing and Preparation for Drying

The next stage in the gel production was the removal of the hydrogel from the organic emulsion. For the production of low volume xerogels, typical of those used in adsorption chromatography, the gel was obtained by repeated washing of the gel with acetone. On addition of the acetone the water associated with the hydrogel became displaced from the pores of the silica gel. Continued washing with acetone was designed to:

- remove traces of petroleum ether from the gel
- remove remaining water from the gel

However the drying of the structure with the loss of supporting liquid as explained in Chapter 4 causes a loss of pore volume, and the xerogel produced even after removal of water by acetone has a lower pore volume than the hydrogel. In Table 6.8, the gels $H .1$ and $H .4$ have been produced by such a method. It is not certain whether the loss of pore volume occured at the drying stage or when the water was replaced by acetone. (The results in Table 6.8 show it must be at the drying stage.)

A series of washing experiments were undertaken to separate the hydrogel from its organic medium and isolate the gel in an aqueous phase as a high - volume gel. Qualitative results shown in Table 6.9 indicated that the gel could be collected in an aqueous layer. This aqueous liquid contained little organic solvent, a high electrolyte concentration to decrease the solubility of the Span, and prevent the formation of an oil in water emulsion. The washing liquor was improved by the lowering of its pH , such that any tendency for dissolution of silica gel was reduced.

Further washing with acidic solutions enabled any last traces of unwanted impurities to be removed. A number of different liquids were used at this stage. FInally dilute nitric acid was used in these secondiry washes in order to avoid introduction of chloride ions into the autoclave mixture.

### 6.6.4. Drying

Several methods have been used for drying silica gel including, rotary evaporation from low boiling point organics, vacuum oven drying and spray drying. For the gels produced in this work the spray drying technique was used. Figure 6.14 shows the spray drying apparatus used.

The hydrogel was pumped as a dilute suspension, by the means of a
peristaltic pump, into a fine atomizer. Once in the capillary a jet of compressed air carried the hydrogel into the body of the drier. The temperature on entering the drier was maintained at $250^{\circ} \mathrm{C}$. At this temperature the water was evaporated from the gel leaving a dry xerogel as the product. The final temperature on entering the cylone was $120^{\circ} \mathrm{C}$

### 6.6.5. Modification to produce Wide Pore Gels

Hydrogels were used as the starting material for the production of wide pore materials. The hydrogels were prepared as described earlier and then by the use of non-organic washing solvents, the hydrogel was transfered to a medium in which HTT could be carried out.

Mild hydrothermal treatment of the hydrogel was undertaken to attempt to increase the degree of primary particle linkage. This increase would increase the resistance to shrinkage on drying. Hydrothermal treatment like other methods of modification does not increase the pore volume. The mild treatment increased the pore diameter as during the process the surface area was lost while the pore volume was maintained. Furthermore the strengthening of the structure would be improved due to the higher area of contact between primary particles in the gel. The strengthening of the material should improve the pressure stability of the high pore volume gels making them suitable for HPLC.

The conditions for mild HTT were as follows:

- A slurry of about $650-700 \mathrm{ml}$ was made up to pH 11 with $2.5 \% \mathrm{v} / \mathrm{v}$ ammonia solution. This was then autoclaved in a stirred autoclave for 18 hours in total. The reaction temperature was $140^{\circ} \mathrm{C}$. After treatment the mix was allowed to settle and then washed with water before spray drying.

The effect of these autoclave treatments on the final structural characteristics of the gel are shown in Table 6.10. Table 6.10 indicates that the HTT has strengthened the gel along the same lines as previous treatments, and that less pore volume has been lost on drying. About $25 \%$ of the maximum pore volume has been lost which compares with the $70 \%$ loss that occurs in the production of Hypersil. Hypersil is made by drying the hydrogel without a hydrothermal treatment. Quite obviously the resistance to particle deformation has been increased by the mild hydrothermal treatment.

This treatment may have produced a gel of suitable surface area and pore volume for chromatography, but any attempt to pack these gels under HPLC conditions failed. It would(seem)that the strengthening; that had occurred was unfortunately insufficient.

The production of these weak but high pore volume gel, indicated that the process could be utilised. The next stage involved an attempt to strengthen the gels by utilising the structural changes that were seen in the preliminary section. That is the gel was subjected to a further strengthening. This secondary strengthening was either through sintering, a further Hydrothermal modification or both. It was thus intended to produce a number of gels of differing pore sizes. The pore sizes being dictated by the degree of treatment.

Furthermore the use of a different starting sol was briefly tried to investigate whether the method could be applied more generally. In Table 6.10 the production details of the high volume gels are outlined.

[^2]a combination of these gels were produced. The starting material for these further modified gels was MB3/4 described in Table 6.10.

Table 6.11 indicates the treatment and properties of all the silica gels made from the starting high volume gel.

The use of these gels in HPLC as explained was dependent on their pressure stability and secondly the pore size had to be of sufficient size to be useful for chromatography of large molecules. These two variables will be dealt with separately.

### 6.7. Examination of the Mechanical Strength of Wide Pore and High Pore

## Volume Silica Gels

For spherical particles within a packed column, through which a viscous fluid is flowing there is a force tending to distort the particles. This force arises from the vis drag. If the pressure drop across the particles becomes too high, they will tend to deform and crush. The bed porosity will drop and the flow resistance will increase in the column. Figure 6.15 shows the how the deformation affects the porosity of the bed. The experiments carried out were designed to examine the effect on flowrate of variation in the value of the pressure drop along the column.

In a packed bed with eluent flowing through it, the eluent velocity is related to the eluent flowrate:

$$
\begin{equation*}
u=f_{v} / \pi R^{2} \tag{6.1}
\end{equation*}
$$

where $u$, is the eluent velocity and $R$, is the column radius.

The eluent velocity is also given by the Kozeny Carmen equation(7), relating
the eluent velocity to the pressure $\operatorname{drop}(\mathrm{dP} / \mathrm{dz}$ ) across the column.

$$
\begin{equation*}
u=d_{p}^{2} / \phi \eta \cdot(d P / d z) \tag{6.2}
\end{equation*}
$$

where $\eta$ is the eluent viscosity, $d_{p}$ is the particle diameter and $\phi$ is the column resistance factor related to the porosity of the column. For impermeable spherical packing the expression becomes:

$$
\begin{equation*}
u=\varepsilon^{2} /(1-\varepsilon)^{2} \cdot d_{p}^{2} / 180 \eta \cdot(d P / d z) \tag{6.3}
\end{equation*}
$$

and for a bed of porous spherical particles

$$
\begin{equation*}
u=\varepsilon^{2} /(1-\varepsilon)^{2}\left(\varepsilon / \varepsilon_{\text {tot }}\right) d_{p}^{2 / 180 \eta(d P / d z)} \tag{6.4}
\end{equation*}
$$

If the particles are deformed in any way the interstitial porosity, $\varepsilon$, will decrease and therefore the value of $\mathrm{u} /(\mathrm{dP} / \mathrm{dz})$ will fall.


However, provided there is no deformation of the particles, it should be possible to obtain a linear plot of flowrate against applied pressure. Deviation from such a plot would indicate that the material within the column was suffering detormation or reorganisation within the column. A factor that was thought relevent to these pressure studies was the variation in particle size. The gels up to this stage had been made of the size range $10-35 \mu \mathrm{~m}$, but for modern HPLC applications it is necessary to have strong particles of $5-10 \mu \mathrm{~m}$ in size.

For a given degree of compression inaparticle the pressure that must be applied to obtain this degree of compression is inversely proportional to the particle size. This can be seen from consideration of a particle within the column as indicated in Figure 6.16. The force on an isolated particle due to the
flow of solvent round it is given by, Srokes' Law;

$$
\begin{equation*}
f=6 \pi \eta r u \tag{6.5}
\end{equation*}
$$

Clearly the flow patterns in a packed bed will not be the same as in an isolated particle, however the force will still be related to the eluent velocity, $u$; and the particle radius, $r$. The value of the constant will be different and therefore we replace $6 \pi$ by $C$.

$$
\begin{equation*}
f=c \eta r u \tag{6.6}
\end{equation*}
$$

If the particle were to suffer a degree of compression, $\alpha$, such that;

$$
\begin{equation*}
r^{\prime}=\alpha r \tag{6.7}
\end{equation*}
$$

where $r^{\prime}$ is the radius of the circle of contact between the particles, as outlined in Figure 6.15. Then the force that must have been exerted to give this compression, is given by;

$$
\begin{equation*}
f=P_{c} \pi \alpha^{2} r^{2} \tag{6.8}
\end{equation*}
$$

where $P_{c}$, is the maximum pressure which the structure can resist before collapse. This will be called the crushing pressure. Thus for a give force at a given degree of compression, equating (6.6) and (6.8):

$$
\mathrm{c} \eta \mathrm{ru}=\pi \alpha^{2} \mathrm{r}^{2} \mathrm{P}_{\mathrm{c}}
$$

thus:

$$
\begin{equation*}
u=\pi \alpha^{2} r P_{c} / C \eta \tag{6.9}
\end{equation*}
$$

This implies that for a given degree of crushing, denoted by, $\alpha$, the linear
flowrate is proportional to the particle size. For porous particles the linear velocity along the packed column is also given by

$$
\begin{align*}
& u=d_{p}^{2} / \phi \eta(d P / d z)  \tag{6.10}\\
& u=\left(4 r^{2} / \phi \eta\right)(d P / d z)
\end{align*}
$$

The pressure gradient is given by $\mathrm{dP} / \mathrm{dz}$. The column resistance factor, $\phi$, can be related to the porosity of the bed:

$$
\begin{equation*}
\phi=1 / 180(\varepsilon / 1-\varepsilon)^{2} \tag{6.11}
\end{equation*}
$$

The value of the porosity, $\varepsilon$, is related to the degree of compression. Therefore for a given degree of compression the value of $\phi$ can be considered constant. Now by equating (6.9) and (6.10):

$$
\begin{gather*}
4(\mathrm{dP} / \mathrm{dz}) \cdot \mathrm{r}^{2} / \phi \quad \eta=\pi \alpha^{2} \mathrm{rP}_{\mathrm{c}} / \mathrm{C} \eta  \tag{6.12}\\
\mathrm{dP} / \mathrm{dz}=\pi \quad \alpha^{2} \mathrm{P}_{\mathrm{c}} \phi / 4 \mathrm{C} r \tag{6.13}
\end{gather*}
$$

Therefore the pressure gradient for a given degree of compression is inversely proportional to the particle size. Thus although larger particles will give higher flowrates for any ( $\mathrm{dP} / \mathrm{dz}$ ), their resistance to compression at a given ( $\mathrm{dP} / \mathrm{dz}$ ) is lower. Now therefore whatever stability of the large particle gels (i.e. $10-35 \mu \mathrm{~m}$ ) show, smaller particles may be expected to have a higher stability. In Figure 6.17 the relation between flowrate and applied pressure is illustrated.

### 6.7.1. Experimental Method and Justification

The samples were tested in $100 \mathrm{~mm} \times 5 \mathrm{~mm}$ Shandon Type columns. The gels were slurry packed using iso-propyl alcohol, at a pressure of between 500 and 1000 psi. After packing the bottom mesh was replaced to decrease the
possibility of the column being blocked by fines. The tests were then carried out as follows:


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- The flowrate was recorded at increments of 500 psi between the applied pressures of 500psi and 6000psi. Between each increase in pressure the applied pressure was returned to 1000 psi and the flowrate measured. Any significant decrease in flowrate at this pressure then indicates a decrease in bed porosity, i.e. particle deformation. For pressures greater than 8000psi the column was transfered to a high pressure Haskell pump and the test continued.


The results obtained were plotted as eluent flowrate against the applied pressure. Five different results were obtained and these are illustrated in Figure 6.18.

- In type (a) the direct linear dependance with equation (6.9) was obtained. The eluent velocity was proportional to the applied pressure. This corresponds to laminar flow through the bed without deformation of the particles.
- In case (b) where the plot tends to be curved, the flow may either be turbulent or the particles may be compressing and the bed porosity decreasing.
- In case (c) the distinction between turbulence and compression was made. Where the flowrate recorded at 1000psi as the test continued as shown by the dotted line the material was compressing. In the case of the dashed line the
column was said to be exhibiting turbulent flow.
- In case (d) repeated measurement of the flowrate (by repeating the pressure test) gives curved/linear plots where each time the test was repeated the measured flowrate at a particular pressure was slightly lower than before. This corresponds to bed settling and will be discussed in Section 6.10. Alternatively compression of the bed may be occuring. Examination of the results as in case (c) would distinguish between the two results.
- In case (e) a flow maxima is obtained, corresponding to a pressure at which crushing of the bed was taking place to such an extent that the bed was said to have collapsed.


### 6.8. Experimental Results

1. As a yardstick the first gel tested was Hypersil, a commercial gel known to be stable for HPLC. Figure 6.19 shows the excellent agreement between the theory discussed in Section 6.7.1, and a near linear plot was obtained.
2. The "origina!" gel (MB3/4) had been made by hydrothermal treatment of hydrogel. The MB3/4 high volume gel thus obtained had neither been sintered nor given a second hydrothermal treatment; it was not mechanically stable enough for use in HPLC and as can be seen in Figure 6.20 bed deformation began to take place very soon after the pressure was applied. The high pore volume gel had been
packed at 500 psi but with a pressure drop of more than 1000 psi across the column, particle breakdown was seen to occur. The hydrothermal treatment although able to strengthen the structure sufficiently for drying, the structure was still unsuithble for use in HPLC. Further reinforcement of the gel was required to make a gel able to withstand the pressures required.
3. In order to further reinforce the silica gels the effect of a second and more vigorous hydrothermal treatment was studied. These gels shown in Figure 6.21 exhibited a definite increase in mechanical strength. The flow maxima were recorded around 4000 psi . This increase in mechanical strength resulted from the increase in the $\qquad$ area of contact between particles within the gel. At the higher treatment temperatures the rate of the solution/deposition process was greater. Although the gel strength had improved, it was still not comparable to that of Hypersil. Further reinforcement was required. It should be noted that these gels were not at any stage subjected to sintering.
4. In this set of experiments a sintering treatment in air at $800^{\circ} \mathrm{C}$ was carried out rather than vigorous HTT. These gels showed a significant improvement over the hydrothermally treated gels displayed in Figure 6.21. As can be seen from Figure 6.22, the flowrate increased linearly with pressure up to around 6000 psi. This although not
more vigorous the treatment the wider the pores produced. These particular results illustrate that the hydrothermal treatment is not in the main responsible for the further strengthening of the structure. Figures 6.23 and 6.24 show the effects of the variation in tertiary hydrothermal treatment. It is the sintering that produces the main strengthening effects on the xerogel. No significant increase in strength was seen by the use of a vigrous hydrothermal treatment if sintering had not been carried out prior to it. The main effect of the more vigorous HTT was in widening the pores.
5. A similar series of experiments to the above were carried out, but on this occasion the temperature of the secondry (sintering) treatment was varied while the temperature of HTT was kept constant. In Figure 6.25 the improvements in mechanical strength can be seen. From the diagram there appears to be little difference in the strength as a result of temperature variation. The important point here was that an improvement in the mechanical strength is seen. The high temperature of sintering provided energy for the rapid depositon of silica around the "necks" of the primary particles. This sintering process is obviously more powerful than the hydrothermal treatment method.
6. In this third set of experiments the treatments carried out at the secondary and tertiary stage were reversed. Figure 6.26 compares the dependance of flowrate against applied


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comparable with Hypersil was stronger than previously obtained. The small decrease in pore volume during this relatively "mild" sintering meant that this form of treatment could be used as the major method of strengthening the high volume xerogels. At the sintering temperatures the increase in contact area between primary particles is greater than can be achieved by hydrothermal treatment alone. As a result the improvement in mechanical stability is greater. For the best results in production of strong wide pore materials, sintering should be used to strengthen the material and hydrothermal treatment to widen the pores.


The combination of the two methods of gel reinforcment was examined as a way of improving the materials still further. The starting material for all these experiments was the same (MB3/4). This gel had been produced by mild hydrothermal treatment (primary treatment) of a hydrogel. The xerogels were given a secondary treatment of either sintering or HTT. After this secondary treatment, a tertiary treatment of either sintering or HTT was given to the gels. A qualitative study examining the degree of secondry and subsequently tertiary treatment was carried out.

> 1. A series of experiments were carried out where the extent of tertiary treatment was varied. In these experiments the secondary treatment involved sintering at a set temperature. These sintered materials were then given a hydrothermal treatment as a tertiary treatment. The temperature of hydrothermal treatment was varied. The surface area and pore volume of these materials are listed in Table 6.11. The


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pressure for the different materials. The sample that had been treated hydrothermally as a secondary treatment and sintered as a final stage was much weaker than in the case where the sintering was carried out as the secondary treatment. The sintering was in effect much less effective on the more uniform structure that was produced by the hydrothermal treatment. If would thus seem that for these treament methods to be combined, sintering should be carried out prior to the vigorous hydrothermal treatment.


### 6.9. Pressure testing of smaller particle Silica Gels

Sintering and HTT have been shown to increase the higher pore volume silica gels' ability to withstand the presures associated with liquid flow through the packed column. As outlined theory suggests that the smaller particles should be stronger still. The next stage in the development procedure saw the production of a number of silica gels of smaller mean particle size ( $5-10 \mu \mathrm{~m}$ ). The objective was to examine whether this increase in strength occured. The gels were produced by the same methods as used in the previous experiments, only for these gels a faster stirring speed of around 4000 rpm was required to make particles of the required size. The production details are given in Table 6.12. These silica gels were then pressure tested as before. There were three main areas of interest.
> 1. A brief study of the absolute effect of gel strengthening on these materials.
2. A study of the variation in particle size, and its effect on gel strength. For this particular set of experiments a
comparison with gels treated in the previous section was made.
3. Examination of the relation between pore volume and mechanical strength.

### 6.9.1. Particle Strength

Figures 6.27 - 6.31 show the results of the pressure tests carried out on these materials. Quite clearly these gels are strong enough for use in HPLC and are suitible for use with high flowrates. The particles dre much stronger than previous materials. The breakdown of the particles now occurs between 10,000 and 15,000 psi, rather than $<8000$ psi as before.

### 6.9.2. Particle Size

As a result of the high pore volume of these gels it was difficult to fractionate them into narrow size ranges. However repeated fractionation produced samples with differing particle size ranges. Using these differing size ranges, pressure tests were carried out. The comparison is made directly between particles from the same batch treatment. These results are given in Figures 6.32 and 6.33. The difference in sizes is rather too small but the underlying trend of the larger particles being weaker is clearly seen. In Figures 6.34 and 6.35 comparison is made against particles whose average particle size was $20-25 \mu \mathrm{~m}$. Here the effect of particle size is even more pronounced. The materials compared had had the same modification treatments. Clearly the larger particles tend to deform and fracture at lower pressures then the smaller ones. Observation of the column packing material after test showed quite clearly that particle fracture occured mainly in the larger paricles. These results are in qualitative agreement with the theory
discussed in Section 6.6.

### 6.9.3. Effect of Pore Volume

Figure 6.36 illustrates the effect of pore volume on the mechanical strength attained. In Table 6.13 a comparison is made of the pressure at which the maximum flow rate was observed. There is a definite link between the pore volume and the mechanical strength as would be expected. These qualitative experiments merely confirmed that the higher pore volume materials are weaker than their low volume counterparts. This is due to the contact area between primary particles being less.

### 6.10. Bed Settling

It was observed that the plots obtained for the first pressure test for almost all samples were not linear. The flowrate against applied pressure graphs were all curved.

The tests illustrated in Figures 6.37-6.40 indicate this curvature. This suggested that either the flow through through the column was turbulent or that the material inside the column was showing some compression under pressure.

- Several of the gel samples were retested at the various
pressures up to 6000 psi. These repeat plots were then
obtained and as can be seen in Figures $6.37-6.40$ the plot
became more linear with each subsequent run. If the
particles were breaking up a linear plot would not be seen.
Instead very low flowrates would be observed due to the
increased resistance within the column.
- It was further observed that during the first column test the


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bed length of the packing materials did decrease. However the bed length during the second and subsequent treatments did not alter. The decrease was regular and related to the applied pressure. This has been illustrated in Figures 6.41-6.43, where the flowrate obtained at 1000 psi is shown for some of the gels examined. It should be noted that even Hypersil gave a decrease in bed length when subjected to the pressure test. A comparison of these changes in bed length in shown in Table 6.14.


These results indicated that there was a certain degree of bed compression, but this seems mainly due to the bed settling. The bed is almost certain to show some compression and reorganisation as the pressures attained in the tests are much higher than the 500 psi used to pack the column.

### 6.11. Discussion of Results

The pressure testing results showed that individually both forms of secondary treatment (HTT and Sintering) do increase the mechanical strength with regard to HPLC applications. The strengthening effect of sintering is greater than that of hydrothermal treatment. Also shown from the results is that the order of the secondary and tertiary treatments was important to the final stability. If the second HTT was carried out prior to sintering, then the resistance of the gel to loss of structure during sintering was increased. As a result higher treatment temperatures would be needed during the sintering stage in order to change the gel structure. This increased resistance to sintering is probably due to the high degree of gel uniformity, due to the extensive HTT, which would have removed the smaller primary particles from the gel. Further resistance to sintering may arise due to the HTT leaching out
the sodium ions that were present in the silica gel. The resulting gel would be purer and would require a higher temperature for effective sintering. Examination of the variation in the degree of each treatment, showed that it made relatively little difference to the mechanical strength. Altering the temperature of HTT affected the surface area and uniformity in the first instance. Altering the temperature of sintering did not seem to make a great difference. The essential point was that it had to be carried out. Furthermore it had to be carried out at such a temperature that slight modification was made to the surface area and pore volume. Thus sintering at temperatures of at least $750^{\circ} \mathrm{C}$ was necessary.

Sintering and HTT have been shown effective in increasing the strength of the gel. As theory suggested, smaller particles $5 \mu \mathrm{~m}$ in diameter, were seen to be stronger than the larger ones examined in the first set of pressure tests where the particle diameter was around $25 \mu \mathrm{~m}$. Even the samples with sizes similar to Hypersil, showed nothing like the strength of Hypersil which has from Figure 6.19 been seen to be exceedingly strong. The higher pore volume materials are weaker due to poorer intraparticle linkage.

The results of the above experiments have shown that

- Silica gels of significantly higher pore volume than gels normally associated with HPLC materials can be produced.
- The gel strengthening techniques used have enabled the consistant production of packing materials that are stable up to 10000 psi .
- These materials have as yet been difficult to fractionate into
the narrow size ranges associated with commercial analytical
packing materials. This has been due to the drop in particle density with the increase in pore volume as listed in Table 6.15.
- The particle size has been shown to be one of the most important factors in the development of material strength. The use of small particles is vital for strong gels to be made.
- There seems to be a limit to the pore volume that can be incorporated within these higher volume materials. Gels were produced with still greater pore volumes (MB5A-1.98cc/g, MB5D-2.10cc/g) but these showed no real ability to withstand high pressures. From the colloidal silicas used to make high volume gels the maximum pore volume was in the range of $1.3-1.5 \mathrm{cc} / \mathrm{g}$.
- The formation of materials with differing pore structures was achieved, although not with the variety that was initially expected. This difficulty in obtaining gels of differing pore size was due primarily to the drastic effects caused by high temperature sintering of the silica gel. However, this sintering had to be carried out to provide the particle strength. The further HTT of the gel provided the pore size variation.

The treatments had been expected to produce materials with a narrow pore size distribution and as will be seen from the Size Exclusion Chromatography Calibration Curves in the next Chapter this was in fact the case.

The modified gels had wider pores, a narrow pore size distribution and a larger pore volume than previous materials.

### 6.12. References

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TABLE 6.1

Preliminary Modification of Low Pore Volume Silica Gel
subjected to Hydrothermal Treatment in a Sealed Vessal

| Material | Treatment Reagent | Duration of Treatment (hours) | Temperature of Treatment ${ }^{\circ} \mathrm{C}$ | Surface Area $m^{2} g^{-1}$ | Pore Volume $\mathrm{cm}^{3} \mathrm{~g}^{-1}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Hypersil | - | - | - | 170 | 0.70 |
| Hypersil | Ammonia pH 11 | 1 | 180 | 117 | - |
| Hypersil | Ammonia pH 11 | 4 | 180 | 68 | - |
| Hypersil | Ammonia pH 11 | 18 | 180 | 54 | 0.65 |
| Hypersil | Ammonia pH 11 | 1 | 250 | 54 | - |
| Hypersil | Ammonia pH 11 | 4 | 250 | 20 | - |
| Hypersil | Ammonia pH 11 | 18 | 250 | 15 | 0.27 |

FIGURE 6.1

Log Plot of Surface Area against Duration of Hydrothermal Treatment


FIGURE 6.2


Diagram shows the difference between two silica gels one of which was hydrothermally treated.

1-PSD with no hydrothermal treatment
2-PSD with hydrothermal treatment at $200^{\circ}$

> - Hydrothermally treated gel
> $\diamond$ Non-hydrothermally treated gel


FIGURE 6.4

## Change in structure

with treatment


The effect of variation of the pH of Hydrothermal Treatment


TABLE 6.2
Variation of Reagent used in Hydrothermal Treatment of Silica Gel.

| Material | Treatment <br> Reagent | Duration <br> of <br> Treatment <br> (hours) | Temperature <br> of <br> Treatment <br> ${ }^{\circ} \mathrm{C}$ | Surface <br> Area** <br> $\mathrm{m}^{2} \mathrm{~g}^{-1}$ | Pore* <br> Volume <br> $\mathrm{cm}^{3} \mathrm{~g}^{-1}$ |
| :--- | :---: | :---: | :---: | :---: | :---: |
| Hypersil | - | - | - | 142.9 | 0.79 |
| Hypersil | Ammonia pH 11 | 18 | 200 | 33.8 | 0.61 |
| Hypersil | $0.01 \mathrm{M} \mathrm{KOH} \mathrm{Sol’n}$ | 18 | 200 | 32.8 | 0.61 |
| Hypersil | 0.01 M Na Silicate | 18 | 200 | 30.4 | 0.57 |
| Hypersil | Water | 18 | 200 | 42.6 | 0.63 |

*     - Measured by Low Pressure Mercury Porosimetry
** - Measured by Gas Adsorption; B.E.T. Method

Table showing the effect of hydrothermal treatment on
a number of different silica gels.

| Sample | Duration (hours) | pH | Temperature $\left({ }^{\circ} \mathrm{C}\right)$ | $\begin{gathered} \text { Surface* } \\ \text { Area } \\ \left(\mathrm{m}^{2} \mathrm{~g}^{-1}\right. \end{gathered}$ | Pore** <br> Volume $\mathrm{cm}^{3} \mathrm{~g}^{-1}$ | Percentage loss of Surface | Starting Material for Gel | Source of Gel |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Hypersil Hypersil | 18 | 11 | 200 | $\begin{gathered} 142 \\ 33 \end{gathered}$ | $\begin{aligned} & 0.79 \\ & 0.61 \end{aligned}$ | 76 | Sol | Wolfson LC Unit |
| Partisil <br> Partisil | - | 11 | 200 | 400 18 | 0.75 0.42 | - 96 | Na Silicate | Whatman |
| Merckosorb Merckosorb | 18 | 11 | 200 | 500 16 | 0.76 0.42 | 97 | Na Silicate | E.I.Merck Darmstadt - |
| "Mihn" Silica <br> "Mihn" Silica | 18 | 11 | 200 | 501 6.4 | 0.50 0.20 | - 99 | $\mathrm{Si}_{(\mathrm{OEt})_{4}}$ | G.Laird, WLCU |
| $732-H K-2 a$ $732-H K-2 a$ | $\overline{18}$ | $\overline{-}$ | 175 | 418 60 | $\begin{aligned} & 2.0 \\ & 1.4 \end{aligned}$ | - 86 | $\mathrm{Si}_{(\mathrm{OEt})_{4}}$ | K.K.Unger Darmstadt |

* Measured by gas adsorption - B.E.T. Method
** Measured by low pressure mercury porosimetry

FIGURE 6.7

Variation in Pore Volume with Sintering Temperature


FIGURE 6.8

Variation in Surface Area with Sintering Temperature


TABLE 6.4
Table showing the properties of a series of Silica

Geis prior to sintering treatment on them.

| Sample | Starting Material | $\begin{aligned} & \text { Surface** } \\ & \text { Area } \\ & \mathrm{m}^{2} \mathrm{~g}^{-1} \end{aligned}$ | Pore ${ }^{* * *}$ Volume $\mathrm{cm}^{3} \mathrm{~g}^{-1}$ | Source of Silica Gel |
| :---: | :---: | :---: | :---: | :---: |
| SSP-WPS2* | $30 \%$ w/v Colloidal Silica | 45 | 1.86 | Shandon Southern Products |
| SSP-WPS3* | $30 \%$ w/v Colloidal Silica | 53 | 1.60 | Shandon Southern Products |
| SSP-WPS4* | $30 \%$ w/v Colloidal Silica | 47 | 0.70 | Shandon Southern Products |
| H. 12 | $30 \%$ w/v Colloidal Silica | 113 | 1.49 | Produced in Edinburgh |
| SX-30A | $30 \%$ w/v Colloidal Silica | 116 | 1.59 | Produced in Edinburgh |
| 732-HK-2a | $\mathrm{Si}(\mathrm{OEt})_{4}$ | 418 | 1.70 | K.K.Unger Darmstadt FRG |
| 732-HK-6a | $\mathrm{Si}(\mathrm{OEt})_{4}$ | - | - | K.K.Unger Darmstadt FRG |

-     * Sintering experiments and measuremens carried out by Dr. Hassan Ceylan at Edinburgh.
_ ** Measured by Gas Adsorption; B.E.T. Method
- *** Measured by Low Pressure Mercury Porosimetry

FIGURE 6.9

Variation in Pore Volume with Sintering Temperature


Variation in Surface Area with Sintering Temperature


FIGURE 6.11

Variation in Surface Area with Sintering Temperature


FIGURE 6.12

Variation in Pore Volume with Sintering Temperature


Hypersil Silica: No Hydrothermal Treatment PLATE 1


PLATE 2


Hypersil silica, HTT at $200^{\circ} \mathrm{C}$ for 18 houcs PLATE 3


PLATE 4


Hypersil Silica: HTT at $220^{\circ} \mathrm{C}$ for 18 hours
PLATE 5


PLATE 6


The specifications for the silica sols used for the production of silica gels are outlined below:

| PROPERTY | Du Pont* <br> Ludox <br> HS-30 | $\begin{aligned} & \text { Monsanto** } \\ & \text { SX-30 } \\ & \text { SYTON } \end{aligned}$ |
| :---: | :---: | :---: |
| Counter Ion | Sodium | Sodium |
| Primary Particle Size | 13-14nm | 11 nm |
| Surface Area $\mathrm{m}^{2} \mathrm{~g}^{-1}$ | 210 | 250 |
| pH | 9.8 | 9.9 |
| $\mathrm{SiO}_{2} / \mathrm{Na}_{2} \mathrm{O}$ | 90 | 88 |
| $\mathrm{SiO}_{2}$ \%wt | 30 | 30 |
| Viscosity (cp) | 4.5 | 5.5 |

[^3]A series of emulsification experiements were carried out; 500 ml of distilled water was mixed at high speed with petroleum ether $(1500 \mathrm{ml})$ containing a set amount of emulsion stabiliser. The stabiliser was Span 80 a sorbitol ester. The properties of the emulsions formed are given below.

| Petroleum Ether bp. ${ }^{\circ} \mathrm{C}$ | Stabiliser Conc'n ( $\mathrm{ml} / \mathrm{I}$ ) | Stirrer Speed (rpm)* | Mesh Size | Particle Size ( $\mu \mathrm{m})^{* *}$ |
| :---: | :---: | :---: | :---: | :---: |
| Varaition in Stirrer Speed |  |  |  |  |
| 100-120 | 30 | 3100 | M | 3-13 |
| 100-120 | 30 | 2130 | M | 5-20 |
| 100-120 | 30 | 1400 | M | 3-38 |
| 100-120 | 30 | 1100 | M | 3-42 |
| Variation in Mesh Size |  |  |  |  |
| 100-120 | 30 | 3300 | F | 5-13 |
| 100-120 | 30 | 3100 | M | 3-13 |
| 100-120 | 30 | 3500 | Si | 4-12 |
| Variation in Dispersant Viscosity |  |  |  |  |
| 60-80 | 30 | 1500 | M | 3-60 |
| 60-80 | 30 | 1400 | M | 3-55 |
| 100-120 | 30 | 1400 | M | 3-38 |
| Variation in Span Concentration |  |  |  |  |
| 100-120 | 30 | 3100 | M | 3-13 |
| 100-120 | 10 | 3150 | M | 4-13 |
| 100-120 | 3 | 3150 | M | Unstable |

[^4]- F; A cylindical shield placed round the stirring rod. Circular holes of 0.5 mm in diameter were cut in this shield at regular intervals. These holes were spaced evenly 1 mm apart.
- M; As above but the hole diameter was 1 mm .
- Si ; In this shield the 1 mm holes were evenly spaced along the top and bottom edges of the shield.

FIGURE 6.13
Gel Time againstpH


TABLE 6.7
Production Details for Spherical Silica Gels

| Sample | Gelling <br> Time <br> (mins) | Bath <br> Temp. <br> $\left({ }^{\circ} \mathrm{C}\right)$ | Stirrer <br> Speed <br> $($ rpm $)$ | Particle <br> Size <br> $(\mu \mathrm{m})$ |
| :---: | :---: | :---: | :---: | :---: |
| H. 1 | 20 | 50 | 1600 | $20-40$ |
| H.4 | 30 | 50 | 1900 | $20-50$ |
| H.5 | 30 | 50 | 1650 | $15-35$ |
| H.6 | 120 | 50 | 1500 | $25-55$ |
| H. 7 | 120 | 50 | 1600 | $20-60$ |
| H.8 | 120 | 50 | 1500 | $30-60$ |
| H.9 | 120 | 50 | $<1400$ | $20-70$ |
| H. 10 | 120 | 50 | $<1400$ | $25-70$ |
| H. 11 | 120 | 50 | 3000 | $10-35$ |
| H.12 | 120 | 50 | 3000 | $10-35$ |

It should be noted that the stirring speeds required to produce particles similar in size to those of Figure 6.14 are significantly faster. This is due to the increased viscosity of the liquid being dispersed.

Production Details for Spherical Silica Gels

| Sample | Washing Method | Wet Gel Treatment | Surface Area $m^{2} g^{-1}$ | Pore Volume $\mathrm{cm}^{3} \mathrm{~g}^{-1}$ |
| :---: | :---: | :---: | :---: | :---: |
| H. 1 | Acetone $\times 2$ <br> Water x 2 | - | 176 | 0.58 |
| H. 4 | Magic Mix <br> Water x 2 | - | 185 | 0.54 |
| H. 5 | Magic Mix 0.1M Nitric Acid | HTT at $140^{\circ} \mathrm{C}$ in Ammonia pH 11 | 118 | 1.39 |
| H. 6 | Acetone, 0.1 M Nitic Acid, Water | HTT at $140^{\circ} \mathrm{C}$ in Ammonia pH 11 | 123 | 1.41 |
| H. 7 | Acetone 0.1M Nitric Acid | - | 185 | 0.58 |
| H. 8 | Magic Mix 0.1M Nitric Acid | HTT at $140^{\circ} \mathrm{C}$ in Ammonia pH 11 | 121 | 1.50 |
| H. 9 | Magic Mix 0.1M Nitric Acid | - | 172 | 0.42 |
| H. 10 | Magic Mix 0.1M Nitric Acid | HTT at $140^{\circ} \mathrm{C}$ in Ammonia pH 11 | 131 | 1.65 |
| H. 11 | Magic Mix 0.1M Nitric Acid | - | 203 | 0.53 |
| H. 12 | Magic Mix 0.1M Nitric Acid | HTT at $140^{\circ} \mathrm{C}$ in Ammonia pH 11 | 113 | 1.70 |

The "Magic Mix" was a 1 M Sodium Nitrate solution in $90 \% 0.1 \mathrm{M}$ Nitric Acid and 10\% Acetone.

Qualatative results of experiments carried out to find the most convenient way of separating the pet. spirit/water emulsion containing the silica hydrogel.

The emulsion contained 500 ml silica sol/hydrogel, 45 ml Span 80 and 1500 ml petroleum ether b.p. $100-120^{\circ} \mathrm{C}$.

|  | Extraction Mixture | Result |
| :---: | :---: | :---: |
| 1 | Distilled Water | No Separation |
| 2 | Distilled Water/Acetone 90/10 | Little Separation |
| 3 | Distilled Water/Acetone 70/30 | Separated |
| 4 | 0.5 M NaCl Distilled Water/Acetone $90 / 10$ | Good Separation |
| 5 | 1 M NaClDistilledWater $/$ Acetone <br> $95 / 5$ | Poor Separation |
| 6 | 1 M NaNO 3 <br> Distilled Water/Acetone <br> 90/10 | Good Separation |
| 7 | $\begin{gathered} 1 \mathrm{M} \mathrm{NaNO} \\ 0.1 \mathrm{M} \mathrm{HNO}_{3} / \mathrm{Acetone} \\ 90 / 10 \end{gathered}$ | Good Separation |
| 8 | $\begin{gathered} 2 \mathrm{M} \mathrm{NaNO} \\ 0.1 \mathrm{M} \mathrm{HNO}_{3} / \mathrm{Acetone}^{2} \\ 90 / 10 \end{gathered}$ | Fair Separation |

Separation mix 7 was used as "Magic Mix" as this provided a good separation using only a small amount of organic solvent. This mixture also maintained the pH of the hydrogel at a low value.

FIGURE 6.14


Properties of High Pore Volume Silica Gels

| GEL | Particle <br> Size <br> $(\mu \mathrm{m})$ | Treatment | Surface <br> Area <br> $\mathrm{m}^{2} \mathrm{~g}^{-1}$ | Pore <br> Volume <br> $\mathrm{cm}^{3} \mathrm{~g}^{-1}$ |
| :--- | :---: | :---: | :---: | :---: |
| MB1 | 20 | HTT at $140^{\circ} \mathrm{C}$ | 139 | 1.20 |
| MB2 | 20 | HTT at $140^{\circ} \mathrm{C}$ | - | 1.25 |
| MB3 | 20 | HTT at $140^{\circ} \mathrm{C}$ | 135 | 1.50 |
| MB4 | 20 | HTT at $140^{\circ} \mathrm{C}$ | 139 | 1.48 |
| SX-30 | 20 | HTT at $140^{\circ} \mathrm{C}$ | 116 | 1.59 |

Gels MB3 and MB4 were combined for use in further experiments.

The following samples were produced from silica gel MB3/4. M83/4 was a high pore volume gel produced by mild hydrothermal treatment $\left(140^{\circ} \mathrm{C}\right)$ of silica hydrogel. The samples listed below have been given a sintering treatment and/or a second more severe hydrothermal treatment.

| Sample Number | Sintering Temperature ( ${ }^{\circ} \mathrm{C}$ ) | Hydrotherma! Temperature $\left({ }^{\circ} \mathrm{C}\right)$ | Surface Area $\left(\mathrm{m}^{2} \mathrm{~g}^{-1}\right)$ | Pore Volume $\left(\mathrm{cm}^{3} \mathrm{~g}^{-1}\right)$ |
| :---: | :---: | :---: | :---: | :---: |
| MB3/4-H4 | - | 140 | 109 | 1.60 |
| MB3/4-H8 | - | 160 | 95 | 1.56 |
| M83/4-H7 | - | 175 | - | - |
| M83/4-H1 | - | 175 | 87 | 1.54 |
| MB3/4-H2 | - | 200 | 71 | 1.56 |
| M83/4-H6 | - | 200 | 71 | 1.54 |
| MB3/4-H5 | - | 220 | 62 | 1.56 |
| MB3/4-S3H1 | 700 | 175 | 95 | 1.51 |
| MB3/4-S19H5 | 700 | 220 | 66 | 1.52 |
| MB3/4-S20H4 | 750 | 140 | 110 | 1.48 |
| MB3/4-S20H8 | 750 | 160 | 100 | 1.53 |
| MB3/4-S9H3 | 750 | 175 | 92 | 1.44 |
| MB3/4-S9H2 | 750 | 200 | 74 | 1.47 |
| MB3/4-S17H4 | 800 | 140 | 110 | 1.49 |
| MB3/4-S29H8 | 800 | 160 | 100 | , |
| MB3/4-S2H1 | 800 | 175 | 87 | 1.39 |
| MB3/4-S22H7 | 800 | 175 | - | - |
| MB3/4-S8H2 | 800 | 200 | 79 | 1.51 |
| MB3/4-S23H6 | 800 | 200 | 73 | 1.48 |
| M83/4-S23H5 | 800 | 220 | 68 | 1.47 |
| MB3/4-S 15 H 4 | 850 | 140 | 108 | 1.37 |
| MB3/4-S27H8 | 850 | 160 | 98 | 1.37 |
| MB3/4-S25H7 | 850 | 175 | - | - |
| MB3/4-S15H3 | 850 | 175 | 89 | 1.23 |
| MB3/4-S24H6 | 850 | 200 | 77 | 1.35 |
| MB3/4-S $14 \mathrm{H}^{2}$ | 850 | 200 | 78 | 1.43 |
| MB3/4-S22H5 | 850 | 220 | 63 | 1.37 |
| M83/4-S18H4 | 900 | 140 | 92 | 0.96 |
| MB3/4-S28H8 | 900 | 160 | 83 | 0.97 |
| M83/4-S6H1 | 900 | 175 | 74 | 1.00 |
| MB3/4-S26H7 | 900 | 175 | 74 | 0.94 |
| MB3/4-S7H2 | 900 | 200 | 74 | 1.22 |
| MB3/4-S21H5 | 900 | 220 | 50 | 0.95 |

The following samples were produced from silica gel MB3/4. MB3/4 was a high pore volume gel produced by mild hydrothermal treatment $\left(140^{\circ} \mathrm{C}\right)$ of silica hydrogel. The samples below were given a second HTT prior to sintering.

| Sample <br> Number | Sintering <br> Temperature <br> $\left({ }^{\circ} \mathrm{C}\right)$ | Hydrothermal <br> Temperature <br> $\left({ }^{\circ} \mathrm{C}\right)$ | Surface <br> Area <br> $\left(\mathrm{m}^{2} \mathrm{~g}^{-1}\right)$ | Pore <br> Volume <br> $\left(\mathrm{cm}^{3} \mathrm{~g}^{-1}\right)$ |
| :---: | :---: | :---: | :---: | :---: |
| MB3/4-H6S1 | 850 | 200 | 76 | 1.56 |
| $\mathrm{MB3} 4-\mathrm{H6S2}$ | 900 | 200 | 75 | 1.57 |
| $\mathrm{MB3} / 4-\mathrm{H} 7 \mathrm{~S} 1$ | 900 | 175 | - | - |

FIGURE 6.15
Bed before Pressure Test


Bed after Pressure Test


FIGURE 6.16

A particle in a packed bed with eluent flowing through it, is subjected to various forces:


Eluent Velocity, u

The force on the particle is:
$\mathrm{f}=C \pi \eta \mathrm{r} \mathrm{u}$

Particles may crush under pressure such that the area of contact between the particles increases.


NO CRUSHING
CRUSHING

FIGURE 6.17

Theory predicts that larger particles will tend todetorm at lower pressures


Radius A > Radius B
Crushing Pressure $P_{B}>$ Crushing Pressure $P_{A}$

FIGURE 6.18





## Pressure Test Result for a column packed with Hypersil



Pressure Test result for Silica Gel MB3/4 (no sintering or second hydrothermal treatment)


FIGURE 6.21Pressure Test result for Silica Gel MB3/4
(no sintering or second hydrothermal treatment)
$\diamond$ Pressure test result for silica Gel MB3/4-H2
(no sintering, HTT at 220 C for 18 hours)
$\triangle$ Pressure test result for Silica GEl MB3/4-H8
(no sintering, HTT at 180 C for 18 hours)


FIGURE 6.22

O prosgure Tost result for silice gol mb3/4
(no sintering or second hydrothormal treatmont)

- Pressure test result for Silice Gel MB3/4-S25
(sintered at 800 C for 16 hours, no second HTT)


O Pressure Test result for Sllica Gel MB3/4-s2H1
(Sintered at 800 C for 16 hours, HTT at 175 C for 18 hours)
$\triangle$ Prossure Test result for Sillca Gel MB3/4-s8H2
(Sintered at 800 C for 16 hours, HTT at 200 C for 18 hours)

- Pressure test result for Sillca Gel MB3/4-S25
(sintered at 800 C for 16 hours, no second HTT)


FIGURE 6.24Pressure Test result for Sllica Gel MB3/4-S22H5
(sintered at 850 C for 18 hours, HTT at 220 C for 18 hours)
© Pressure test result for Silica Gel MB3/4-Si5H3
(sintered at 850 C for 18 hours, HTT at 175 C for 18 hours)Pressure Test result for Silica Gel MB3/4-S14 2
(sintered at 850 C for 16 hours, HTT at 200 C for 18 hours)


O Pressure Test result for silica Gel MB3/4-H2 (no sintering, HTT at 200 C fo 18 hours)
$\triangle$ Pressure Test result for Sllica Gol MB3/4-S8H2
(Sintered at 800 C for 16 hours, HTT at 200 C for 18 hours)

- Pressure test result for Silica Gel MB3/4-S7H2
(sintered at 900 C for 18 hours, HTT at 200 C for 18 hours)Pressure Test result for Silica Gel MB3/4-Si4H2
(sintered at 850 C for 16 hours, HTT at 200 C for 18 hours)


OPressure Test result for Silica Gel MB3/4-H2S12 (HTT at 200 C for 18 hours, then sintered at 900 C for 18 hours)
$\diamond$ Pressure test result for Silica Gel MB3/4-S7H2
(sintered at 900 C for 18 hours, HTT at 200 C for 18 hours)


TABLE 6.12
Production Conditions

The three treatments given to the gels were:

1. Mild Hydrothermal Treatment in Ammonia solution pH 11 ; Temperature of treatment indicated on table.
2. Sintering Treatment in air; Temperature of Treatment indicated on table.
3. Hydrothermal treatment in Ammonia solution pH 11 ; Temperature of treatment indicated on table.

| Sample | Particle Size ( $\mu \mathrm{m}$ ) | ```Treatment 1``` | $\begin{gathered} \text { Treatment } \\ 2 \end{gathered}$ | $\begin{gathered} \text { Treatment } \\ 3 \end{gathered}$ | Surface <br> Area <br> $\left(m^{2} g^{-1}\right)$ | Pore Volume $\mathrm{cm}^{3} \mathrm{~g}^{-1}$ ) |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| MB5J | 3-15 | 140 | 800 | 300 | 14 | 0.70 |
| MB5S | 3-10 | 140 | 800 | 160 | 90 | 1.28 |
| MB5T | 3-10 | 140 | 800 | 220 | 50 | 1.55 |
| MB5 U | 3-15 | 140 | 800 | 220 | 50 | 1.30 |
| MB5B | 3-15 | 140 | 800 | 220 | 56 | 1.35 |
| MB5L | 3-15 | 140 | 800 | 160 | 91 | 1.15 |
| MB5A | - | 140 | 800 | 200 | 76 | 1.93 |
| MB5D | - | 140 | 800 | 180 | 91 | 2.10 |
| MB5C | - | 140 | 800 | 240 | 38 | 1.22 |

O Pressure test result for Silica Gel MB5B (Sintered at $800^{\circ} \mathrm{C}, \mathrm{HTT}$ at $220^{\circ} \mathrm{C}$ )


FIGURE 6.28
$\bigcirc$ Pressure test result for Silica Gel MB5J (Sintered at $800^{\circ} \mathrm{C}$, HTT at $300^{\circ} \mathrm{C}$ )


FIGURE 6.29

O Pressure test result for Silica Gel MB5U (Sintered at $800^{\circ} \mathrm{C}, \mathrm{HTT}$ at $220^{\circ} \mathrm{C}$ )



[^5]FIGURE 6.31



Comparison of Pressure Test data for experimental materials of different particle size.

## $\diamond$ Pressure Test result for stlica Gel MB5U (smaller particles) <br> O Pressure test result for silica Gel MBSU (larger particles)



FIGURE 6.34
$\rangle$ Pressure Test result for silica Gel mB5S (6-10رm)
O Pressure test result for silica Gel MB3/4-s2Hi (25 $\mu \mathrm{m}$ )
Both gels were sintered at 800 C and then hydrothermally treated.


FIGURE 6.35
$\diamond$ Pressure Test result for silica Gel MB3/4-S22H5 (25 $\mu \mathrm{m}$ )
O Pressure test result for Silica Gel MB5U (6-10 $\mu \mathrm{m}$ )
Both gels were aintered at $850^{\circ} \mathrm{C}$ and Hydrothermally treated at $220^{\circ} \mathrm{C}$ for 18 hours


FIGURE 6.36
Pressure against flowrate for silica gels of different pore volume;

```
O MB5T (1.55cccg-1)
\diamondMB5B (1.35ccg-1)
\nablaMB5S (1.28ccg-1)
\squareMB5U (1.30ccg-1)
```



TABLE 6.13
Comparison of Pressure Test Data

| Sample | Particle <br> Size <br> $(\mu \mathrm{m})$ | Surface <br> Area <br> $\left(\mathrm{m}^{2} \mathrm{~g}^{-1}\right)$ | Pore <br> Volume <br> $\left(\mathrm{cm}^{3} \mathrm{~g}^{-1}\right.$ | Pressure at <br> Flow Maxima <br> $(\mathrm{psi})$ |
| :--- | :---: | :---: | :---: | :---: |
| MB5S | $3-7$ | 90 |  | 1.28 |
| MB5S | $6-10$ | 90 | 1.28 | 15000 |
| MB5S | $3-15$ | 90 | 1.28 | 10000 |
| MB5T | $3-6$ | 50 | 1.55 | 13000 |
| MB5T | $5-10$ | 50 | 1.55 | 2500 |
| MB5T | $4-8$ | 50 | 1.55 | 2500 |
| MB5U | $3-5$ | 50 | 1.30 | 2500 |
| MB5U | $5-12$ | 50 | 1.30 | 10000 |
| MB5U | $3-15$ | 50 | 1.30 | 6500 |
| MB5L | $3-6$ | 91 | 1.15 | 10000 |
| MB5L | $5-10$ | 91 | 1.15 | 12000 |
| MB5L | $3-10$ | 91 | 1.15 | 13000 |
| MB5J | - | 14 | 0.70 | 13000 |
| MB5J | - | 14 | 0.70 | 9000 |
| MB5B | $3-15$ | 56 | 1.35 | 10000 |
| Hypersil | 5 | 180 | 0.68 | 8000 |

FIGURE 6.37

Plot shows that during the first run through the pressure test the results are not linear.

- Pressure test result for Silica Gel MB3/4-S8H2
$\diamond$ Pressure test result for Silica Gel MB3/4-S15H3 Pressure test result for Silica Gel MB3/4-S29H8


FIGURE 6.38

## Repeat Pressure Tests for Silica Gel MB3/4-S15H3

O First Test
$\diamond$ Second Test
Third Test


## Repeat Pressure Tests for Silica Gel MB3/4-S29H8

O First Test
$\diamond$ Second Test
Third Test


FIGURE 6.40

Repeat Pressure Tests for Silica Gel MB3/4-S8H2

- First Test
$\diamond$ Second Test
Third Test


Diagram shows the results obtained from pressure tests of experimental materials. The flowrates indicated are those obtained at 1000psi after a pulse of applied

FIGURE 6.41 pressure.


Diagram shows the results obtained from pressure tests of experimental materials. The flowrates indicated are those obtained at 1000 psi after a pulse of applied pressure.
$\begin{array}{ll}\circ \mathrm{MB3} / 4-\mathrm{H} 8 \mathrm{~S} 2 & \mathrm{HTT} \text { at } 200^{\circ} \mathrm{C} \text {, Sintered } 900^{\circ} \mathrm{C} \\ \diamond \mathrm{MB3} / 4-\mathrm{S} 7 \mathrm{HZ} & \text { Sintered } 900^{\circ} \mathrm{C} \text {, HTT at } 200^{\circ} \mathrm{C}\end{array}$


Diagram shows the results obtained from pressure tests of experimental materials. The flowrates indicated are

FIGURE 6.43 those obtained at 1000 psi after a pulse of applied pressure.


TABLE 6.14

A comparison of the bed length of different silica gels. All tests were carried out in stainless steel columns of $100 \times 5 \mathrm{~mm}$.

| Sample | Pore <br> Volume <br> $\left(\mathrm{cg}^{-1}\right)$ | Bed Length <br> after application of <br> 6000psi as a fraction <br> of total bed length. |
| :---: | :---: | :---: |
| Hypersil | 0.70 | $96 \%$ |
| MB5L | 1.15 | $91 \%$ |
| MB5S | 1.28 | $90 \%$ |
| MB5U | 1.30 | $92 \%$ |
| MB5B | 1.35 | $91 \%$ |
| MB5T | 1.55 | $83 \%$ |

TABLE 6.15

Data showing how the Particle Density is
related to the Pore Volume of the Silica Gel

| Pore <br> Volume <br> $\mathrm{cm}^{3} \mathrm{~g}^{-1}$ | Particle <br> Density <br> in air <br> $\mathrm{g} . \mathrm{cm}^{-3}$ | Particle <br> Density <br> in water <br> g.cm |
| :---: | :---: | :---: |
|  |  |  |
| 0.0 | 2.2 | 2.2 |
| 0.5 | 1.05 | 1.58 |
| 1.0 | 0.70 | 1.38 |
| 1.5 | 0.51 | 1.28 |
| 2.0 | 0.41 | 1.22 |

## DETERMINATION OF S E C CALIBRATION CURVES

### 7.1. Introduction

The Size Exclusion Chromatography Calibration (SECC) curve of a packing material can be used to give information on the pore structure of the material within the column. Once packed with material the total column volume, $V_{c}$, can be divided as follows:

$$
\begin{equation*}
V_{c}=V_{o}+V_{p}+V_{s} \tag{7.1}
\end{equation*}
$$

where $V_{0}$ is the void volume between the particles of the packing material. $V_{p}$ is the volume of the pores of the packing material and $V_{s}$ is the volume of the solid material in the column. In Chapter 4 the retention volume of a solute, $V_{R}$, is described as:

$$
\begin{equation*}
V_{R}=V_{0}+K \cdot V_{p} \tag{7.2}
\end{equation*}
$$

where $K$ is the permeation coefficient, and is related to the fraction of pore volume that is accessible to the solute under examination.

$$
\begin{equation*}
K=V_{\text {access }} / V_{p} \tag{7.3}
\end{equation*}
$$

A SECC curve is a semi-logarithmic plot where the solute size is related to the retention of the various samples. In Chapter 4 the SECC curve is outlined and explained in detail. The solute size parameter is usually either Log (Molecular Weight) of Log (Solute Radius), and the retention is usually described either by the permeation coefficient $(K)$, or the retention volume $V_{R}$, or
as a fraction of the total column volume $\left(V_{R} / V_{c}\right)$.

In this chapter the data obtained from High performance Size Exclusion Chromatography (HPSEC) of different materials is given together with the SECC curves plotted from the data. The materials studied were all silica gels and were of four main catagories.

- Experimental silica gels made during this project
- Commercial and experimental silica gels examined by Shandon Southern Products
- Commercial materials examined during this project
- Data obtained from the literature

In the presentation of this data the solute size parameter was the solute radius. The solute radius was approximated using the van Kreveld(1) equation, where the solute radius, r, was

$$
\begin{equation*}
r=0.123 \times(\text { Molecular Weight })^{0.588} \AA \tag{7.4}
\end{equation*}
$$

The retention of each solute was described by the permeation coefficient, where:

$$
\begin{equation*}
K=\left(V_{R}-V_{\min } / V_{\max }-V_{\min }\right) \tag{7.5}
\end{equation*}
$$

$V_{\text {min }}$ was the retention volume of the largest solute molecule and $V_{\text {max }}$ was the retention volume of the smallest solute used. For all curves experimentally obtained $V_{\text {max }}$ was the retention volume of benzene.

The solute with the smallest retention volume was taken to have failed to permeate any of the pore volume and thus was termed "totally excluded" and assigned a permeation coefficient of zero $(K=0)$. The smallest solute experimentally examined (Benzene) was assumed to fully permeate all the pore volume within the packing material and thus has a permeation coefficient equal to one ( $K=1$ ).

The retention volume of the largest solute was taken as being equivalent to the void volume of the column. The retention volume of the smallest solute was taken to correspond to total permeation of the pores. The difference between the void volume and the volume of total permeation was equivatent to the volume of pores within the column. The volume of the solid packing material was obtained from:

$$
\begin{equation*}
V_{s}=V_{c}-\left(V_{o}+V_{p}\right) \tag{7.6}
\end{equation*}
$$

From the volume of the solid packing, the mass of the packing material was calculated.

$$
\begin{equation*}
M_{s}=V_{s} / V_{s p} \tag{7.7}
\end{equation*}
$$

where $M_{s}$ is the mass of the solid material in the column and $V_{s p}$ is the volume of 1 g of solid silica. For this work $V_{s p}$ was $0.45 \mathrm{~cm}^{3}$. From the mass of silica in the column the pore volume of the packing material was determined by HPSEC where:

$$
\begin{equation*}
\text { Specific Pore Volume }=V_{p} / M_{s} \tag{7.8}
\end{equation*}
$$

The work in this thesis has attempted to produce silica gel packing materials with a relatively higher pore volume than normally associated with silica gels used for HPSEC. This higher pore volume was required to improve the resolution of the column. The data in Table 7.1 compares the pore volume obtained by SEC for all the materials studied.

A second objective of the work presented was to produce silica gels with wider pores than normally associated with HPLC materials used in retentive LC. The SECC curve can be used to give an indication of the pore size distribution of the column packing. This topic will be fully discussed in Chapter 8, fowever the vertical displacement of the SECC curve does give an indication of pore size. The
method of Pore Size Distribution (PSD) determination developed by Halasz and Martin(2) although containing a fundamental error does give a good approximation to the mean pore size of an HPSEC material. In Table 7.3 the mean pore size of each material is listed according to the Halasz method where the mean pore radius is found from the solute having a permeation coefficient of 0.5 . The mean pore radius was then given by

$$
\begin{equation*}
\text { Mean Pore Radius }=2.5 \times r_{0.5} \stackrel{\circ}{\AA} \tag{7.9}
\end{equation*}
$$

where $r_{0.5}$ was the solute radius whose permeation coefficient was 0.5 .

### 7.2. Experimental Procedures

The chromatographic system, solutes and solvents used for the determination of SECC curves was given in detail in Chapter 5 . The column packing method was also outlined in Chapter 5. The column sizes used for individual tests are outlined in Table 7.1. The eluent flowrate was maintained at $1.0+/-0.05 \mathrm{ml} / \mathrm{min}$. On elution from the column the solutes were detected by ultra-violet absorbance at 254 nm .

The retention volume $\left(V_{R}\right)$, of each solute was determined from measuring the retention time ( $t_{R}$ ) of each solute and multiplying this by the volume flowrate of the eluent $\left(f_{v}\right)$. Thus

$$
\begin{equation*}
V_{R}=f_{v} \times t_{R} \tag{7.10}
\end{equation*}
$$

This method was used for all samples.

The determination was made by studying the retention volume of each solute separately. Each solute was injected in a dilute solution and the retention volume determined for five separate injections. The average was then calculated.

### 7.3. Experimental Results

The data obtained from this experimental HPSEC was as folllows:

1. In Figure 7.1 the SECC curve of Hypersil a typical HPLC packing material used in retentive HPLC is shown. In particular the point at which the permeation coefficient is zero should be noted.
2. In Figures 7.2 - 7.6 the SECC curves for experimental silica gels produced during the preliminary studies outlined in Chapter 6 are shown. These gels were all made from batch MB3/4 and had an average particle diameter of around $25 \mu \mathrm{~m}$. The curves show that these materials have pores that are significantly larger than those of Hypersil.
3. In Figures $7.7-7.12$ further experimental materials are shown. These gels were the second set of gels produced in Chapter 6. This series of materials were all made from batch MB5S. The average particle diameter was $5-10 \mu \mathrm{~m}$. Once again the curves illustrate significantly enlarged pores.
4. In Figures $7.13-7.16$ experimental SECC curves were obtained for a selection of commercially available silica gels are shown.
5. In Figures 7.17-7.21 the curves for experimental materials examined by John Spencer of Shandon Southern Products.
6. In Figures $7.22-7.23$ the data used was obtained from Knox and $S \operatorname{cott}(3)$.

### 7.4. Pore Volume by HPSEC

The data listed in Table 7.2 compares the pore volume of different silica gels examined by HPSEC. Also outlined in Table 7.2 is the ratio of pore volume to interstitial space as experimentally obtained by HPSEC (i.e. the value of $\mathrm{V}_{\mathrm{p}} / \mathrm{V}_{\mathrm{o}}$ )

The table divides into three sections. In section A the gels typically used in retentive HPLC are shown. In particular the low pore volume should be noted. In Section B the experimental gels of higher pore volume produced during this project are shown. here we see that the materials generally have a larger value of $V_{p} N_{o}$, which impies a higher pore volume. In Section $C$ a number of commercial silica gels are shown, these indicate the variety of materials available.

Generally the pore volume measured by mercury porosimetry is higher than the value obtained by HPSEC. The HPSEC pore volume is calculated to include pores between the radius of the totally excluded solute and that of total permeation, while in mercury porosimetry all pores with a radius smaller than say $800 \AA$. The pore radius corresponding to total exclusion in these HPSEC studies was around $600 \AA$.

### 7.5. Conclusions

The different methods of pore volume analysis give different values for the gel pore volume. The value of pore volume according to HPSEC is dependant on the quality of column packing. The problem of packing does not affect the value obtained by mercury porosimetry, however the HPSEC method may give a more realistic picture $\hat{f}$ the column pore volume.

The experimental gels of batches MB3/4 and MB5 generally show a higher pore volume than other materials currently available. This high pore volume is supported by the higher value of $V_{p} N_{o}$ in the experimental gels than in the
commercially available materials. This has been achieved in both $25 \mu \mathrm{~m}$ and $5 \mu \mathrm{~m}$ materials.

The shape of the SECC curves in the experimental materials produced in this project is generally much flatter than the other materials; indicating a narrower PSD. In Chapter 8 the question of determination of PSD by SEC will be dealt with in full.

A series of chromatograms shown in Figures 7.25-7.28 show examples of typical chromatograms obtained from mixtures of different polystyrene standards. In Figure 7.25 a SEC chromatogram obtained with Hypersil is shown - the resolution between the solute is poor. In Figures 7.26 and 7.28 chromatograms examining samples in a similar molecular weight range are shown to have much improved resolution. This improved resolution has been achieved with experimental gels. These Figures provide a clear indication of how the HPSEC of polymers, particularly of high molecular weight, may be improved by using a higher pore volume support with a wider mean pore diameter.

### 7.6. References

1. M.E.Van kreveld and N.van den Hoed: J. Chrom. 83, 111, (1973)
2. I.Halasz and K.Martin: Angew. Chem. Int. Ed. 17, 901, (1978)
3. J.H.Knox and H.P.Scott: J. Chrom. 316, 311, (1984)

In this Table the volume of the packed SEC column is broken down into three fractions; the volume of the interstitial space ( $V_{0} N_{c}$ ), the volume of the pores ( $V_{p} / N_{c}$ ), and the volume of the solid silica gel $\left(V_{s} / V_{d}\right) \cdot V_{c}$ is the volume of the empty column.

| Silica <br> Gel <br> Sample | Dimensions <br> of <br> Column <br> $(m m)$ | $v_{o} N_{c}$ | $v_{p} N_{c}$ | $v_{s} N_{c}$ |
| :--- | :--- | :--- | :--- | :--- |
| MB3/3-S2H1 | $100 \times 5$ | 0.33 | 0.46 | 0.21 |
| MB3/4-S8H2 | $100 \times 5$ | 0.36 | 0.41 | 0.23 |
| MB3/4-S15H3 | $100 \times 5$ | 0.36 | 0.43 | 0.21 |
| MB3/4-S14H2 | $100 \times 5$ | 0.36 | 0.41 | 0.23 |
| 732HK2a-H3 | $100 \times 5$ | 0.33 | 0.38 | 0.29 |
| MB5J | $100 \times 5$ | 0.52 | 0.25 | 0.23 |
| MB5T | $100 \times 5$ | 0.41 | 0.39 | 0.20 |
| MB5B | $100 \times 5$ | 0.37 | 0.37 | 0.26 |
| MB5U | $100 \times 5$ | 0.35 | 0.44 | 0.21 |
| MB5S | $100 \times 5$ | 0.33 | 0.44 | 0.22 |
| MB5L | $100 \times 5$ | 0.37 | 0.39 | 0.23 |
|  |  |  |  |  |
|  |  |  |  |  |
| PR185 | $250 \times 5$ | 0.42 | 0.29 | 0.30 |
| PR183 | $250 \times 5$ | 0.41 | 0.31 | 0.29 |
| PR179 | $250 \times 5$ | 0.43 | 0.29 | 0.29 |
| SSP501 | $250 \times 5$ | 0.42 | 0.23 | 0.35 |
| WP1004 | $250 \times 5$ | 0.43 | 0.26 | 0.31 |
| VYDAC | $160 \times 4.6$ | 0.45 | 0.27 | 0.27 |
| HYPERSIL | $100 \times 5$ | 0.41 | 0.31 | 0.28 |
| Li-Si1000 | $160 \times 4.6$ | 0.45 | 0.39 | 0.16 |
| PSM 60 | $250 \times 6.4$ | 0.34 | 0.19 | 0.47 |
| PSM 1000 | $250 \times 6.4$ | 0.41 | 0.28 | 0.32 |
|  |  |  |  |  |

Table shows the pore volume obtained by HPSEC and the ratio of pore volume to void space in the series of gel species examined.

| A - | Sample | Pore Volume* $\mathrm{cm}^{3} \mathrm{~g}^{-1}$ | $\underline{V}_{p}$ | $\begin{gathered} \text { Pore } \\ \text { Volume } \\ \mathrm{cm}^{3} \mathrm{~g}^{-1} \end{gathered}$ |
| :---: | :---: | :---: | :---: | :---: |
|  | Hypersil <br> PR185 <br> PR183 <br> PR179 <br> SSP501 <br> WP 1004 | $\begin{aligned} & 0.49 \\ & 0.43 \\ & 0.49 \\ & 0.45 \\ & 0.31 \\ & 0.39 \end{aligned}$ | $\begin{aligned} & 0.76 \\ & 0.69 \\ & 0.76 \\ & 0.67 \\ & 0.55 \\ & 0.62 \end{aligned}$ | $\begin{aligned} & 0.69 \\ & 0.58 \\ & 0.64 \\ & 0.63 \\ & 0.51 \\ & 0.53 \end{aligned}$ |
|  | MB3/4-S2H1 <br> MB3/4-S8H2 <br> MB3/4-S14H2 <br> MB3/4-S15H3 <br> 732HK2a-H3 <br> MB5J <br> MB5T <br> MB5B <br> MB5U <br> MB5S <br> MB5L | $\begin{aligned} & 1.00 \\ & 0.79 \\ & 0.79 \\ & 0.94 \\ & 0.61 \\ & 0.49 \\ & 0.86 \\ & 0.64 \\ & 0.93 \\ & 0.90 \\ & 0.76 \end{aligned}$ | $\begin{aligned} & 1.39 \\ & 1.14 \\ & 1.14 \\ & 1.19 \\ & 1.15 \\ & 0.48 \\ & 0.95 \\ & 1.00 \\ & 1.26 \\ & 1.33 \\ & 1.05 \end{aligned}$ | $\begin{gathered} 1.39 \# \\ 1.15 \# \\ 1.43 \# \\ 1.23 \# \\ -\quad \\ 0.70 \# \\ 1.55 \# \\ 1.35 \# \\ 1.30 \# \\ 1.28 \# \\ 1.15 \# \end{gathered}$ |
|  | VYDAC Lichrospher /PSM 60 PSM 1000 | $\begin{aligned} & 0.45 \\ & 1.10 \\ & 0.20 \\ & 0.39 \end{aligned}$ | $\begin{aligned} & 0.60 \\ & 0.87 \\ & 0.56 \\ & 0.68 \end{aligned}$ | $\begin{aligned} & 0.46 \\ & 0.98 \\ & 0.47 \\ & 0.36 \end{aligned}$ |

*     - Pore Volume by HPSEC
** - Pore Volume by Hg Porosimetry
\# - Pore Volume by Low Pressure Hg Porosimetry

Mean Pore Diameter using Halasz factor of 2.5

| Sample | Mean <br> Pore <br> Diameter <br> A |
| :---: | :---: |
| MB3/4-S2H1 | 380 |
| MB3/4-S8H2 | 500 |
| MB3/4-S15H3 | 360 |
| MB3/4-S14H2 | 510 |
| 732HK2a-H3 | 410 |
| MB5J | 1000 |
| MB5T | 700 |
| MB5B | 380 |
| MB5U | 630 |
| MB5S | 340 |
| MB5L | 340 |
| PR185 | 350 |
| PR183 | 360 |
| PR179 | 450 |
| SSP501 | 450 |
| WP1004 | 310 |
| HYPERSIL | 780 |
| Lichrospher | 150 |
| PSM 60 1000 | 820 |

## S.E.C. Calibration Curve



## DATA TAKEN FROM REfERENCE 3



HPSEC calibration data for MB3／4－S2H1

Column Dimensions $100 \times 5 \mathrm{~mm}$ Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log（Sol．Rad） | Retention <br> Volume <br> $(\mathrm{ml})$ | Permeation <br> Coefficient |
| :--- | :--- | :--- |
| 6.938 | 0.65 | 0.0 |
| 6.322 | 0.69 | 0.044 |
| 5.874 | 0.70 | 0.056 |
| 5.626 | 0.70 | 0.056 |
| 5.020 | 0.85 | 0.222 |
| 4.512 | 1.05 | 0.444 |
| 4.120 | 1.25 | 0.667 |
| 3.456 | 1.39 | 0.822 |
| 2.779 | 1.50 | 0.944 |
| 1.831 | 1.55 | 1.00 |
| 0.466 | 1.55 | 1.00 |



HPSEC calibration data for MB3/4-S8H2

Column Dimensions $100 \times 5 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log | Retention | Permeation |
| :--- | :--- | :--- |
| Solute <br> Radius | Volume <br> $(\mathrm{ml})$ | Coefficient |
| 6.938 | 0.70 |  |
| 6.322 | 0.70 | 0.0 |
| 5.874 | 0.70 | 0.0 |
| 5.626 | 0.77 | 0.0 |
| 5.020 | 0.95 | 0.313 |
| 4.512 | 1.15 | 0.563 |
| 4.120 | 1.28 | 0.725 |
| 3.456 | 1.45 | 0.938 |
| 2.779 | 1.50 | 1.00 |
| 1.831 | 1.50 | 1.00 |
| 0.466 | 1.50 | 1.00 |




HPSEC calibration data for MB3/4-S 14 H 2

Column Dimensions $100 \times 5 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log | Retention | Permeation |
| :--- | :--- | :--- |
| Solute <br> Radius | Volume <br> $(\mathrm{ml})$ | Coefficient |
| 6.938 | 0.70 |  |
| 6.322 | 0.70 | 0.0 |
| 5.874 | 0.70 | 0.0 |
| 5.626 | 0.80 | 0.0 |
| 5.020 | 1.00 | 0.125 |
| 4.512 | 1.15 | 0.375 |
| 4.120 | 1.30 | 0.563 |
| 3.456 | 1.40 | 0.75 |
| 2.779 | 1.50 | 0.875 |
| 1.831 | 1.50 | 1.00 |
| 0.466 | 1.50 | 1.00 |
|  |  | 1.00 |

S.E.C. Calibration Curve


HPSEC calibration data for $732 \mathrm{HK} 2 \mathrm{a}-\mathrm{H} 3$

Column Dimensions $100 \times 5 \mathrm{~mm}$ Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log(Sol. Rad) | Retention Volume (ml) | Permeation Coefficient |  |
| :---: | :---: | :---: | :---: |
| 6.938 | 0.65 | 0.0 | N |
| 6.322 | 0.67 | 0.027 | $\checkmark$ |
| 5.874 | 0.69 | 0.053 |  |
| 5.626 | 0.70 | 0.067 |  |
| 5.020 | 0.80 | 0.20 |  |
| 4.512 | 1.00 | 0.467 |  |
| 4.120 | 1.20 | 0.733 |  |
| 3.456 | 1.30 | 0.867 |  |
| 2.779 | 1.40 | 1.00 |  |
| 1.831 | 1.40 | 1.00 |  |
| 0.466 | 1.40 | 1.00 |  |
|  |  |  | - |
|  |  |  | v |



HPSEC calibration data for MB5 J

Column Dimensions $100 \times 5 \mathrm{~mm}$ Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log | Retention | Permeation |  |
| :---: | :---: | :---: | :---: |
| Solute | Volume | Coefficient |  |
| Radius | (mI) |  |  |
| 6.6 .23 | 1.02 | 0.0 | N |
| 6.217 | 1.02 | 0.0 | $\infty$ |
| 6.146 | 1.02 | 0.0 |  |
| 6.061 | 1.06 | 0.08 |  |
| 5.829 | 1.08 | 0.12 |  |
| 5.703 | 1.14 | 0.25 |  |
| 5.255 | 1.28 | 0.53 |  |
| 4.881 | 1.38 | 0.73 |  |
| 4.591 | 1.44 | 0.85 |  |
| 4.072 | 1.48 | 0.93 |  |
| 3.628 | 1.49 | 0.95 |  |
| 2.681 | 1.50 | 0.97 |  |
| 0.466 | 1.52 | 1.00 |  |
|  |  |  |  |
|  |  |  |  |

## S.E.C. Calibration Curve



HPSEC calibration data for MB5 T

Column Dimensions $100 \times 5 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log(Sol. Rad) | Retention Volume (ml) | Permeation Coefficient |  |
| :---: | :---: | :---: | :---: |
| 7.251 | 0.80 | 0.00 |  |
| 6.879 | 0.79 | -0.01 |  |
| 6.322 | 0.80 | 0.00 | N |
| 6.226 | 0.82 | 0.03 | 6 |
| 6.110 | 0.83 | 0.04 |  |
| 5.882 | 0.91 | 0.14 |  |
| 5798 | 0.88 | 0.10 |  |
| 5.581 | 0.83 | 0.04 |  |
| 5.026 | 1.15 | 0.48 |  |
| 4.756 | 1.23 | 0.56 |  |
| 4.512 | 1.29 | 0.64 |  |
| 4.284 | 1.34 | 0.71 |  |
| 4.119 | 1.38 | 0.76 |  |
| 3.632 | 1.41 | 0.76 |  |
| 3.417 | 1.48 | 0.89 |  |
| 2.817 | 1.53 | 0.96 |  |
| 2.430 | 1.53 | 0.96 | $\overline{0}$ |
| 1.990 | 1.54 | 0.97 | $\bigcirc$ |
| 1.781 | 1.53 | 0.96 | m |
| 1.535 | 1.54 | 0.97 | $\cdots$ |
| 0.466 | 1.57 | 1.00 | $\infty$ |



HPSEC calibration data for MB5 B

Column Dimensions $100 \times 5 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log <br> Solute <br> Radius | Retention <br> Volume <br> (mI) | Permeation <br> Coefficient |
| :--- | :--- | :--- |
| 7.620 | 0.73 |  |
| 7.251 | 0.73 | 0.00 |
| 6.879 | 0.75 | 0.00 |
| 6.612 | 0.74 | 0.03 |
| 6.322 | 0.73 | 0.01 |
| 6.110 | 0.75 | 0.03 |
| 5.797 | 0.74 | 0.01 |
| 5.584 | 0.79 | 0.08 |
| 5.020 | 0.93 | 0.28 |
| 4.756 | 0.98 | 0.35 |
| 4.512 | 1.09 | 0.50 |
| 4.120 | 1.15 | 0.58 |
| 3.742 | 1.23 | 0.69 |
| 3.417 | 1.30 | 0.80 |
| 2.817 | 1.36 | 0.87 |
| 2.430 | 1.40 | 0.93 |
| 1.777 | 1.43 | 0.97 |
| 1.535 | 1.43 | 0.97 |
| 0.466 | 1.45 | 1.00 |



HPSEC calibration data for MB5 U

Column Dimensions $100 \times 5 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log | Retention | Permeation |  |
| :---: | :---: | :---: | :---: |
| Solute | Volume | Coefficient |  |
| Radius | (mi) |  |  |
| 7.251 | 0.68 | 0.00 |  |
| 6.938 | 0.69 | 0.01 |  |
| 6.879 | 0.69 | 0.01 |  |
| 6.322 | 0.72 | 0.03 |  |
| 6.226 | 0.71 | 0.02 |  |
| 6.110 | 0.72 | 0.03 | $N$ |
| 5.882 | 0.76 | 0.09 | $\stackrel{\square}{\square}$ |
| 5.797 | 0.79 | 0. 12 |  |
| 5.626 | 0.85 | 0.19 |  |
| 5.020 | 1.06 | 0.42 |  |
| 4.756 | 1.16 | 0.53 |  |
| 4.512 | 1.25 | 0.63 |  |
| 4.284 | 1.29 | 0.67 |  |
| 4.120 | 1.34 | 0.73 |  |
| 3.632 | 1.41 | 0.80 |  |
| 3.417 | 1.48 | 0.91 |  |
| 2.817 | 1.52 | 0.84 | 7 |
| 2.430 | 1.48 | 0.91 | $\bigcirc$ |
| 1.990 | 1.53 | 0.94 | D |
| 1.777 | 1.50 | 0.90 | $v$ |
| 1.535 | 1.56 | 0.97 | $\stackrel{\rightharpoonup}{\square}$ |
| 0.466 | 1.58 | 1.00 | O |

S.E.C. Calibration Curve


HPSEC calibration data for MB5 S

Column Dimensions $100 \times 5 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log <br> Solute <br> Radius | Retention <br> Volume <br> $(\mathrm{ml})$ | Permeation <br> Coefficient |
| :--- | :--- | :--- |
|  |  |  |
| 6.938 | 0.65 | 0.00 |
| 6.322 | 0.65 | 0.00 |
| 6.226 | 0.65 | 0.00 |
| 6.110 | 0.65 | 0.00 |
| 5.797 | 0.66 | 0.01 |
| 5.584 | 0.67 | 0.02 |
| 5.020 | 0.80 | 0.19 |
| 4.756 | 0.89 | 0.28 |
| 4.512 | 0.96 | 0.37 |
| 4.120 | 1.13 | 0.57 |
| 3.480 | 1.27 | 0.73 |
| 2.817 | 1.37 | 0.84 |
| 1.535 | 1.49 | 0.98 |
| 0.466 | 1.50 | 1.00 |

S.E.C. Calibration Curve


HPSEC calibration data for MB5 L

Column Dimensions $100 \times 5 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log | Retention | Permeation |  |  |
| :---: | :---: | :---: | :---: | :---: |
| Solute | Volume | Coefficient |  |  |
| Radius | (ml) |  |  |  |
| 7.620 | 0.73 | 0.00 |  | N |
| 7.251 | 0.73 | 0.00 |  | $\omega$ |
| 6.879 | 0.73 | 0.00 |  |  |
| 6.612 | 0.73 | 0.00 |  |  |
| 6.322 | 0.73 | 0.00 |  |  |
| 6.110 | 0.71 | -0.03 |  |  |
| 5.797 | 0.72 | -0.01 |  |  |
| 5.584 | 0.75 | 0.03 |  |  |
| 5.020 | 0.80 | 0.09 |  |  |
| 4.756 | 0.85 | 0.16 |  |  |
| 4.512 | 0.97 | 0.32 |  |  |
| 4.120 | 1.16 | 0.56 |  |  |
| 3.742 | 1.22 | 0.65 | 7 |  |
| 3.417 | 1.31 | 0.76 | $\overline{0}$ |  |
| 2.817 | 1.39 | 0.87 | ${ }_{5}$ |  |
| 2.430 | 1.42 | 0.91 | m |  |
| 1.777 | 1.48 | 0.99 | $\stackrel{N}{\sim}$ |  |
| 1.535 | 1.49 | 1.00 | $\stackrel{\rightharpoonup}{N}$ |  |
| 0.466 | 1.49 | 1.00 |  |  |

S.E.C. Calibration Curve


HPSEC calibration data for PSM 1000

Column Dimensions $250 \times 6.4 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log | Retention | Permeation |  |
| :---: | :---: | :---: | :---: |
| Solute | Volume | Coefficient |  |
| Radius | (mI) |  | N D |
| 7.251 | 3.09 | 0.00 |  |
| 6.938 | 3.09 | 0.00 |  |
| 6.322 | 3.28 | 0.092 |  |
| 6.110 | 3.52 | 0.209 |  |
| 5.797 | 3.63 | 0.262 |  |
| 5.626 | 3.79 | 0.340 |  |
| 5.020 | 4.17 | 0.524 |  |
| 4.512 | 4.48 | 0.675 |  |
| 4.120 | 4.75 | 0.806 |  |
| 3.417 | 4.95 | 0.903 | 7 |
| 2.817 | 5.01 | 0.932 | $\stackrel{\square}{\square}$ |
| 2.430 | 5.05 | 0.951 | D |
| 1.781 | 5.09 | 0.971 | $v$ |
| 1.535 | 5.14 | 0.995 | $\stackrel{\rightharpoonup}{\square}$ |
| 0.466 | 5.15 | 1.00 | $\omega$ |



HPSEC calibration data for PSM 60

Column Dimensions $250 \times 6.4 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log <br> Solute <br> Radius | Retention <br> Volume <br> $(\mathrm{ml})$ | Permeation <br> Coefficient |
| :--- | :--- | :--- |
| 7.620 | 2.52 | 0.00 |
| 7.251 | 2.54 | 0.015 |
| 6.938 | 2.57 | 0.036 |
| 6.226 | 2.63 | 0.072 |
| 5.797 | 2.65 | 0.095 |
| 5.626 | 2.73 | 0.153 |
| 5.020 | 2.75 | 0.168 |
| 4.512 | 2.83 | 0.226 |
| 4.120 | 2.87 | 0.255 |
| 3.742 | 2.96 | 0.321 |
| 3.632 | 3.06 | 0.394 |
| 3.417 | 3.20 | 0.496 |
| 3.159 | 3.30 | 0.569 |
| 2.817 | 3.33 | 0.591 |
| 2.430 | 3.42 | 0.657 |
| 1.781 | 3.69 | 0.854 |
| 1.535 | 3.67 | 0.839 |
| 0.466 | 3.89 | 1.00 |

 . ................

HPSEC calibration data for Lichrospher Si 1000

Column Dimensions $250 \times 4.6 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log | Retention | Permeation |  |
| :---: | :---: | :---: | :---: |
| Solute | Volume | Coefficient |  |
| Radius | (mI) |  |  |
| 7.620 | 1.89 | 0.00 | N |
| 7.251 | 1.95 | 0.037 | $\stackrel{\circ}{\circ}$ |
| 6.938 | 1.92 | 0.019 |  |
| 6.226 | 2.03 | 0.086 |  |
| 5.797 | 2.30 | 0.253 |  |
| 5.626 | 2.36 | 0.290 |  |
| 5.020 | 2.72 | 0.512 |  |
| 4.512 | 2.98 | 0.673 |  |
| 4.120 | 3.11 | 0.753 |  |
| 3.742 | 3.27 | 0.852 |  |
| 3.632 | 3.26 | 0.846 |  |
| 3.417 | 3.28 | 0.858 |  |
| 3.159 | 3.42 | 0.944 |  |
| 2.817 | 3.38 | 0.920 |  |
| 2.430 | 3.42 | 0.944 | $\bar{\pi}$ |
| 1.781 | 3.46 | 0.969 |  |
| 1.535 | 3.41 | 0.938 |  |
| 0.466 | 3.51 | 1.00 | $\checkmark$ |
|  |  |  | $\vec{\pi}$ |



## HPSEC calibration data for Vydac

Column Dimensions $160 \times 4.6 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$



HPSEC calibration data for SSP 501

Column Dimensions $250 \times 5 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log | Retention | Permeation |  |
| :---: | :---: | :---: | :---: |
| Solute | Volume | Coefficient |  |
| Radius | (mI) |  |  |
| 6.938 | 2.0 | 0.00 | N |
| 6.322 | 2.0 | 0.00 | $\infty$ |
| 5.874 | 2.05 | 0.04 |  |
| 5.626 | 2.20 | 0.17 |  |
| 5.020 | 2.40 | 0.33 |  |
| 4.512 | 2.60 | 0.50 |  |
| 4.120 | 2.80 | 0.67 |  |
| 3.417 | 2.95 | 0.79 |  |
| 2.817 | 3.00 | 0.83 |  |
| 1.781 | 3.18 | 0.98 |  |
| 0.466 | 3.20 | 1.00 |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |



HPSEC calibration data for PR 179

Column Dimensions $250 \times 5 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log | Retention | Permeation |  |
| :---: | :---: | :---: | :---: |
| Solute | Volume | Coefficient |  |
| Radius | (mI) |  |  |
| 6.938 | 2.0 | 0.00 | $N$00 |
| 6.322 | 2.0 | 0.00 |  |
| 5.874 | 2.10 | 0.07 |  |
| 5.626 | 2.20 | 0.13 |  |
| 5.020 | 2.45 | 0.30 |  |
| 4.756 | 2.60 | 0.40 |  |
| 4.512 | 2.75 | 0.50 |  |
| 4.280 | 2.85 | 0.57 |  |
| 4.120 | 3.00 | 0.67 |  |
| 4.050 | 3.25 | 0.83 |  |
| 3.630 | 3.25 | 0.83 |  |
| 3.417 | 3.20 | 0.80 |  |
| 3.160 | 3.30 | 0.87 |  |
| 2.817 | 3.35 | 0.90 |  |
| 2.430 | 3.40 | 0.93 | TOCD |
| 1.990 | 3.40 | 0.93 |  |
| 1.781 | 3.45 | 0.97 |  |
| 0.466 | 3.50 | 1.00 | $\checkmark$ |
|  |  |  | $\infty$ |

S.E.C. Calibration Curve


HPSEC calibration data for PR 183

Column Dimensions $250 \times 5 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log | Retention | Permeation |  |
| :---: | :---: | :---: | :---: |
| Solute | Volume | Coefficient |  |
| Radius | (ml) |  |  |
| 6.938 | 2.0 | 0.00 | N |
| 6.322 | 2.0 | 0.00 |  |
| 5.874 | 2.00 | 0.00 |  |
| 5.626 | 2.10 | 0.07 |  |
| 5.020 | 2.25 | 0.17 |  |
| 4.756 | 2.35 | 0.23 |  |
| 4.512 | 2.70 | 0.47 |  |
| 4.280 | 2.75 | 0.50 |  |
| 4.120 | 2.90 | 0.57 |  |
| 4.050 | 2.90 | 0.60 |  |
| 3.630 | 3.10 | 0.73 |  |
| 3.417 | 3.15 | 0.77 |  |
| 3.160 | 3.20 | 0.80 |  |
| 2.817 | 3.35 | 0.90 | $\cdots$ |
| 2.430 | 3.40 | 0.93 | $\stackrel{\square}{\square}$ |
| 1.990 | 3.40 | 0.93 | m |
| 1.781 | 3.45 | 0.97 | $v$ |
| 0.466 | 3.50 | 1.00 | $\stackrel{\rightharpoonup}{0}$ |



HPSEC calibration data for PR 185

Column Dimensions $250 \times 5 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$

| Log | Retention | Permeation |  |
| :---: | :---: | :---: | :---: |
| Solute | Volume | Coefficient |  |
| Radius | (ml) |  | N |
| 6.938 | 1.90 | 0.00 |  |
| 6.322 | 1.95 | 0.03 |  |
| 5.874 | 2.05 | 0.10 |  |
| 5.626 | 2.10 | 0.13 |  |
| 5.020 | 2.35 | 0.29 |  |
| 4.756 | 2.40 | 0.32 |  |
| 4.512 | 2.60 | 0.45 |  |
| 4.280 | 2.65 | 0.48 |  |
| 4.120 | 2.80 | 0.58 |  |
| 4.050 | 2.80 | 0.58 |  |
| 3.630 | 2.95 | 0.68 |  |
| 3.417 | 3.05 | 0.74 |  |
| 3.160 | 3.10 | 0.77 |  |
| 2.817 | 3.15 | 0.81 | 7 |
| 2.430 | 3.25 | 0.87 | $\stackrel{\square}{\square}$ |
| 1.990 | 3.35 | 0.94 | - |
| 1.781 | 3.40 | 0.97 | $\checkmark$ |
| 0.466 | 3.45 | 1.00 | N |

S.E.C. Calibration Curve


HPSEC calibration data for WP 10-04

Column Dimensions $250 \times 5 \mathrm{~mm}$

Eluent $\mathrm{CH}_{2} \mathrm{Cl}_{2}$


FIGURE 7.22



## FIGURE 7.25

Chromatogram of Polystyrene Standards on HYPERSIL

Column : $5 \mu \mathrm{~m}$ HYPERSIL
Dimensions: $250 \times 5 \mathrm{~mm}$
Flowrate: $1 \mathrm{ml} / \mathrm{min}$
Eluent: $\mathrm{CH}_{2} \mathrm{cćl}_{2}$
Detector: ÚV 254 nm
Injection: $20 \mu 1$

## Solutes:

PS 4250000
PS 505000
PS 111000
PS 20500
PS 480


FIGURE 7.26
Chromatogram of Polystyrene Standerds on MB5S

Column : $5-10 \mu \mathrm{~m}$ MB5S
Dimensions: $250 \times 5 \mathrm{~mm}$
Flowrate: $1 \mathrm{ml} / \mathrm{min}$
Eluent: $\mathrm{CH}_{2} \mathrm{e}^{\prime} \mathrm{l}_{2}$
Detector: ƯV 254 nm
Injection: 20رl

## Solutes:

PS 4250000
PS 180000
PS 115000
PS 51500
PS 11800
Benzene

mins

FIGURE 7.27
Chromatogram of Polystyrene Standards on MB5B

Column : 5-10 $\boldsymbol{\mu m}$ MB5B
Dimensions : $250 \times 5 \mathrm{~mm}$
Flowrate: $1 \mathrm{ml} / \mathrm{min}$
Eluent: $\mathrm{CH}_{2} \mathrm{Cl}_{2}$
Detector: UV 254 nm
Injection : $20 \mu$ I
Solutes:

| PS | 4250000 |
| :--- | :---: |
| PS | 505000 |
| PS | 111000 |
| PS | 20500 |
| PS | 4800 |



FIGURE 7.28
Chromatogram of Polystyrene Standards on MB5U

Column : $5-10 \mu \mathrm{~m}$ MB5U
Dimensions: $250 \times 5 \mathrm{~mm}$
Flowrate: $1 \mathrm{ml} / \mathrm{min}$
Eluent: $\mathrm{CH}_{2} \mathrm{Cl}_{2}$
Detector: UV 254 nm
Injection: 20رI

## Solutes:

PS 8000000
PS 675000
PS 180000
PS 76000
PS 39000
PS 7600
Benzene

mins

## CHAPTER 8

## DETERMINATION OF PORE SIZE DISTRIBUTIONS

### 8.1. Introduction

Porous solids are of vital importance in many fields, as chromatographic supports, catalysts and adsorbents(1). It is necessary to know the stuctural characteristics of the materials as these properties (surface area, pore volume, pore diameter, pore size distribution and pore shape) are all relevant to the performance of the material (2). In HPLC the separation of biopolymers peptides and proteins is now more common with the use of High Performance Size Exclusion Chromatography (HPSEC) and Reversed, 3 Phase HPLC (RP-HPLC). High Performance Liquid Chromatography with its increased application in the separation of biopolymers, peptides and proteins (see Chapter 3) requires supports with well defined pore systems allowing access to the particle surface. It is possible as described earlier by variation of synthesis techniques to alter the structural parameters of silica gel packing materials. This chapter deals with the methods currently used for PSD determination and develops the theory of Knox and $S \operatorname{cott}(3)$ where the PSD can be obtained from a Size Exclusion Chromatography Calibration (SECC) curve.

Although various classifications of pore sizes have been given by different authors, it is now generally agreed that pores can usefully be considered to fall into three distinct bands(4):

- Micropores: less than $20 \AA$ in radius
- Mesopores: radiưS between $20 \AA$ and $500 \AA$
- Macropores: radius,greater than $500 \AA$

In order to determine this pore size distribution of any material, the material must be tested by a method which does not alter the PSD during the test. The following techniques are used:

1. Gas adsorption/desorption isotherms(4)
2. Mercury Penetration $(5,6)$
3. Exclusion Chromatography $(3,7,8)$
4. Electron Microscopy $(9,10)$
5. Low angle $X$-ray techniques(11)
6. Permeability to gases(12)

### 8.2. Gas Adsorption/Desorption Isotherms

The amount of particular gas adsorbed by a given solid at a given temperature is dependant on the pressure of that gas.

$$
\begin{equation*}
X_{a}=f\left(P / P_{o}\right) \tag{8.1}
\end{equation*}
$$

where $X_{a}$, is the amount of gas adsorbed $P_{1}$ is the pressure of the gas $P_{0}$, is the saturation vapour pressure of the gas. This adsorption of gas may be expressed as an isotherm where the amount of gas adsorbed is plotted as a function of the relative pressure at a given temperature. The many records of these isotherms have been classified by Brunauer Demming Demming and Teller(13) and also by Brunauer Emmett and Teller(14) The classification was based on the isotherm shape and the three adsorption phenomenon taking place:

- Monolayer adsorption
- Multilayer adsorption
- Capillary condensation within the pores of the material

The isotherms breakdown into five types as shown in Figure 8.1

## TYPE 1

The Type I isotherms are exhibited by microporous materials. Here monolayer physical adsorption takes place. Initially there is a rapid rise in the amount adsorbed up to a limiting value corresponding to monolayer coverage of the surface.

## TYPE II

For a Type II isotherm multilayer adsorption takes place on top of an initial monolayer. This tends to be exhibited by non porous solids.

## TYPE III \& V

In Types III and $V$ the initial forces of adsorption tend to be weak. These isotherms tend to be rare.

## TYPE IV

These isotherms tend to level off near the saturated vapour pressure of the adsorbent. Thereafter it is the final rise in the isotherm that is considered to reflect capillary condensation within porous solids. The presence of the hysteresis loop corresponding to the occurence of mesopores within the solid is often a distinctive feature of the isotherm. Figure 8.2 shows schematically the cross section through a mesoporous adsorbent indicating the extent of coverage by adsorbate at various stages in the isotherm. from Figure 8.2 it can
be seen that initially $(A \rightarrow B)$ monolayer adsorption occurs in the same way as with a non porous material. The step BCD corresponds to the build up of a multilayer, until at ' $D$ ' all the mesoporés are filled with adsorbate molecules in a liquid like state. Between $D$ and $E$ further adsorption may take place but only on the outside grain of the particle. On desorption the isotherm proceeds DFB. as the relative pressure is reduced the adsorbate will evaporate from the meniscus which stretches across the pore.

The Type I-V classification is based on differing isotherm shapes caused by differing structures. For the determination of PSD a classification based on pore size may be more useful. For this purpose the isotherms divide into three groups as shown in Figure 8.3. These three isotherms give classification of materials by the differing adsorption mechanisms that are occurring within the pores. The microporous material show a sharp increase in adsorption, due to the gradual pore filling. This adsorption proceeds until all pores are filled corresponding to the long flat branch of the isotherm. no hysteresis is observed. In the mesoporous system the filling of the pores by adsorbate is not purely by adsorption but also by capillary condensation. the relative pressure at which this takes place depends on pore diameter and shape. The flat branch at high relative pressure indicates complete filling of the pores. The desorption isotherm is also related to the pore size distribution of the material. It is interpretation of these isotherms (Type IV) that is the basis of PSD by Nitrogen adsorption.

### 8.2.1. The Calculation of PSD based on the Kelvin Equation

The hysteresis associated with Type IV isotherms is not seen in microporous materials where the pores are filled at very low relative pressure and in macroporous materials the condensation is often not seen as it takes
place at a relative pressure of near unity. The theory of capillary condensation(4) can be descibed by the Kelvin Equation which relates the relative pressure to the radius of pore entered for a particular system. The equation is given by

$$
\begin{equation*}
\ln \left(P / P_{0}\right)=-2 \gamma V \cos \theta / r R T \tag{8.2}
\end{equation*}
$$

where

- $V$ is the molar volume of the gas
- $\theta$ is the contact angle
- $\gamma$ is the surface tension
$-r$ is the radius of the pore
- $R$ is the Gas constant
- $T$ is the temperature

Several authors have subsequently developed calculation methods relating the volume adsorbed at a particular pressure to the pore size distribution. In 1945 Wheeler (15) recognised that as the pressure increased, not only were different pores filled with condensed gas, but also the thickness of the multilayers on other pores would alter. This was represented by

$$
\begin{equation*}
v_{s}-v=\int_{R}^{\infty}(R-t)^{2} L(R) d R \tag{8.3}
\end{equation*}
$$

where $t$ is the thickness of the adsorbed multilayer, $R$ is the pore radius, $L(R) d R$ is the length of pores of size between $R$ and $R+d R$, and $V$ is the volume of gas adsorbed at a $\left(P / P_{0}<1\right)$ The volume of gas adsorbed at a relative pressure of unity is $\mathrm{V}_{\mathrm{s}}$.

The calculation methods of Barrett, Joyner and Halenda(16) and then Cranston and Inkley(17) developed the process for cylindrical pores. Other
authors(18) made further studies in the calculation for pores of different shapes.

In systems like silica gel where the material is made up of coalesced spherical units or has a sponge like structure the behaviour of the material with regard to capillary condensation in unlikely to resemble cylindrical pores. This is because in many cases the entrances to the pores may be significantly narrower than the cavity itself(19). As condensation is dependent on the radii of the pores then as the relative pressure rises gas will condense at the appropriate radii. However on desorption of the gas, the desorption is limited, and will not occur until the widest channel into the cavity has been emptied. This will lead to adsorption - desorption hysteresis. In Figure 8.4 examples of typical distributions are shown. The relative pressure at which the hysteresis occurs is dependent on the width of the pore entrance. The size of the hysteresis loop is dependent of the internal pore width relative to the pore opening.

### 8.2.2. Thickness of the Adsorbed Layer

Schull(20) showed that the volume adsorbed : volume of monolayer could be plotted as a function of relative pressure then he assumed the packing of the nitrogen to arrive at the thickness of a unimolecular layer of nitrogen on the surface ( $4.3 \AA$ ). Barrett et al(16) used these calculations by Schull but corrected the multilayer adsorption effects to allow for close packing of the nitrogen molecules. The difference between the two treatments is shown in Figure 8.5, where the close packed system has much lower $t$ values, where $t$ is the thickness of the adsorbed layer. In fact for a close packed system where the adsorbate density is the same as that of normal liquid phase the $t$ value for a given amount of adsorbed material can be found from

$$
\begin{equation*}
t=X / S \cdot 10^{4}=M \cdot V_{p} / 22414\left(V_{a} / S\right) \cdot 14 \AA \tag{8.4}
\end{equation*}
$$

Where $X$ is the adsorbed volume of liquid adsorbate per gram of adsorbent (ml), $S$ is the specific surface area of the adsorbent $\mathrm{m}^{2} \mathrm{~g}^{-1}, \mathrm{~V}_{a}$ is the adsorbed volume of adsorbate (ml), $M$ is the molecular weilght of the adsorbate, and $V p$ is the specific volume of the adsorbate $\left(\mathrm{ml} \mathrm{g}^{-1}\right)$. For nitrogen the expression reduces to

$$
\begin{equation*}
t=15.47 \mathrm{~V}_{\mathrm{a}} / \mathrm{S} \AA \tag{8.5}
\end{equation*}
$$

This experimental $t$ curve has been calculated by various workers Barrett et al, de Boer(21), Cranston and Inkley have produced the major work.

### 8.2.3. Limitations

Inherent within the calculation, surface tension $(\gamma)$, contact angle $(\theta)$ and the pore shape are all assumed to be constant.

1. The thickness of the adsorbed layer will obviously effect the calculated radius as

$$
\begin{equation*}
R_{p}=R_{k}+t \tag{8.6}
\end{equation*}
$$

(Rp - pore radius, $t$ - thickness of adsorbed layer, Rk Kelvin Radius) Schull(20) produced a multilayer picture of the adsorbed layer allowing close packing only in the initial layer. Berrett et al (16) adapted this to encompass close packing throughout the multilayer. Necessarily a close packed multilayer will have a different thickness. The determination of this thickness is normally carried out on non-porous solids and exactly how this relates to porous materials will be important. Narrow pores can result in
constrictions being imposed on the adsorbing material.
2. The pore shape will also affect the final result. Other pore systems studied including eg. parallel fissures, spherical cavities or inverse cubics will produce different results(18) the differences in the calculated radii perhaps being as much as two fold.
3. The surface tension is a function of the pore size at values less than $500 \AA$ and at values of less than $50 \AA$ this can introduce large errors.

### 8.2.4. Conclusions

Allowing for the uncertanties involved the method can be used to compare materials with pore sizes in the range of $15 \AA$ to $150 \AA$ with an upper limit of about 400 A. Above this level it is not possible to observe the hysteresis easily as the relative pressures involved are large. The hysteresis loop that appears in mesoporous materials shows that the pores are not uniform in shape. The loop itself is not required, in principle we only require an up turn in the isotherm at high $\mathrm{P} / \mathrm{P}_{\mathrm{o}}$. This up turn in the isotherm may be interpreted in terms of capillary condensation.

It is not necessary for the material to have a high mechanical stability.

### 8.3. Mercury Porosimetry

When compared with gas adsorption for PSD determination, the vital requirement of the Mercury porosimetry method is that the particles must be rigid and able to withstand high pressures. This method of determination of PSD is perhaps the most commonly used. It relies on the fact that when a non-wetting liquid is brought in contact with a porous material the pressure required to force the liquid into a pore is a function of the pore radius. This was first shown by Wasburn $(22,23)$ in 1921 , and quantified by the Washburn Equation.

$$
\begin{equation*}
\Delta P=-2 \gamma \cos \theta / r \tag{8.7}
\end{equation*}
$$

The equation was based on the Young and Laplace equation relating the pressure drop on either side of a curved meniscus to the surface tension and radius of the meniscus(4) In 1945 Ritter and Drake(5) produced the first calculation method from the equation of Washburn. This method assumed that the surface tension between liquid and vapour and also the contact angle were constant throughout the determination. Figure 8.6 illustrates the intrusion of the non-wetting liquid into the pores.

The procedure was derived as follows: Let the total volume of all pores having radii between $R$ and $R+d R$ be

$$
\begin{equation*}
d V=D(r) d r \tag{8.8}
\end{equation*}
$$

where $D(r)$ is the distribution function for the pore size. From the Washburn Eq'n ( $\gamma$ and $\theta$ are constant)

$$
\begin{equation*}
p d r+r d p=0 \tag{8.9}
\end{equation*}
$$

Now by combining the equations, the volume of pores is qiven by

$$
\begin{gather*}
d V=2 \gamma \cos \theta \cdot D(r) \cdot \Delta p / p  \tag{8.10}\\
d V=-D(r) r / p \Delta p \tag{8.11}
\end{gather*}
$$

As mercury is intruded into the porous material at each stage the volume measured is the total pore volume minus that intruded so far (i.e. $V_{0}-V$ )

$$
\begin{equation*}
d(V o-V) / \Delta p=-d V / \Delta p \tag{8.12}
\end{equation*}
$$

and the final equation then becomes

$$
\begin{equation*}
D(r)=p / r \cdot d\left(V_{o}-V\right) / \Delta p \tag{8.13}
\end{equation*}
$$

By differentiation of a plot of $\left(\mathrm{V}_{\mathrm{o}}-\mathrm{V}\right)$ against applied pressure ( p ) is obtained. The plot of $V_{0}-V$ against applied pressure or pore radius is called the cumulative Pore Size Distribution. It is posible to obtain a second plot of $D(r)$ against r. i.e. The Differential Pore Size Distribution. A typical PSD obtained by mercury porosimetry is shown in Figure 8.7.

### 8.3.1. Limitations

The mercury porosimetry method suffers from the following limitations.

1. Assumption of Pore Shape. The pores are assumed cylindrical, however pores with narrow openings (spheroidal cavities or ink bottles) although of large diameter will appear to have the radius of their entrance ports.
2. Assumption of contact angle.
..... Recorded values of mercury on glass vary from 112 degrees to 180 degrees although it is now accepted that the value is in the region 130-142 degrees. The contact
angle tends to vary with temperature, surface roughness, and humidity(24). The effect of variation in $\theta$ is shown in Table 8.1. Incomplete evacuation can result in variation of contact angle. A variation of 1 degree may be responsible for about a $1.5 \%$ error in the results(4)
3. Assumption of Surface Tension. As previously mentioned surface tension may well be a function of pore size at low radius(25).
4. Particle Damage. As the pressures involved in reaching small pores are extremely high (eg. 60,000 psi for $r=15 \AA$ ) and particles are sometimes damaged. This damage can lead to sudden intrusions and thus an over estimate of the volume of pores of a particular small size in the system(25).

### 8.3.2. Conclusions

This method is absolute in that the pore volume is measured directly. It is only the precise relation of the pressure to pore radius which is somewhat variable. Thus although the method is time consuming it can provide a more complete picture of the meso-macro pores within a material. Thus its major drawback is that the particle have to have a high mechanical stability. The method works well within the range from $75 \AA$ up to several thousand angstroms. Finally the material must be reproducibly dried before applying this method.

### 8.4. PSD from Size Exclusion Chromatography

The theoretical basis of SEC has been dealt with in detail in Chapter 4. The retention of a solute is assumed to be entirely due to steric effects. These effects introduce three main variables to the retention.

- The Solute Shape
- The Pore Size and Shape.
- The Pore Size Distribution.

The extent of the interaction between solute and pore structure determines the degree of exclusion by the column. The breakdown of the column volume is shown in Figure 8.8. The column volume $V_{c}$, is made up as follows:

$$
\begin{equation*}
V_{c}=V_{o}+V_{p}+V_{s} \tag{8.14}
\end{equation*}
$$

where $V_{0}$, is the void volume made up of the spaces between the particles and voids within the column. $\mathrm{V}_{\mathrm{p}}$, is the volume of the pores within the packing material and $\mathrm{V}_{\mathrm{s}}$, is the solid volume of the packing material.

The retention is given by $V_{r}$, where:

$$
\begin{equation*}
V_{r}=V_{o}+K . V_{p} \tag{8.15}
\end{equation*}
$$

and $K$, is the distribution coefficient, or permeation coefficient. It is equivalent to the fraction of pore volume which is accessible to the solute.

$$
K=V_{\text {accessible }} / V_{\text {pores }}
$$

In SEC the permeation coefficient is often plotted as a function of solute radius or molecular weight. The well known semi logarithmic plot is often called a Size Exclusion Chromatography Calibration Curve (SECC curve) as shown in Figure 8.9.

In order to determine the SECC Curve molecules of assumed radii are used to probe the pores within the packing material. These solutes are usually random chain polymer molecules such as polystyrenes or polyethylene glycols. As explained in Chapter 4 it is assumed that these polymers are assumed to have a spherical shape. This is only an approximation as the polymers are sufficiently flexible to enter pores with entrances slightly smaller than the average probe diameter.

Probes small enough to enter the entire pore space will elute in a volume,

$$
\begin{equation*}
V_{r}=V_{0}+V_{p} \tag{8.16}
\end{equation*}
$$

Probes too large to enter any of the pores elute in a volume,

$$
\begin{equation*}
V_{r}=V_{0} \tag{8.17}
\end{equation*}
$$

while probes with intermediate size elute in a volume,

$$
V_{r}=V_{0}+K \cdot V_{p}
$$

where $0<K<1$.

### 8.4.1. Solute and Pore Shape

In order to model the SEC process the size and shape of the probe solutes has to be assumed. For the purposes of this method we have assumed, in accordance with previous workers, that the effective radius of the polystyrene molecules is proportional to its hydrodynamic radius. The effective radius is then given by

$$
\begin{equation*}
r / \AA=0.123 \times M w t .^{0.588} \tag{8.18}
\end{equation*}
$$

where Mwt, is the molecular weight of the polymer solute.

In Gas Adsorption methods it is possible to make some comment on the pore shape according to the hysteresis observed in the isotherm. However in Mercury Porosimetry the pores are assumed to be cylindrical. Various models of the pore shapes within silica gels have been described $(26,27)$ however, these shapes make only a small difference to the Exclusion Calibration curves. If cylindrical pores are used as a model then the mathematics involved in the model is much more straightforward. The method of PSD determination of Knox and $\operatorname{Scott}(3)$ used a cylindrical pore network.

### 8.4.2. PSD from SECC Curves using the Knox and Scott Method

In this method polymer solutes are assigned molecular radii as described in the previous section, and the pores are assumed to be cylindrical. In Figure 8.10 an illustration of the solute within a cylindrical pore is outlined.

As before the degree of retention or permeation is given by:

$$
\begin{equation*}
K=V_{\text {accessible }} / V_{\text {pores }} \tag{8.19}
\end{equation*}
$$

For a simple system where the pores are of one size then for a pore of length L , and radius R , as shown in Figure 8.10 The volume of the pores is

$$
\begin{equation*}
V_{\text {pores }}=\pi R^{2} L \tag{8.20}
\end{equation*}
$$

If a solute of radius reenters the pores then the solute is restricted in its volume to which it can move. As outlined in Figure 8.10 the centre of mass of the solute cannot permeate the entire pore space. It can only move to a distance, $r$, from the pore wall. In effect the solute is excluded from this region and thus has access to a volume, $\mathrm{V}_{\text {accessible, }}$ where,

$$
\begin{equation*}
V_{\text {accessible }}=\pi(R-r)^{2} L \tag{8.21}
\end{equation*}
$$

when $r>R$ then $V_{\text {accessible }}=0$. Thus the permeation coefficient is given by:

$$
\begin{gather*}
K=(R-r)^{2} / R^{2}  \tag{8.22}\\
K=(1-r / R)^{2} \tag{8.23}
\end{gather*}
$$

For a series of cylindrical pores of differing radii the expression is summed to give a step function where:

$$
\begin{equation*}
K=\sum_{R \cdot r}^{\infty} f(R)(1-r / R)^{2} \tag{8.24}
\end{equation*}
$$

and $f(R)$ is the fraction of pores of radius $R$. For a continuous function where $f^{\prime}(R) d R$ is the fraction of pores within the range $R$ up to $R+d R$, the permeation coefficent is given by:

$$
\begin{equation*}
K=\int_{\tau}^{\infty} f^{\prime}(R)(1-r / R)^{2} d R \tag{8.25}
\end{equation*}
$$

It should be noted that the summation or integral for any molecular radius $r$, contains pores with radius greater than $r$, and then $K=0$ when the pore radius is less than $r$. The cumulative PSD is given for a step function as:

$$
\begin{equation*}
G(R)=\sum_{0}^{R} f(R) \tag{8.26}
\end{equation*}
$$

and for a continuous distribution function as:

$$
\begin{equation*}
G(R)=1-\int_{0}^{R} f^{\prime}(R) d R \tag{8.27}
\end{equation*}
$$

Knox and Scott differentiated the expression in Equation(8.25) to give an expression where the fraction of each pore in the distribution and hence the cumulative PSD could be described in terms of the permeation coefficient, K .



First differentiation

$$
\begin{equation*}
\frac{d K}{d r}=f(R)[1-r / R]_{R \cdot r}^{2}-\int_{r}^{\infty} 2 / R \cdot f^{\prime}(R)[1-r / R]^{2} d R \tag{8.28}
\end{equation*}
$$

Second differentiation

$$
\frac{d^{2} K}{d r^{2}}=2 / R \cdot f^{\prime}(R)[1-r / R]_{R=r}-\int_{r}^{\infty} 2 / R^{2} f^{\prime}(R) d R
$$

Third differentiation

$$
\frac{d^{3} K}{d r^{3}}=2 / R^{2} \cdot f^{\prime}(R)_{R=r}
$$

In terms of $f^{\prime}(R)$,

$$
\begin{equation*}
f^{\prime}(R)=R^{2} / 2\left(d^{3} K / d r^{3}\right) \tag{8.29}
\end{equation*}
$$

evaluated at $r=R$ this becomes

$$
\begin{equation*}
f^{\prime}(r)=r^{2} / 2\left(d^{3} K / d r^{3}\right) \tag{8.30}
\end{equation*}
$$

The cumulative PSD is then

$$
\begin{equation*}
G(R)=1-\int_{0}^{R} r^{2} / 2 d^{3} K / d r^{3} d r \tag{8.31}
\end{equation*}
$$

On integration of this expression the cumulative PSD is

$$
\begin{equation*}
G(R)=R^{2} / 2 d^{2} K / d r^{2}-R \cdot d K / d r+K \tag{8.32}
\end{equation*}
$$

For convenience the $K$ values are usually plotted against $\log _{\boldsymbol{n}} r$ values rather than $r$ values. The expression then translates to

$$
\begin{equation*}
G(R)=0.5\left[d^{2} K / d(\ln r)^{2}\right]-1.5 d K / d(\ln r)+K \tag{8.33}
\end{equation*}
$$

The above expression relates the cumulative PSD to the permeation coefficient
as a function of $r$. Thus it appears that from a direct plot of permeation coefficient against $\log _{h} r$ and the evaluation of first and second derivatives, the PSD can be calculated.

### 8.4.3. Limitations

On application of Equation (8.33) to the PSD determination it was found that the experimental error in obtaining data points and the large error introduced on differentiation made the method as it stood too sensitive for reproducible determinations to be made.

The experimental work carried out in this Chapter first showed that the assumptions of cylindrical pores and spherical solutes was acceptable and secondly that the PSD could be determined effectively using the Knox and Scott method A series of different methods were tried to produce the PSD from the SECC curve and these are outlined in the following sections.

### 8.5. Validity of SECC Curves for PSD Determination

In order to check that the assumption regarding the use of cylindrical pores was correct, the method was compared with Mercury Porosimetry. If the PSD model of the pores being an assembly of cylinders of different radii was realistic then SECC curves should be readily calculable from the Pore Size Distribution obtained in mercury porosimetry. Equation (8.24) was used to carry out this check

$$
K=\sum_{r}^{\infty} f(R)(1-r / R)^{2}
$$

In Figure 8.11 the experimental SECC Curve and the SECC curve obtained using the PSD experimentally obtained by mercury porosimetry for a silica gel packing material is shown.

From Figure 8.11 it is clear that the assumption of cylindrical pores as a model for the pore structure is indeed a satisfactory one. Mercury porosimetry of a silica gel gave a good agreement with experimental SECC curves. The contact angle between the silica gel and mercury alters the displacement of the curve but not the general shape or gradient of the linear section of the curve.

The method used to determine PSD from SECC curves using the cylindrical pore approximation for the pore structure of the material and the hard sphere for the solute structure must take into account two problems linking the experimental and theoretical values of the permeation coefficient.

Firstly, in deriving the experimental values of the permeation coefficient it is normal to make $K=1$ for the smallest solute, which in these studies was benzene. This solute is assumed to permeate all the pore volume $\left(V_{0}+V_{p}\right)$. However this is not the case as even benzene has a finite radius which makes the values of $r / R$ for the solute/pore system greater than 0 and thus the value of $K$ is accordingly reduced. For example in a system of uniform pores of size $150 \AA$, benzene would have a permeation coefficent of 0.93 . In the modelling of the porous material it must be recognised that some flexibility is required in assigning the theoretical $K$-value to a so-called fully permeating solute.

Secondly, for the large solute molecules that are excluded from the pores of the packing material, the permeation coefficient is nominally assigned as $K=0$. However, this is an over simplification of the system. It is not possible to make a clear distinction between large pores within the particles and the interparticle space that exists between the particles of the packing materials. This problem is clearly indicated in the typical mercury porosimetry trace, shown in Figure 8.7. The first step in the curve is due to the interparticle space
while the second is due to the intraparticle pores. It is commonplace for the distinction between the two types of pore space to be unclear and the two merge into one. This is especially true in HPLC materials where the smallest particles are of the order of $2 \mu \mathrm{~m}$ in diameter. As molecules with small values of $r / R$ are significantly excluded it is necessary when modelling the SECC curve to include pores with diameters at least 10 times larger than the largest probe molecule and these pores may well include part of the extraparticle void space. In such a way the permeation coefficient for the largest solutes is also arbitrary and will depend on the range of pore sizes used in the model. In Figure 8.13 these scaling factors for the value of the permeation coefficient are shown. The expression that relates this rescaling of the $K$ values is:

$$
\begin{equation*}
K_{\mathrm{adj}}=\alpha+\beta \mathrm{K}_{\mathrm{exp}} \tag{8.34}
\end{equation*}
$$

where $K_{\text {adj, }}$ is the altered permeation coefficient, $\alpha$ allows for the permeation of large pores, $B$ allows for the exclusion from small pores and $K_{\text {exp }}$ is the experimentally obtained value for the permeation coefficient. Thus for the largest probe

$$
\begin{equation*}
K_{\text {largest probe }}=\alpha \quad\left(K_{\text {exp }}=0\right) \tag{8.35}
\end{equation*}
$$

and for the smallest probe

$$
\begin{equation*}
\mathrm{K}_{\text {smallest probe }}=\alpha+\beta \quad\left(K_{\exp }=1\right) \tag{8.36}
\end{equation*}
$$

### 8.5.1. The Halasz Method for PSD Determination

The method of Halasz and Martin $(7,8)$ used the SECC curve directly as a measure of the cumulative PSD. For each solute the radius $\mathbf{r}$ was assumed to be the radius of the smallest pore that the solute could enter. In practice these
radii were found to be about 2.5 times the radii given by the van Kreveld(28) calculation. (This does not in itself disprove the Halasz and Martin theory)

The procedure according to Halasz was as follows:

If the elution volume of two solutes of molecular weights $M_{1}$ and $M_{2}$ where $\left(M_{1}>M_{2}\right)$ gave,

$$
\begin{equation*}
\Delta V_{\text {elution }}=\left|V_{1}-V_{2}\right| \tag{8.37}
\end{equation*}
$$

then $\Delta V$, is the difference in the elution volumes of the two solutes. This difference was stated to be equal to the volume of pores in the material that had a size ranging from $r_{1}$ to $r_{2}$. In this way the PSD was built up comparing the difference in elution volumes of different pairs of solutes. An illustrative example of the PSD obtained using this method is outlined in Figure 8.14. This has been the main SECC method to date.

However, this method, where the difference in elution volumes is assumed to be a direct measure of the pore fraction is invalid. Were the key assumption. correct then a material of uniform pore size would have a horizontal exclusion curve. In practice a uniform pore material does not give a horizontal exclusion curve. Figure 8.15 illustrates the different shapes of the PSD curves obtained from the different SEC methods.

The Halasz method does however give a good estimate of the mean pore size, that is the $R$-value when $G(R)=0.5$, which may account for its popularity. However, the complete PSD curve often bears little resemblance to that obtained by mercury porosimetry, and shows a much wider pore size range than that given by mercury porosimetry.

### 8.5.2. Trial Procedures for the Knox and Scott Calculation of PSD.

A number of procedures for obtaining the PSD from the SECC curves have been attempted using the Knox and Scott theory. The degree of success being. determined by the correlation between the experimental SECC curve and the SECC curve obtained using the trial PSD. That is for each solute a trial function, $f(R)$, was developed, from which the $K_{r}$ value for each solute could be calculated.

$$
\begin{equation*}
K_{r}=\sum_{r}^{\infty} f(R)(1-r / R)^{2} \tag{8.38}
\end{equation*}
$$

The calculated $K_{r}$ values were then compared with the experimental $K_{r}$ values and the $f(R)$ adjusted to minimise a control function. The control function used in all calculation models was $V$, which measured the variance of the theoretical from the adjusted $K_{r}$ values.

$$
\begin{equation*}
V=1 / N \sum_{i}^{N}\left(K_{\text {theoretical }}-K_{\mathrm{adj}}\right)^{2} \tag{8.39}
\end{equation*}
$$

where $N$ is the number of solutes examined. In the initial calculations $K_{\text {adj }}$ was simply taken as $K_{\text {exp }}$ but in the more successful later calculations the adjustments to the experimental values were made, allowing adjustment of the $K$ values of very large and very small pores. i.e.:

$$
K_{\mathrm{adj}}=\alpha+\beta K_{\exp }
$$

### 8.5.3. Polynomial fit to SECC Data

Here the experimental SECC curve is fitted by a polynomial of the general form

$$
K_{a d j}=a_{o}+a_{1}(\ln r)+a_{2}(\ln r)^{2}+\ldots+a_{n}(\ln r)^{n}
$$

where $r$ is the solute radius and $K_{\text {adj }}$ is the permeation coefficient. This
polynomial expression is then used to derive the Pore Size Distribution of the material. The method used to derive the PSD is obviously crucial to the results obtained. Warren and Bidlingmeyer(29) used the Halasz assumption and in effect, (as discussed in Section 8.5.1 this simply shifted the the $K$ versus In $r$ curve to higher radii to give the $f(R)$ versus $R$ curve. In spite of this error the work is of interest in showing that it was very difficult to fit a polynomial to a typical SECC Curve. In our method the $K$ versus In r curve: is differentiated according to the equations of Knox and Scott.

The polynomial fits were carried out using the Edinburgh Multi-Access System with standard curvefitting routines( 30,31 ). For the purposes of this method the values of $\alpha$ and $\beta$ of equation (8.34) were taken as 0 and 1 respectively. Polynomials of degrees 3 to 8 were obtained for the SECC data as illustrated in Figures 8.16 and 8.17. As the degree of the polynomial increased progressively better fits were obtained for the experimental data. In Table 8.2 the improvement in fit for increasing degree is listed. However as the degree of polynomial fit was increased, unacceptable waves were then present in the calculated SECC curve.

Having obtained the best fits for the data, the polynomial expression for $\mathrm{K}_{\mathrm{adj}}$ for each fit was differentiated as required by equation (8.33) to give a value for the PSD according to the Knox and Scott method.

$$
\mathrm{G}(\mathrm{R})=0.5\left(\mathrm{~d}^{2} \mathrm{~K} / \mathrm{d}(\mathrm{ln} r)^{2}\right)-1.5 \mathrm{~d} K / \mathrm{d}(\ln r)+K
$$

As the SECC fit improved, the theoretical SECC curve obtained from these expressions developed waves which were unacceptible. Furthermore the differentiation of the expression to obtain the PSD accentuated the waves seen in the original polynomial data. This resulted in unacceptable PSD curves where
values of $G(R)>1$ and $G(R)<0$ were obtained.

It was possible to avoid these unacceptible values by choosing the correct degree of polynomial fit. However, there did not appear to be one particular degree that gave acceptible PSDs for all materials.

In Figures 8.18 and 8.19 the PSD curves obtained are shown for two different gels. The polynomial fit followed by differentiattion was unacceptible as the errors associated with the raw SECC data were exaggerated by the process. If the experimental data were more extensive and accurate it should be possible to obtain polynomial fits without waves.

As will be seen in Section 8.8 the polynomial fit can be modified to produce an acceptible PSD if truncation of the curve is carried out when the $G(R)$ function has a value greater than 1 or less than 0 . This is not satisfactory as a general method to be applied to all cases, even if it does give PSDs in agreement with Mercury Porosimetry in the cases we have examined.

### 8.5.4. Optimisation of Assumed Analytical Expressions for the PSD

Here an optimisation procedure was carried out as outlined in Section 8.5.2 where an analytical expressions for the pore size distribution function was used to give the best fit curve to the SECC data. The best fit was again taken as that which minimised the control function $V$, of equation (8.39). Again the values of $\alpha$ and $\beta$ were taken as 0 and 1. Initial studies used a simple Gaussian as the distribution function, such that

$$
\begin{equation*}
f(R)=1 / \sqrt{2 \pi \sigma^{2}} \exp \left(-(\ln R-\ln \mu)^{2} / 2 \sigma^{2}\right) \tag{8.40}
\end{equation*}
$$

where the SECC data was calculated from the cylindrical pore expression. $f(R)$ was the fraction of pores of radius, $R$. The mean pore radius was, $\mu$, and the
standard deviation was $\sigma$. To obtain different distributions the values of $\mu$ and $\sigma$ could be input by the operator. This method was quite capable of producing different SECC curves, as illustrated in Figure 8.20. It was however, too restrictive to give a general fit and it was not able to fit the experimental data with any real success.

As a way of making the Gaussian curve more flexible, an exponentially modified Gaussian distribution was examined. In this system illustrated in Figure 8.21, a series of Gaussian curves were used. Each curve in the envelope had a general Gaussian shape. The expression describing this type of distribution was:

$$
y=\frac{\exp -(\alpha \beta n) \cdot \exp \left(-(x-\beta \sigma n)^{2} / 2 \sigma^{2}\right)}{\sqrt{2 \pi \sigma^{2}}}
$$

where

- $\alpha$ gives the steepness of fall off of successive gaussians
- $\beta$ gives the number of standard deviations between each gaussian
- $n$ is the number of gaussian curves
$-1 / \sqrt{2 \pi \sigma^{2}}$ is the normalising factor, and
$-e^{-\alpha \beta n}$ gives a measure of the steepness of fall off of the gaussian maxima.

In order to give a complete envelope of a series ofcurves then the separation between individual Gaussian curves must decrease such that $\beta \rightarrow 0$ and $n_{\text {total }} \rightarrow \infty$.

As such the total area under the curve in Figure 8.21 is then given by:

$$
y_{\text {sum }}=\Sigma e^{-\alpha \beta n} e^{\left[-(x-n \beta \sigma)^{2} / 2 \sigma^{2}\right]} \cdot \sum_{n=1}^{\infty} \frac{1}{e^{-\alpha \beta n} \sqrt{2 \pi \sigma^{2}}}
$$

The expression $1 / \Sigma e^{-\alpha \beta n} \cdot / 2 \pi \sigma^{2}$ normalises the area under the curve such that $\int y_{\text {sum }} d x=1$. This corresponds to

$$
\begin{equation*}
\int_{0}^{\infty} f(R) d R=i \tag{8.41}
\end{equation*}
$$

in the expression for PSD.

Now the value of $y_{\text {sum }}$ is a function of $x$ and depends only on $\alpha, \beta$ and $\sigma$. As $\beta$ decreases the terms in the summation become closer and the summation may be expressed as the integral. The $\mathrm{y}_{\text {sum }}$ curve obtained versus x for a series of Gaussians where $\alpha$ represents the skewing factor and $\sigma$ a half width parameter of the function when x is less than zero, i.e. on the Gaussian side of the function. This is illustrated in Figure 8.22. When $\alpha$ is very large the curve will tend to the original gaussian and when $\alpha$ is small a highly skewed distribution is obtained.

The normalised function relating the fraction of each pore, $f(R)$, to the pore radius, $R$, when $\beta \rightarrow 0$ is given by:

$$
f(R)=\frac{\alpha}{\sqrt{\pi \sigma}} \exp -\left[\frac{2 \alpha(\operatorname{Ln} R-\operatorname{Ln} \mu)-\alpha^{2} \sigma}{2 \sigma}\right] \cdot \operatorname{erfc}\left[\frac{\alpha \sigma-(\operatorname{Ln} R-\operatorname{Ln} \mu)}{\sqrt{2 \sigma^{2}}}\right]
$$

The derivation of this expression has been outlined in Appendix 2.1.

The expression contains three adjustible parameters, $\mu$, the position of the maxima of the major gaussian. The width of each gaussian is given by $\sigma$, and $\alpha$ is the factor describing the skewness of the distribution.

Unfortunately this again proved insufficientiy flexible for the PSD fit, and variation of $\mu, \alpha$ and $\sigma$ could not produce an accurate fit to the SECC data. In Figure 8.23 results from a typical material are shown. The best fit to the data was still not satisfactory.

### 8.6. Microcomputer Optimisation of Pore Fractions

The analytical forms were unable to give good correlation to the SECC curves it was decided to use methods where a set of pores of arbitrary radii were selected and the volume fractions of each pore optimised to give a distribution of pores that would give a SECC curve similar to the experimental data. In the initial stage of the attempt the values of $\alpha$ and $\beta$ were 0 and 1 respectively. The equation that was optimised was;

$$
\begin{equation*}
V=\sum_{1}^{N}\left(K_{\exp }-\sum f(R)(1-r / R)^{2}\right)^{2} \tag{8.42}
\end{equation*}
$$

A direct interactive method was first examined where the various trial PSDs were tested. The PSD was built up by the operator around a "mean" pore. This "mean" pore size was the pore size that produced the best single pore fit to the data. The operator was also able to change the pore size distribution by:

1. Altering the fraction of the different pores in the distribution.
2. Altering the number of pores in the distribution.
3. Altering the spacing of the pore radii in the distribution.

The simple flowchart of this interactive program was outlined in Figure 8.24. Several procedural observations were made;

- There was a stage when addition of further pore radii resulted
in no significant decrease in the value of the variance, $V$, described in Equation (8.42)
- The number of pore sizes was limited by the computer time and the number of data points.
- The SECC curves produced were most sensitive to change at the large pore sizes.

The distributions that were obtained for the different materials examined are given in full in Appendix. 2.2

A fully automatic program was then developed from this interactive system. In this program the pores of the distribution were arranged such that they were in geometrical progression. The best distributions obtained are listed in Appendix. 2.3. The flow diagram in Figure 8.25 outlines the procedure used by the automatic program.

During the development of the program the values of $\alpha$ and $\beta$ were 0 and 1 in the early work. However after further development the program was able to take into account the $\beta$ parameter which corrected the data to account for partial exclusion of even the smallest probe solutes. This correction was done after the best single pore had been determined.

As in the earlier interactive program the best single pore fit to the first to be selected; this was radius $R_{o}$. It was obtained on the first pass through the program. In the subsequent rounds new pores were added for each pass through the program until seven pores had been added. These pores were added in the following order:

| Original Pore Radius | $R_{o}$ |
| :---: | :---: |
| Second Pore Radius | $R_{o} \times F$ |
| Third Pore Radius | $R_{o} / F$ |
| Fourth Pore Radius | $R_{o} \times F^{2}$ |
| Fifth Pore Radius | $R_{0} / F^{2}$ |
| Sixth Pore Radius | $R_{o} \times F^{3}$ |
| Seventh Pore Radius | $R_{0} \times F^{4}$ |

When a new pore was added to the distribution the fraction of each pore in turn was altered on passage through the program. On each passage the best pore size distribution was that distribution with the lowest value of the variance, $V$. A typical output from this automatic program is shown if Appendix. 2.4

The ássymetric distribution of pore sizes around the optimum single pore was arrived at from experience of the interactive program, where it had been seen that the pores of small radii had little effect on the SECC curve. The experimental data was usually well fitted at the low molecular weight end, and it was at the high molecular weight end that the greatest flexibility was required.

The common factor, $F$, by which the spacing of the pores in the geometric progression was arranged, was input by the operator and in this way the wider and narrower ranges of pore sizes could be tried. As expected differing materials required differing arrangments of pores. In Table 8.3 the variance for each sample material in the different programs are compared. The single pore is as expected not as good as other fits and the interactive results are obviously the most successful as it was the most flexible routine. The automatic results were successful and produced distributions similar to the
interactive program. The distributions obtained for these materials by either interactive or automatic methods were in very good agreement with the mercury porosimetry results obtained for these materials. The comparison can. be seen in Appendix. 2.5 where the porosimetry results are listed.

These methods were purely iterative and involved repetitive calculation, often examining distributions that were quite obviously incorrect. Although success had been achieved in predicting the PSD further development was still required. This was achieved with the mainframe computer.

### 8.7. Optimisation of PSD using a Mainframe Computer

As explained in the previous section the microcomputer iterative methods used a long calculation procedure with a number of unlikely distributions being tried. In order to make the procedure more efficient a more powerful system was used; the Edinburgh Multi Access System of the Edinburgh Regional Computing Centre. The optimisation program was named "MINUIT", and was developed by James and Roos(32). In its most basic level the program was capable of optimising up to 15 variables.

The control function that was optimised was again the variance, $v$. On this occasion allowances were made for large pores ( $\alpha$ ) and partial exclusion of small solutes ( $B$ ). The expression of the variance was now written as:

$$
\begin{equation*}
\left.\underset{n=1}{V=\sum_{n}^{N}}\left[\left[\sum_{R=r}^{\infty} f(R)(1-r / R)^{2}\right]-\left(\alpha+B K_{\text {ex }}\right)\right]\right]^{2} \tag{8.43}
\end{equation*}
$$

where $N$, is the number of solutes and $r_{n}$ is the radius of solute $n$. The above equation is merely an expansion of the expression where

$$
\begin{equation*}
V=\sum_{n=1}^{N}\left[\sum_{R=r}^{\infty}\left(K_{\text {theoretical }}-K_{\text {exp }}\right)\right] 2 \tag{8.44}
\end{equation*}
$$

Now optimisation was also made not only of the $f(R)$ but also of $\alpha$ and $\beta$. The
pore sizes for each distribution were in geometrical progression as in the previous method. The sizes for each sample could have been optimised using the "MINUIT" program, but for ease of computing the pore sizes used for the automatic micro-computer method were used. Therefore in this system we optimised $\alpha, \beta$ and the seven pore fractions. As $\sum f(R)=1$ only six pore fractions were optimised, the seventh being

$$
\begin{equation*}
F_{7}=1-\left(F_{1}+F_{2}+F_{3}+F_{4}+F_{5}+F_{6}\right) \tag{8.45}
\end{equation*}
$$

Eight parameters were optimised. The conditions being that

$$
0<F_{i}<1 \quad \text { for } i=1,7
$$

The initial values of the variables were given to the computer together with limits within which the final values should fall. In Appendix 2.6 a listing of the computer program is given, together with the output for the different materials.

The program used two main optimisation routines:

### 8.7.1. SIMPLX Optimisation Routine

The SIMPLX subroutine calculates a series of simplices to minimise a function in $N$ variables. For this particular operation $N$ was eight. A simplex is a figure in N -dimensional space defined by a convex hull of $(\mathrm{N}+1)$ points. For example a triangle in two dimensions and a tetrahedron in three dimensions. Each point represents one possible set of N parameters and corresponds to a single value of V .

In order to construct the initial simplex, $S_{0}$, from which the minimisation procedure starts, a particular set of the N parameters is chosen to define a starting point $P_{0}$. The remaining $N$ points are then found by proceeding from $P_{o}$ in the directions of the N co-ordinate axes and determining the positions of
minimum V in each of these N directions.

At each performance of the subroutine a new simplex, $S_{i}$, is generated from simplex $S_{(i-1)}$. The routine first of all identifies $P_{w}$ which has the highest value of $V$. The point $P_{w}$ is then reflected in the $(N+1)$ dimensional hyperplane through the remaining $N$ points of the simplex to give $P^{*}$, and $V\left(P^{*}\right)$ is calculated.

Various alternatives are then open depending upon whether or not $V$ is sufficiently reduced by the procedure. These procedural options are ranked in the following order:

1. Replace $P_{w}$ by $P^{*}$
2. If $\mathrm{V}\left(\mathrm{P}^{*}\right)$ is not a sufficient improvement on $\mathrm{V}\left(\mathrm{P}_{\mathrm{w}}\right)$ then find the point $P^{* *}$ on the line $P_{w} P^{*}$ which gives a minimum $V$; replace $P_{w}$ by $P^{* *}$
3. If $\mathrm{V}\left(\mathrm{P}^{* *}\right)$ is not a sufficient improvement on $\mathrm{V}\left(\mathrm{P}_{\mathrm{w}}\right)$ then find the point $\mathrm{P}^{* * *}$ on the parabola through $\mathrm{P}_{\mathrm{w}} \mathrm{P}^{*} \mathrm{P}^{* *}$ which has a minimum $V$; replace $P_{w}$ by $P^{* * *}$
4. If improvement is still not sufficient then chose the point $P_{L}$ giving the lowest $V$ in the simplex and construct a new simplex with all the dimensions reduced by a factor of 2 .
5. If improvrement still inadaquate then construct a new simplex starting at $P_{\mathrm{L}}$.

Following the above routines successive simplices produce a convergence of V towards a minimum and the algorithm terminates when the values of $V$ at the
$P_{w}$ and $P_{B}$ (the worst and best points of the simplex) coincide to within the required accuracy.

### 8.7.2. MIGRAD Optimisation Routine

The second minimisation subroutine which was used was named "MIGRAD". This was a second order "steepest descents" algorithm. From a starting set of parameters giving a particular value of V , the routine calculates matrices of gradients and second derivatives of.$V$ with respect to the $N$ parameters. From this the direction of steepest descent and the estimated position of the minimum $V$ are found. The procedure is then repeated starting at this new position in the $N$-dimensional parameter space. "MIGRAD" tends to find a local minimum and is most useful when performed after the completion of the simplex routine.

As a final check the routine moves a large distance from the previously found minimum to check that there are no better solutions. In practice the SIMPLX subroutine provided satisfactory minima and the MIGRAD used subsequently produced only marginal improvements.

### 8.8. Discussion

The Halasz method of PSD determination has been widely used for silica gel packing materials(29,33-37). Our work has been aimed at the correction of the error embodied in the method of PSD determination from SECC data of Halasz and Martin $(7,8)$. The effect of the error is shown in Figure 8.26 where the PSDs obtained from three different methods are compared. As outlined earlier the Halasz method merely involves the displacement of the SECC curve to values of pore radii that are around 2.5 times greater than the molecular radii. In Figure 8.26 the PSD derived by the Halasz method is shown to be very much wider than the PSD obtained from mercury porosimetry or that obtained using
the Knox and Scott method. The reason for this difference has already been explained. The Halasz method could however be used to give a starting point for selecting the trial pore radii $R_{o}$ used in the computer methods described above. In that the mean pore diameter is approximately $2.5-->3$ times the radius of the molecules for which $K_{\text {exp }}=0.5$. Table 8.4 demonstrates this difference in pore sizes.

The initial attempts to fit analytical expressions to the SECC data so that they could be twice differentiable over the range of the SECC curve proved unsuccessful due to the lack of flexibility of these methods. However these methods did bring out the fact that the curve was much more difficult to fit at high molecular weights than at low molecular weights. The modelling methods using the BBC microcomputer were not particularly efficient but they did give the PSD and not simply a displacement of the SECC curve. The non-interactive method of PSD determination described in Section 8.6 also gives a good correlation to mercury porosimetry. Further comparison in Figures 8.27 to 8.33 shows that the more powerful mainframe computer also gave good agreement with the mercury porosimetry methods.

In Figure 8.27 to 8.30 a number of different pore structures were observed. The experimental gels based on Hypersil all show very narrow PSDs with $95 \%$ of the pores within a ten fold range. Lichrosphere Si1000 has a wider distribution but the agreement between mercury porosimetry and the computer method is still good. The Zorbax PSM materials in Figures 8.31 and 8.32 show a very wide distribution with pores stretching over a 50-fold range. This difference in pore structure arises from the different methods of production.

Two samples showed poor agreement Vydac and WP1004, the computed PSD covered a higher range of pore sizes than the mercury intrusion method.

This is shown in Figures 8.33 and 8.34. An explanation for this may be the presence of ink-bottle pores, i.e. pores where the mean diameter of the pore is greater than the entrance diameter. In such conditions the mercury porosimetry would tend to give higher porportions of smaller pores.

The method of polynomial fitting which produced waved SECC curves and PSD curves with $G(R)<0$ and $G(R)>1$ has already been discounted as an unsatisfactory technique. However, if the PSD curve calculated from the polynomial fit is truncated when it exceeds the bounds of the cumulative PSD function, it is possible to obtain PSD curves in good agreement with the computed PSD. This agreement is illustrated for two materials in Figure 8.35.

### 8.8.1. Summary

- The Halasz method has been shown to be based upon a fundimentally incorrect premise.
- The iterative method of PSD determination on the BBC microcomputer gives good agreement with mercury porosimetry.
- The optimisation routine using a mainframe computing system gives good agreement with mercury porosimetry.
- The different silica gels studied had both narrow and wide PSDs, this difference in distribution did not affect the agreement between the methods.
- The determination of PSD by curvefitting polynomials was possible, although not satisfactory.
- The determination of PSD by SECC methods gives a measure of the PSD of the column itself rather than a "representative" sample which mercury porosimetry would measure.


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The five isotherm types





The Schematic filling of pores.



Stages in adsorption of vapor and filling of pores with liquid with increasing parial pressure.

Taken from reference 2

FIGURE 8.3

The different isotherms for materials with differing pore sizes.

: A . Vitrogen sotphon isotherms at 77 K on a purely (a) microporous, (b) mesoporous and (c) macroporous silica.

Taken from reference 1


Pore size distribution from the adsorption isotherm of nitrogen at $-195^{\circ}$
(a) Adsorption isotherm. O, adsorption; © desorption. (b) Pore size distribution curve.



Pore size distribution from the adsorption isotherm of nitrogen at $-195^{\circ}$
(a) Adsorption isotherm. O, adsorption; ©, desorption.
(b) Pore size distribution curve.

Distribution of nitrogen molecules according to Schüll


Distribution of nitrogen molecules according to Barrett


Taken from reference 5

Intrusion of Mercury into a porous structure



FIGURE 8.7

## High Pressure Mercury Porosimetry Curve for PR-179



Variation in Pore Radius for several values
of contact angle

| Pressure <br> psi | Pore Radius for contact angle of: |  |  |
| :---: | :---: | :---: | :---: |
|  | $112^{\circ}$ | $140^{\circ}$ | $180^{\circ}$ |
|  | A | A | A |
| 25 | 20840 | 42680 | 55680 |
| 100 | 5210 | 10670 | 13920 |
| 500 | 1040 | 2135 | 2780 |
| 1000 | 521 | 1067 | 1392 |
| 2000 | 260 | 533 | 696 |
| 5000 | 104 | 214 | 278 |
| 10000 | 52 | 107 | 139 |

Taken from Reference 1.


FIGURE 8.9

SECC Curve


Illustrative diagram of a spherical solute within a cylindrical pore.



## Scaling of a SECC Curve




FIGURE 8.15

Illustration of the way that the PSD determined by HPSEC varies with the calculation method.
1-SECC Curve
2-Knox and Scott PSD
3-Halasz and Martin PSD


POLYNOMIAL FIT DEGREE 3
POLYNOMIAL FIT DEGREE 4
GEL: HR-WPS-2


POLYNOMIAL FIT DEGREE 7
GEL: HR-TPS-2


POLYNOMIAL FIT DEGREE 5
GEL: HR-WPS-2


POLYNOMLAL FTT DEGREE 8
GEL: HR-MPS-2


POLYNOMIAL FIT DEGREE
GEL: PSM1000


POLYNOMIAL FIT DEGREE 6
GEL: PSM1000


POLYNOMIAL FIT DEGREE 4
GEL: PSM1000


POLYNOMIAL FIT DEGREE 9 GEL: PSM1000


POLYNOMIAL FTT DEGREE 5
GEL: PSM1000


POLYNOMIAL FIT DEGREE 8
GEL: PSM1000


The value of the control function, V , for curvefitted data

| Degree | PSM 1000 | $H R-W P S-2$ |
| :---: | :---: | :--- |
| 3 | $4.72 \times 10^{-2}$ | $4.36 \times 10^{-2}$ |
| 4 | $1.86 \times 10^{-2}$ | $3.04 \times 10^{-2}$ |
| 5 | $1.54 \times 10^{-2}$ | $2.47 \times 10^{-2}$ |
| 6 | $1.43 \times 10^{-2}$ | $1.21 \times 10^{-2}$ |
| 7 | $1.28 \times 10^{-2}$ | $1.15 \times 10^{-2}$ |
| 8 | $1.28 \times 10^{-2}$ | $1.00 \times 10^{-2}$ |

Pore Size Distribution Curve
from Polynomial Degree-4
Sample HR-WPS-2


Pore Size Distribution Curve
from Polynomial Degree-5
Sample HR-WPS-2


Pore Size Distribution Curve from Polynomial Degree-6

Sample HR-WPS-2


Pore Size Distribution Curve from Polynomial Degree-8

Sample HR-WPS-2


Pore Size Distribution Curve
from Polynomial Degree-4
Sample PSM1000


Pore Size Distribution Curve
from Polynomial Degree-5
Sample PSM1000


Pore Size Distribution Curve
from Polynomial Degree-6
Sample PSM1000


Pore Size Distribution Curve from Polynomial Degree-8

Sample PSM 1000





Results of attempt to fit the SECC curve with a modified Gaussian.


## Interactive Program



## Non-interactive Program



|  | PORE SPACING FACTOR |  |  |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| SAMPLE | SINGLE <br> PORE | MANUAL <br> FIT | F=0.25 | F=0.50 | F=0.75 | F=1.00 | F=1.50 | EMAS <br> FIT |
| Hypersil | 42 | 29 | 28 | 28 | 28 | 27 | 28 | 20 |
| Vydac | 118 | 108 | 107 | 108 | 110 | 110 | 111 | 74 |
| Lichro | 119 | 39 | 81 | 43 | 33 | 38 | 40 | 34 |
| PSM60 | 852 | 92 | 556 | 299 | 121 | 125 | 132 | 102 |
| PSM1000 | 177 | 49 | 101 | 47 | 49 | 57 | 73 | 38 |
| WP1004 | 276 | 66 | 188 | 93 | 62 | 50 | 59 | - |
| PR183 | 61 | 45 | 47 | 45 | 45 | 44 | 45 | 42 |
| PR179 | 106 | 26 | 36 | 34 | 43 | 44 | 57 | 22 |
| SSP501 | 255 | 160 | 126 | 90 | 97 | 98 | 100 | - |
| PSM50 | 461 | 37 | 265 | 85 | 40 | 42 | 53 | - |
| PSM1500 | 156 | 2 | 77 | 25 | 21 | 24 | 35 | - |
| $732 H K 2 a$ | 367 | - | 350 | 350 | 350 | 350 | 350 | - |
| HRWPS2 | 42 | 23 | 27 | 25 | 23 | 24 | 29 | 24 |
| HRWPSU | 156 | - | 154 | 157 | 159 | 162 | 168 | 99 |

various different methods used. All data was obtained using
a BBC Microcomputer except the "EMAS FIT" which was obtained
with the mainframe system.
This table gives the values of the control function, $V$, for

FIGURE 8.26

Comparison of PSD Curves obtained for Silica Gel HR-WPS-2 using
three different methods
$\triangle$ SECC Curve
O Computer Predicted PSD
$\square$ Mercury Porosimetry PSD
$\triangle$ PSD from Halasz Method


Ln Solute Rad r/A
Ln Pore Rad R/A

| Material <br> (Figure) | Mean Solute Radius $\bar{r} / A$ <br> (a) | Mean Pore Radius $\overline{\mathrm{R} / \AA}$ |  | Halasz Factor$H=\bar{R} / \bar{r}$ |
| :---: | :---: | :---: | :---: | :---: |
|  |  | by Hg Porisimetry <br> (b) | by Computation <br> (b) |  |
| Hypersi] | 45 | 110 | 110 | 2.4 |
| PR179 | 95 | 230 | 270 | 2.9 |
| PR183 | 70 | 280 | 180 | 2.7 |
| WP-10-04 | 65 | 130 | 220 | 3.3 |
| HR-WPS-2 | 70 | 270 | 270 | 2.9 |
| Lichrosphere Si1000 | 165 | 300 | 470 | 2.8 |
| Vydac | 80 | 140 | 280 | 3.5 |
| PSM60 | 25 | 60 | 60 | 2.3 |
| PSM1000 | 170 | 630 | 630 | 3.7 |

(a) $\bar{r}$ is value of $r$ at which $K(r)=0.5$
(b) $\bar{R}$ is value of $R$ at which $G(R)=0.5$

FIGURE 8.27

## SECC and PSD Curves for Hypersil <br> $\triangle$ SECC Curve <br> O Computer Predicted PSD <br> $\square$ Mercury Porosimetry PSD



Ln Solute Rad r/A Ln Pore Rad R/A

SECC and PSD Curves for Silica Gel HR-WPS-2
$\triangle$ SECC Curve
O Computer Predicted PSD
$\square$ Mercury Porosimetry PSD


## SECC and PSD Curves for Silica Gel PR-179 <br> $\triangle$ SECC Curve <br> O Computer Predicted PSD <br> $\square$ Mercury Porosimetry PSD



Ln Solute Rad r/A
Ln Pore Rad R/A

FIGURE 8.30

## SECC and PSD Curves for Lichrosphere Si-1000 <br> $\triangle$ SECC Curve <br> O Computer Predicted PSD <br> $\square$ Mercury Porosimetry PSD



FIGURE 8.31

SECC and PSD Curves for Silica Gel PSM1000
$\triangle$ SECC Curve
O Computer Predicted PSD
$\square$ Mercury Porosimetry PSD


## SECC and PSD Curves for

 Silica Gel PSM 60$\triangle$ SECC Curve
O Computer Predicted PSD
$\square$ Mercury Porosimetry PSD


FIGURE 8.33

SECC and PSD Curves for Silica Gel Vydac
$\triangle$ SECC Curve
O Computer Predicted PSD
$\square$ Mercury Porosimetry PSD


Ln Solute Rad r/A
Ln Pore Rad R/A

FIGURE 8.34

SECC and PSD Curves for Silica Gel WP-10-04
$\triangle$ SECC Curve
$\square$ PSD from Mercury Porosimetry
O PSD from BBC Microcomputer


Ln Solute Rad r/A
Ln Pore Rad R/A
Permeation Coefficient, K

© Cumulative PSD, G(R)

000 INSd

Z-SdM- CH


Permeation Coefficient, K

## CHAPTER 9

## CONCLUSIONS

During this work a number of different aspects of the production, development and characterisation of silica gel packing materials for use in High Performance Liquid Chromatography were studied. The work has been undertaken as current trends in HPLC required the use of wide pore packing materials for improved separation in polymer and biopolymer analysis.

In the first part of this research existing silica gel materials were modified by hydrothermal treatment to produce wider pored packing materials. The effect of hydrothermal treatment on the structure of these materials was recorded. Following on from this, hydrothermal treatment was used as a method of improving silica hydrogel strength and then combined with sintering of the silica gel in air to give a packing material with wider pores ( $300-800 \mathrm{~A}$ in diameter). This packing material could be made in a variety of particle sizes with sufficient mechanical strength to make them suitable for HPLC. The improvement in the stability of the silica hydrogel enabled xerogels to be produced with a higher pore volume than previously attainable. The hydrothermal treatment enabled significant increase in pore diameter to be obtained without less in pore volume while the sintering of the material altered the strength of the silica gel particles.

The materials with high pore volume and wide pores were chromatographically tested by High Performance; Size Exclusion Chromatography of polystyrene standards. This revealed firstly that the materials did possess a high pore volume, than the existing material examined. From the HPSEC it was also evident that the mean pore diameter of the materials produced in this work were significantly
increased during the production method. Furthermore the Pore Size distribution was narrowed considerably by the production method.

In the second part of the work, a method for the determination of HPLC packing material pore size distribution was developed. This was primarily developed to give a better characterisation of the experimental materials produced. This method used Size Exclusion chromatography, where the retention of a solute is proportional to its size to determine the Pore size Distribution (PSD). The method was initially calculated by hand but during the course of the project computer routines to carry out the calculations required were developed. The technique enabled the PSD of the material in the column rather than a "representative" sample to be determined. Results obtained by this method were in excellent agreement with high pressure mercury porosimetry a major method currently used for PSD determination. The method developed is cheap, quick and simple to use and furthermore provides a PSD for the packing material in its working environment without the use of extremely high pressures as in mercury porosimetry.

Appendix

Fortran program for the calculation of B.E.T. Surface Area

C PROGRAM DESIGNED TO INPUT DATA OBTAINED FROM THE C SURFACE AREA MACHINE STORED IN ROOM 254 AND THEN C CALCULATE THE SURFACE AREA OF THE SAMPLE USING THE C B.E.T. METHOD C THIS PROGRAM ALSO STORES THE DATA REQUIRED FOR THE C B.E.T. PLOT FROM WHICH THE SURFACE AREA IS CALCULATED C FILENAME - GRAPH
C THE INFORMATION REQUIRED FOR AN ADSORPTION ISOTHERM PLOT
C IS ALSO STORED
C FILENAME - ADSORP
C
INTEGER D,B1,C1
DIMENSION XVAL(25),YVAL(25)
CALL EMASFC('DEFINE',6,'5,.IN',5)
CALL EMASFC('DEFINE',6,'6,.OUT',6)
CALL EMASFC('DEFINE',6,'7,.LP20',7)
CALL EMASFC('DEFINE',6,'21,GRAPH,, $\left.\mathrm{C}^{\prime}, 10\right)$
CALL EMASFC('DEFINE',6,'22,ADSORP,,C',11)
CALL FPRMPT('RUN NO.?',8)
READ $(5,509) \mathrm{D}, \mathrm{B} 1, \mathrm{C} 1$
509 FORMAT(A4,A4,A2)
WRITE $(7,716) \mathrm{D}, \mathrm{B} 1, \mathrm{C} 1$
716 FORMAT(////,30X,' $X X X X X X X X X X X X X X X X X X X X X X X X X X X X X X X X X^{\prime}, /$ .30X,' $X$ ',31X,' $X$ ',/,30X,' $X$ CALCULATED DATA FOR BET $X ', /$
.30X,' $X \quad$ RUN NO. ',A4,A4,A2, $7 \times,{ }^{\prime} X^{\prime}, /, 30 X,{ }^{\prime} X^{\prime}, 31 X,{ }^{\prime} X^{\prime}, /$
$\left.30 \times,{ }^{\prime} \times X X X X X X X X X X X X X X X X X X X X X X X X X X X X X X X X ', / / / / / /\right)$
WRITE $(7,717)$
717 FORMAT(1X,'INPUT DATA',/,1X,'----------',
CALL FPRMPT('PD ? ', 3)
READ*,PD
WRITE $(7,701)$ PD
701 FORMAT(1X,'PRESSURE IN DOZER = ',F5.1,'MV')
CALL FPRMPT('PO ? ',4)
READ*,P0
WRITE $(7,702)$ PO
702 FORMAT(1X,'P0 = ',F6.3,'MV')
CALL FPRMPT('SAMPLE WEIGHT ? ',14)
READ*,W
WRITE $(7,703)$ W
703 FORMAT(1X,'SAMPLE WEIGHT = ',F6.4,'G')
CALL FPRMPT('ROOM TEMPERATURE ? ',11)
READ*,T 1
WRITE $(7,704)$ T1
704 FORMAT( $1 \times$, 'ROOM TEMPERATURE $={ }^{\prime}, F 5.1,{ }^{\prime}{ }^{\prime}$ ')

```
    CALL FPRMPT('VO ? ',4)
    READ*,V0
    WRITE(7,650) V0
    650 FORMAT(1X,V0 = ',F5.3,'ML')
    CALL FPRMPT('NO. OF DOSES ? ',9)
    READ*,NDOZES
    WRITE(7,706) NDOZES
    706 FORMAT(1X,'TOTAL NO. OF DOSES = ',I2)
    WRITE(7,715)
    715 FORMAT(1X,////)
    CUMDO=0.0
    CONSTS=(273.0/T1)*(8.75/760.0)*(1.0/W)
    WRITE(7,710)
    710 FORMAT(1X,'DOZE NO. DOZE VOL. PN GAS IN DOZE NO. CU
    .M DOZE AMOUNT ADS. VOL ADS(STP) X Y')
    WRITE(7,711)
    71 FORMAT(1X,' /ML /MV /ML
        ./ML /MLMV /MLG**-1',/)
        DO 50 J=1,NDOZES
        CALL FPRMPT('VD PN ?',6)
        READ*,VD,PN
        AGD=(VD*PD)-(VD*PN)
        CUMDO=CUMDO+AGD
        AGLISV=V0*PN
        AMGA=CUMDO-AGLISV
        VOLAD=AMGA*CONSTS
        X=100.0*PN/PO
        Y=1000.0*PN/((P0-PN)*VOLAD)
        WRITE(7,705) J,VD,PN,AGD,CUMDO,AMGA,VOLAD,X,Y
    705 FORMAT(5X,I2,7X,F5.2,5X,F5.2,9X,F8.2,8X,F8.2,5X,F8.2,11X,F6.2,14X,
        .F5.2,6X,F5.2)
        WRITE(21,721) X,Y
    721 FORMAT(1X,F5.2,1X,F5.2)
    WRITE(22,722) X,VOLAD
    722 FORMAT(1X,F5.2,1X,F6.2)
        XVAL(J)=X
        YVAL(J)=Y
    50 CONTINUE
        XSUM=0.0
        YSUM=0.0
        XYSUM=0.0
        X2SUM=0.0
        CALL SUM(XVAL,XSUM,NDOZES)
        CALL SUM(YVAL,YSUM,NDOZES)
        CALL SQSUM(YVAL,XVAL,NDOZES)
        CALL SQSUM(XVAL,XVAL,NDOZES)
        CALL SUM(YVAL,XYSUM,NDOZES)
        CALL SUM(XVAL,X2SUM,NDOZES)
C
C TO EVALUATE THE GRADIENT
C
    GRAD=((FLOAT(NDOZES)*XYSUM)-(XSUM*YSUM))
    ./((X2SUM*FLOAT(NDOZES))-(XSUM**2))
```

C
c to evaluate the constant
C
CONST=(YSUM-(GRAD*XSUM))/NDOZES WRITE $(7,5)$ GRAD,CONST
5 FORMAT(1X,////,5X,'Y=',F10.3,5X,'X + ',F10.3)
GRAD $=$ GRAD/ 10.0
CONST=CONST/ 1000.0
$\mathrm{V}=1.0 /(\mathrm{GRAD}+\mathrm{CONST}$ )
$\mathrm{A}=4.35 * \mathrm{~V}$
WRITE $(7,12)$ V,A
12 FORMAT( $1 \mathrm{X}, / / / / /, 5 \mathrm{X}, \mathrm{V}=\mathrm{V}, \mathrm{F} 10.3, '$ SURFACE AREA $=$ ',F10.3,'M**2G-1') STOP
END
SUBROUTINE SUM (VAL,VSUM,N)
C THIS ROUTINE SUMS THE CONTENTS OF THE ARRAY VAL, OF LENGTH N,
C AND PUTS THE RESULT INTO VSUM
C
DIMENSION VAL(N)
DO $1 \mathrm{I}=1, \mathrm{~N}$
VSUM=VSUM + VAL(I)
1 CONTINUE
RETURN
END
SUBROUTINE SQSUM (SOVAL1,SQVAL2,N)
C
C
C This routine takes the PRODUCT OF THE ELEMENTS IN ARRAYS SQVAL1 AND
C SQVAL2 UP TO INDEX N AND STORES EACH ONE
AS A MEMBER OF THE ARRAY SQVAL. 1
C
DIMENSION SOVAL1(N),SOVAL2(N)
DO 1 I=1,N
SQVAL1(1)=SQVAL1(I)*SQVAL2(I)
1 CONTINUE
RETURN
END

## BBC Basic program for the calculation of B.E.T. Surface Area

```
VDU23,225,0,0,126,126,126,126,0,0
FEM SURFACE AREA FROGFIAM
REM ALTERED FDF NEW FFEESSURE TRANSDUCEF
FEM FFKOGRAIH------BETAFEA
FEM
    DIM X(40):DIM Y(40):DIM V(40):DIM E(40) :DIM VOL(4O)
    T=O: B=0
    Cl.S
INFUT"DATE. .",D;
IF LEN(D:5)>3 THEN130
SOUND1,-15,53,20
gOTO 90
INFUT"SAMPLE NUMEER...",A末
IF LEN(AF)>S THEN170
SDUND1,-15,53,20
GOTO 130
INFUT"INITIAL DOSER FFESSSURE";FD
INFUT"VAF' FRESSURE DF NITF:OGEN",FO
INFUT"SAMFLE HEIGHT (GFAMS)";SW
IMFUT"FOOM TEMFERATURE (K)";FOOMT
INFUT"SAMFLE VOLUME(ML)";SV
INFUT"NO. OF DOSES MADE..";ND%
CONST = (273/FOOMT )*(38.55/760)
CONST=CONST* (1/SW)
FOF J%=1 TO NT%
    INFUT"DOSE VOL. (ML)";V(J%)
    INFUT"EQ. FRESSURE (MV)"; E (J%)
    AGD=(V(J%)*FD)-(V(J%)*E(J%))
    CUMDO=CUMDO+AGD
    AGLISV=SV*E (J%)
    AMGA=CUMDD-AGLISV
    YOLAD=AITGA*CONST
    X(J%)=100*E (J%)/FO
    Y(J%)=1000%E (J%)/((FO-E (J%))*VOLAD)
    VOL (J%)=VOLAD
    NEXT J%
    FOF I=1 TO ND%
        SUMX=SUMXX+X (I)
        SUHTY=SUMY+Y(I)
        XY:=X(I)*Y(I)
        SUMXY=SUHAXY+XY
        SQX=SQX+X(I)<2
        NEXT I
        T=(ND%*SLMAXY) - (SUMAX*SUNV)
    E=(HD%*SQX)-(SUMX`2)
    GFAOD=T/E
FEFH Calculation of area from gradient.
INCFT=(SUMY-(GRAD*SUHX))/ND%
    grdnt=GRAAD: intcpt=INCF'T
    G=GFiAD/10
    INCFT = INCFTT / 1000
    A=4.35*(1/(G+INCPT))+0.5
    G=INT (A)
    MODEO
    VDUZ
    FFINT TAB(2O);"---------------------------------------------------
    FFINT TAE(5);DF;TAE(25);"SAMFLLE NUMEEF ":AF
    FRINT TAE(2O);"
    FEINT
        FRINT"Initial Doser Fressure........";FD;"mv"
        FFINT"Vapour Fressure of Nitrogen..";FO;"mV"
        FFi|NT"Sample Weight (g)..............";SW
        FRINT"Fioom Temperature (K)..........."; FOONT
    FFINT"Sample Volume (ml).............";SV
    Ffint"Number of Doses...............";'ND%
```

```
    GEO FTINT
    G70 FFiNNT
    GBO FFINTTAE(1)"DOSE VOl."TAB(14);"Eq. Fressure"TAE(34);"X";TAE(50);":";TrE(G
4);"OO1 Ad="
    6?O m-1:INT:FRINT
    OO fr
    710 FRINT: e%=131850
    7O FOOR H=1 TO ND%
    7SO FRINTTAE(S);V(M);TAE(18);E(11);TAB(SJ);X(M);TAE(48);Y(H);TAB(6S);VOL(M)
        HEXT H
    7EO FISNNT:G%=10
    760 FFIHT"---
    770 F-RINT"SURFACE AFEA......";A;"sq. metres per gram"
    7OO FRENT
    7% FRINT"
    BOG FRINT:FFINT:FFINT
    81O VDUF
    B20 11ODEO
    gFO vDU4
    840 CLS
    650 K%=0
    EGO HLOAD codeFX
    870 Y%=5
    800 FFINTTAB(15,5)"Do you Fieuqire a plot?"
    890 FRINTTAB(15,8) "B.E.T. FLOT --Enter 1"
    900 FFINTTAB(15,10)"ISOTHEFM --Enter 2"
    710 FriNTTAE(15,15) "No Flot --Enter 9"
    g20 INFUT" 1,2 or 9 ? "nmbr
    90 Cl_S
    740 IF nmbr=1 GOT0970
    950 IF nmbr=2 GOTO1230
    760 IF nmbr=9 G0T01690
    770 FROCchart
    QGO FFINTTAB(2O,\Omega)"B.E.T. FLOT
    9 9 0 ~ F F I N T T A B ~ ( 2 0 , 4 ) ~ " - - - - . - - - - - - - - - - " ,
1000 FFINTTAB(38,30) "Fielative Fressure 100:F/Fo"
1010 FFRINTTAB (5,27) "0.0"
1000 FRINTTAE (2,10) "A":FRINTTAB(2,11)"d":FFINTTAB(2,12)"s"
10%0 FRINTTAB (2,13)"0":FFINTTAB (2,14)"r":FFINTTAB (2,15)"p"
1040 PFINTTAB(2,16)"t":FRINTTAB(2,17)"i":FRINTTAB(2,18)"o"
"0
1060 FOF: I%=1 TO ND%
1070 YDUS:MOVE(X(I%)*40+94), (Y(I%)*40+118)
1080 FRINTCHFRE (225)
10%0 NEXT I%
1100 MOVE O,O
1.10 FEEM FLOT LINE
1120 Y1=(intcpt*40)+110: %1=110
1.80 X2=1110
11.40 T=grdnit*25+intcept
1150 Y 2=T*40+110
11&0 HOVEX1,Y1:DFAWX2,Y2
11%% vou2S,1,0;0;0;0;
118O CALLEAOO
1190 VDU23,1,1;0;0;0;
1200 YDU4
1210 CLS
12%O GOTO880
1230 VDU4
```

```
1240 CLS
1%50 x%=0
1:60 *LDAD codeFX
1270 %%=5
12GO FFOCchart
1290 FFINTTAB (20,3) "Adsorption Isotherm"
1200 FFINTTAB (20,4) "-..--.-------------
1310 FRINTTAR (3O,SO)"Fi, "ative Fressure 100mF;Fo"
1צ20 FRINTTAB(2,3)"V":FFRINTTAB(2,4)"o":FRINTTAB(2,5)"l":FRINTTAB(2,b)"u":FRINTT
AE:2,7)"m":FFINTTAE (2, 日) "e"
    1830 FRINTTAE(2,10)"A":FFINTTAB(2,11)"d":FFINTTAB(2,12)"5"
    1.34 FRINTTAB(2,13)"口":FRINTTAB(2,14)"r":FRINTTAE (2,15)"b"
    150 FRINTTAE (2,16)"e":FRINTTAE (2,17)"d"
    186O FOR J%=1 TO ND%
    1%70 VNW=VDL(J%)*BOO/VOL (ND%)
    1>EO FHAX=1/((FD-E (ND%)/E(ND%))* (FO/E (ND%))*grdnt+intcpt)
    1%%O IF E(J%)<=0 THEN 1400 ELSE 1410
    1400 FNW=0:GOTO1420
    1410 FNN=1/((FO-E(J%)/E(J%))*(FO/E (J%))*grdntrintcpt)*(1000/FHAX)
    1420 UDUS:MOVE (X(J%)*40+94),(VNW+118)
    14.0 FFINTCHF*(225)
    1440 NEXTJ%
1.&ETO MOYE11O,110
14A0 VISMAX=(4000/(FO-4))*(1/(grdnt*(400/FO)+intcpt))
1470 FISMAX=E(ND%)/FO
14E0 FORFISO= 0.01 TO 4 STEF 0.01
1450-VISO=((1000*FISO)/(FO-FISO))/((grdnt*(100*FISO/FO)+int[Pt))*gOO/VISMAX
    FIST=(FISO/FO)*1000/FISMAX
            DFAWFIST+110,VISO+110
        NEXT
    CALL&AOO
    CHAIN"HEADEF":END
    DEFFROCchart
    MOVE 10,10:DFAW1O,960
    MOVE 10,10:DRAW1210,10
    MOVE10,960: DFAN1210,960
    DFAN1210,10
    HIVE 100,100: DFAW100,900
    MOVE100,100: DFAWW1100,100
    FORI%=100 TO 1100 STEF 100
        MOVEI%,105: DFAWI%,90
        NEXTI%
        FOFI%=100 TO 900 STEF 80
        MOVE1OS, 1%: DRAW9O, 1%
        NEXTI%
        ENDFFROC
    1670 GOTO1540
```

$B B C$ Basic program for the calculation of Pore Volume.

```
    10 FEM --- FOFE VOLUME CALCULATION FGF
    2O FEM --- LOW FFESSUFE MEFICUFY FOFOSIMETFIY
    O
    40
5 0
60 INFUT"DATE "D$
70 INFUT"SAMFLE ND. "S$
80 CLS
90 INFUT"Weight of tube "wt
100 INFUT"Weight + silica gel "ws
110 INFUT"WEight + glass "wg
120 INFUT"Weight + mercury "whg
13O INFUT"Calibration mark: "cm
140 INFUT"Mercury Level "ml
150 INFUT"Mark: Level "mb:
16O FEEAD LO,L1,L2,LS,L4
170 DATA 0. 9395,0.8376,0.7373,0.6356,0.5342
100 FEAD VO1,V12,V2S,VZ4
190 DATA 0.1017,0.100S,0.1017,0.1014
2OO FEAD HO1,H12,H2S,H34
210 DATA 1.681,1.720,1.708,1.692
220 CLS
2%0
240 IF cm=1 THEN FFOCpore(L1,VO1,HO1)
250 IF Cm=12 THEN FFOCpore(L1,V12,H12)
260 IF cm=2 THEN FFOCpOre(L2,V12,H12)
270 IF cm=2\Xi THEN FROCpore(L2,V2S,H2S)
200 IF cm=S THEN FFOCpore (LS,V2S,H2S)
290 IF Cm=S4 THEN FFOCpore(LS,VS4,HS4)
300 IF cm=4 THEN FFOCpore(L4,VJ4,HS4)
30
320 FV=porvol/(ws-wt)
30 VDU2
S40 FFINT:FFINT:FFINTTAE(10)"Date":FRINT
S50 FFINTTAB(10)D*
300 FFINT:FFIINT:FFIINTTAB(10)"Sample Number":FFINT
\Xi70 FRINTTAB(10):S车
SBO FFIINT:FFIINT
390 @%=131850
400 FFINTTAE(10) "Fore Volume ":FV: "cc/g"
410 G%=10
420 VDUS
4.0 FESTORE
440 INFUT"Another Sample ? (Y/N) "w$
450 IF W专="Y" THEN 10
460 END
470 DEF FFROCpOrE ( }x,y,z
480 VOL=((whg-wg)/1S.594)+((wg-wt)/2.2)
490 extra=((ml-mb)/z)*y
500 porvol=(x+extra)-VOL
510 ENDFFRC
5 2 0 ~ E N D
```


## DERIVATION OF MODIFIED GAUSSSIAN EXPRESSION

For a typical peak shown in Figure 8.21,

$$
Y_{n}=\frac{\exp [-\alpha \beta n] \exp \left[-(x-\beta n \sigma)^{2} / 2 \sigma^{2}\right]}{\sqrt{2 \pi \sigma^{2}}}
$$

$-e^{\alpha \beta n}$ represents the fall off of the maxima of successive gaussians

- n is the number of gaussian curves
- $\alpha$ gives the steepness of fall off
- $\sigma$ is the standard deviation of the gaussian peak
- $B$ gives the distance between successive gaussians.
$-1 \sqrt{2 \pi \sigma^{2}}$ is a normalising factor

As the distance between successive gaussian curvess gets smaller i.e. $\beta \longrightarrow 0$ and the number of curves increases $n_{\text {total }}{ }^{-->\infty}$ the envelope under the curves may be given as the sum of all the curves so that

$$
Y_{\text {sum }}=\frac{\sum_{n=0}^{\infty} e^{-\alpha n \beta} e^{-(x-\beta n \sigma)^{2} / 2 \sigma^{2}}}{\sqrt{2 \pi \sigma^{2}} \cdot \sum_{0}^{\infty} e^{-\alpha n \beta}}
$$

The expression $\left(e^{-\alpha \beta n}\right) \sqrt{2 \pi \sigma^{2}}$ normalises the area under the curves, so that

$$
\int y_{\text {sum }} d x=1
$$

The value of $y_{\text {sum }}$ is now a function of $x$ and depends on the values of $\alpha, \beta$ and $\sigma$.
As $\beta$ gets smaller the terms of the summation get closer together and the sum may be replaced an integral.

The final plot of $y_{\text {sum }}$ versus $x$ illustrated in Figure 8.22 will now be a function of $\alpha$ and $\sigma$ only. The area under the curve will be unity. $\alpha$ becomes the skewing factor and $\sigma$ a half width of the portion of the curve at $x<0$. When $\alpha$ is very large the slope is similar to the original Gaussian and when $\alpha$ is $\tilde{s} m a l l$ we get a peak with an exponential tail.

The Denominator of the expression.

$$
\begin{aligned}
\sqrt{2 \pi \sigma^{2}} \sum_{n=0}^{n=\infty} e^{-\alpha n \beta} \rightarrow & \sqrt{2 \pi \sigma^{2}} \int_{0}^{\infty} e^{-\alpha n \beta} d n \\
& =\frac{\sqrt{2 \pi \sigma^{2}}}{\alpha \beta}
\end{aligned}
$$

## The Numerator of the expression.

$$
\begin{aligned}
& \sum_{n=0}^{n=\infty} e^{-\alpha n \beta} e^{-(x-\beta n \sigma)^{2} / 2 \sigma^{2}} \\
& \int_{0}^{\infty} e^{-\left(\frac{\left.2 \sigma^{2} \alpha \beta n+x^{2}-2 \beta \sigma x n+\beta^{2} \sigma^{2} n^{2}\right)}{2 \sigma^{2}} d n\right.} \\
&\left.\longrightarrow \int_{0}^{\infty} e^{-(A}+2 B n+n^{2} n^{2}\right) d n
\end{aligned}
$$

where

$$
\begin{gathered}
A=x^{2} / 2 \sigma^{2} \\
B=\left(\alpha \beta \sigma^{2}-\beta \sigma x\right) / 2 \sigma^{2} \\
B=(\alpha \beta \sigma-\beta x) / 2 \sigma \\
C \neq \beta^{2} / 2=\beta / \sqrt{2}
\end{gathered}
$$

$A+2 B n+C^{2} n^{2}$ may be written as

$$
(C n+B / C)^{2}+\left(A-B^{2} / C^{2}\right)
$$

The numerator may then be written as

$$
\begin{aligned}
& =\int_{0}^{\infty} \exp \left[-(C n+B / C)^{2}-\left(A-B^{2} / C^{2}\right)\right] d n \\
& =\exp \left[-\left(A-B^{2} / C^{2}\right)\right] \cdot \int_{0}^{\infty} \exp \left[-(C n+B / C)^{2}\right] d n \\
& =1 / C \exp \left[-\left(A-B^{2} / C^{2}\right)\right] \int_{B / C}^{\infty} \exp \left[-(C n+B / C)^{2}\right] d(C n+B / C)
\end{aligned}
$$

Let $z=(C n+B / C)$, then

$$
\begin{aligned}
& (C n+B / C) \text {, then } \\
& =1 / C \exp \left[-\left(A-B^{2} / C^{2}\right)\right] \int_{B / C}^{\infty} e^{-z^{2}} d z
\end{aligned}
$$

Combining the numerator and the denomenator

$$
Y_{S U T}=\frac{\alpha \beta}{\sqrt{2 \pi \sigma^{2}} C} \exp \left[-\left(A-B^{2} / C^{2}\right)\right] \int_{B / C}^{\infty} e^{-z^{2}} d z
$$

Now by replacing the substitution $A, B$ and $C$, we get the following expression.

$$
Y_{\text {Sum }}=\frac{\alpha}{\sqrt{\pi \sigma}} \exp \left[-\left(\frac{2 \alpha x-\alpha^{2} \sigma}{2 \sigma}\right)\right] \int_{\left(\frac{\alpha \sigma-x}{\sqrt{2} \sigma}\right)}^{e^{-z^{2}} d z}
$$

The value of $x$ is the distance from the maximum value of $y_{\text {max }}$ along the
$x$-axis. In the use of this expression the $y_{\text {max }}$ at the mean pore size, $\mu$, and the pores are arranged in a geometrical progression so that for the purposes of this application $x$ may be replaced by the expression ( $\operatorname{Ln} \mathrm{R}-\operatorname{Ln} \mu$ ) where R is the pore size and $\mu$ is the mean pore size. The final expression as quoted in Chapter. 8 is then;

$$
f(R)=\frac{\alpha}{\sqrt{\pi \sigma}} \exp \left[-\left(\frac{2 \alpha(\ln R-\ln \mu)-\alpha^{2} \sigma}{2 \sigma}\right)\right] \text { erfc. } \frac{\alpha \sigma-(\ln R-\ln n \mu)}{\sqrt{2 \sigma}}
$$

```
ClS
VDU 23,224,24,60,102,195,195,102,60,24
UDU 23,225,0,60,102,195,195,102,60,0
VDU23,226,0,0,24,60,60,24,0,0
VDU23,227,0,0,0,24,24,0,0,0
DIM K(50):DIM RDUS(50):DIM W(50):DIM C(5O)
DIM PT (SO), ECK(SO),FORE (5O),FR(50)
N=1:VV=0.00S
INPUT"Run Number...." RUND%
INPUT"Name of datafile..?"N:
Y=OPENIN NF
INPUTEY,N:
PRINT N*
REPEAT
    INPUTEY,K(N),W(N)
            RDUS (N)=EXP(W (N))
IF K(N)}>=1.0001 THEN 23
@%=131850
    PRINT TAB(10); K(N);TAB(3O);W(N)
G%=10
N=N+1
UNTIL EOFEY
CLOSEf Y
PRINT N-1
NN=N-1
@%=10
V=10
FOR SIZE=3 TO & STEFO.1
    TVAR=0
        FGZ=EXF(SIZE)
    FOR N=1 TO NN
            PT(N)=RDUS (N)/PSZ
            VAR=O
            IF FT (N)>=1 THEN 350 ELSE 360
            PT (N)=0:GOTO 370
            PT (N)=(1-PT(N))心2
            VAR=(K(N)-PT (N))^2
                    TVAR=TVAR+VAR
            NEXTN
    IF TVAR<U THEN 42O ELSE 470
    PRINTV,TVAR
    V=TVAR
    FOR N=1 TO.NN
            ECK(N)=FT (N)
            PORE(1)=PSZ:FR(1)=1
            NEXT
            NEXTSIZE
NUM%=1
VDU2
PRINTTAB(5)"****************************************"
PRINTTAB (5) "*****************************************"
PRINTTAB(5) "****";TAB(41)"****"
PRINTTAB (5) "****"; TAB (18);N#; TAB(41) "****"
PRINTTAB (5) "****";TAB (41) "****"
PRINTTAB (5) "*****************************************"
PRINTTAB(5) "******************************************"
PRINT:PRINTTAB(5);"Fiun Number...";RUND%
PRINT:PRINT
VDU3
OTO 840
    CLS
    NFUT"NO. of pore sizes.?" "NUM%
FOR S=1 TO NUM%
    INPUT"Pore size.? "POFE(S)
    INPUT"Fraction.? "FR(S)
    NEXTS
    TDEV=0
FOR N=1 TO NN
            DEV }=
    SUM2=0
    FOR S=1 TO NUM%
            PT2=0
            PT2=RDUS (N)/PORE (S)
            IF PT2>=1 THEN 750 ELSE760
            PT2=0 : GOTO 770
            PT2=FF(S)*((1-PT2)^2)
            SUM2=SUM2+PT2
            NEXTS
    (N) =SUM2
    DEV=(K(N)-C (N))^2
```

```
                                    TDEV=TDEV+DEV
        NEXT N
    V=TDEV
FEM AXIS
    MODEO
    MOVE 100,100
    DRAW 100,920
    MDVE 910,100
    DRAW 100,100
    FOR I%=100 TO 910 STEFGO
        MDVE I%,100
        DRAW 1%,80
        NEXTI%
    FOR I%=100 TO 920 STEF100
        MOVE1OO,I%
        DRAW 80, I%
        NEXT I%
    VDU 5
    MOVE 80,80:PFINT"O.0"
    MOVE 480,GO:PRINT"O.5"
    MOVE 650,30; PRINT"K-Coefficient"
    MOVE 880,80:PRINT"1.0"
    MOVE 10,110:PRINT"O.0"
    MOVE 10,510:PRINT"4.0'
    MOVE O,710:FRINT"LM.R""
    MOVE 10,910:FRINT"8.0"
    VDU4
    FRINTTAE(15,1)"S E C Calibration Curve"
    PRINTTAB (50,1)"FILE.. "N直
    PRINTTAB (50, 2) "Vari ance="V/NN
    FOR S=1 TO NUM%
        FRINTTAB(5O,3+S)FORE(S)" "FR(S)
        NEXTS
    UDU 5
    FOR N%=1 TO NN
                MOVE (K(N%)*BOO+92),(W(N%)*100+116)
        FRINT CHR名(22S)
        NEXT
    FGR N%=1 TO NN
        MOVE (C (N%)*800+92),(W(N%)*100+116)
        PRINT CHR:$(226)
    NEXT N%
    VDU4
    IF (V/NN)}>VV\mathrm{ GOTO 1340
    VV=V/NN
    SOUND1,-15,53,10
    SOUND1, -15,69,10
    SOUND1,-15,81,10
    SOUND1,-15,101,10
    SOUND1,-15,81,10
    SOUND1,-15,69,10
    SOUND1,-15,53,10
    VDU23,1,0;0;0;0;
    REF'EAT:UNTIL GET=32
    VDU23,1,1;0;0;0;
    VDU28,61,31,79,10
    INPUT"Printed Dutput?"CF
    IF C&="Y" THEN1400 ELSE1560
    VDUZ
    PRINTTAB(5);"VARIANCE=";V/NN:PFINT
    PRINT:PRINTTAB(10);"PORE SIZE";TAB(25);"PEFCENTAGE"
    FRRINTTAB(10);"-----------";TAB.(25);"--------------
    FOR S=1 TO NUM%
    RINTTAB(14);(INT(PORE (S) +0.5)); TAB(29); INT((FR(S)*100)+0.5
450 NEXTS
460 PRINT
4 7 0 ~ P R I N T T A B ( 2 ) ; ~ " K - E X P T " ; T A B ( 2 0 ) ; " K - C A L C " ; T A B ( 3 B ) ; " S D L U T E ~ R A D I U S " ,
480 PRINTTAB(2);"------";TAB (20);"------";TAB(38);"----------------
490 @%=&2030A
1500 FOR N%=1 TO NN
1510 FRINTTAB(3);K(N%);TAB(21);C(N%);TAB(42); INT (EXP(W(N%))+0.5)
    NEXT N%
530 PRINTTAE(15);"
```



```
                                    ": FRINT
```

$\qquad$

```
540 UDU3
550 @%=10
1560 INFUT"Continue.? "T索
1570 IF T:="Y" GOTO 610
1580 VDU26
```

| MB5S2 Percentage |  |  |
| :---: | :---: | :---: |
|  | Pore Diameter | Percentage |
|  | 400 | - 5 |
|  | 350 | 5 |
|  | 300 | 20 |
|  | 250 | 45 |
|  | 200 | 20 |
|  | 150 | 5 |
| PR183 |  |  |
|  | Pore Diameter | Percentage |
|  | 850 | 2 |
|  | 700 | 5 |
|  | 550 | 5 |
|  | 400 | 20 |
|  | 250 | 40 |
|  | 150 | 26 |
|  | 100 | 2 |
| WP 1004 |  |  |
|  | Pore Diameter | Percentage |
|  | 700 | 7 |
|  | 500 | 17 |
|  | 350 | 11 |
|  | 250 | 30 |
|  | 125 | 30 |
|  | 75 | 5 |
| Lichrosphere |  |  |
|  | Pore Diameter | Percentage |
|  | 2500 | 1 |
|  | 2000 | 4 |
|  | 1500 | 6 |
|  | 1000 | 13 |
|  | 600 | 40 |
|  | 400 | 20 |
|  | 200 | 16 |
| Hypersil Percentage |  |  |
|  | Pore Diameter | Percentage |
|  | 100 | 5 |
|  | 150 | 85 |
|  | 200 | 5 |
|  | 250 | 5 |
| PSM 60 |  |  |
|  | Pore Diameter | Percentage |
|  | 1500 | 5 |
|  | 1000 | 10 |
|  | 500 | 10 |
|  | 100 | 30 |
|  | 50 | 30 |
|  | 20 | 15 |


| PSM 1000 |  |  |
| :---: | :---: | :---: |
|  | Pore Diameter | Percentage |
|  | 3000 | 1 |
|  | 2000 | 5 |
|  | 1000 | 25 |
|  | 650 | 40 |
|  | 300 | 14 |
|  | 400 | 15 |
| SSP 501 |  |  |
|  | Pore Diameter | Percentage |
|  | 640 | 5 |
|  | 540 | 5 |
|  | 440 | 25 |
|  | 340 | 50 |
|  | 240 140 | 10 5 |
| PR179 |  |  |
|  | Pore Diameter | Percentage |
|  | 680 | 10 |
|  | 580 | 10 |
|  | 480 | 15 |
|  | 330 | 35 |
|  | 230 | 25 |
|  | 140 | 5 |

```
REM DISTRIEUTION
    REH Pores incremented by 0.75
    REM Log units **HRFSD6**
REM HR 12/02/8S
REM UPDATAED 19/03/85
REM
TIME=0
DIM KEXP(50):DIM RDUS(50):DIM WDTH(50):DIM CALCK(50)
    DIM PORE(30):DIM FR(30):DIM KCHEW(50)
    DIM SUM1(100):DIM BCK(50):DIM TK(50):DIM FRI(50)
    N=1
    INFUT"Kun Number..." RUND%
    INPUT"Name of datafile..?'N*
    Y=OPENIN NS
INFUTEY,N:
FRINT N:
REFEAT
    INPUTEY,KEXP(N),WDTH(N)
IF KEXF(N)>=1.0001 THEN 250
E%=131850
    FFINT TAB(10); KEXF(N);TAB(30);WDTH(N)
6%=10
N=N+1
UNTIL EOFEY
CLOSEf Y
PFINT N-I
8%=10
REM Calculation for single pore.
LET V=2
PRINT
vDU3
    PRINT TIME/100;"SECONDS"
FOR SIlE=3 TO 8 STEF 0.1
    SQDEV=0
            FOR NN=1 TO N
            RDUS(NN)=EXP(HDTH(NN)
            PSIZE=EXP(SIZE)
                IF PSIZES=RDUS(NN) THEN 390 ELSE400
                CALCK (NN)=0:G0T0410
            CALCK(NN)=(1-(FDUS(NN)/PSIZE))^2
            DEVIAT = (KEXP (NN)-CALCK (NN) ):2
                IF KEXF(NN)>=2 THEN 440 ELSE 430
            SQDEV=SQDEV +DEVIAT
                DEVIAT=0
            NEXI NN
        F SQDEV<=V THEN470 ELSESIO
        Y=SQDEV
        BESTSI=SIZE
        FORE(1)=PSILE
            FK(1)=1
        DEVIAT=0
            FOR G=1 TO N
            BCK(G)=0
            NEXT G
        NEXT SIZE
        FRINI TIME/100;"SECONDS"
        YDU2
        FRINTTAE(10);"Run Number....";FUND%
        FRINT:PRINT
        FRINTTAB(10); *****************************************"
        FRINTTAB(10);"***";TAB(24);N%;TAB(47);"***"
        FRINTTAR(10);"*****************************************"
        PRINTTAR(4);"Logn Fore Size";TAE(23);"Pore Size";TAB(37);"DEVIATION":FRINI
        e%=131850
        FFINT TAB(10); BESTS2;TAB(25);FORE(1);TAB(40);V
        FORE(2)=EXP((LN(PORE(1))+0.75))
        FORE (3)=EXF((LN(PORE(1))-0.75))
        FORE(4)=EXP({LN(PORE(1))+1.5))
        FORE(5)=EXP((LN(PORE(1))-1.5))
        FORE(6) EXP((LN(PORE(1))+2.25))
        FORE(7)=EXP((LN(PORE(1))+3.0))
        FEAD NUMP%.
        DATA 1,PORE(2),Y,Y
        DATA 2,PORE (3), Y,Y
        DATA 3,PORE (4), Y,Y
        DATA 4,PORE(5),Y,Y
        DATA S,FORE(b),Y,Y
        DATA 6,PORE(7),Y,N
        READ HIGHF%
        REM Attempt to find best pore size
        VLUE=2
        e%=10
```

```
FOR A=1 TO NUMF%
    PORI=PORE (A)
    FF!(A)=FR(A
    NF=HIGHP%
    FOR DST=0.01 TO 0.35 STEF 0.01
        TVRT=0
        IF OST>FRI(A) GOTO 1210
        FOR B=1 TO N-1
            PTI=RDUS (B)/NF
            |F FTI>=1 THEN 930 ELSE 940
            FII=0:GOIO 950
            PTI=0ST*((1-PT1)^2)
            PT2=RDUS(B)/PORI
            IF PT2>=1 THEN 970 ELSE 980
            PT2=0:60T0 990
            PT2=(FRI(A)-DST)*((1-PT2)*2)
            SUMM=0
            FDR M=1 TO NUMP%
                    IF FORE(M)=PORI GOTO1070
                    PT3=RDUS (B)/PORE (M)
                    IF PTJ>=1 THEN 1040 ELSE 1050
                    FT3=0:G0T01060
                    PT3=FR(M)*((1-PTJ)^2)
                    SUMM=SUMM+PIJ
                    NEXT M
            BCK(B)=SUMM+FT2+FT!
            VRT=(KEXF(B)-BCK(B))^2
            TVRT = TVRT + VRT
            NEXT E
            TVRT=TVRT/(N-1)
            IF TVRT<U THEN 1140 ELSE 1200
            FOR B=1 TO N-I
                    TK(B)=BCK(B)
                    NEXT B
            V=TVRT
            AA=A
            VLUE=1:BDST=DST:BNP=NF
            NEXT DST
    NEXT A
    IF VLUE<>1 THEN 1230 ELSE 1240
    FRINT"The variance has not improved.":GOTO 1490
    FORE (NUMF%+1)=ENF
    SOUND 1;-15,33,20
    FR(AA) =FRI (AA)-RDST
    FR(NUMF%+1)=BDST
    SOUND 1,-15,149,20
    READY$
    IF Y$="Y" THEN 1310 ELSE 1490
    PRINTTAB(5);"
            "------------------------------
                            __"
    FRINTTAB(5);"#--VE---"च
    FRINTTAB(S); "------------------------------------------------------
    FRINTTAB(6);"Pore Size";TAB(26);"Percentage"
    PRINTTAB(6);"---------";TAB(2b);"---..------""
    FOR M=1 T0 NUMP%+1
        FRINTTAB(10);INT(FORE(M)+0.5);TAB(30);INT((FR(M)*100)+0.5)
        NEXT M
    FRINTTAB(5);"--------K.TAB(18);"CALC-K";TAB(J1)"nSOL.RAD"
    PRINTIAR(5);"EXP-K*;TAB(1B); CALC-K;'TAB(31)",
    PRINITAB(5);"-----";TAB(18);"------";TAB(31);
    E%=131594
    FOR B=1 TO N-1
        FRINTTAB(6);KEXP(B);TAB(20);TK(B);TAB(33);INT(RDUS(B)+0.5)
        NEXT B
    FRIHTTAB(5);
```

$\qquad$

```
                            --"
    PRINTTAB(5);
    e%=10
    READV:
    IF V$="Y" GOTO 720
```

| MB5U |  |  |
| :---: | :---: | :---: |
|  | Pore Diameter | Percentage |
|  | 602 | 89 |
|  | 772 | 1 |
|  | 468 | 10 |
| MB5S2 |  |  |
|  | Pore Diameter | Percentage |
|  | 245 | 90 |
|  | 518 | 4 |
|  | 115 | 0 |
|  | 1096 | 2 |
|  | 54 | 4 |
| 732HK2a-H3 |  |  |
|  | Pore Diameter | Percentage |
|  | 403 | 94 |
|  | 854 | 6 |
| PSM 1500 |  |  |
|  | Pore Diameter | Percentage |
|  | 602 | 74 |
|  | 1274 | 4 |
|  | 284 | 3 |
|  | 2697 | 7 |
|  | 134 | 11 |
|  | 5710 | 1 |
| PSM 50 |  |  |
|  | Pore Diameter | Percentage |
|  | 49 | 65 |
|  | 104 | 0 |
|  | 23 | 13 |
|  | 221 | 0 |
|  | 11 | 4 |
|  | 468 | 14 |
|  | 992 | 4 |
| PR183 |  |  |
|  | Pore Diameter | Percentage |
|  | 245 | 94 |
|  | 665 | 2 |
|  | 90 | 1 |
|  | 1808 | 3 |


| WP1004 |  |  |
| :---: | :---: | :---: |
|  | Pore Diameter | Percentage |
|  | 221 | 75 |
| --- - | 601 | 7 |
|  | 81 | 1 |
|  | 1635 | 6 |
|  | 22 | 10 |
|  | 4447 | 1 |
| Lichrosphere |  |  |
|  | Pore Diameter | Percentage |
|  | 602 | 80 |
|  | 1274 | 2 |
|  | 284 | 1 |
|  | 2697 | 6 |
|  | 134 | 10 |
|  | 5710 | 1 |
| Hypersil |  |  |
|  | Pore Diameter | Percentage |
|  | 148 | 94 |
|  | 403 | 6 |
| PSM 60 |  |  |
|  | Pore Diameter | Percentage |
|  | 99 | 65 |
|  | 210 | 0 |
|  | 46 | 1 |
|  | 445 | 0 |
|  | 22 | 18 |
|  | 943 | 0 |
|  | 1998 | 16 |
| PSM 1000 |  |  |
|  | Pore Diameter | Percentage |
|  | 665 | 66 |
|  | 1096 | 9 |
|  | 403 | 0 |
|  | 1808 | 7 |
|  | 244 | 16 |
|  | 2980 | 2 |

Vydac
Pore Diameter ..... 299 ..... 86 ..... 383 ..... 0

$$
232
$$

$$
492
$$ ..... 2

$$
181
$$

$$
632
$$ ..... 632 ..... 5

SSP 501
Pore Diameter Percentage
365 ..... 61
601 ..... 7

$$
221
$$

$$
992
$$ ..... 

134 ..... 18
1635 ..... 3
PR179
Pore Diameter Percentage
330 ..... 73
594 ..... 0
200 ..... 10
897 ..... 12
121 ..... 3
1480 ..... 2
Fun Number. ... 303
********************************
*** ${ }^{W P 1004}$ Logn 5.400 221.40 O
$5.400 \quad 221.406 \quad 0.047$


| Pore 8120 |  | Parcentage |
| :---: | :---: | :---: |
| 221 |  | 93 |
| 601 |  | 7 |
| EXP-K | CALC-K | sol. RAD |
| 0.00 | 0.00 | 1033.00 |
| 0.00 | 0.00 | 536.00 |
| 0.07 | 0.01 | 354.00 |
| 0.11 | 0.02 0.13 | 279.00 $1=1.00$ |
| 0.21 0.25 | 0.13 0.25 | 151.00 <br> 117.00 <br> 1 |
| 0.36 | 0.37 | 97.00 |
| 0.43 | 0.48 | 72.10 |
| 0.50 | 0.54 | $\stackrel{62.00}{5700}$ |
| 0.54 | 0.57 | 57.00 |
| 0.64 | 0.70 | 36.00 24.00 |
| 0.71 0.82 | 0.86 | 17.00 |
| 0.86 | 0.90 | 11.00 |
| 0.89 | 0.94 | 7.00 |
| 0.93 1.00 | 0.95 0.99 | 6.00 2.00 |
|  |  |  |
|  |  |  |
|  | Porcentage |  |
| Pore Sizo |  |  |
| 221 601 | $\frac{82}{7}$ |  |
| 601 91 |  |  |
| Exp-K | CALC-K | SOL. RAD |
| 0.00 | 0.00 | 1033.00 556.00 |
| 0.00 .0 .07 | 0.00 0.01 | - 354.00 |
| 0.11 | 0.02 | 279.00 |
| 0.21 | 0.12 | 151.00 |
| 0.25 0.36 | 0.23 0.34 0.3 | 117.00 91.00 |
| 0.36 0.43 | 0.34 0.43 | 71.00 72.00 |
| 0.50 | 0.49 | 62.00 |
| 0.54 | 0.52 | 57.00 |
| 0.64 0.78 | 0.66 | 30.00 24.00 |
| 0.71 0.62 | ${ }_{0}^{0.84}$ | 17.00 |
| 0.86 | 0.89 | 11.00 |
| 0.89 | 0.93 0.94 | 7.00 6.00 |
| 0.95 1.00 | 0.98 | 2.00 |


|  |  |  |
| :---: | :---: | :---: |
| Pore Stze |  | Percentage |
| 221 |  | 76 |
| 601 |  | 7 |
| ${ }_{161}^{165}$ |  | 11 |
| Exp-k | CALC-K | SLL. RAD |
| 0.00 | 0.01 | 1033.00 |
| 0.00 | 0.03 | \$56.00 |
| 0.07 | 0.05 | 354.00 |
| 0.11 | 0.06 | 279.00 |
| 0.21 | 0.16 | 151.00 |
| 0.25 | 0.27 | 117.00 |
| 0.36 | 0.37 | 91.00 |
| 0.43 | 0.46 | 72.00 |
| 0.50 0.54 | 0.51 0.54 | 62.00 57.00 |
| $\stackrel{0}{0.64}$ | 0.67 | 37.00 |
| 0.71 | 0.78 | 24.00 |
| 0.82 | 0.84 | 17.00 |
| 0.86 | 0.89 | ${ }^{11.00}$ |
| 0.89 | 0.93 | 7.00 |
| 0.93 | 0.94 | 6.00 |
| 1.00 | 0.98 | 2.00 |
|  |  |  |
|  |  |  |
| Pore size | Percentage |  |
| 221 | $\begin{aligned} & 76 \\ & 7 \\ & 1 \\ & 6 \\ & 10 \end{aligned}$ |  |
| ${ }_{61}^{601}$ |  |  |
| 81 1635 |  |  |
| 29 |  |  |
| Exp-K | CALC-K | SQL. RAD |
| 0.00 | 0.01 | 1033.00 |
| 0.00 | 0.03 | 556.00 |
| 0.07 | 0.05 | 354.00 |
| 0.11 0.21 | 0.16 0.16 | 279.00 151.00 |
| 0.25 | 0.27 | 117.00 |
| 0.36 | 0.37 | 97.00 |
| 0.43 | 0.45 | 72.00 |
| 0.50 | 0.51 | 62.00 |
| 0.54 0.64 | 0.53 | \$57.00 |
| 0.64 0.71 | 0.64 0.74 | 38.00 24.00 |
| 0.71 0.82 | 0.74 0.60 | 24.00 17.00 |
| 0.86 | 0.85 | 11.00 |
| 0.89 | 0.90 | 7.00 |
| 0.93 | 0.92 | 6.60 |
| 1.00 | 0.98 | 2.00 |

Run Number..... 304
*** **************1500

$6.400 \quad 601.845 \quad 0.036$


|  |  |  |
| :---: | :---: | :---: |
| Pore size |  | Parcentage |
| 602 |  | -3 |
| 1635 |  | 4 |
| 221 |  | 13 |
| Exp-k | CALC-K | sol. rad |
| 0.00 | 0.00 | 1607.00 |
| 0.00 | 0.00 | 1302.00 |
| 0.02 | 0.00 | 1060.00 |
| 0.05 | 0.01 | 792.00 |
| 0.99 | 0.02 | 624.00 |
| 0.18. | 0.10 | 415.00 |
| 0.24 | 0.19 | 336.00 |
| -.30 | 0.27 | 276.00 |
| 0.40 | 0.38 | 204.00 |
| 0.48 | 0.48 | 161.00 |
| 0.61 | 0.63 | 107.00 |
| 0.68 | 0.69 | 97.00 |
| 0.74 | 0.74 | 71.00 |
| 0.80 | 0.80 | 53.00 |
| 0.89 | 0.89 | 28.00 |
| 0.91 | 0.91 | 22.00 |
| 0.92 | 0.93 | 18.00 |
| 0.94 | 0.95 | 14.00 |
| 0.97 | 0.97 | 7.00 |
| 0.98 | 0.98 | 6.00 |
| 0.98 | 0.98 | 5.00 |
| 0.99 | 0.99 | 4.00 |
| 0.99 | 0.99 | 2.00 |


|  |  |  |
| :---: | :---: | :---: |
| Pare size |  | Percentage |
| 602 |  | 78 |
| 1635 |  | 4 |
| ${ }_{4447}^{221}$ |  | ${ }_{5}^{13}$ |
|  |  |  |
| ExP-K | CALC-K | SOL.RAD |
| 0.00 | 0.02 | 1607.00 |
| 0.00 | 0.03 | 1302.00 |
| 0.02 | 0.03 | 1068.00 |
| 0.05 | 0.04 | 792.00 |
| 0.09 | 0.05 | 624.00 |
| 0.18 | 0.14 | 415.00 |
| 0.24 0.30 | 0.22 0.30 | 335600 |
| 0.30 | 0.30 | 276.00 |
| 0.40 | 0.42 | 204.00 |
| 0.49 | 0.51 | 161.00 |
| 0.61 | 0.64 | 107.00 |
| 0.68 | 0.70 | 87.00 |
| 0.74 0.80 | 0.75 0.81 | 71.00 53.00 |
| $\stackrel{8}{0.88}$ | 0.81 0.90 | 28.00 |
| 0.91 | 0.92 | 22.00 |
| 0.92 | 0.93 | 18.00 |
| 0.94 | 0.95 | 14.00 |
| 0.97 | 0.97 | 7.00 |
| 0.98 | 0.98 | 6.00 |
| 0.98 | 0.98 | 5.00 4.00 |
| 0.99 0.99 | , $\begin{array}{r}0.99 \\ 0.98\end{array}$ | 4.00 2.00 |
|  |  |  |
|  |  |  |
| Pore 5120 | Percentage |  |
|  |  |  |
| 602 | $75$ |  |
| ${ }_{2635}^{1635}$ |  |  |
|  | 4135 |  |
| $4447$ |  |  |
| ExP-K | calc-k | SOL. RAD |
| 0.00 | 0.02 | 1607.00 |
| 0.00 | 0.03 | 1302.00 |
| 0.02 | 0.03 | 1068.00 |
| 0.05 0.09 | 0.04 0.05 | 792.00 624.00 |
| 0.09 0.18 | 0.05 0.14 | 624.00 415.00 |
| 0.24 | 0.21 | 336.00 |
| 0.30 | 0.29 | 276.00 |
| 0.40 | 0.40 | 204.00 |
| 0.48 | 0.49 | 161.00 |
| 0.61 | 0.62 | 107.00 |
| 0.68 | 0.68 | 67.00 |
| 0.74 | 0.73 | 71.00 |
| 0.80 | 0.79 | 53.00 |
| 0.88 | 0.80 | ${ }^{28.00}$ |
| 0.91 | 0.90 | 22.00 |
| 0.92 | 0.92 | 18.00 |
| 0.94 | 0.94 | 14.00 |
| 0.97 | 0.97 | 7.00 |
| 0.98 0.98 | 0.97 0.98 | 6.00 5.00 |
| -0.98 | 0.98 0.98. | 5.00 4.00 |
| 0.99 | 0.99 | 2.00 |



## Pore Size Distribution Data obtained by High Pressure Mercury

 Porosimetry. This datawas used for the comparison with SEC PSD determination.

Pore Size Distribution Curve
Mercury Porosimetry


Pore Size Distribution Curve


Pore Size Distribution Curve


Pore Size Distribution Curve
Mercury Porosimetry


Pore Size Distribution Curve
Mercury Porosimetry


Pore Size Distribution Curve


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Listing and output from the Minuit optimisation

```
Listing of the program that was used in the optimisation routine "MINUIT". The program calculates the variance descrbed in Chapter 8 using the cylindrical pore model.
```

program minimise
call mintld
end
subroutine fcn(nvpar, deriv, funcvalue, parameters, iflag)
implicit double precision(a-h, o-z)
real*8 deriv(14), crck(40),parameters(14),xobs(40),yobs(40),
+pore(10),fct(10), excok(40),part2,aval,valk,tval,alpha,beta,
+aval2,fract,fract2,fract3,part3,aval3,aval4,aval5,aval6,
+part 1,aval7,part4,part5,part6,part7,
$+\quad$ fract4,fract5,fract6,fract7
if (iflag.eq.1) then
C Input the SECC data as ' $K$ ' and ' $\mathrm{Ln} R$ ' followed by
$C$ the number of pores and their radii in angstroms.
read $(8, *)$ no of values do $100 i=1$, no of values
read( $8, *$ ) xobs(i),yobs(i)
yobs(i)=exp(yobs(i))
continue
Read(8,*) no pores
do $150 \mathrm{j}=1$, no pores
read( $8,{ }^{*}$ ) pore(j)
150 continue
end if

C Define the various parameters.
func value $=0.0$
fract $=$ parameters(1)
fract2 = parameters(2)
fract3 = parameters(3)
fract4 = parameters(4)
fract5 = parameters(5)
fract6 $=$ parameters(6)
alpha = parameters(7)
beta $=$ parameters(8)

C Calcuate the contribution each pore makes to the
C K-value of each solute. Then sum these contributions
C to give the total $K$-value for each solute.
do $200 i=1$, no of values
aval $=0.0$
part $1=0.0$
part2 $=0.0$
part3 $=0.0$
part4 $=0.0$
part5=0.0
part6=0.0
part7 $=0.0$
corrk=0.0
valk=0.0
tval $=0.0$
aval=yobs(i)/pore(1)
if (aval .ge. 1.0) then
part $1=0.0$
else
if (fract .LT. 0.0) then fract=0.0
endif
part $1=$ fract*(1-aval)**2
endif
aval2=yobs(i)/pore(2)
if (aval2 ge. 1.0) then
part2=0.0
else
if (fract2 LTT. 0.0) then fract2=0.0
endif
part2=fract2*(1-aval2)**2
endif
aval3=yobs(i)/pore(3)
if (aval3 .ge. 1.0) then
part3=0.0
else
if (fract3 LT. 0.0) then
fract $3=0.0$
endif
part3=fract3*(1-aval3)**2
endif
aval4=yobs(i)/pore(4)
if (aval4 .ge. 1.0) then
part4=0.0
else
if (fract4 LTT. 0.0) then
fract4=0.0
endif
part4=fract4*(1-aval4)**2
endif

```
aval5=yobs(i)/pore(5)
if (aval5 .ge. 1.0) then
part5=0.0
else
if (fract5 .LT. 0.0) then
fract5=0.0
endif
part5=fract5*(1-aval5)**2
endif
aval6=yobs(i)/pore(6)
if (aval6 .ge. 1.0) then
part6=0.0
else
if (fract6 .LT. 0.0) then
fract6=0.0
endif
part6=fract6*(1-aval6)**2
endif
fract7=1-fract-fract2-fract3-fract4-fract5-fract6
aval7=yobs(i)/pore(7)
if (aval7 .ge. 1.0) then
part7=0.0
else
part7=fract7*(1-aval7)**2
endif
valk=part 1+part2+part3+part4+part5+part6+part7
```

C Now optimise the values of alpha and beta. This will
$C$ rescale the $K$-values still keeping them between 0 and 1

```
    delta=alpha+beta
```

    if (delta .GT. 1.0) then
    alpha=0.0
    beta=1.0
    endif
    corrk=alpha+beta*xobs(i)
    C Calculate the variance from the calculated $K$-value and
$C$ the rescaled $K$-value
tval=(valk-corrk)**2
func value=func value+tval
continue
func value $=$ func value / no of values
if (iflag.eq.3) then
fract7=1-fract2-fract3-fract4-fract5-fract6-fract
C Print out the pore radii $(A)$ the fraction of each pore $C$ and the values of alpha and beta. Also output the values
$C$ the solute radii and the experimental, the rescaled and
$C$ the calculated $K$-values.
write $(9,400)$ func value

```
400 format(///,10X,'Minimum Squares Value: ',f11.7)
    write(9,500) pore(1),fract,pore(2),fract2,pore(3),fract3
        write(9,550) pore(4),fract4,pore(5),fract5,pore(6),fract6
    + ,pore(7).fract7
500 format(//,10X,'1st Pore Size: ',d11.4,10x,'Fraction: ',d15.4,
    + /,10X,'2nd Pore Size: 'd11.4,10X,'Fraction: ',d15.4,
    + /,10X,'3rd Pore Size: ',d11.4,10X,'Fraction: ',d15.4)
550 format(10X,'4th Pore Size: ',d11.4,10X,'Fraction: ',d15.4,
    + /,10X,'5th Pore Size: ',d11.4,10X,'Fraction: ',d15.4,
    + /.10X,'6th Pore Size: ',d11.4,10X,'Fraction: ',d15.4,
    + /,10X,'7th Pore Size: ',d11.4,10X,'Fraction: ',d15.4)
        write(9,600) alpha,beta
600 format(//.10X,'Alpha: ',f8.4,10X,'Beta: ',f8.4)
```

    write \((9,590)\)
    590 format(//9x,'Solute Radius', 8x,'Experimental K',9x
+,'Corrected K', $8 \mathrm{X},{ }^{\prime} \mathrm{Calculated} \mathrm{K}^{\prime}, /$ )
fct(1)=fract
fct(2)=fract 2
$\mathrm{fct}(3)=\mathrm{fract} 3$
$\mathrm{fct}(4)=\mathrm{fract} 4$
$\operatorname{fct}(5)=\mathrm{fract} 5$
$\mathrm{fct}(6)=\mathrm{fract} 6$
fct(7)=fract7
do $300 \mathrm{i}=1$, no of values
tpiece $=0.0$
do $350 \mathrm{j}=1$, no pores
piece $=0.0$
b=yobs(i)/pore(j)
if (b .GE. 1.0) then
piece $=0.0$
else
piece $=\mathrm{fct}(\mathrm{j}) *\left((1-\mathrm{b})^{* * 2}\right)$
endif
tpiece $=$ tpiece + piece
350 continue
excok(i)=tpiece
crck(i)=alpha+beta*xobs(i)
write $(9,700)$ yobs(i),xobs(i),crck(i),excok(i)
700 format ( $10 x, f 10.2,12 x, f 7.4,16 x, f 7.4,16 \times, f 7.4$ )
300 continue
end if
return
ènd

## HYPERSIL




## VYDAC



| 15 | Puri Sizu | O． $18151 / \cdots 00$ |  | Fraction： | 0． $1428 \mathrm{CD}+00$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 2n | かure $\because$ ¢ze： | 0．230419＋03 |  | Fraction： | 0．5459D－0．4 |
| 3 r | rors Sixe： | （1．2．790．40 | ： | Fiaction： | 0． $80760+00$ |
| 4 t |  |  | $\because$ | Frartion： | $0.68700-05$ |
| 5 t | F6ro Silze： | ¢） 4920 |  | Fraction： | 0．38300－67 |
| ¢ち！ | 9are Size： | 3） $60 \% 0003$ |  | Fraction： | $0.63010-05$ |
| 7も1 | Pone Sixat | 0． $31.205+170$ | ： | Fraction： | 0． $47460-01$ |

Alpha：©．000：Eatil 0． 5597

Solute Radius

| 7813.40 |
| :---: |
| 1777.70 |
| 犬57． 81 |
| 34\％． 87 |
| 150.57 |
| 171． 63 |
| 98． 78 |
| 63． 56 |
| 37.34 |
| 1\％．48 |
| S：Se |

i． 2000
0.0000
o．0002．
0．0002
0.0000
0.0074
0.0074
0.0155
$\begin{array}{ll}0.0002 & 0.0155 \\ 0.0336 & 0.0198\end{array}$
0.03836
0.1352

0． 1392
0.2984
0.4311
0.4311
0.6303

0． 7536
0． 8801
0.9536

Miniment Squaves Valua: O. OOIOR.11


PSM 1000



| Sotuteramius | Fipuerimentai in | Corrgetsat $k$ | Calculated $X$ |
| :---: | :---: | :---: | :---: |
| $140 \%$ 5! | © 00ts | 0.0819 | 0.0367 |
| 1030.71 | 0.0000 | 0.0819 | 0.07e6 |
| 558.6 | a. Ocia | O. $16.5 \%$ | 0. 1655 |
| 750.34 | 0. a\%\% | 0. 272 | 0. 2306 |
|  | \% ce2\% | 0. 3206 | 0. 3334 |
| -7\% 5\% | O. 3*0: | 0. 3915 | 0. 3884 |
| 131. | D. 52ais | 0. 5.573 | 0. 5797 |
| 91.15 | O. 6750 | 0. 8749 | 0. 7213 |
| 61.66 30.48 | 0. 8060 | O. Eline | 0.8034 |
| 16. 168 | 0. 7030 | 0. 7046 | 0.8983 |
| 11.3x | 0. 9320 | 0.93i6 | 0. 9431 |
| 5.97 | -. 7710 | 0. 9483 | 0.9611 |
| 4. 64 | 9. 9950 | 0. 98634 | 0.9.95 |
| 1.59 | 1.0000 | 0. 9930 | 0. 9745 |




| Solubi ！nujus |  | Corrected K | Calculated K |
| :---: | :---: | :---: | :---: |
| $103 \%$ | 0． 0 00 | 0． 0190 | 0.0096 |
| 「ぜ，5\％ | ¢． 0000 | 0． 0190 | 0． 0373 |
| －iS4． 25 | Q． 6700 | 0．0967 | 0． 0694 |
|  | a． 1300 | 0． 1447 | 0． 0996 |
| 1：i3．${ }^{1} 1$ | $\therefore .3000$ | 0． 3090 | 0． 3027 |
| 113．75 | 9． 900 | 0． 4057 | 0． 4142 |
| \％0．${ }^{\text {\％}}$ | 0． 5000 | 0．502\％ | 0． 5158 |
| $\because \mathrm{F}$ 2 4 | O． 6000 | O． 5990 | 0． 5992 |
| 勺L．5\％ | O． 6700 | 0． 6667 | 0． 6506 |
| 37． 71 | 0． 8000 | 0． 7924 | 0． 7752 |
| $30.5 \%$ | 0． 8300 | 0． 8214 | 0.8152 |
| $\therefore 3.57$ | ¢． $3 \% 6$ | 0.8800 | 0． 8556 |
| 18．74 | 0． 9050 | 0.8934 | 0． 8958 |
| 11.36 | 9． $9+10$ | 0．92\％ | 0． 9287 |
| 7．38 | a． 7000 | 0.9470 | 0． 9538 |
| 5.75 | 0.7700 | 0． 9567 | 0． 9635 |
| 1． 59 | 1． 0000 | 0.9857 | 0.9898 |

PR 183



Alpho：0．日ige ligty：0．98в7

Bolute tientiv：
Enverinertal $K$
Corrected K
Calculated K

| 1ray | 13． 0000 | J． 0005 |  | 0.0077 |
| :---: | :---: | :---: | :---: | :---: |
| 565.5 | Q． 0000 | 0.0005 | ． | 0.0121 |
| 3i4． | O． 0300 | 0． 0302 |  | 0.0207 |
| 27\％．¢ | 0． 0600 | 0.0599 |  | 0． 0257 |
| 15i．41 | c． 1700 | O． 1686 | － | 0． 1703 |
| i16．$\%$ | 9． 2300 | －． 2280 |  | 0． 2913 |
| 70.92 | c． 4500 | 0.4455 | － | 0． 4054 |
| \％2 24 | O． 5000 | 0． 4949 |  | 0． 5019 |
| 61.56 | c． 5700 | 0． 5641 |  | $0 . .5629$ |
| \＃\％ 40 | 0． 8000 | D． 5938 | ． | 0． 5878 |
| $3 \% .71$ | 0.7300 | －． 7223 | － | 0.7146 |
| $30.5 \%$ | O． 7700 | O． 7619 |  | 0.7642 |
| 23． $5 \%$ | c． 8100 | 0． 8014 |  | 0． 8148 |
| 16． 76 | 0．8700 | 0． 8608 |  | 0． 8658 |
| 11.36 | 0． 9200 | 0.9102 |  | 0.9079 |
| 7.32 | －0．9400 | 0.9300 | $\therefore \quad \vdots$－． | 0.9401 |
| 5． 93 | 0． 9700 | 0.9596 |  | 0.9512 |
| 1． 59 | 1． 0000 | 0． 9893 | $\because \because$ | 0.9868 |

MB5－U


|  |  | Fraction： | 2． $20795-01$ |
| :---: | :---: | :---: | :---: |
|  |  | Fraction： | 0． $59 \% 4 \mathrm{D}-01$ |
|  |  | Fraction： | \％90920 00 |
| Ath 以igut Siz： | ¢ \％－¢ | Fraction： | 0．21000－04 |
| 5in irare Sus： |  | Fraction： | －． $60000+00$ |
| $\therefore$ 他 |  | Frastion： | 0．7e000－04 |
| 7th forx $\mathrm{O}_{6} \mathrm{y}$ |  | Fraction： | 9．16：30－15 |
| Alpin：\％¢：\％ | 1）： 1 ！： | D． 7502 |  |



| \％5 36 | $\therefore$ avo |
| :---: | :---: |
|  | $: 8 \mathrm{BO}$ |
| 752． 20 | a \％000 |
| $\because 01.20$ | （3）030 |
| 4こ大． 55 | 0． 0 0 |
| 12－3． 30 | 4． 200 |
|  | （6． 1704 |
| 3A\％． 02 | c．1300 |
| こ\％\％．77 | 8． 2000 |
| 191．52 | （\％） 4.700 |
| 157.75 | $\therefore 560 \%$ |
| 131．76 | 0． $710 \%$ |
| 98.59 | 2． $3.40 \%$ |
| 63.7 | c）secoo |
| 33．6\％ | （i） $18.40 \%$ |
| 23． 28 | （1． 9100 |
| 17． 51 | a． 7600 |
| 14． 60 | 0.7700 |


| 0.0126 | 0.0000 |
| :--- | :--- |
| 0.0126 | 0.0000 |
| 0.0126 | 0.0000 |
| 0.0414 | 0.0255 |
| 0.0417 | 0.0459 |
| 0.06 .26 | 0.0758 |
| 0.0890 | 0.1507 |
| 0.1374 | 0.1776 |
| 0.2046 | 0.2389 |
| 0.4351 | 0.4519 |
| 0.5503 | 0.5326 |
| 0.6944 | 0.5974 |
| 0.7232 | 0.6905 |
| 0.8000 | 0.7782 |
| 0.6192 | 0.8088 |
| 0.9056 | 0.9053 |
| 0.7344 | 0.9408 |
| 0.9440 | 0.9505 |

MB5－S2



Soloteprotiou

Coracetay ix
Calculated K．
1032.77
355.57
307.76
450.37
35.25
363.07
151.41
116.75
91.10
63.18
33.46
15.63
4.31
1.39
9.0000
9.0000
6.0000
0.0000
0.0100
0.0300
0.1900
0.2800
6.3700
6.5700
6.7300
0.8400
9.9800
1.0000
0.0050
0.0050
0.0050
0.0050
0.0147
0.0347
0.1932
0.2823
0.3714
0.5695
0.7280
0.3765
0.9756
0.9754
0.0002
0.0156
0.0185
0.0185
0.0223
0.0295
0.0370

0． 1771
0.2942
0.2942
0.4051

0． 5512
0.7460

0． 8638
0.9634
0.9864

## Lichrosphere



# DETERMINATION OF PORE SIZE DISTRIBUTION CURVES BY SIZEEXCLUSION CHROMATOGRAPHY 

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#### Abstract

SUMMARY The mathematical formulation of the relationship between the pore size distribution (PSD) and the size-exclusion chromatography calibration (SECC) curve for a rigid porous material originally developed by Knox and Scott has been extended and computerized.

Knox and Scott [J. Chromatogr., 316 (1984) 311-332] showed that the SECC could be accurately predicted from the PSD obtained by mercury porosimetry on the assumption that the pores of the material were cylindrical and that the probe molecules (polystyrenes) could be regarded as spheres, and that the reverse procedure was also possible by a simple mathematical procedure. We have found that their original procedure cannot unfortunately be computerized because of the difficulty of fitting a smooth mathematical function to the experimental SECC, and we have examined several alternative methods. Of these the most successful selects an optimum single pore size and then builds around this a distribution of pores whose sizes fall in geometrical progression. The program can be implemented by a microcomputer using up to seven pore contributions, or in a more powerful procedure by a mainframe computer which can also allow for incomplete penetration of the smallest sample probe and incomplete exclusion of the largest sample probe.

We recommend adoption of our procedure in place of the widely used method of Halasz and Martin [Ber. Bunsenges. Phys. Chem., 79 (1975) 731-4; Angew: Chem., Int. Ed. Engl., 17 (1978) 901-908].


## INTRODUCTION AND THEORY

Porous solids are of prime importance in chromatography, as catalysts, as catalyst supports and as adsorbents generally. In relating their performance to their physical and chemical properties, it is important to understand as much as possible about their internal structure including their surface area, mean pore size and pore size distribution (PSD).

This paper is devoted to determining PSDs of rigid porous materials which are suitable for modern high-performance liquid chromatography (HPLC) using data from size-exclusion chromatography calibration (SECC) curves. Typical of such ma-
terials are silica gels, rigid macroporous polystyrenes, porous glasses, and other materials containing mainly meso- and macropores. In these materials there is a clear distinction between the solid structure and the pore space so that the internal surface area and the pore volume are both well defined. In general pores may be grouped into three classes ${ }^{1}$ : (a) micropores, having radii below $10 \AA$; (b) mesopores, having radii between 10 and $500 \AA$; (c) macropores, having radii larger than $500 \AA$.

PSDs in the mesopore range and above can be found by three main methods: (a) gas adsorption-desorption isotherms at high relative pressures of adsorbate; (b) mercury porosimetry; (c) size exclusion chromatography (SEC).

The determination of PSDs by gas adsorption relies on interpretation of the adsorption isotherm of a gas such as nitrogen. Materials which contain mesopores give type IV isotherms according to the Brunnauer-Emmett-Teller classification ${ }^{2}$ and exhibit a hysteresis loop at relative vapour pressures of adsorbate, $p / p_{0}$, between 0.4 and 1.0 . The method depends upon the fact that the vapour pressure, $p$, of a liquid above a curved concave of radius, $R$, is less than the vapour pressure, $p_{0}$, above a flat surface, being given by the Kelvin equation:

$$
\begin{equation*}
\ln \left(p / p_{0}\right)=-2 V_{\mathrm{m}} \gamma \cos \theta /(R \mathrm{R} T) \tag{1}
\end{equation*}
$$

where $\theta$ is the contact angle, $\gamma$ the surface tension of liquid adsorbate, $V_{\mathrm{m}}$ the molar volume of liquid adsorbate, R the universal gas constant and $T$ the absolute temperature. When $R$ falls within the mesopore range, $10-500 \AA$, capillary condensation occurs within such pores at pressures which are significantly below $p_{0}$. Accordingly the volume of pores up to a particular radius, $R$, can be found from the volume of liquid adsorbate taken up by the material when the pressure is increased up to the pressure, $p$, given by eqn. 1. Generally type IV adsorption isotherms show a hysteresis loop; that is, the plot of amount adsorbed against relative pressure $p / p_{0}$ follows a lower curve during adsorption than during desorption. There is considerable argument about the interpretation of the hysteresis loop and this is discussed in detail by Gregg and Sing ${ }^{3}$. The most plausible explanation when dealing with materials made up of coalesced spherical colloidal units seems to be that initial capillary condensation occurs around the cusp-shaped regions where the colloidal spheres touch. This condensation is reversible and so does not enter into the hysteresis loop. At higher relative pressures condensation occurs in the narrow channels formed by trios of spheres. These channels lead into larger cavities. It is likely that at a certain stage these cavities become isolated by condensation in all the channels which lead into them ${ }^{4}$. Subsequently these cavities are filled at relative pressures which are appropriate to their radii. However, on reducing the relative pressure to determine the desorption branch of the isotherm, any particular cavity will not empty until the widest channel leading into it has emptied. Cavity filling and emptying are not therefore reversible so a well defined hysteresis loop is produced which does not depend upon the time allowed for equilibration. If this explanation is correct then the pore size distribution should be determined from the adsorption branch of the isotherm not from the desorption branch.

The method of determining the PSD using eqn. 1 makes several assumptions. It assumes, first that the pores are cylindrical; secondly that the contact angle is known; and thirdly that the surface tension of the liquid adsorbate is constant down
to very small radii of curvature of the liquid surface. All three assumptions are suspect. In addition, by the stage that capillary condensation occurs, the internal surface of the adsorbent will already be covered by at least a monolayer of adsorbate which will reduce the radii of all pores by the monolayer thickness leading to low values of the pore size unless a correction is made.

The determination of the PSD by mercury porosimetry is based upon eqn. 2 due to Washburn which gives the excess pressure required to maintain a convex spherical surface of radius, $R$

$$
\begin{equation*}
\Delta p=2 \gamma \cos \theta / R \tag{2}
\end{equation*}
$$

Mercury is commonly used as the non-wetting liquid, and with pressures up to 5000 bar it is possible to penetrate pores with radii as small as $10 \AA$. The method again assumes that the pores are cylindrical, that the contact angle is known and that the surface tension of mercury is independent of the radius of its surface. Since the mercury porosimetry method depends upon penetration of cavities through their entrance channels, the penetration of any cavity will occur at a pressure determined by the radius of the widest entry channel rather than the radius of the cavity itself. On reduction of pressure these cavities may not all.empty, leading to the so-called "inkbottle" effect. Mercury porosimetry will give PSDs which are directly comparable to those obtained from the desorption branch of the adsorption isotherm, and should give_slightly smaller pore radii than those determined from the adsorption branch of the isotherm.

Methods based upon SEC use molecules of known or assumed radius to probe the pores of the material. Random coil polymers such as polystyrenes are commonly used. As Casassa ${ }^{5}$ has shown such molecules can be regarded for this purpose as behaving as if they were rigid spheres. Nevertheless because of their real flexibility they are able to pass through channels whose radii are actually smaller than the effective sphere-radius of the polymer molecule itself, and can therefore explore the entire pore structure of a material even when it contains ink-bottle pores.

In practice one measures the retention volume, $V_{\mathbf{R}}$, of a range of polymers of different molecular weights, the polymer samples having low polydispersities. Care is taken to avoid adsorption by using a good solvent as eluent. The retention volume will then lie between the extra-particle void volume, $V_{0}$, and the total volume of eluent within the column, $V_{\mathrm{m}}$. The degree of permeation into the pores of the material (pore volume, $V_{\mathrm{p}}=V_{\mathrm{m}}-V_{0}$ ) is characterised by the permeation coefficient, $K$, defined by eqn. 3 :

$$
\begin{equation*}
\text { . } K=\left(V_{\mathbf{R}}-V_{0}\right) /\left(V_{\mathrm{m}}-V_{0}\right) \tag{3}
\end{equation*}
$$

$V_{\mathrm{m}}$ is normally found using a small molecule as probe, for example benzene, while $V_{0}$ is found using a molecule of sufficiently high molecular weight that it will be excluded from all the pores of the material. Both of these assignments are somewhat arbitrary and, as we point out later, this has to be allowed for in fitting theoretical to experimental values of $K$.

Most recent work on the determination of PSD curves from SECC curves ${ }^{6-10}$ uses the method of Halasz and Martin ${ }^{11,12}$ which assumes that for a uniform array
of cylindrical pores of radius $R$, any molecule of radius greater than $R$ will have $K=0$, while any molecule of radius less than $R$ will have $K=1$. As pointed out by Knox and Scott ${ }^{13}$ the second of these assumptions is incorrect, and it forced Halasz and Martin to assume that in SEC polystyrene molecules had an apparent radius 2.5 times greater than that given in well established formulae ${ }^{14,15}$ of which we use that of Van Krefeld and Van den Hoed ${ }^{15}$

$$
\begin{equation*}
r / \AA=0.123 M^{0.588} \tag{4}
\end{equation*}
$$

(where $M$ is the relative molecular weight of the polystyrene molecule). The correct formulation for the permeation coefficient of a spherical molecule of radius, $r$, into cylindrical pores is given by eqns. 5,6 and 8 .

For an array of uniform cylinders of radius $R$,

$$
\begin{equation*}
K(r)=\left(1-\frac{r}{R}\right)^{2} ; r<R: K=0 ; r>R \tag{5}
\end{equation*}
$$

For an assembly of cylinders with a continuous distribution of radii, such that a fraction $G^{\prime}(R) \mathrm{d} R$ of the pore volume is taken up by cylinders of radii between $R$ and $R+$. $R$

$$
\begin{equation*}
K(r)=\int_{r}^{\infty} G^{\prime}(R)\left(1-\frac{r}{R}\right)^{2} \mathrm{~d} R \tag{6}
\end{equation*}
$$

The differential pore size distribution function, $G^{\prime}(R)$, is related to the cumulative pore size distribution function, $G(R)$, by eqn. 7 :

$$
\begin{equation*}
G(R)=\int_{0}^{R} G^{\prime}(R) \mathrm{d} R \tag{7}
\end{equation*}
$$

For an assembly of cylindrical pores with discrete radii where a fraction $F(R)$ of the total pore volume is taken up in pores of radii $R$,

$$
\begin{equation*}
K(r)=\sum_{R=r}^{R=\infty} F(R)\left(1-\frac{r}{R}\right)^{2} \tag{8}
\end{equation*}
$$

Knox and Scott ${ }^{13}$ showed that for a continuous distribution of pore diameters, $G(R)$ could be obtained from the plot of $K(r)$ again $\ln r$ by a simple differentiation procedure as given in eqn. 9.

$$
\begin{equation*}
G(R)=K(r)-\frac{3}{2}\left[\frac{\mathrm{~d} K(r)}{\mathrm{d} \ln r}\right]+\frac{1}{2}\left[\frac{\mathrm{~d}^{2} K(r)}{\mathrm{d}(\ln r)^{2}}\right] \tag{9}
\end{equation*}
$$

They used a graphical method to differentiate the plot of $K(r)$ against $\ln r$. Unfor-
tunately this method was time consuming, subjective and showed instability. In this paper, inter alia, we have sought a procedure whereby an analytical expression could be computer fitted to the $[K(r), \ln r]$ curve and from which the $[G(R), \ln R]$ curve could be derived by eqn. 9 .

Unlike the previous methods, the SECC method for determining the PSD of a material requires no assumptions about contact angle or surface tension at low radii of curvature, but it does assume that the polymer probe has zero enthalpy of transfer from the bulk solvent outside the particles to the solvent within the pores of the SEC material. Accordingly, the SEC method should provide PSDs with fewer assumptions than the adsorption and mercury porosimetry methods. PSDs derived from SEC curves should be comparable to those obtained from the adsorption branch of the hysteresis loop of the adsorption isotherm and may indicate slightly larger pores than those given by mercury porosimetry, since the latter sees ink-bottle pores as having the radii of their entrance channels.

We would argue that PSDs derived from SECC curves are likely to be the most relevant if the PSD information is actually required for a chromatographic application.

## PROCEDURES AND GENERAL CONSIDERATIONS

In devising any procedure for matching SECC and PSD curves one has to recognise two problems in fitting theoretical and experimental $K(r)$ values. First of all, the small molecule which is assigned an experimental $K(r)$ value of unity will, having a finite radius, be slightly excluded from the pore space and will therefore have a theoretical $K(r)$ value just below unity. Secondly, the largest probe molecule, which is assigned an experimental $K(r)$ value of zero may have a non-zero theoretical $K(r)$ value. This can arise as follows. As shown by Fig. 1, there is no clear distinction


Fig. 1. High-pressure mercury porosimetry data for a typical $5-\mu \mathrm{m}$ silica gel PR-179 having a narrow pore size distribution.
between the intra-particle pore volume and the inter-particle void space. There can thus be no sense in which a large probe molecule can be said to be totally excluded from a clearly defined set of intra-particle pores. Thus any calculated value of $K(r)$ for a large molecule of a size comparable with the largest intra-particle pores using a trial PSD will most likely be non-zero. We allow for these possibilities by defining an adjusted $K(r)$ value which is related to the experimental $K(r)$ value by eqn. 10 :

$$
\begin{equation*}
K(r)_{\mathrm{adj}}=\alpha+\beta K(r)_{\mathrm{exp}} \tag{10}
\end{equation*}
$$

$\alpha$ is now the adjusted $K(r)$ value for the "excluded" solute, and $(\alpha+\beta)$ the adjusted $K(r)$ value for the "fully permeating" solute. The constants $\alpha$ and $\beta$ may be arbitrarily chosen or may be optimised along with the PSD curve to achieve the best fit between theoretical and experimental $K(r)$ data.

We have examined three procedures for deriving PSDs from SECC curves. In each case we optimise the values of a number of parameters to obtain the best fit between calculated and experimental $K(r)$ values. The control function, which is minimised, is the variance, $V$, between calculated and experimental $K(r)$ values: $V$ is evaluated by

$$
\begin{equation*}
V=\sum_{\substack{\text { all } \\ \text { data } \\ \text { points }}}\left[K(r)_{\mathrm{adj}}-K(r)_{\mathrm{th}}\right]^{2} \tag{11}
\end{equation*}
$$

In general $K_{\text {adj }}$ will be given by eqn. 10 but where it is not convenient to make any adjustment to the experimental values $\alpha$ is taken as zero and $\beta$ as unity so that $K_{\exp }$ is used directly without adjustment.

In the first procedure we attempted to improve the manual method of Knox and Scott ${ }^{13}$ by fitting a polynomial to the $[K(r), \ln r]$ data. The coefficients of the polynomial were adjusted to minimise $V$ before applying eqn. 9 .

The second procedure assumed a mathematical expression for $G^{\prime}(R)$ as a function of $R$ (for example a skewed Gaussian) and a theoretical $[K(r), \ln r]$ curve was derived using eqn. 6. The coefficients in the trial function for $G^{\prime}(R)$ were adjusted to minimise $V$.

In the third and most general procedure a step-function PSD was assumed comprising a set of discrete pores of radii $R_{1}$ to $R_{N}(N \leqslant 7)$. $K(r)$ was then found for each $r$ value using eqn. 7, and the volume fractions $F_{1}$ to $F_{N}$ for each pore size were adjusted to minimise $V$ : optimisation of $\alpha$ and $\beta$ could also be included.

## Method 1. Best fit of a polynomial to $[K(r), \ln r]$ Data

Polynomials of degrees from 3 to 8 , given by eqn. 12 were fitted to typical exclusion data

$$
\begin{equation*}
K_{\mathrm{adj}}=a_{0}+a_{1}(\ln r)+\mathrm{a}_{2}(\ln r)^{2}+\ldots .+\mathrm{a}_{n}(\ln r)^{n} \tag{12}
\end{equation*}
$$

As the degree of the polynomial was increased, progressively lower values of $V$ were obtained but, as seen from Fig. 2 and 3, the higher degree polynomials gave unacceptable $[K(r), \ln r$ ] curves with waves, a well known feature of this method of curve


Fig. 2. Best fits of polynomials of degrees 3 to 8 to $[K(r), \ln r]$ data for silica gel HR-WPS-2, an experimental wide-pore silica gel (method I).
fitting. Differentiation according to eqn. 9 accentuated these waves and gave unacceptable PSD curves with both negative $G(R)$ values and values exceeding unity as seen in Figs. 4 and 5. Such effects could be avoided by limiting $n$ but comparing Figs. 4 and 5 , it is seen that no unique degree of polynomial gives an adequate fit for these two particular materials.

We conclude, as did Warren and Bidlingmeyer ${ }^{16}$, that a polynomial cannot in general be successfully fitted to $[K(r), \ln r]$ data, and therefore that the approach, using eqn. 9 to obtain the PSD, is not likely to be successful. Nevertheless, as shown later, a reasonable PSD can be obtained, at least in some cases, if $G(R)$ values outside the limits 0 and 1 are simply ignored. It would, however, be dangerous to assume that this could be applied to all cases.

## Method 2. Optimisation of an assumed function for $G^{\prime}(R)$

In this procedure a skewed Gaussian distribution was assumed for the differential PSD curve. $\alpha$ and $\beta$ of eqn. 10 were taken as zero and unity respectively and eqn. 7 used to compute the $[K(r), \ln r]$ data. The skewed Gaussian is formed by
adding together a series of Gaussian profiles whose heights decrease exponentially with distance from the centre of the first member of the series. The primary Gaussian curve is given by

$$
\begin{equation*}
G^{\prime}(R)=\frac{1}{\sqrt{2 \pi \sigma^{2}}} \exp \left[-(\ln R-\ln \mu)^{2} / 2 \sigma^{2}\right] \tag{13}
\end{equation*}
$$

where $\mu$ is the radius of the central pore and $\sigma$ is the standard deviation of the curve in units of $\ln R$. The formula for the skewed Gaussian is given by eqn. 14 where $\alpha$ is the exponential decay factor.

$$
\begin{equation*}
G^{\prime}(R)=\frac{\alpha}{\sqrt{\pi \sigma^{2}}} \exp \left[-\frac{2 \alpha(\ln R-\ln \mu)-\alpha^{2} \sigma}{2 \sigma}\right] \cdot \operatorname{erfc}\left[\frac{\alpha \sigma-(\ln R-\ln \mu)}{\sqrt{2 \sigma^{2}}}\right] \tag{14}
\end{equation*}
$$

An example of the best fit obtained by optimizing $\mu, \sigma$ and $\alpha$ is shown in Fig. 6. The fit to the SECC data is good at low values of $r$ but a systematic deviation is seen at intermediate values. This is observed even with a material having a very narrow pore


Fig. 3. As for Fig. 2. Data for silica gel PSM 1000 (DuPont material).


Fig. 4. PSD curves derived from best fit polynomials of degree 4, 5, 6 and 8 to data for silica gel HR-WPS-2 (method 1). Note the excursions of $G(R)$ beyond the limits zero and one.


Fig. 5. As for Fig. 4 but for silica gel PSM 1000.


Fig. 6. Best correlation of a skewed Gaussian PSD function with the experimental [ $K(r), \ln r]$ data obtained for Hypersil (silica gel marketed by Shandon Southern Products) (method 2). Points are experimental data with calculated line superimposed: full line is $G^{\prime}(R)$ versus $R$ curve; broken line is $G(R)$ versus $R$ curve.
size range. The fit is likely to be much worse when the method is applied to materials with wide PSDs. Evidently restriction to three adjustable parameters, at least in this form, is too severe. We conclude that an adequate fit could be found only when the chosen expression for $G(R)$ or $G^{\prime}(R)$ contained at least four adjustable parameters.

Method 3A. Optimisation of the volume fractions of a set of pores of preselected radii using a microcomputer

With a failure of analytical forms adequately to represent either the SEC curve or to predict the SEC curve from an assumed form of PSD curve, methods were examined in which a set of pores of arbitrary radii was selected and the volumefraction of each pore optimized by microcomputer to provide the best fit to the experimental data using eqns. 8 and 11.

A direct interactive method was first examined in which various trial PSDs were tested following determination of the single pore size $R_{0}$ which gave the best fit to the $[K(r), \ln r$ ] data. Intervention by the operator enabled changes in $V$ to be examined which were brought about by:
(1) altering the PSD for a given selection of pore sizes;
(2) changing the number of pores in the PSD;
(3) changing the spacing of the pore radii in the PSD.

At this stage $\alpha$ and $\beta$ of eqn. 10 were taken as zero and one respectively.
After some practice the operator was able to determine an optimum PSD relatively quickly by what was essentially a trial and error procedure. It soon became clear that the number of different pore radii which could usefully be optimized was limited to about seven after which addition of further pores of new radii led to little or no improvement in the variance.

A flow diagram for the interactive program is given in Fig. 7. This led to a non-interactive program which used a group of pores whose radii were in geometric progression with a common ratio $F$. As shown in the flow diagram given in Fig. 8, the program first of all determined the single pore radius $R_{0}$, which gave the best fit to the $[K(r), \ln r]$ data and then determined $\beta$ taking $\alpha$ as unity. Additional pores


Fig. 7. Flow diagram for interactive computation of optimum PSD using a set of pores of assumed radii.
were then added successively in the order $R_{0} F, R_{0} / F, R_{0} F^{2}, R_{0} / F^{2}, R_{0} F^{3}, R_{0} F^{4}$.
This asymmetric distribution of pore sizes around the optimum single pore was used as experience showed that the low radius end of the $[K(r), \ln r]$ curve was generally well fitted even by a single pore radius whereas the high radius end of the curve nearly always required additional pore sizes. It is at the high molecular weight end near the exclusion limit that the greatest flexibility is required.

Method 3B. Optimisation of $\alpha, \beta$ and volume fractions of a set of pores of preselected radii using EMAS

The use of the Edinburgh Multi-Access System (EMAS) mainframe computer at the Edinburgh Regional Computing Centre (ERCC, Edinburgh, U.K.) greatly accelerated the optimisation procedure and provided access to the highly sophisticated routines available from the ERCC library, in particular the program named MINUIT described in detail by James and Roos ${ }^{17}$, which enabled up to 15 parameters to be optimised. Eqn. 11 for the variance $V$ was expanded by incorporating eqns. 8 and 10 to give eqn. 15 :

$$
\begin{equation*}
V=\sum_{\substack{\text { data } \\ \text { points }}}^{\left.\sum\left\{\alpha+\beta K(r)_{\mathrm{exp}}\right]-\sum_{\substack{R=r \\ \text { pore } \\ \text { sizes }}}^{\substack{R=\infty}} \left\lvert\, r(R)\left(1-\frac{r}{R}\right)^{2}\right.\right\}^{2}} \tag{15}
\end{equation*}
$$



Fig. 8. Flow diagram for non-interactive computation of optimum PSD using a set of.pores of assumed radii.
where the first term in the major summation is $K_{\text {adj }}$ and the second term is $K_{\mathrm{th}}$. Using eqn. 15 the values of $\alpha$ and $\beta$ plus those of the $F(R)$ could all be optimized to give the lowest value of $V$. With seven discrete pore radii, as used in procedure 3 A , only six are independent since

$$
\begin{equation*}
\sum_{i=1}^{7} F_{i}=1 \tag{16}
\end{equation*}
$$

Optimisation was therefore carried out for the eight parameters $\alpha, \beta, F_{1}, F_{2}, F_{3}$, $F_{4}, F_{5}, F_{6}$, under the conditions that $(\alpha+\beta)$ and all seven $F_{i}$ must lie between 0 and 1 and that neither $\alpha$ nor $\beta$ can be negative. Initially the computer was provided with starting trial values of the eight parameters along with limits within which the final values should fall. MINUIT contains a number of optional subroutines of which we have used SIMPLX and MIGRAD.

SIMPLX is a simplex routine which minimises a function of $N$ variables. In our case the function was $V$.of eqn. 15 and $N$ was eight. A simplex is a figure in $N$ dimensional space defined by a convex hull of $(N+1)$ points (for example a triangle in two dimensions). Each point represents one possible set of the $N$ parameters and corresponds to a single value of $V$. To construct the initial simplex $\mathrm{S}_{0}$ from which the minimisation procedure starts, a particular set of the $N$ parameters is chosen to define a starting point $\mathrm{P}_{0}$. The remaining $N$ points, $\mathrm{P}_{1}$ to $\mathrm{P}_{N}$, are then found by proceeding from $\mathrm{P}_{0}$ in the directions of the $N$ coordinate axes and determining the positions of minimum $V$ in each of these directions.

At each performance of the subroutine a new simplex $S_{i}$ is generated from simplex $\mathrm{S}_{(i-1)}$. The routine first of all identifies $\mathrm{P}_{\mathrm{w}}$ which has the highest value of $V$. The point $\mathrm{P}_{\mathrm{w}}$ is then reflected in the $(N+1)$ dimensional hyperplane through the remaining $N$ points of the simplex to give $\mathrm{P}^{*}$, and $V\left(\mathrm{P}^{*}\right)$ is then calculated.

Various alternatives are now open depending upon whether or not $V$ is sufficiently reduced by the procedure. The procedures in ranking order are as follows:
(a) replace $\mathrm{P}_{\mathrm{w}}$ by $\mathrm{P}^{*}$.
(b) IF $V\left(\mathrm{P}^{*}\right)$ is not a sufficient improvement on $V\left(\mathrm{P}_{\mathrm{w}}\right)$ THEN find the point $\mathrm{P}^{* *}$ on the line $\mathrm{P}_{\mathrm{w}} \mathrm{P}^{*}$ which gives minimum $V$; replace $\mathrm{P}_{\mathrm{w}}$ by $\mathrm{P}^{* *}$.
(c) IF $V\left(\mathrm{P}^{* *}\right)$ is not a sufficient improvement on $V\left(\mathrm{P}_{\mathrm{w}}\right)$ THEN find the point $\mathrm{P}^{* * *}$ on the parabola through $\mathrm{P}_{\mathrm{w}} \mathrm{P}^{*} \mathrm{P}^{* *}$ which has minimum $V$; replace $\mathrm{P}_{\mathrm{w}}$ by $\mathrm{P}^{* * *}$.
(d) IF improvement is still inadequate THEN choose the point $P_{L}$ giving the lowest $V$ in the simplex and construct a new simplex with all dimensions reduced by a factor of 2 .
(e) IF improvement still inadequate THEN construct a new simplex starting at $P_{L}$.

Following the above routine successive simplices produce a convergence of $V$ towards a minimum and the algorithm terminates when the values of $V$ at the $\mathrm{P}_{\mathrm{w}}$ and $\mathrm{P}_{\mathrm{B}}$ (the worst and best points in the simplex) coincide within the required accuracy.

The second minimisation subroutine named "MIGRAD" is a second order "steepest descents" algorithm. From a starting set of parameters giving a particular value of $V$, the routine calculates matrices of gradients and second derivatives of $V$

Hypersil


Silica Gel PR-183


Lichrosphere Si 1000


Fig. 9.

Silica Gel PR-179


Silica Gel HR-WPS-2


Silica Gel Vydac

(Continued on p. 80)

## Silica Gel PSM 60



Silica Gel PSM1000


Fig. 9. Optimum PSDs derived from SECC data for eight silica gel samples using mainframe computer and MINUIT optimisation routine (method 3B). $\triangle$, SECC data; $\square$, PSD by Mercury porosimetry; O, PSD by computation. Silica gels were: Hypersil (Shandon Southern Products); PR-179 and PR-183 (Experimental materials, Shandon Southern Products); HR-WPS-2 (Experimental material, this work); LiChrosphere Si 1000 (Merck); Vydac (Varian Assoc.); PSM60 and PSM 1000 (DuPont SEC materials).
with respect to the $N$ parameters. From this the direction of steepest descent and the estimated position of the minimum $V$ are found. The procedure is then repeated starting at this new position in $N$-dimensional parameter space. MIGRAD tends to find local minima within a "minimum trough" and is most useful when performed after completion of the simplex routine as a form of fine tuning.

As a final check the "MIGRAD" routine moved a large distance from the position of the previously found optimum to check that no better solutions existed. In practice, SIMPLX provided satisfactory minima and MIGRAD used subsequently produced only marginal improvements.

The results obtained using these procedures are shown in Fig. 9 where the derived PSDs are compared to those found by mercury porosimetry for eight representative silica gels. Reasonable agreement is found in most cases.

## Discussion

The major contribution made by Knox and Scott ${ }^{13}$ to the derivation of PSDs from SECC data was to correct the error embodied in the method of Halasz and Martin ${ }^{11,12}$. The effect of this error is shown in Fig. 10 where three methods of deriving the PSD are compared. The Halasz method involves the simple displacement of the SECC curve to values of pore radii equal to 2.5 times the molecular radii. The PSD so obtained bears little relation to the PSD derived by mercury porosimetry or by computation according to method 3B except that it gives a reasonable value for the mean pore diameter [that is the pore diameter at $G(R)=0.5$ ].

Fig. 11 shows that the non-interactive program using method 3 A with a BBC microcomputer gives a good correlation between the PSD from mercury porosimetry and from computation, while Fig. 9 shows similar good agreement using method 3B with a mainframe computer for a range of commercial and experimental silica gels.

We have commented adversely on method 1 whereby a polynomial is fitted to


Fig. 10. Comparisons of PSDs derived by the Halasz method ( $\mathbf{A}$ ), by method 3 B of this work ( O ), by Mercury porosimetry ( $\square$ ). SECC data represented by $\triangle$.

Fig. 11. Example of optimised PSD derived from SECC data for silica gel WP-10-04 (experimental wide pore material, Shandon Southern Products) using BBC Microcomputer (method 3A). $\triangle$, SECC data; $\square$, PSD by Mercury porosimetry; O, PSD by computation.
the $[K(r), \ln r$ ] data and this subsequently differentiated to give the PSD. However, Fig. 12 shows that if the PSDs derived using a 6 th degree polynomial fit to the $[K(r)$, $\ln r$ ] data are simply truncated when $G(R)$ goes outside the range 0 to 1 the resulting curve is fairly close to that obtained by method 3 . While this technique is not intellectually satisfying and has not been extensively studied by us it could nevertheless provide an acceptable method of obtaining a reasonable PSD fairly simply.

Table I compares the mean pore radius, $\bar{R}$, defined as the value of $R$ for $G(R)=0.5$ with the manufacturer's stated pore radius, and with the mean molecular radius, $\bar{r}$, defined as the value of $r$ for which $K(r)=0.5$. The ratio $\bar{R} / \bar{r}$ is the "Halasz Factor'' giving the shift in the $K(r)$ curve required to give the best fit to the true PSD curve: This appears to be in the range 2.5 to 3.5 with a mean of about 3.0 rather than the value given by Halasz and Martin of 2.5 .

Bearing in mind that the values of $\bar{R}$ and $\bar{r}$ are somewhat subjective, with uncertainties of around $10 \%$, there is reasonable agreement for the majority of materials between the $\bar{R}$ values determined by mercury porosimetry and by unfolding the SECC curve. However both values are often at substantial variance with those quoted by the manufacturers even if there is broad agreement in so far as the supposed wide-pore materials do indeed have much wider pores according to our method than the supposed narrow-pore materials.

Use of the SECC unfolding technique clearly distinguishes between the different types of PSD exhibited by different materials. As can be seen from Figs. 9-11. The first five materials in Table I have a narrow PSD with $95 \%$ of the pores within a ten-fold range of diameter. LiChrosphere Si 1000 has a somewhat wider PSD with $95 \%$ of the pores within a roughly twenty-fold range, while the PSM materials have substantially wider PSDs with $95 \%$ of the pores within a fifty-fold range. Vydac and WP-10-04 are unusual in exhibiting a large difference between positions of the mercury porosimetry and SECC calculated PSD curves. This may indicate ink-bottle


Fig. 12. Comparison of PSD derived by method 1 after truncation of fitted curve (see text) with that derived by method 3 for silica gel HR-WPS-2 (experimental batch this work) and PSM1000 (DuPont). $\triangle$, SECC data $[K(r), \ln r] ; \square$, PSD by method $1 ;$, PSD by method 3 .
pores or that the particles have an outer surface layer with smaller pores than those inside the particles.

## EXPERIMENTAL

## Materials

Both commercial silica gels and batches of experimental materials were examined. Their physical properties along with the dimensions of the columns used for obtaining the SECC curves are listed in Table II.

## TABLE I

MEAN PORE RADII FOR SEC'MATERIALS

| Material (Fig.) | Mean solute radius $\bar{r}$ $(\dot{A})^{\star}$ | Mean pore radius $\bar{R}(\stackrel{\circ}{A})$ |  |  | Halasz factor $H=\tilde{\bar{R}} / \bar{r}$ <br> ( $\bar{R}$ is computed) |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | $\begin{aligned} & \text { By } \mathrm{Hg} \\ & \text { porosimetry }{ }^{\star \star} \end{aligned}$ | By computation** | Manufacturers value ${ }^{\star \star \star}$ |  |
| Hypersil (9) | 45 | 110 | 110 | 65 | 2.4 |
| PR-179 (9) | 95 | 230 | 270 | - | 2.9 |
| PR-183 (9) | 70 | 280 | 180 | - | 2.7 |
| WP-10-04 (11) | 65 | 130 | 220 | 150 | 3.3 |
| HR-WSP-2 (9) | 70 | 270 | 270 | - | 2.9 |
| LiChrosphere Si 1000 (9) | 165 | 300 | 470 | 500 | 2.8 |
| Vydac (9) | 80 | 140 | 280 | - | 3.5 |
| PSM60 (9) | 25 | 60 | 60 | 30 | 2.3 |
| PSM 1000 (9) | 170 | 630 | 630 | 500 | 3.7 |
|  |  |  |  | Mean | 2.94 |

[^6]Hypersil is a commercial silica gel manufactured by Shandon Southern Products. PR-179, PR-183 and WP-10-04 were experimental batches of wide-pore silica gels from the same supplier. HR-WPS-2 was an experimental wide pore material made for this work in the University of Edinburgh Chemistry Department. LiChrosphere Si 1000 is a commercial silica gel manufactured by Merck (Darmstadt, F.R.G.). Vydac is a commercial silica gel obtained from Varian Assoc. PSM60 and PSM1000 materials were obtained as packed columns from DuPont.

## Equipment and chromatography

Surface areas were determined with nitrogen using a laboratory constructed BET equipment. Pore volumes and PSDs were determined by mercury intrusion porosimetry using a Micromeritics pore sizer, Model 9305. The data handling package calculated the pore size using eqn. 2 with a contact angle of $130^{\circ}$.

High-performance SEC was performed on home assembled equipment comprising an Altex 110A high-pressure pump, a Rheodyne 7125 injection valve and a Spectroflow 773 ultraviolet photometric detector (Kratos, Manchester, U.K.).

Details of the column and particle dimensions for each material are given in Table II. The Zorbax materials were supplied prepacked in DuPont columns. The remainder of the materials were packed into Shandon type columns using a Shandon packing system. Isopropanol was used as the dispersing liquid for the slurry and methylene chloride as the follower liquid. The packing was carried out at 200 bar. Solutes were polystyrene standards of polydispersity less than 1.1, obtained from Polymer Labs. (Church-Stretton, U.K.). All solvents were of HPLC-grade supplied by Rathburn Chemicals (Walkerburn, U.K.).

## Computing equipment

The microcomputer was a BBC Model B made by Acorn Computers (Cambridge, U.K.). The more powerful optimization was carried out using a program named MINUIT devised by the Cern Computer Centre (Cern, Switzerland). This program was operated on the Edinburgh Multi-Access System (EMAS) at the Edinburgh Regional Computing Centre (ERCC).

TABLE II
PARTICLE PROPERTIES AND COLUMN DIMENSIONS

| Material | Particle <br> size <br> $(\mu m)$ | Surface <br> area <br> $\left(m^{2} \mathrm{~g}^{-1}\right)$ | Pore <br> volume <br> $\left(\mathrm{cm}^{3} \mathrm{~g}^{-1}\right)$ | Dimensions of <br> test column <br> $(\mathrm{mm})$ |
| :--- | :---: | :--- | :--- | :--- |
| Hypersil | 5 | 176 | 0.68 | $250 \times 4.6$ |
| PR-179 | 5 | 59 | 0.63 | $250 \times 5.0$ |
| PR-183 | 5 | 74 | 0.64 | $250 \times 5.0$ |
| WP-10-04 | 5 | 93 | 0.53 | $250 \times 5.0$ |
| HR-WPS-2 | $5-10$ | 90 | 1.30 | $100 \times 4.6$ |
| LiChrosphere Si 1000 | 10 | - | 0.98 | $250 \times 4.6$ |
| Vydac | 5 | - | 0.46 | $250 \times 5.0$ |
| Zorbax PSM60 | 5 | - | 0.47 | $250 \times 6.2$ |
| Zorbax PSM1000 | 5 |  | 0.37 | $250 \times 6.2$ |

## CONCLUSIONS

We have demonstrated that PSDs of silica gels used for HPLC may be derived by "unfolding" SECC data.

The best procedure optimises the volume fractions of a group of up to seven pores whose radii are in geometric progression. This can be achieved using a microcomputer, or more efficiently using a mainframe computer.

Computed PSDs agree well with those found by mercury porosimetry.
The method of Halasz and Martin ${ }^{1,12}$ gives a reasonable value for the mean pore size of a material but too small a slope for the PSD curve, especially for materials with a narrow PSD. The "Halasz Factor" should however be taken as 3.0 rather than 2.5.

We recommend that the methods developed in this paper be employed to determine PSDs of mesoporous materials especially when they are to be employed for size exclusion and other forms of liquid chromatography.

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Fig. 2. (a) The surface ( $\leqslant$ ) and interfacial tension ( $\square$ ) behavior of a $30 \%$ fixed water and $70 \%$ methanol and acetonitrile mixture, as a function of acetonitrile $\%(v / v)$ change. (b) The density behavior at two different mixtures of fixed $30 \%$ and $70 \%$ water (the rest methanol and acetonitrile mixture) as a function of acetonitrile $\%(\mathrm{v} / \mathrm{v})$ change. For more examples on the above physical properties, contact the principal investigator directly.

Fig. 2a the surface tension and interfacial tension for a mixture containing 30\% (v/v) water have been plotted against volume percent of acetonitrile replacing $70 \%$ methanol. In Fig. 2b the density for two mixtures of 30 and $70 \%$ water have been plotted. Fig. 3 represents the behavior of the distribution coefficients for three different solutes in a ternary solvent system of $30 \%$ water and $70 \%$ methanol and acetonitrile combined. In Fig. 4a-c the concentration of free (unassociated) water, acetonitrile and methanol at three different starting water compositions ( 30,40 and $70 \%$ ) are plotted against their corresponding acetonitrile volume fraction in the ternary mixtures, respectively.


Fig. 3. The partition coefficient behavior of three different solutes between a $30 \%$ fixed water, $70 \%$ methanol and acetonitrile and a $n$-hexadecane paraffinic phase, as a function of acetonitrile $\%$ ( $\mathrm{v} / \mathrm{v}$ ) change. Solutes: benzene ( $\square$ ); benzonitrile ( $\bullet$ ); nitrobenzene ( $\square$ ).


Fig. 4. The volume \% of "free" (unassociated) water (a), acetonitrile (b) and methanol (c) as a function of experimental acetonitrile $\%(\mathrm{v} / \mathrm{v})$ in three different ternary mixtures of the above solvents, respectively. Water contents: 30 ( $\square$ ); $40(\leftrightarrow) ; 70 \%$ $\square$

## DISCUSSION

In a ternary solvent system when going from a high polar to a much less polar solvent mixture one anticipates a relatively smooth and monotonic decrease in the retention time in RPLC. Contrary to this expectation, a very irregular trend appears in the behavior of the capacity factor, when at a fixed value of water content methanol is being replaced by a much less polar organic modifier such as acetonitrile or THF, as can be seen in Fig. la and b. In the search for an answer to what causes such an anomaly in retention behavior, one needs to know more about the nature of the eluent, specifically, and the structure of the bonded phase which, indeed, is directly affected by the mobile phase properties.

In RPLC, surface tension of the mobile phase, $\gamma_{\mathrm{s}}$, and the interfacial tension, $\gamma$, between the mobile phase and the bonded phase are considered to be important factors in interactions of a solute in the column ${ }^{14,15}$. Accordingly, these two param-
eters were measured in a few ternary solvent mixtures where unusual results were observed in these cases also (see Fig. 2a). At a constant water composition, as methanol is replaced by acetonitrile the surface and the interfacial tensions tend to go to a maxima then start dropping continuously. In determination of the interfacial tension values, one needs to know the density of each participating phase; the mixture and the paraffin. Once these values were measured, it seemed appropriate to look at the behavior of the density as a function of solvent composition. It was astonishing to see that the density follows more or less the same trend as the surface or interfacial tension. Again, as the amount of acetonitrile is increased in the ternary mixture, the density reaches a maxima and then tends to descend continuously, as seen in Fig. 2 b . This trend seems very peculiar, since at a constant concentration of water with the highest density $\left(0.9982 \mathrm{~g} / \mathrm{ml}\right.$ at $\left.20^{\circ} \mathrm{C}\right)$, as acetonitrile with the lowest density $\left(0.7857 \mathrm{~g} / \mathrm{ml}\right.$ at $20^{\circ} \mathrm{C}$ ) is replacing methanol with a density between water and acetonitrile, one simply expects a continuous decrease in the density of the mixture.

As can be seen in Fig. 4a-c, in a ternary solvent system, at a constant water composition, as the methanol is replaced by acetonitrile the amount of free acetonitrile increases gradually, as expected (Fig. 4b), and free methanol content decreases very rapidly, indeed, much faster than appearance of added acetonitrile (Fig. 4c). The result is an unexpected increase in the concentration of free water as acetonitrile is added, and almost at the same rate as acetonitrile is introduced into the mixture. This behavior can be explained by the strength of the association between each pair. As discussed before (see Table I), water and methanol show the highest degree of association compared to water-acetonitrile and methanol-acetonitrile pairs. Naturally, as methanol is being replaced by acetonitrile, the acetonitrile concentration would increase rapidly since it has a very weak association capability with either water or methanol. Methanol tends to disappear at a much faster rate than the appearance of acetonitrile since first, it is being replaced, and second because it has a strong tendency to associate with water. Hence, a fair amount of methanol would be used up to associate with water, and as a result not much free methanol can be expected to remain in the mixture. In the case of water, even though its starting concentration is kept constant, its free concentration tends to increase almost at the same rate as the disappearance of methanol. This is the case, since the water-acetonitrile pair do not associate very strongly (see Table I), on one hand, and no matter how much acetonitrile is added a very small fraction of water would associate with acetonitrile and the rest remains free or complexed with the methanol in the mixture. On the other hand, since methanol is being replaced by acetonitrile, continously lesser and lesser methanol is going to be available to associate with water and consequently more free water is going to be released into the mixture (see Fig. 4a).

Considering the above findings, the content in terms of free water seems very important, since one can correlate the behavior of the surface tension, interfacial tension, density, partition coefficient and the capacity factor to it in various mixtures. The density, surface tension and interfacial tension tend to increase at first, when acetonitrile starts replacing methanol in the mixture of water-methanol (while the starting composition of water is kept fixed). This replacement results in the release of some water due to the existence of a lesser amount of methanol to associate with water, on one hand and a much lower degree of association of water with acetonitrile, on the other. The result would be the addition of more free water into the solution.

Water with the highest value in density, surface and interfacial tensions, compared to methanol and acetonitrile, would force these quantities to rise, as seen in Fig. 2a and $b$. As the replacement of methanol with acetonitrile continues, the amount of free acetonitrile with a lower value in density, surface and interfacial tensions, compared to water, increases high enough to overcome the rate of release of water in the mixture. This would make acetonitrile to become the dominant component in determining the physical properties of the mixture and the descending path of the above quantities would be a direct result of this change, as seen in Fig. 2a and b). The same argument applies in the cases of partition coefficient and capacity factor (since they behave relatively similar to density and surface tension, see Fig. la and b and 3). One observes a lag or even an increase in the capacity factor (retention time) and partition coefficient, depending on the solute, at the beginning when acetonitrile starts to replace methanol (in a fixed starting composition for water) in the mixture of watermethanol. This, again, seems to be the case because more free water gets released into the mixture. Introduction of more water into the mixture tẹnds to reduce or even cancel the effect of added acetonitrile, which is a better organic solvent, and consequently resulting in a lag or even an increase in the retention time (capacity factor) and partition coefficient. As this process continues the acetonitrile concentration increases enough to overcome the undesired effect of the released free water and that is when the retention time starts to descend.

It appears then that the anomalous behaviour of solute retention found experimentally can be explained by a judicious extension of the approach by Katz et $a l .{ }^{9}$ to the competitive equilibria occurring in ternary mixtures. The measurement of other physical parameters such as density and surface tension supports the model.

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[^0]:    A paper describing the determination of Pore Size Distributions using the methods described in this thesis has been accepted by the Journal of Chromatography and will appear in January 1987.

[^1]:    1. Gel Reinforcement, where the structure is made more rigid without significant alteration to the skeleton by the deposition of additional silica. Silicic acid or low molecular weight polysilicic acids may be introduced into a saturated solution and deposited evenly on the gel surface
[^2]:    From the previous work, variation in structure using HTT could be accomplished over:a range of temperature from $120-250^{\circ} \mathrm{C}$. Variation in structure could also be achieved by sintering treatment from $750-900^{\circ} \mathrm{C}$. Using

[^3]:    *     - produced by E.l. Du Pont de Nemours \& Co. Wilmington, Delaware. U.S.A.
    ** - produced by Monsanto Limited, London. England.

[^4]:    * Measured using a stroboscope
    ** Measured using an optical microscope
    Mesh Sizes.

[^5]:    

[^6]:    * $\vec{r}$ is value of $r$ at which $K(r)=0.5$.
    ** $\bar{R}$ is value of $R$ at which $G(R)=0.5$.
    *** Manufacturers' normally quote the pore diameter, these values are half the diameter.

