

THEORETICAL STUDIES IN
HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

by

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DECLARATION

This thesis is the original work of the author, unless otherwise stated, and has not been submitted previously for any other degree. The work in chapter six was begun as an undergraduate project, but was fully revised and significantly extended before inclusion in this thesis.

A list of the postgraduate courses attended during the period of this research is given on piii.

Hugh P. Scott B.Sc.

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ABSTRACT OF THESIS (Regulation 6.9)

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ABSTRACT

The various modes of chromatography, particularly liquid chromatography are discussed. Various theories of band broadening in chromatography are described, and equations suitable for testing against experimental data are devised.

In order to facilitate the interpretation of experimental data, diffusion coefficients for a number of solute/eluent pairs were measured directly by the chromatographic broadening technique using glass capillary tubing and HPLC detection technology.

Band broadening by axial diffusion was examined by the interrupted flow technique. This enabled determination of the obstructive factor for both excluded and retained solutes. The relative rate of diffusion in the stationary phase was also obtained from these experiments.

Band broadening by slow mass transfer was examined by determining plate heights using large (50 - 540 μm) particles and high reduced velocities. Although it was not possible to ignore flow effects in this experiment, reasonable assumptions bring the experimental data into the range predicted by the non-equilibrium theory. The retention dependence of the data was also in agreement with this theory.

In a completely different vein, a computer model was developed to investigate the mechanism of size-exclusion chromatography. This model consisted of randomly-sized touching non-overlapping spheres. Although the model did not adequately fit the results, this was probably due to differences in porosity and pore-size distribution than to any effect of pore shape. Pore shape was not found to be critical in these studies.

An alternative model consisted of cylindrical pores whose radii were determined by the pore-size distribution of the material in question. Initial results were encouraging, and better data may lead to a good model for SEC.

COURSES ATTENDED

The following courses were attended during the period of postgraduate study.

Detergency and the Design of Surface Active Agents

Dr. W.D. Cooper (Autumn 1978)

The Neutron in Chemistry

Professor R.A. Cowley and others

(Edinburgh University Physics Department) (December 1978)

High Performance Liquid Chromatography in Clinical and Biomedical Chemistry

Various Speakers

(Chemical Society Analytical Division/Association of Clinical Biochemists) (February 1979)

Catalysis and Chemical Productivity

Dr. N.J. Sampson and Others

(ICI Petrochemicals Division) (March 1979)

Advanced Seminar Course in HPLC

Various Speakers

(Chemical Society/Wolfson Liquid Chromatography Unit) (July 1979)

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Dr. C.N.M. Pounder

(Spring 1980)

Advances in Applied HPLC

Various Speakers

(Chemical Society Analytical Division/Association of Clinical Biochemists) (February 1980)

Industrial Inorganic Chemistry

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(ICI Mond Division) (March 1980)

Courses attended (contd.)

- A New Approach to Teaching Quantum Mechanics to Chemists
Dr. K.P. Lawley (Autumn 1980)
- Chromatography, Equilibria and Kinetics
Various Speakers
(15th Faraday Symposium)
(at University of Sussex) (December 1980)
- Electronics and Microprocessors
Mr. A. King (Spring 1981)
- Clinical and Biochemical Advances in HPLC
Various Speakers
(Chemical Society Analytical Division/Association of Clinical
Biochemists) (February 1981)
- Introduction to Management
Various Speakers
(Careers Recruitment and Advisory Service)
(at University of York) (September 1981)

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1.1. DEFINITION OF CHROMATOGRAPHY

Chromatography is the name given to a method of separation of different molecules, solids. The molecules of separation of the participating of the molecules between two phases of matter in contact, one of which is stationary and the other continuously moving. If the molecules have different distribution coefficients between the two phases, the one which has a greater proportion of its molecules in the mobile phase will be moved further from the starting point than the other in the same time.

CHAPTER 1

INTRODUCTION

The name 'chromatography' was given by Michael Szwed, who was working with plants. In 1906 he gave the name of the technique which was used in his experiments. From the above definition of the word, it is obvious that chromatography is the separation of substances by migration to some phase.

1.1. Scope of Chromatography

Various chromatographic techniques are possible depending on the choice of mobile and stationary phases. The mobile phase may be a gas or a liquid, and the stationary phase may be a liquid (supported on a solid) or a solid. When the mobile phase is a gas, the technique is known as gas chromatography. Gas liquid chromatography (GLC) and gas solid chromatography (GSC) are the two main forms of this technique with the different types of stationary phase. The terms 'partition' and 'adsorption' describe the mechanism of separation and are used in the stationary phase with a liquid as the mobile phase. The technique is known as

CHAPTER 1

INTRODUCTION

1.1. DESCRIPTION OF CHROMATOGRAPHY

Chromatography is the name given to a method of separation of different molecular species. The mechanism of separation is the partitioning of the molecules between two phases or zones in intimate contact, one of which is stationary and the other continuously moving. If two compounds have different distribution coefficients between the two phases, the one which has a greater proportion of its molecules in the mobile phase will be moved further from its starting point than the other in the same time.

The name 'chromatography' was given by Tswett (1), who was working with plant pigments. The name was given because of the coloured bands which appeared in his experiment. From the above description of the process, it is obvious that colour is not a necessary requirement for separation to take place.

1.2. MODES OF CHROMATOGRAPHY

Various chromatographic techniques are possible, depending on the choice of mobile and stationary phases. The mobile phase may be a gas or a liquid, and the stationary phase may be a liquid (supported on a solid) or a solid. When the mobile phase is a gas, the technique is known as gas chromatography. Gas liquid partition chromatography (2) and gas solid adsorption chromatography (3) are the two main forms of this technique with the different types of stationary phase. The terms 'partition' and 'adsorption' describe the mechanism whereby molecules are held in the stationary phase. With a liquid as the mobile phase, liquid liquid partition

chromatography and liquid solid adsorption chromatography are the corresponding modes, but other modes such as ion exchange, bonded phase and exclusion chromatography are also possible. A more complete analysis of the modes of liquid chromatography is given below.

For most chromatographic techniques, both stationary and mobile phases are held in a column. The stationary phase for liquid chromatography, however, may be in the form of a sheet of paper (paper chromatography) (4) or a thin layer of material deposited on a glass plate (thin-layer chromatography) (5).

This thesis deals primarily with column liquid chromatography. The other techniques described above will be mentioned in passing only.

1.3. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Modern column liquid chromatography is generally known as high-performance liquid chromatography (HPLC), since by using high pressures and sophisticated equipment good separations of compounds can be obtained in much shorter times than with traditional liquid chromatographic techniques using gravity feed. This is achieved by packing small (3-10 μ m) particles into fairly short (100-250mm) columns to form the stationary phase, and pumping the mobile phase through this at high (20-200 bar) pressures. Separations of many mixtures may thus be obtained in times of about 15-20 minutes. The pioneering work permitting such high performance was carried out by Lipsky (6), Huber (7) and Kirkland (8).

Various modes of HPLC are possible, depending on the exact nature of the stationary and mobile phases. A brief description

of the most common modes is given below.

The first mode of chromatography used was liquid-solid chromatography (1), more commonly known as adsorption chromatography. In this mode, the solute is partitioned between a non-polar mobile phase and the surface of an adsorbate, such as silica or alumina, which is highly polar. This technique requires careful control of the adsorbent activity. This is achieved by adding a small percentage of polar modifier to the eluent. A detailed treatment of this technique is given by Snyder (9).

Liquid-liquid partition chromatography was invented by Martin and Synge (10). In this mode, the stationary phase is a liquid supported on an inert solid support. Wide pore silica gel is often used as the support. The stationary liquid may be either polar (such as triethylene glycol) or non-polar (such as squalane). In the former case the eluent used would be non-polar and saturated with stationary phase to maintain the surface coating. Polar eluents would be used with non-polar stationary phases. Because of the difficulty of holding the stationary phase in the column, these systems are little used now, having been largely superseded by systems using chemically bonded stationary phases.

Chemically bonded stationary phases are prepared by bonding organic molecules to the surface of an adsorbent, normally silica gel. Various types of phase can be prepared, but the most common are hydrophobic phases, polar phases or ion-exchange phases.

Hydrophobic phases are prepared by using groups such as octadecyl (C_{18}), octyl (C_8) or other short non-polar chains. Polar phases are prepared by using groups such as aminopropyl and

cyanopropyl, and ion-exchange phases by using groups such as sulphonate and quaternary ammonium. These phases, especially the hydrophobic type, are now very widely used in HPLC. A review of reversed phases is given in reference (11).

When a hydrophobic stationary phase is used with a polar eluent the system is referred to as a reversed-phase system, since the polarity of the phases is reversed with respect to adsorption chromatography. The eluent used in such systems is normally a mixture of water and a water-soluble organic liquid, methanol or acetonitrile being the most common.

Reversed-phase liquid chromatography probably involves some extraction of the less polar component of the eluent onto the stationary phase, thus forming an organic-rich layer at the surface. The solute is partitioned between this layer and the eluent (12,13). Other suggested mechanisms are referred to in reference (11).

In order to separate ionisable compounds, ion exchange chromatography may be used (14). The stationary phase contains fixed ionised groups, such as sulphonic acid groups (for separation of cations), or quaternary ammonium groups (for separation of anions). Counter ions must also be present to preserve electrical neutrality. Solute ions exchange with the counter ions and are thus retained. The eluent must be buffered to control the acid-base equilibria in the column, in order to avoid peak tailing.

An alternative approach is to use either a normal phase or reverse phase system, and to include an ion-pairing agent in the system (15). Typical pairing agents are cetyltrimethyl ammonium bromide and naphthalene sulphonate. The former forms ion pairs with cations; the latter with anions. The charges on the ions in

an ion pair are shielded from the eluent by the large non-polar parts of the molecules. The ion pairs thus behave like large non-polar molecules and are predominately found in the organic phase. Once formed, the ion pairs may be separated by either normal or reverse-phase chromatography; the latter is becoming the more popular.

Large molecules may be separated on the basis of size by a technique known as size-exclusion chromatography (16). In this technique, there is no chemical interaction between the sample and the stationary phase. The separation is determined by the degree to which sample molecules can penetrate the pores of the support. Large molecules cannot enter the pores and thus elute more quickly than smaller molecules which can enter the pores. Control of pore size is thus necessary to achieve good separations.

1.4. DEFINITIONS

When a solute is eluted from a chromatographic system, having been introduced as a sharp concentration pulse, the eluted band for a sufficiently efficient system will be Gaussian in shape. If two solutes are introduced at the same time, but have different distribution coefficients between the two phases, the maxima of the eluted bands will appear at different times. But since the concentration pulse of each sample broadens as elution proceeds, it is possible that the bands will still overlap, and a useful degree of separation will not have been achieved.

The degree of separation may be quantified by the resolution factor R_s :

$$R_s = \frac{\Delta z}{4\sigma} \quad 1.4. - 1a$$

$$R_s = \frac{\Delta z/z}{4\sigma/z} \quad 1.4. - 1b$$

where z is the distance travelled from injection, Δz is the distance between the two peaks, and σ is the mean standard deviation of the two peaks. All three quantities must be measured in the same units, but any convenient units may be used. The separation of the two peaks may be said to be satisfactory if $R_s > 1.5$; this corresponds to nearly baseline separation of the components.

Resolution depends on two parameters, as may be seen from equation (1.4.-1). The first is the separation of the two peaks, which depends on their relative distribution coefficients (see equation 1.4.-1b). The second is the relative peak width which depends on the kinetics of the processes occurring during elution.

Since molecules are washed down the column only while in the mobile phase, each molecule spends the same time t_m in this phase, within statistical limits. Under ideal conditions, when no band broadening occurs, all molecules would spend exactly t_m in the mobile phase, but the kinetics of the processes involved under real conditions lead to this being an average time, the statistical limits being set by the kinetics of the processes going on. This time t_m may be measured as the time taken for elution of a substance which is neither retained by the stationary phase nor excluded from the pores of the support. If a solute is retained, it will elute in a time t_r , which is the time taken for the mean of the peak to elute. It thus spends a time $t_r - t_m$ in the stationary phase, again within statistical limits.

The ratio of the time spent in the stationary phase to that spent in the mobile phase is given by the phase capacity ratio, k' :

$$k' = \frac{t_r - t_m}{t_m} \quad 1.4. - 2$$

This quantity is known as the phase capacity ratio since it may be shown to correspond to the ratio of the amount of solute in the stationary phase to the amount in the mobile phase. It is closely related to the distribution coefficient, and is a useful way of characterising the degree of retention of a solute in a system. Indeed it is readily shown that k' is related to the equilibrium distribution coefficient for the solute between the mobile and stationary phases by equation 1.4. - 3:

$$k' = D \frac{V_s}{V_m} \quad 1.4. - 3$$

where V_s is the volume of the stationary phase, V_m is the volume of the mobile phase, and D is the distribution coefficient.

Sometimes we wish to measure the ratio of the amount of solute actually moving to that which is stationary. This is the case in size-exclusion chromatography, where molecules are retained in stagnant mobile phase which is held in pores of the support material. From a theoretical standpoint, it is also often more useful to distinguish between moving and stationary molecules than between molecules in the mobile and stationary phases, since again some of the mobile phase is stagnant in the pores of the support. In these cases, the zone capacity ratio k'' is used. This is defined as -

$$k'' = \frac{t_r - t_o}{t_o} \quad 1.4. - 4$$

where t_0 is the mean time spent in the column by molecules of a solute confined to the mobile zone. This may be measured as the elution time of a solute which is totally excluded from the pores of the material.

The distinction between the phase capacity ratio and the zone capacity ratio is very important in some parts of this thesis. The difference is perhaps best seen by writing equations 1.4. - 4 and 1.4 - 5 in terms of volumes rather than times:

$$k' = \frac{V_r - V_m}{V_m} \tag{1.4. - 6}$$

$$k'' = \frac{V_r - V_0}{V_0} \tag{1.4. - 7}$$

Here V_r is the volume of eluent required to elute the solute. V_m is the volume of mobile phase in the column, and V_0 is the volume of mobile phase actually flowing in the column. The distinction between these two is made clear in figure 1.4 - 1, which schematically shows the important volumes which may be defined in a column.

These capacity ratios are used to express the position of a peak relative to the time taken for elution. Since they are ratios, they are independent of flowrate. The greatest difficulty associated with their use is the measurement of the time spent in the mobile phase or mobile zone.

The peak width is normally taken as being four times the standard deviation of the peak, since if tangents are drawn to the points of inflection of a Gaussian curve, the distance between the points where these tangents cross the baseline is four standard deviation units:

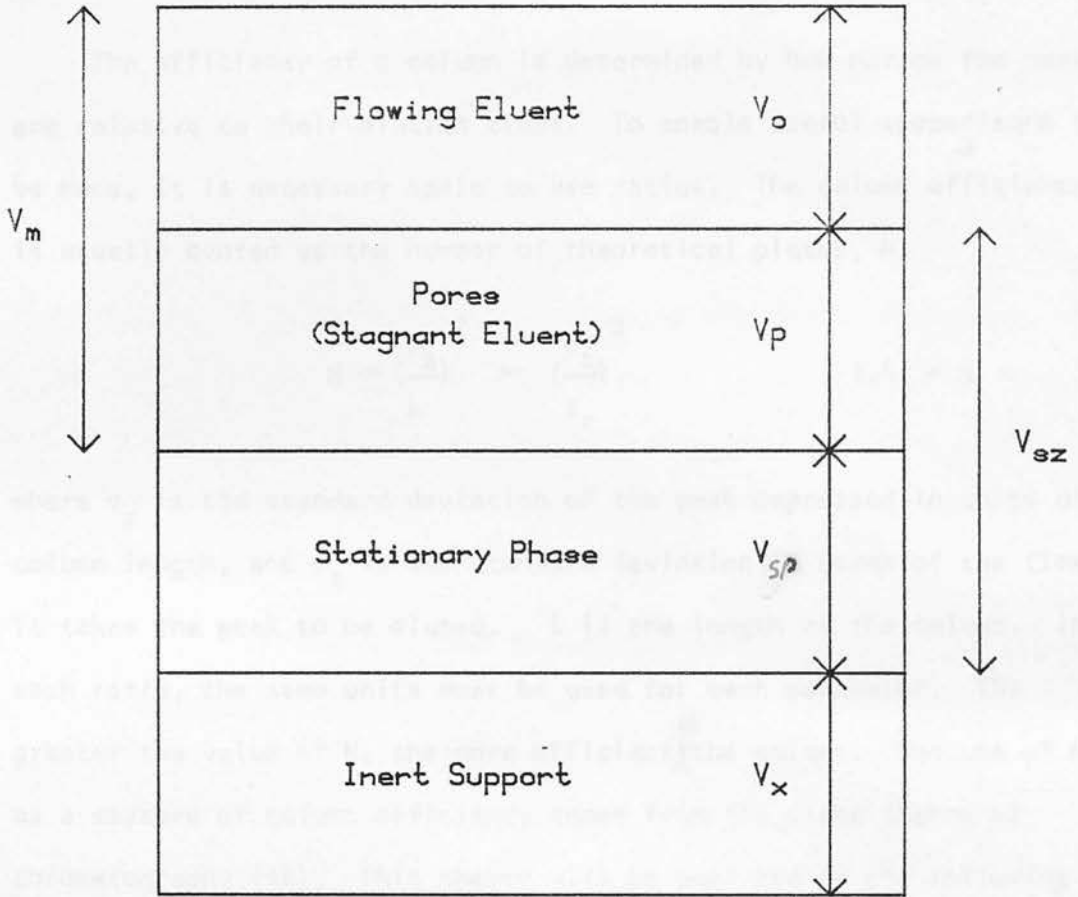


FIGURE 1.4. - 1.

Schematic Diagram of Volumes within a Column.

V_o = volume of mobile zone

V_p = volume of pores

V_{sp} = volume of stationary phase

V_x = volume of inert support

V_m = volume of mobile phase = $V_o + V_p$

V_{sz} = volume of stationary zone = $V_{sp} + V_p$

$$W_b = 4\sigma \quad 1.4. - 8$$

The efficiency of a column is determined by how narrow the peaks are relative to their elution times. To enable useful comparisons to be made, it is necessary again to use ratios. The column efficiency is usually quoted as the number of theoretical plates, N:

$$N = \left(\frac{\sigma_z}{L}\right)^2 = \left(\frac{\sigma_t}{t_r}\right)^2 \quad 1.4. - 9$$

where σ_z is the standard deviation of the peak expressed in units of column length, and σ_t is the standard deviation in terms of the time it takes the peak to be eluted. L is the length of the column. In each ratio, the same units must be used for each parameter. The greater the value of N, the more efficient ^{is} the column. The use of N as a measure of column efficiency comes from the plate theory of chromatography (10). This theory will be outlined in the following chapter.

It is useful to characterise columns by the 'height equivalent to a theoretical plate', H:

$$H = L/N \quad 1.4. - 10$$

since this indicates the inherent efficiency of the system. A more efficient separation could be achieved by, for example, increasing the length of the column, but such an increase in N would not indicate any improvement in the efficiency of the processes involved, whereas if a column is shown to have a lower value of H, the separating potential of the system is increased. Increasing efficiency by increasing

column length is an approach limited by factors such as pumping pressure; if H can be decreased, better separations can be achieved with the same length of column.

Another useful measure of the column efficiency is the reduced plate height h :

$$h = H/d_p \quad 1.4. - 11$$

where d_p is the particle diameter. This is the height equivalent to a theoretical plate expressed in terms of particle diameters. This allows a comparison between systems with different particle sizes, since many of the processes which lead to band broadening can be scaled to the particle size (17).

The efficiency of a column depends on the velocity of the eluent passing through it. The mobile phase velocity u may be obtained from the equation

$$u = L/t_m \quad 1.4. - 12$$

It is often useful to express this velocity in a reduced form, independent of particle size or the rate of diffusion of solute in the eluent. The reduced velocity, v , is used for this purpose (17):

$$v = u d_p/D_m \quad 1.4. - 13$$

where D_m is the diffusion coefficient of the solute in the eluent. If we are interested in the velocity of the mobile zone rather than the mobile phase, the reduced velocity to use is

$$v_o = \frac{u_o d_p}{D_m} = \frac{L d_p}{t_o D_m} \quad 1.4. - 14$$

The most important reason for the introduction of these reduced variables is that they allow the variation of column efficiency with eluent velocity to be expressed in universal equations, independent of particle size, solute type, or even the type of eluent (liquid or gas) used. Such an equation is the Knox equation (18):

$$h = Av^{1/3} + B/v + Cv \quad 1.4. - 15$$

The reasons for the form of this equation will be discussed in the following chapters.

CHAPTER 2

KINETICS

2.1. INTRODUCTION

It is important, for efficient chromatography, that the plates pack up as narrow as possible. It is therefore important to understand the nature of band broadening. The width of the elution band is related to the kinetics of the sorption processes. Several chapters have been devoted to explain the relationship between these processes and the ultimate peak widths. There are few, if any, types of theory or text) need: the Theoretical Plate model, the Van Deemter model, the theory of mass balance and the Porosity Diffusion Theory. These will be outlined below.

CHAPTER 2

KINETICS

2.2. PARTICULATE DIFFUSION

One cause of band broadening which is also caused by any of the above theories, but which is often neglected or overlooked, is the broadening due to resistance to diffusion. It is known that the rate of change of concentration with time is proportional to the square of the distance.

Chromatography is a process of mass transfer. The concentration of solute will depend on the amount of solute which has been adsorbed. Diffusion is a process of mass transfer. It is known that the rate of change of concentration with time is proportional to the square of the distance. The rate of change of concentration with time is proportional to the square of the distance. The rate of change of concentration with time is proportional to the square of the distance.

CHAPTER 2

KINETICS

2.1. INTRODUCTION

It is important, for efficient chromatography, that the eluted peaks be as narrow as possible. It is therefore important to understand the causes of band broadening. The width of the eluted band is related to the kinetics of the on-column processes. Several theories have been proposed to explain the relationship between these processes and the observed peak width. There are four main types of theory or model used: the Theoretical Plate model, the Random Walk model, the theory of Mass Balance and the Nonequilibrium Theory. These will be outlined below.

2.2. MOLECULAR DIFFUSION

One cause of band broadening which is not covered by any of the above theories, but which is often included as an additional term, is broadening due to molecular diffusion. Diffusion is discussed in chapter (3) below; only a few relevant details will be mentioned here.

A chromatographic band is a region of increased concentration of solute with respect to the concentration in the remainder of the column. Diffusional spreading will therefore take place about the concentration maximum. If the band is moving, diffusional spreading will take place at the same rate as if the band were stationary, but it will take place about the moving concentration maximum. If a coordinate system is chosen whose origin is at this maximum, the zone profile due to molecular diffusion will be independent of flow-rate.

The variance σ^2 of the distribution due to longitudinal molecular diffusion may be calculated from the Einstein equation (19):

$$\sigma^2 = 2Dt \quad 2.2. - 1$$

where D is the effective diffusion coefficient and t is the time for which diffusion has been taking place.

In the mobile phase, the value of the effective diffusion coefficient is given by

$$D = \gamma D_m \quad 2.2. - 2$$

where γ is an obstructive factor introduced to take account of the tortuosity and constriction of the channels in the column (20,21). The value of the time t in equation 2.2-1 is the time t_m spent in the mobile phase. This may be obtained from the column length and the eluent velocity by the equations

$$t_c = L/u \quad 2.2. - 3a$$

$$t = \frac{L}{v} \frac{d_p}{D_m} \quad 2.2. - 3b$$

The plate height is given by the equations

$$H = \sigma^2/L \quad 2.2. - 4a$$

$$h = \sigma^2/L d_p \quad 2.2. - 4b$$

When the value of σ^2 from equation 2.1. is used in this equation, with the values of D and t as given in equations 2.2. - 2 and 2.2.-3, the following equations for the plate-height result:

$$H = \frac{2\gamma D_m}{u} \quad 2.2. - 5a$$

$$h = \frac{2\gamma}{v} \quad 2.2. - 5b$$

For diffusion in the stationary phase, the diffusion coefficient to be used in equation 2.2. - 1 may be written as D_s where D_s is the diffusion coefficient in the stationary phase, which includes the effects of any stationary phase obstructive factor. The time to be used is t_s , which is related to t_m by the capacity ratio:

$$t_s = k' t_m \quad 2.2. - 6$$

The plate height is thus given by

$$H = \frac{2D_s k'}{u} \quad 2.2. - 7a$$

$$h = \frac{2}{v} \frac{D_s}{D_m} k' \quad 2.2. - 7b$$

2.3. THE THEORETICAL PLATE MODEL

The theoretical plate model was first used to describe distillation processes. Because of the analogy between distillation and chromatography, Martin and Synge (10) used this model to describe what happens to a band of solute as it migrates down the column.

Martin and Synge define a plate as 'the thickness of the layer such that the solution issuing from it is in equilibrium with the mean concentration of solute in the non-mobile phase throughout the

layer', although a better definition would refer to the thickness of the layer such that the concentration of the solution issuing from it is the same as that in the eluent in the plate if the plate were allowed to come to equilibrium, since the original definition does not allow for solutes which do not enter the stationary phase. From the second definition it is obvious that the number of plates in the column is related to the degree of departure from equilibrium. If equilibrium were obtained, the plate-height would be infinitesimal.

By considering what happens to the solute as it is washed from plate to plate, and equilibrates within each plate, the concentration within each plate after the passage of a given amount of eluent may be calculated. It is found that a binomial distribution results:

$$Q_{r+1} = \frac{n! (1 - \delta V/V)^{n-r} (\delta V/V)^r}{r! (n-r)!} \quad 2.3. - 1$$

where r is the serial number of the plate, and n is the number of incremental volumes δV of mobile phase which has passed. Q_{r+1} is the amount of solute in the $(r+1)^{\text{th}}$ plate, and V is the volume of a plate. When n and r are large (e.g. at the end of a fairly efficient column), the binomial distribution may be approximated by a Gaussian distribution:

$$Q_r = \frac{1}{(2\pi\sigma^2)^{\frac{1}{2}}} \exp\left(\frac{-(r - \bar{r})^2}{2\sigma^2}\right) \quad 2.3. - 2$$

where \bar{r} is the plate containing the maximum amount of solute and σ is obtained from the formula

$$\sigma^2 = n (1 - \delta V/V) (\delta V/V) \qquad 2.3. - 3$$

Most peaks obtained in efficient chromatography are practically Gaussian in shape so the plate theory successfully predicts peak shape. It is possible, from the above comments on the degree of non-equilibrium, to see a relationship between plate-height and eluent velocity and particle size, since large velocities and large particles lead to large deviations from equilibrium. These relationships appear more naturally from other theories.

One of the most lasting contributions of plate theory to chromatography is the use of the number of theoretical plates, and the height equivalent to a theoretical plate, as measures of column efficiency.

2.4. THE RANDOM WALK MODEL

A random walk is a process in which a series of steps are taken, the direction of each step being completely independent of the direction of the previous step. The associated problem is to determine the probability of being a certain distance from the starting point after a given number of steps. Such a problem is posed by Moore (22): 'A sailor leaves a waterfront bar still able to walk but unable to navigate. He is just as likely to take a step east as a step west, i.e. each step has a probability $\frac{1}{2}$ of being in either direction. After he has taken N steps, what is the probability that he is n steps away from his starting point?'.

The above process is analogous to that of a molecule migrating in a gas or liquid, where each step is the distance travelled between

collisions. The analogy breaks down since in this case the length of each step is not constant. Such a process approximates to a random walk where the step length is the root mean square distance between collisions.

Molecular diffusion may also be modelled by a random walk. If a large number of molecules of one species are present at a given position in a sea of molecules of a different species, the probability of a molecule reaching a certain position after a certain time is given by the random walk model, and so the resulting concentration profile may be calculated (23). The same result applies to the diffusion of a concentration pulse as it is washed down a chromatographic column, if the origin of the random walk is taken to move with the mean velocity of the pulse.

The same model may be used to describe the effects on such a pulse of sorption-desorption kinetics (17), since when a molecule is sorbed into the stationary phase, it takes a step back relative to the zone centre, as it is immobile, whereas when it is desorbed it takes a step forward relative to the zone centre, as it then moves at the velocity of the mobile phase which is faster than the velocity of the band as a whole.

Flow phenomena may be described in the same way (17). A step back from the band centre occurs when a molecule moves to a region where the eluent velocity is slower than the mean eluent velocity, and a step forward occurs when the molecule moves to a region where the eluent velocity is above the mean velocity.

The solution to the random walk problem is that the probability of being a distance of n steps from the origin of the walk after taking a total of N steps is given by a Gaussian distribution (23)

$$p(n) = \left(\frac{2}{\pi N}\right)^{\frac{1}{2}} \exp\left(\frac{-n^2}{2N}\right) \quad 2.4. - 1$$

If each step is of length ℓ , the probability of being a distance L from the origin is given by

$$p(L) = \left(\frac{2}{\pi N \ell}\right)^{\frac{1}{2}} \exp\left(\frac{-n^2}{2N \ell}\right) \quad 2.4. - 2$$

where $L = n\ell$.

The variance of this distribution is given by

$$\sigma^2 = \ell^2 N \quad 2.4. - 3$$

thus the standard deviation is given by

$$\sigma = \ell N^{\frac{1}{2}} \quad 2.4. - 4$$

If two random processes take place independently, either simultaneously or consecutively, the final distribution is still Gaussian with a variance given by

$$\sigma^2 = \sigma_1^2 + \sigma_2^2 \quad 2.4. - 5$$

where σ_1^2 and σ_2^2 are the variances of the distributions due to the two contributing processes. This result may be extended for any number of independent processes. If the processes are not independent this result cannot be applied.

To obtain useful results from the random walk model, it is necessary to find some way of estimating the length of each step and the number of steps executed during the period of interest. Examples of how this may be done in chromatography are given below.

ADSORPTION-DESORPTION KINETICS

For simple one-step adsorption-desorption processes there is a rate constant k for each process which expresses the rate of adsorption or desorption. This is given by

$$k = \frac{d \ln x}{dt} \quad 2.4. - 6$$

where x is the amount of solute per unit length of column. The value of k will be different for adsorption and desorption. The rate constant for adsorption will therefore be written as k_a , and the constant for desorption will be written as k_d . The mean time constant for such processes is the inverse of the rate constant. Thus

$$t_a = 1/k_a \quad 2.4. - 7$$

$$t_d = 1/k_d \quad 2.4. - 8$$

The phase capacity ratio is related to these quantities by the equation

$$k' = \frac{t_d}{t_a} = \frac{k_a}{k_d} \quad 2.4. - 9$$

On average a molecule will remain in the mobile phase for a time t_a before being adsorbed. During this time it will move ahead of the main band of eluting solute. It will also travel a distance l down the column:

$$l = u t_a \quad 2.4. - 10$$

where u is the eluent velocity. The number of adsorptions experienced is thus given by

$$n = L/\lambda \quad 2.4. - 11$$

While a molecule is adsorbed, on average for a time t_d , it will lag behind the main band, so these periods also count as steps in the random walk. The number of adsorption steps is equal to the number of desorption steps, since each period for which the molecule is adsorbed is terminated by a desorption. Thus the number of steps in the random walk is

$$n = \frac{2L}{\lambda} = \frac{2L}{u t_a} \quad 2.4. - 12$$

The length of each step is the distance moved relative to the zone centre during that step. The velocity of the zone centre is lower than the eluent velocity because all the molecules of the zone spend a fraction $k'/(1 + k')$ of their time in the column in the stationary phase. The velocity of the zone is given by

$$u_z = \frac{L}{t_r} = \frac{L}{(1+k')t_m} = \frac{1}{1+k'} u \quad 2.4. - 13$$

Therefore for a molecule in the mobile phase with move a distance $\bar{\lambda}$ ahead of the zone centre during its time of residence in that phase, where $\bar{\lambda}$ is given by

$$\bar{\lambda} = (u - u_z) t_a \quad 2.4. - 14$$

From equation 2.4. - 13 this may be written as

$$\bar{\lambda} = \frac{k'}{1+k'} u t_a \quad 2.4. - 15$$

It may readily be shown that the step length for a molecule in the stationary phase is also given by equation 2.4. - 15. While this molecule is stationary, the zone moves with a velocity u_z as given by equation 2.4. - 13. The distance the zone moves is therefore given by

$$\bar{l} = \frac{1}{1+k'} u t_d = \frac{k'}{1+k'} u t_a \quad 2.4. - 16$$

which agrees with equation 2.4. - 15. Equation 2.4. - 9 has been used to relate t_a and t_d .

Equation 2.4. - 12 gives an expression for the number of steps taken in the random walk, and equation 2.4. - 15 gives the length of each step. The peak variance can therefore be calculated by using these values in equation 2.4. - 3 as

$$\sigma^2 = \left(\frac{k'}{1+k'}\right)^2 u^2 t_a^2 \frac{2L}{u t_a} = \left(\frac{k'}{1+k'}\right)^2 2L u t_a \quad 2.4. - 17$$

This may be written in terms of the mean desorption time as

$$\sigma^2 = \frac{2k'}{(1+k')^2} L u t_d \quad 2.4. - 18$$

again using 2.4. - 9 to relate the two time constants. The plate height may thus be written as

$$H = \frac{2k'}{(1+k')^2} u t_d \quad 2.4. - 19a$$

$$h = \frac{2k'}{(1+k')^2} \frac{D_m}{d_p^2} t_d v \quad 2.4. - 19b$$

by applying equation 2.2. - 4 and introducing the reduced velocity into equation 2.4. - 19b. Equation 2.4. - 19 has been shown to be correct for adsorption-desorption kinetics by the stochastic theory and by the non-equilibrium theory in spite of the approximate nature of some of the assumptions used.

DIFFUSION CONTROLLED KINETICS

The above approach can also be used when the stationary phase is a bulk phase, and separation proceeds by a partition mechanism. Equation 2.4. - 19 may still be used, with a suitable value for t_d . An approximation to t_d may be obtained in terms of the dimensions of the stationary phase and the diffusion coefficient D_s of the solute in this phase. If the stationary phase is present as spherical particles of diameter d_p , we may assume that a molecule must diffuse over a distance $d_p/2$ to escape from this phase. The time required to diffuse over this distance is given by the Einstein equation as

$$t_d = d_p^2 / 4 D_s \quad 2.4. - 20$$

When this value is substituted into equation 2.4. - 19, we obtain the following equations for plate height:

$$H = \frac{1}{4} \frac{k'}{(1+k')^2} \frac{d_p^2}{D_s} u \quad 2.4. - 21a$$

$$h = \frac{1}{4} \frac{k'}{(1+k')^2} \frac{D_m}{D_s} v \quad 2.4. - 21b$$

The form of these equations is correct, but the factor $1/4$ (known as the configuration factor, since it is determined by the configuration of the stationary phase) ought to be $1/30$. This error is due to an overestimation of the distance over which molecules must diffuse to escape from the stationary phase; few molecules will diffuse to the centre of the sphere, thus only a few will need to diffuse over a distance $d_p/2$ to escape from the mobile phase. The correct value of the configuration factor may be obtained from the non-equilibrium theory.

MOBILE PHASE MASS TRANSFER

The mobile phase will flow at different speeds at different position within the column. As molecules of solute alternately and randomly experience velocities greater than and less than the mean band velocity, the band will spread. Band broadening by this mechanism may also be treated by the random walk model. In this case, the steps are between regions of different velocity.

Giddings (17) proposes five types of velocity difference in the mobile phase:

1. The transchannel velocity difference, i.e. the velocity difference between eluent in the centre of a channel formed by the particles of the support and at the walls due to the parabolic flow velocity profile.
2. The transparticle velocity difference, i.e. the velocity difference between stagnant mobile phase within the pores of a particle and the streaming mobile phase outwith the particle.
3. A short-range interchannel velocity difference will exist between smaller and larger channels in fairly close proximity.

4. A long-range interchannel velocity difference similar to 3 will exist over a longer range.
5. A trans-column velocity difference will exist between the central and wall regions of the column, or between opposite walls, due to bending or coiling of the column.

Each of the above differences will make a contribution to the plate height. These contributions may be evaluated by identifying the number of steps taken between the different velocity regions in each effect, and the length of each step relative to the band maximum. The way in which this is done is best illustrated by example.

Consider the transchannel effect described above. The time required for a molecule to diffuse from one velocity region to another is given by

$$t_e = \omega_\alpha^2 d_p^2 / 2D_m \quad 2.4. - 22$$

where ω_α is the distance between these regions in units of particle diameter. Since a typical channel has a diameter of about $d_p/3$, this distance is approximately $d_p/6$, thus $\omega_\alpha = 1/6$.

The length of the step for this process is the distance gained by a molecule in the centre of the channel, or lost by a molecule near a channel wall, with respect to the mean velocity. This may be expressed as $\Delta u t_e$, where Δu is the difference between the mean velocity and the velocity at the extremes. Δu will be some fraction ω_β of the mean velocity, i.e. $\Delta u = \omega_\beta u$. Thus the step length is given by

$$l = \omega_\beta u t_e \quad 2.4. - 23$$

In the present case, $\omega_\beta \approx 1$, since the mean flow velocity is u , and for a parabolic flow profile the velocity is $2u$ at the channel centre and 0 at the walls.

The number of steps taken may be calculated as the number of times a solute molecule spends a time t_e moving at the mean eluent velocity u in travelling down the column:

$$n = L/u t_e \quad 2.4. - 24$$

From equation 2.4. - 3 the variance due to such a process is given by

$$\sigma^2 = \ell^2 n = \frac{\omega_\beta^2 \omega_\alpha^2}{2} \frac{d_p^2}{D_m} L u \quad 2.4. - 25$$

from whence

$$H = \frac{\omega_\beta^2 \omega_\alpha^2}{2} \frac{d_p^2}{D_m} u = \omega_i^2 \frac{d_p^2}{D_m} u \quad 2.4. - 26a$$

$$h = \frac{\omega_\beta^2 \omega_\alpha^2}{2} v = \omega_i^2 v \quad 2.4. - 26b$$

Values of ω_α and ω_β are calculated by Giddings for each of the velocity differences considered by him. These are listed in table (2.4. - 1).

If a molecule follows a single flow-path throughout the column, rather than diffusing between different paths, it will still experience different flow velocities because of the random nature of the chromatographic bed. This provides a second mechanism for transfer

TABLE 2.4. - 1

MOBILE PHASE EDDY DIFFUSION PARAMETERS

i	DESCRIPTION	ω_α	ω_β	ω_i	ω_λ	λ_i
1	Transchannel	1/6	1	0.01	1	0.5
2	Transparticle	1/2	1	0.1	10^4	10^4
3	Short-range interchannel	1.25	0.8	0.5	1.5	0.5
4	Long-range interchannel	10	0.2	2	5	0.1
5	Transcolumn	m/2	0.1	$0.001m^2$	$20m^2$	$0.02m^2$

m is the number of particles across the column diameter.

For the meanings of the various parameters, see text.

between the different types of velocity region listed above.

The velocity difference between the extremes of a flow regime will be the same as outlined above, since only the method of exchange has changed. The number of steps between these extremes will, however, be different. There will be some distance S over which the velocity of a solute molecule will remain approximately constant. This distance may be written in terms of the particle diameter:

$$S = \omega_{\lambda} d_p \quad 2.4. - 27$$

where ω_{λ} is the number of particle diameters over which the velocity remains constant. For the trans-channel effect considered above, this may be approximated by $\omega_{\lambda} \approx 1$.

The number of steps to be used in equation 2.4. - 3 is given by the number of such constant flow regimes experienced by a molecule in travelling the length of the column:

$$n = L/S \quad 2.4. - 28$$

The time of exchange is the time spent in each constant flow regime:

$$t_e = S/u \quad 2.4. - 29$$

Thus from equation 2.4. - 23 the length of each step is

$$l = \omega_{\beta} S \quad 2.4. - 30$$

The contribution to the variance is thus

$$\sigma^2 = \omega_{\beta}^2 \omega_{\lambda} d_p L \quad 2.4. - 31$$

and thus the plate-height contribution is

$$H = \omega_{\beta}^2 \omega_{\lambda} d_p \quad 2.4. - 32a$$

$$h = \omega_{\beta}^2 \omega_{\lambda} = 2\lambda_i \quad 2.4. - 32b$$

where λ_i is given by

$$\lambda_i = \omega_{\beta}^2 \omega_{\lambda} / 2 \quad 2.4. - 33$$

Values of ω_{λ} and ω_i are also given in table 2.4. - 1.

For many of the processes which lead to band broadening in chromatography, the plate-height contributions are additive.

From equation 2.4 - 5 it is possible to write

$$h_{\text{tot}} = h_{1d} + h_{sm} + h_{mm} \quad 2.4. - 34$$

where h_{tot} is the total reduced plate height, h_{1d} is the reduced plate height contribution from longitudinal diffusion, as given by equation 2.2. - 5, h_{sm} is the stationary phase mass transfer term as given by equation 2.4. - 19 or 2.4. - 21, and h_{mm} is the mobile phase mass transfer term. This term cannot be obtained from

$$h_{mm} = h_d + h_f \quad 2.4. - 35$$

where h_d is the contribution from the diffusion mechanism of mass transfer between velocity regimes and h_f is the contribution from the flow mechanism described above. Equation 2.4. - 35 does not hold because variances are only additive for independent processes, and the two processes involved are not independent. A molecule's stay in a flow regime may be terminated either by diffusing out of

that flow path, or by the flow path transporting it into a different velocity regime. The two mechanisms are thus said to be coupled. In such a case the random walk theory requires that the number of steps from each cause must be added. Thus the persistence of velocity span S when both mechanisms operate is given by

$$S = L / (n_f + n_d) \quad 2.4. - 36$$

where n_f is the number of exchanges due to the flow mechanism of transfer, and n_d the number of exchanges due to the diffusion mechanism of transfer. The value of n_f may be obtained from equation 2.4. - 28, with the value of S given by equation 2.4.- 27. Similarly, the value of n_d may be obtained from equation 2.4. - 24, with the value of t_e given by equation 2.4. - 32. The step-length l is therefore given by

$$l = \omega_\beta S \quad 2.4. - 37$$

where S is given by equation 2.4. - 36. The resulting equation for the variance when both processes are operating is therefore given by

$$\sigma^2 = \frac{\omega_\beta^2 L}{\frac{1}{2\lambda_i d_p} + \frac{D_m}{\omega_i d_p^2 u}} \quad 2.4. - 38$$

The value of h_{mm} to be used in equation 2.4. - 34 is thus

$$h_{mm} = \sum_i \frac{1}{2\lambda_i + 1/\omega_i v} = \sum_i \frac{1}{1/h_{fi} + 1/h_{di}} \quad 2.4. - 39$$

where h_f and h_d are the plate-height contributions from the two transfer mechanisms, and the summation is over the five different types of velocity inequality.

Although the random walk model is imperfect, it leads to an equation of an acceptable form for the variation of reduced plate-height with eluent velocity. Such an equation may be written as

$$h = \frac{B}{v} + C_s v + \frac{1}{1/A + 1/C_m v} \quad 2.4. - 40$$

The B term in this equation comes from longitudinal diffusion. From equations 2.2. - 5 and 2.2. - 7 this may be written as

$$B = 2\gamma_m + 2\gamma_s \frac{D_s}{D_m} k' \quad 2.4. - 41$$

The C_s term comes from slow mass transfer between the mobile and stationary phases. For example, for adsorption-desorption kinetics it may be evaluated as

$$C_s = \frac{2k'}{(1+k')^2} \frac{D_m}{d_p^2} t_d \quad 2.4. - 42$$

as shown in equation 2.4. - 19. The C_m term describes the slow mass transfer in the mobile phase and is given by

$$C_m = \sum_i \omega_i \quad 2.4. - 43$$

where the values of ω_i refer to specific velocity differences. The A term refers to the contribution from structural effects and is given

by

$$A = \sum_i \lambda_i \quad 2.4. - 44$$

The nature of equation 2.4. - 39 is such that for any particular value of v , the flow mechanism or the diffusion mechanism of transfer may dominate the contribution from mobile phase mass transfer. If $2\lambda_i \ll \omega_i v$, the plate-height contribution due to mobile phase mass transfer may be written as

$$h_i = 2\lambda_i \quad 2.4. - 45$$

If $2\lambda_i \gg \omega_i v$, this contribution may be written as

$$h_i = \omega_i v \quad 2.4. - 46$$

If $2\lambda_i$ and $\omega_i v$ are of the same order of magnitude, then the equation 2.4. - 39 must be used as it stands. Equation 2.4 - 40 may thus be written as

$$h = A_1 + \frac{B}{v} + C_1 v + C_2 v + \sum_i \frac{1}{1/A_2 + 1/C_2 v} \quad 2.4. - 47$$

where A_1 represents the contribution of the type which may be expressed by equation 2.4. - 45, and C_1 represents the contribution which may be expressed by equation 2.4. - 46. A_2 and C_2 represent the mobile phase terms for which the full coupling expression must be used.

The random walk model gives a valuable insight into the processes which cause band spreading. For adsorption-desorption kinetics it

does yield the same equations as may be derived from more rigorous theories. For diffusion-controlled mass transfer, the equation derived is correct apart from the configuration constant, which can only be roughly estimated. The most serious fault with the model lies in its treatment of mobile phase mass transfer, although no other model does better.

One reason for failure in this area is that there is a continuous change in velocity across a channel so examining only transfer between the centre of the channel and the walls is an oversimplification. A similar criticism of the other velocity inequalities may also be made.

2.5. THE STOCHASTIC THEORY

A more rigorous approach than the random walk model, still based on ideas of probability, is the stochastic theory (25). This is described by Giddings (17) as follows:-

'Due to the random events there is a certain probability, which can be explicitly written, that after a time t the molecule will have arrived somewhere in the interval from z to $z + \delta z$ along the column'.

From the probability described above, it is possible to derive the concentration profile after a given time.

This theory is well suited to the study of adsorption-desorption kinetics, but it is not so suitable for diffusion controlled kinetics, because the probabilities involved in describing the continuous process of diffusion are much less tractable than those used to describe the discrete adsorption-desorption processes. One

advantage of the stochastic theory is that it does not require the assumption of long time periods. This theory has been applied to one, two and multisite adsorption processes (25,26,27) and gives a good explanation of kinetic peak tailing due to different kinds of adsorption sites (26).

2.6. THE MASS BALANCE THEORY

A similar approach to the stochastic theory is the 'mass balance' approach (28,29). Equations may be developed which describe the concentration of solute at any distance along the column in terms of the processes by which a molecule may enter or leave a part of the column. A molecule may be transported into or out of a segment of mobile phase between $z = \ell$ and $z = \ell + \delta \ell$ by three different mechanisms: diffusion, flow or transfer to the stationary phase. The rate of change of concentration in this segment may thus be written as

$$\frac{\partial c_m}{\partial t} = D \frac{\partial^2 c_m}{\partial z^2} - u \frac{\partial c_m}{\partial z} + k_a (K c_s - c_m) \quad 2.6. - 1$$

where the first term on the right-hand side describes transfer by diffusion, the second describes transfer by flow, and the third describes transfer to the stationary phase. c_m is the concentration in the mobile phase, and c_s is the concentration in the stationary phase. The rate constant for transfer to the stationary phase is again k_a . K is the distribution factor, which is given by the ratio of c_m to c_s at equilibrium. This equation is intended to allow the calculation of c_m as a function of t at the end of the column. To achieve this, the value of c_s must be known. This may be found by

considering the processes which transport solute molecules to and from the stationary phase. Only one-mass transfer from the mobile phase-is normally considered. The value of c_s may be found by solving the differential equation

$$\frac{\partial c_s}{\partial t} = k_d (c_m - K c_s) \quad 2.6. - 2$$

where k_d is the rate constant for transfer to the mobile phase. Since this equation involves c_m , equations 2.6 - 1 and 2.6 - 2 are said to be coupled. Thus the solution of 2.6. - 1 is possible only after simplifying assumptions have been made. This approach was the basis for the Van Deemter equation (30).

2.7 THE NON-EQUILIBRIUM THEORY

The most powerful modern theory of band broadening in chromatography is the non-equilibrium theory developed by Giddings (17). According to this theory, band broadening occurs because, due to the relatively slow rate of the mass transfer processes compared to the eluent velocity, equilibrium between solutes in the mobile and stationary zones is never established. The concentration in the stationary zone always lags behind the equilibrium concentration because during the time required to establish equilibrium the solute concentration in the column element under consideration either rises as more solute is transported into that element than is transported out, or falls as there is a net movement of solute out of the element. The first of these cases describes the situation before the band maximum passes through the element, and the second describes the situation after the band maximum has passed. In the same way, the mobile phase concentration

leads the equilibrium concentration because insufficient solute is transported to (or from) the stationary zone to allow equilibrium to be attained. As a result of this, molecules of the solute in the part of the chromatographic band ahead of the band maximum migrate along the column at a slightly higher velocity than the band maximum itself, while molecules behind the band maximum migrate more slowly. This results in band broadening.

Giddings' theory, outlined below, is based on the premise that the departure from equilibrium is small. With this assumption, the solution of equations 2.6. - 1 and 2.6. - 2 may be simplified, and expressions for the degree of band broadening may be found for a variety of mass transfer processes.

A general outline of the procedure for solution of these equations is given below, illustrated by example where appropriate. The theory is then used to derive an expression for band broadening due to diffusional mass transfer in spherical particles.

Let c_m be the mobile zone concentration of solute at any distance along the column, expressed as amount of solute per unit volume of column packing, and let c_s be the corresponding stationary zone concentration. Let the concentrations in these zones if the column element were isolated and allowed to come to equilibrium be denoted by c_m^* and c_s^* . The particular concentration units chosen allow the concentrations in the two zones to be added. Thus the overall concentration at that distance along the column, c is given by

$$c = c_m + c_s = c_m^* + c_s^* \qquad 2.7. - 1$$

c_m and c_s may be written in terms of the equilibrium concentrations and equilibrium departure terms ϵ_m and ϵ_s . Thus

$$c_m = c_m^* (1 + \epsilon_m) \quad 2.7. - 2a$$

$$c_s = c_s^* (1 + \epsilon_s) \quad 2.7. - 2b$$

These equations may be taken as defining the equilibrium departure terms. From the above equations, it may readily be seen that

$$c_m^* \epsilon_m + c_s^* \epsilon_s = 0 \quad 2.7. - 3$$

This equation allows either departure term to be written in terms of the other, and a ratio of equilibrium concentrations.

One reason for defining these equilibrium departure terms is that ϵ_m may be related to the plate-height by a fairly simple expression. This may be derived as follows:

The solute flux J across the column is given by

$$J = c_m u \quad 2.7. - 4$$

From equation 2.7. - 2 this may be written as

$$J = c_m^* u + c_m^* \epsilon_m u \quad 2.7. - 5$$

The first term on the right-hand side of this equation is an equilibrium term. Since band broadening would not occur if equilibrium were found throughout the column, this term contributes

nothing to the plate height. Band broadening is caused by the second term in equation 2.7. - 5 which may be written as a flux ΔJ :

$$\Delta J = c_m^* \epsilon_m u \quad 2.7. - 6$$

This flux causes the band to broaden in a way which is analogous to diffusion. ΔJ may therefore be written in terms of Fick's first law (33)

$$\Delta J = - D \partial c / \partial z \quad 2.7. - 7$$

where D is an apparent diffusion coefficient. An expression for D may be obtained by equating expressions 2.7. - 6 and 2.7. - 7:

$$D = \frac{-c_m^* \epsilon_m u}{\partial c / \partial z} \quad 2.7. - 8$$

c_m^* may be eliminated from this equation since $c_m^* = c / (1+k'')$. Thus

$$D = \frac{-\epsilon_m u}{\partial \ln c / \partial z} \frac{1}{1+k''} \quad 2.7. - 9$$

D is related to the bandwidth by equation 2.2. - 1. Thus

$$\sigma^2 = - \frac{2\epsilon_m u}{\partial \ln c / \partial z} \frac{1}{1+k''} t \quad 2.7. - 10$$

where t is the time spent in the column. The speed at which the band moves is $1/(1+k'')$ that of the eluent. From this and the length of the column, equation 2.7. - 9 may be written as

$$\sigma^2 = \frac{-2 \epsilon_m L}{\partial \ln c / \partial z} \quad 2.7. - 11$$

The plate-height contribution may now be obtained from this and equation 2.2. - 4:

$$H = \frac{-2 \epsilon_m}{\partial \ln c / \partial z} \quad 2.7. - 12a$$

$$h = \frac{-2 \epsilon_m}{\partial \ln c / \partial z} \frac{1}{d_p} \quad 2.7. - 12b$$

Thus if ϵ_m may be obtained in terms of measurable parameters, the plate-height may be readily calculated.

ADSORPTION-DESORPTION KINETICS

Again, the simplest case is that of single-site adsorption-desorption kinetics. To find an expression for ϵ_m , we consider first the rate of mass transfer from the stationary to the mobile zone. This is denoted by s_m , and is given by the following equation:

$$s_m = k_d c_s - k_a c_m \quad 2.7. - 13$$

where k_d and k_a are the rate constants for adsorption and desorption as defined above. By equation 2.7. - 2 this may be expanded to

$$s_m = k_d c_s^* - k_a c_m^* + k_d c_s^* \epsilon_s - k_a c_m^* \epsilon_m$$

The first two terms in this equation are equilibrium terms. At equilibrium there is no net mass transfer between zones so these terms cancel. Thus

$$s_m = k_d c_s^* \epsilon_s - k_a c_m^* \epsilon_m \quad 2.7. - 15$$

ϵ_s may be written in terms of ϵ_m according to equation 2.7. - 3

Thus

$$s_m = - c_m^* (k_d + k_a) \epsilon_m \quad 2.7. - 16$$

This gives s_m in terms of the equilibrium concentration and the equilibrium departure term for the mobile zone. A second equation for s_m may be found from the mass balance equation 2.6. - 1, which is rewritten here without the diffusion term:

$$\frac{\partial c_m}{\partial t} = - u \frac{\partial c_m}{\partial z} + s_m \quad 2.7. - 17$$

The diffusion term has been ignored because at reasonable velocities band broadening due to diffusion is negligible. Although s_m may be obtained directly from equation 2.7. - 17, it is more useful to recast this equation so that the time derivative on the left hand side is replaced by a distance derivative. This may be done in the following manner:

For the overall concentration c , there is no mass transfer term, and equation 2.7. - 17 may be written as

$$\frac{\partial c}{\partial t} = - u \frac{\partial c_m}{\partial z} \quad 2.7. - 18$$

c_m appears on the right-hand side since only mobile zone flow can change the overall concentration at any distance along the column.

It is assumed throughout this treatment that, although not at equilibrium, we are close to it. This allows us to make the approximation:

$$\frac{\partial c_m}{\partial z} \cong \frac{\partial c_m^*}{\partial z} \quad 2.7. - 19$$

Thus equation 2.7. - 18 may be written as

$$\frac{\partial c}{\partial t} = -u \frac{\partial c_m^*}{\partial z} \quad 2.7. - 20$$

We know that c_m^* is directly proportional to c :

$$c = (1 + k'') c_m^* \quad 2.7. - 21$$

Thus

$$\frac{\partial c_m^*}{\partial t} = \frac{-u}{1+k''} \frac{\partial c_m^*}{\partial z} \quad 2.7. - 22$$

A further approximation allowed by the near equilibrium approximation is

$$\frac{\partial c_m}{\partial t} \cong \frac{\partial c_m^*}{\partial t} \quad 2.7. - 23$$

From this and equation 2.7 - 19, equation 2.7. - 17 may be written as

$$\frac{\partial c_m^*}{\partial t} = -u \frac{\partial c_m^*}{\partial z} + s_m \quad 2.7. - 24$$

to a good approximation. The left-hand side of this equation may be obtained from equation 2.7. - 22. Thus our equation for s_m may be written as

$$s_m = \frac{k''}{1+k''} u \frac{\partial c_m^*}{\partial z} \quad 2.7. - 25$$

after some rearrangement. Equating this and equation 2.7 - 16 leads to the following equation:

$$-c_m^* (k_d + k_a) \epsilon_m = \frac{k''}{1+k''} u \frac{\partial c_m^*}{\partial z} \quad 2.7. - 26$$

which may be solved for ϵ_m :

$$\epsilon_m = -\frac{k''}{1+k''} u \frac{1}{(k_d + k_a)} \frac{\partial \ln c_m^*}{\partial z} \quad 2.7. - 27$$

From equation 2.7. - 12 the plate-height is immediately given as

$$H = \frac{2k''}{1+k''} \frac{1}{k_d+k_a} u \quad 2.7. - 28a$$

$$h = \frac{2k''}{1+k''} \frac{1}{k_a+k_d} D_m v \quad 2.7. - 28b$$

Since $k_a + k_d$ are related by the expression

$$k'' = k_a/k_d \quad 2.7. - 29$$

the equation 2.7. - 28b may be simplified to either

$$h = \frac{2k''}{(1+k'')^2} \frac{1}{k_d} D_m v \quad 2.7. - 30a$$

or

$$h = \frac{2k''^2}{(1+k'')^2} \frac{1}{k_a} D_m v \quad 2.7. - 30b$$

The above approach may be extended from simple single-site one-step kinetics to cover cases where there are two or more types of adsorptive sites on the surface (31, 32) or where the adsorptive process involves several consecutive steps (17).

DIFFUSION CONTROLLED KINETICS

For partition chromatography, the approach is broadly similar, in that an expression is sought for ϵ_m , from which the plate height may be calculated by equation 2.7. - 12. However, since diffusion within the stationary zone is possible, it is necessary to examine what happens in this zone more closely.

At any point in the stationary zone there will be a local concentration of solute, which we denote by c_s' . It should be noted that whereas c , c_m , c_s etc. above are amounts per unit volume of column packing, c_s' is given by the amount per unit volume of stationary zone. c_s' is related to the equilibrium concentration at that point c_s^{*} by a local equilibrium departure term ϵ_s' :

$$c_s' = c_s^{*'} (1 + \epsilon_s') \quad 2.7. - 31$$

The mean of this local departure term over the column is the same as the overall departure term:

$$\overline{\epsilon_s'} = \epsilon_s \quad 2.7. - 32$$

Thus $\overline{\epsilon_s'}$ may be related to ϵ_m :

$$\overline{\epsilon_s'} = \frac{-1}{k''} \epsilon_m \quad 2.7. - 33$$

The approach now adopted is analogous to that in equations 2.7. - 13 - 2.7. - 27, in that two expressions for s_m are found and equated, and thus an equation for ϵ_m is found. Our attention, however, is focussed on the stationary zone.

The rate of solute flux at a given point in this zone is determined by diffusion:

$$s_s' = D_s \nabla^2 c_s' \quad 2.7. - 34$$

where D_s is the molecular diffusion coefficient in this zone, and ∇^2 is the Laplacian operator. Using equation 2.7. - 31 for c_s' this becomes:

$$s_s' = D_s c_s^{*'} \nabla^2 \epsilon_s' \quad 2.7. - 35$$

This is one equation for the solute flux. The mass balance equation for the stationary zone is simply

$$\frac{\partial c_s^i}{\partial t} = s_s^i \quad 2.7. - 36$$

The time derivative may again be replaced by a distance derivative. The details are as follows:

Applying the near-equilibrium approximation 2.7. - 23, equation 2.7. - 36 may be written as:

$$\frac{\partial c_s^{*i}}{\partial t} = s_s^i \quad 2.7. - 37$$

The left-hand side of this equation may be expressed as:

$$\frac{\partial c_s^{*i}}{\partial t} = \frac{c_s^{*i}}{c} \frac{\partial c}{\partial t} \quad 2.7. - 38$$

since c_s^{*i} is directly proportional to the overall concentration c . The second term on the right-hand side may be obtained from equation 2.7. - 20, yielding

$$\frac{\partial c_s^{*i}}{\partial t} = -u \frac{c_s^{*i}}{c} \frac{\partial c_m^*}{\partial z} \quad 2.7. - 39$$

It is preferable to have the same type of concentration variable on both sides of this equation. This is possible because of the proportionality of c_m^* and c_s^{*i} . Thus

$$\frac{\partial c_s^{*'}}{\partial t} = -u \frac{c_s^{*'}}{c} \frac{c_m^*}{c_s^{*'}} \frac{\partial c_s^{*'}}{\partial z} \quad 2.7. - 40$$

This equation may be simplified to:

$$\frac{\partial c_s^{*'}}{\partial t} = -u \frac{1}{1+k''} \frac{\partial c_s^{*'}}{\partial z} \quad 2.7. - 41$$

From this and equation 2.7. - 37, the second equation for solute flux is:

$$s_s' = -u \frac{1}{1+k''} \frac{\partial c_s^{*'}}{\partial z} \quad 2.7. - 42$$

From this and equation 2.7. - 35, the following differential equation for ϵ_s' may be found:

$$\nabla^2 \epsilon_s' = -\frac{u}{D_s} \frac{1}{1+k''} \frac{\partial \ln c}{\partial z} \quad 2.7. - 43$$

Having obtained this equation, the strategy adopted is to solve it for ϵ_s' by integrating and applying the appropriate boundary conditions. The average value of ϵ_s' must then be obtained, and thus ϵ_m may be found from equation 2.7. - 33. The plate height may then be calculated from equation 2.7 - 12. This method is best illustrated by example.

CASE OF UNIFORM LAYER OF STATIONARY ZONE

The simplest example is when the stationary zone is a uniform layer of depth d held on an impermeable support. This case is illustrated in Figure 2.7. - 1.

Let x be the perpendicular distance from the support. Equation 2.7. - 43 may then be written as

$$\frac{\partial^2 \epsilon_s'}{\partial x^2} = \frac{u}{D_s} \frac{1}{(1+k'')} \frac{\partial \ln c}{\partial z} \quad 2.7. - 44$$

Integrating once with respect to x yields

$$\frac{\partial \epsilon_s'}{\partial x} = - \frac{u}{D_s} \frac{1}{1+k''} \frac{\partial \ln c}{\partial z} x + g_0 \quad 2.7. - 45$$

where g_0 is a constant of integration. A second integration yields

$$\epsilon_s' = - \frac{u}{D_s} \frac{1}{1+k''} \frac{\partial \ln c}{\partial z} \frac{x^2}{2} + g_0 x + g_1 \quad 2.7. - 46$$

where g_1 is a second constant of integration. These constants may be evaluated by applying the appropriate boundary conditions. These are that there is no solute flux from the stationary zone to the support, and that there is equilibrium at the interface between the stationary and mobile zones. The first condition may be written as

$$\frac{\partial \epsilon_s'}{\partial x} = 0 \text{ at } x = 0 \quad 2.7. - 47$$

and the second as

$$\epsilon_s' = \epsilon_m \text{ at } x = d \quad 2.7. - 48$$

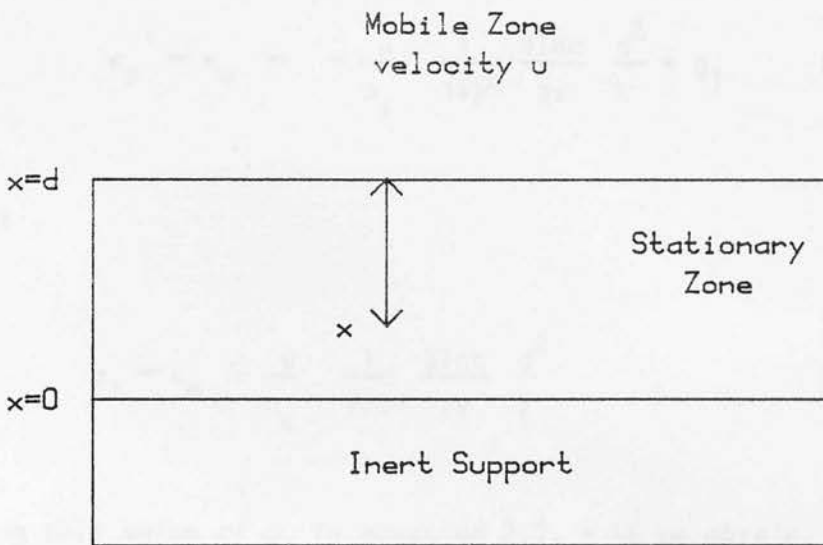


FIGURE 2.7. - 1. Schematic Diagram of Uniform Stationary Zone. Diffusion over distance x into and out of stationary zone leads to band broadening by slow mass transfer.

Equation 2.7. - 47 with equation 2.7. - 45 shows that $g_0 = 0$. At $x = d$, equation 2.7. - 46 becomes

$$\epsilon_s' = \epsilon_m = - \frac{u}{D_s} \frac{1}{1+k''} \frac{\partial \ln c}{\partial z} \frac{d^2}{2} + g_1 \quad 2.7. - 49$$

Thus

$$g_1 = \epsilon_m - \frac{u}{D_s} \frac{1}{1+k''} \frac{\partial \ln c}{\partial z} \frac{d^2}{2} \quad 2.7. - 50$$

Using this value of g_1 in equation 2.7. - 46 we obtain

$$\epsilon_s' = \epsilon_m - \left(\frac{u}{D_s} \frac{1}{1+k''} \frac{\partial \ln c}{\partial z} \right) \left(\frac{x^2 - d^2}{2} \right) \quad 2.7. - 51$$

The value of $\overline{\epsilon_s'}$ may be obtained by finding the mean value of ϵ_s' . This may be calculated from the equation

$$\overline{\epsilon_s'} = \frac{\int_0^d \epsilon_s' dx}{\int_0^d dx} \quad 2.7. - 52$$

The result of this operation is:

$$\overline{\epsilon_s'} = \epsilon_m + \frac{u}{D_s} \frac{1}{1+k''} \frac{\partial \ln c}{\partial z} \frac{d^2}{3} \quad 2.7. - 53$$

$\overline{\epsilon_s'}$ is also given in terms of ϵ_m by equation 2.7. - 33. From these two equations $\overline{\epsilon_s'}$ may be eliminated, yielding the following for ϵ_m :

$$\epsilon_m = - \frac{u}{D_s} \frac{k''}{(1+k'')} \frac{\partial \ln c}{\partial z} \frac{d^2}{3} \quad 2.7. - 54$$

Thus by equation 2.7. - 12 the plate-height is given by:

$$H = \frac{2}{3} \frac{k''}{(1+k'')^2} \frac{d^2}{D_s} u \quad 2.7. - 55a$$

$$h = \frac{2}{3} \frac{k''}{(1+k'')^2} \frac{D_m}{D_s} \frac{d^2}{d_p^2} \quad 2.7. - 55b$$

by introducing the appropriate particle diameter and diffusion coefficient.

CASE OF STATIONARY ZONE IN SPHERICAL UNITS

The assumption that the stationary zone exists as a layer of uniform depth is reasonable only for pellicular particles covered with a shallow uniform layer of stationary phase. A more realistic model, especially when the column is packed with spherical particles, is that the stationary zone exists in uniform spherical units. Even if the support particles comprise microporous beads, these will be loaded with immobile liquid, and there will exist an effective diffusion coefficient within the bead. This will apply even if the interior of the bead is heterogeneous on a molecular scale due to the presence of stationary phase layers and adsorbed components of eluent, since on a macroscopic scale the bead is still effectively homogeneous.

For spherical units, equation 2.7. - 43 may be written as:

$$\frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial \epsilon_s'}{\partial r} \right) = - \frac{u}{D_s} \frac{1}{1+k''} \frac{\partial \ln c}{\partial z} \quad 2.7. - 56$$

Angular coordinates are absent because of the spherical symmetry of the particles. When this equation is integrated, the following equation is obtained:

$$\epsilon_s' = - \frac{u}{D_s} \frac{1}{1+k''} \frac{\partial \ln c}{\partial z} \frac{r^2}{6} - \frac{g_0}{r} + g_1 \quad 2.7. - 57$$

where again g_0 and g_1 are constants of integration. The boundary conditions used to evaluate these constants are:

$$\frac{\partial \epsilon_s'}{\partial r} = 0 \text{ at } r = 0 \quad 2.7. - 58$$

and

$$\epsilon_s' = \epsilon_m \text{ at } r = r_p \quad 2.7. - 59$$

where r_p is the radius of the particle. These equations are analogous to equations 2.7. - 47 and 2.7. - 48. Equation 2.7. - 58 yields the constant $g_0 = 0$. After evaluating g_1 by using conditions 2.7. - 59, the following equation for ϵ_s' is obtained:

$$\epsilon_s' = \epsilon_m + \frac{1}{1+k''} \frac{u}{D_s} \frac{\partial \ln c}{\partial z} \left(\frac{r_p^2 - r^2}{6} \right) \quad 2.7. - 60$$

The mean value of ϵ_s' is found from the equation

$$\overline{\epsilon_s'} = \frac{\int_0^{r_p} 4\pi r^2 \epsilon_s' dr}{\int_0^{r_p} 4\pi r^2 dr} \quad 2.7. - 61$$

When the expression for ϵ_s' is substituted into this equation, the value of $\overline{\epsilon_s'}$ is found to be

$$\overline{\epsilon_s'} = \epsilon_m + \frac{1}{15} \frac{1}{1+k''} \frac{u}{D_s} \frac{\partial \ln c}{\partial z} r_p^2 \quad 2.7. - 62$$

From this and equation 2.7. - 33, the following equation for ϵ_m is found:

$$\epsilon_m = - \frac{1}{15} \frac{k''}{(1+k'')^2} \frac{u}{D_s} \frac{\partial \ln c}{\partial z} r_p^2 \quad 2.7. - 63$$

Hence by equation 2.7. - 12, the plate-height is

$$H = \frac{2}{15} \frac{k''}{(1+k'')^2} r_p^2 \frac{u}{D_s} \quad 2.7. - 64$$

If particle diameter, rather than particle radius is used, the reduced plate-height may be written as:

$$h = \frac{1}{30} \frac{k''}{(1+k'')^2} \frac{D_m}{D_s} v \quad 2.7. - 65$$

This is one of the most important equations in this thesis. It will be examined further in Chapter 5.

CHAPTER 3

DIFFUSION

INTRODUCTION

Diffusion is the process by which particles of a substance move from a region of high concentration to a region of low concentration. This process occurs in all states of matter: solids, liquids, and gases. In solids, diffusion is slow because particles are closely packed and can only move by jumping from one position to another. In liquids, diffusion is faster because particles are more loosely packed and can move more freely. In gases, diffusion is the fastest because particles are widely spaced and move rapidly in all directions.

CHAPTER 3

DIFFUSION

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From the above considerations, it is obvious that the rate of transfer of particles depends on the distance between the two regions, or the quantity of the substance

CHAPTER 3

DIFFUSION

3.1. INTRODUCTION

Diffusion is the name given to the process whereby initially separate components intermix, or a concentrated zone of one component spreads out into another, becoming diluted by it, without any external agent being responsible for the mixing. This phenomenon may be explained by the random motion of molecules or ions. In what follows we consider only diffusion in liquids.

All molecules in a liquid are in a state of random motion. If the liquid is at rest there is an equal probability of a molecule moving in any given direction; if the liquid as a whole is in motion, this motion will be superimposed on the above random motion.

Consider two adjacent elements of a liquid, where there is a slightly higher concentration of solute in one element than in the other. In a given time, the same fraction of solute molecules will transfer from the region of high concentration to the region of lower concentration as will transfer in the opposite direction. However, since there were a greater number of solute molecules in the region of high concentration, a greater number will transfer out of this element to the other than will transfer from the low concentration element to that of higher concentration. This explains the overall transfer of solute from regions of high concentration to those of low concentration.

From the above considerations, it is obvious that the rate of transfer of solute depends on the difference in concentration between the two elements, or more generally on the concentration

gradient. This fact is expressed by Fick's first law of diffusion (33).

$$J = -D \partial c / \partial z \qquad 3.1. - 1$$

where J is the amount of solute passing across a unit area perpendicular to the z-axis in unit time, $\partial c / \partial z$ is the concentration gradient along the z-axis, and the proportionality factor D is known as the diffusion coefficient. D is a positive quantity; the negative sign indicates that the flux is in the opposite direction to the concentration gradient, i.e. there is a net transfer of solute to the region of lower concentration.

The above argument is based on diffusion in one dimension only. Equation 3.1. - 1 may be generalised to

$$\underline{J} = -D \nabla c \qquad 3.1. - 2$$

using the normal notation of vector calculus.

The rate of change of concentration with time may be found from Fick's second law

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial z^2} \qquad 3.1. - 3$$

This equation assumes that D is independent of concentration. Otherwise, this equation should be written as

$$\frac{\partial c}{\partial t} = \frac{\partial c}{\partial z} D \frac{\partial c}{\partial z} \qquad 3.1. - 4$$



These equations may also be generalised to three dimensions as

$$\frac{\partial c}{\partial t} = D \nabla^2 c \quad 3.1. - 5$$

or

$$\frac{\partial c}{\partial t} = \nabla \cdot (D \nabla c) \quad 3.1. - 6$$

For the remainder of this discussion, we will assume that D is independent of concentration, and we will consider diffusion in one dimension only. Thus the relevant equations are 3.1. - 1 and 3.1. - 3.

The solution of equation 3.1. - 3 depends on the initial conditions chosen. In most of the situations met with in this work the initial concentration profile is assumed to be a sharp spike. In this case, a solution of 3.1. - 3 is

$$c = \frac{M}{t^{\frac{1}{2}}} \exp\left(\frac{-z^2}{4Dt}\right) \quad 3.1. - 7$$

(see ref. (34)). Thus a sharp spike develops into a Gaussian profile. Such a profile is normally written in terms of the peak variance σ^2 :

$$c = \frac{M}{t^{\frac{1}{2}}} \exp\left(\frac{-z^2}{2\sigma^2}\right) \quad 3.1. - 8$$

A comparison of equations 3.1. - 7 and 3.1. - 8 reveals the relationship

$$\sigma^2 = 2Dt \quad 3.1. - 9$$

This equation is known as the Einstein equation (19). It enables the diffusion coefficient to be determined from the width of a Gaussian peak.

3.2. ESTIMATION OF DIFFUSION COEFFICIENTS IN LIQUIDS

The rate of diffusion in a liquid is obviously related to the viscosity of the liquid since both processes involve transport of molecules within the liquid. Both processes involve the breaking of bonds within the liquid, and it is found that the activation energy required to break the bonds and so allow diffusion or viscous flow to take place is approximately equal for both processes. Since viscosity data are much more easily obtainable than diffusion data, many equations for estimating the diffusion coefficient in liquids introduce the activation energy by a term $1/\eta$.

The Stokes-Einstein equation (35) for estimating diffusion coefficients in liquids is

$$D = \frac{RT}{6\pi \eta r N} \quad 3.2. - 1$$

where R is the gas constant, T is the temperature, r is the solute radius and N is Avogadro's number. This equation may be derived by assuming a frictional resistance term given by Stokes law. Since Stokes law assumes the solvent to be continuous, this equation only holds when the solute molecules are much larger than the solvent molecules.

By applying reaction-rate theory to the diffusion process in liquids, modelling the liquid by the 'hole theory', Eyring (36)

derived the equation

$$D = \lambda^2 \kappa \quad 3.2. - 2$$

where λ is the distance between molecules at equilibrium, and κ is the specific reaction rate for diffusion, given by

$$\kappa = \frac{(kT) (F^\ddagger)}{(h) (F)} \exp \left(\frac{-\epsilon_0}{kT} \right) \quad 3.2. - 3$$

F^\ddagger and F are partition functions, and ϵ_0 is the activation energy for diffusion per degree of freedom.

A similar treatment when applied to viscosity yields

$$\eta = \frac{\lambda_1}{\lambda_2 \lambda_3} \frac{kT}{\lambda^2 \kappa_{\text{visc}}} \quad 3.2. - 4$$

where λ_1 , λ_2 and λ_3 are intermolecular distances which come from the theory of viscosity. Assuming that the value of λ in equation 3.2. - 4 is the same as that in equation 3.2. - 2, and that the value of κ is the same for diffusion and viscosity, by combining equations 3.2. - 2 and 3.2. - 4 we obtain

$$D = \frac{\lambda_1}{\lambda_2 \lambda_3} \frac{kT}{\eta} \quad 3.2. - 5$$

It is difficult to apply this equation because of the need to estimate values for λ_1 , λ_2 and λ_3 .

Other theoretical equations have been derived from the random walk theory, hydrodynamics, thermodynamics, irreversible process

thermodynamics and absolute rate theory. These are reviewed by Johnson and Babb (37). All suffer from the same drawback as the Eyring theory, namely that it is difficult to assign values to some of the parameters used in the equations, and thus the equations have little predictive value.

To allow the estimation of diffusion coefficients from more readily available data, several empirical (or semi-empirical) formulae have been developed. Typical of these, and perhaps the most widely known, is the Wilke-Chang equation (38, 39)

$$D = \frac{7.4 \times 10^{-15} (\psi M)^{\frac{1}{2}} T}{\eta V_s^{0.6}} \quad 3.2. - 6$$

Where M is the molecular weight of the eluent, T is the absolute temperature in Kelvin, V_s is the molar volume of solute in $\text{cm}^3 \text{mol}^{-1}$ and η is the solvent viscosity in N s m^{-2} . ψ is an association constant, whose value is unity for unassociated liquids. The values of ψ for some common associated liquids are as follows: water, 2.6; methanol, 1.9; ethanol, 1.5. When the above units are used, the diffusion coefficient is given in $\text{m}^2 \text{s}^{-1}$.

This equation was obtained as follows. From equations 3.2. - 1 and 3.2. - 5 it appears that the group $D\eta/T$ is a constant, independent of temperature. These equations also show that the value of the diffusion coefficient depends in some way on the size of the solute molecule. A correlation of experimental values of D and solute molal volume gave a best fit when this variation was of the form

$$D \propto V^{-0.6} \quad 3.2. - 7$$

Of the various solvent properties examined, the best correlation of experimental values of the diffusion coefficient was the correlation

with molecular weight. The best form of this correlation was found to be

$$D \propto M^{\frac{1}{2}} \quad 3.2. - 8$$

This worked well for non-polar liquids, but when the constant derived in this way was used with polar liquids, the diffusion coefficient was underestimated. The association constant ψ was introduced to correct for this. The constant appearing in equation 3.2. - 6 was calculated to give the best correlation between experimental values of the diffusion coefficient and values calculated by an equation of the form of 3.2. - 6.

For mixed solvents, equation 3.2. - 6 may still be used, if the value of (ψM) used is the volume average of (ψM) for the components of the solvent (40). Thus for a solvent consisting of 60% methanol and 40% water, (ψM) is calculated as

$$(\psi M) = (0.6 \times 2.6 \times 18) + (0.4 \times 1.9 \times 32) = 52.4 \quad 3.2. - 9$$

With single-component solvents, the Wilke-Chang equation often gives results differing from the experimental values by up to 30% (41). For mixed solvents, there is little experimental data available, but the equation is unlikely to perform better for these than it does for pure solvents.

Other semi-empirical equations for the calculation of D have been proposed. Some of these are described briefly below; fuller details are given by Reid and Sherwood (41).

Scheibel (42) proposed an equation similar to that of Wilke and Chang which does not include a factor ψ , but which has several

recommendations for the value of the constant depending on the solvents being used.

Othmer and Thakar (43) proposed an equation which includes the heat of vaporisation of the solvent as a parameter. This may be considered as an alternative way of obtaining a factor like (ψM) in equation 3.2. - 6.

Sitaraman et al (44) proposed an equation which includes the heats of vaporisation of both solvent and solute. The form of this equation differs considerably from that of Othmer and Thakar.

Over a wide variety of systems there is little to choose in accuracy among the various equations proposed. The Wilke-Chang equation has been singled out since it appears most often in the chromatographic literature, and uses the most readily accessible data.

3.3. TEMPERATURE DEPENDENCE OF DIFFUSION COEFFICIENTS

To allow values of D at one temperature to be calculated from values at another temperature for which the experimental data is available, we assume that the group $D\eta/T$ is constant, as suggested by equations 3.2. - 1 and 3.2.-5. However, this is only an approximation as indicated by Innes and Albright (45). It should be adequate for extrapolation over small ranges of temperature.

3.4. MEASUREMENT OF DIFFUSION COEFFICIENTS

To obtain values of diffusion coefficients, many different experimental methods have been used. The most popular are summarised below.

One of the most popular experimental methods is the diaphragm cell technique. This was originally used by Northup and Anson (46), and significantly improved by Stokes (47). In the Stokes diffusion cell, the two solutions are separated by a horizontal diaphragm (typically of porous glass), with the more dense solution in the lower part of the cell. Both solutions are stirred so that there is no concentration gradient within either compartment, and the only gradient is across the diaphragm. The apparatus must be thermostatted. Once the compartments are filled with the solutions of interest, diffusion proceeds across the diaphragm. After a certain time (typically several days), the experiment is stopped and the concentration of the two solutions are measured. From a knowledge of the initial and final concentrations, the volumes of the solutions used and the effective path length of the diaphragm, the rate of solute flux and the diffusion coefficient may be calculated. The effective cross-sectional area and path length of the diaphragm must be found by calibration with a system for which ^{the} diffusion coefficient is known.

The other popular method, for which no calibration is required, is the free diffusion method (48). In this method, the two solutions of interest are placed in direct contact. It is vital that an initial sharp boundary is formed between the two solutions. Again the more dense solution is placed beneath the less dense. The composition of the liquid at any time and at any position within the cell may be determined by measuring the refractive index at that position. Various optical techniques have been used to determine this property. For example, the 'Gouy fringes' produced by shining monochromatic light through a medium containing a refractive index gradient may be

recorded on photographic film. Alternatively, a light beam may be split so that one beam passes through the sample, and the other through a reference cell. When the beams are recombined, the refractive index of the sample may be calculated from the interference pattern produced.

To use this method, the relationship between refractive index and composition must be known. The chief experimental drawbacks are the formation of the initial boundary, and the avoidance of mixing by methods other than diffusion.

Further information on these and other techniques may be found in Tyrell (48) or in Johnson and Babb (37) as well as in the original references.

THE CHROMATOGRAPHIC BROADENING TECHNIQUE

A further method to measure diffusion coefficients in liquids is the chromatographic broadening technique. This method is based on the role played by diffusion in the broadening of chromatographic bands as they are eluted from open tubular columns which do not retain the solute. This problem was first analysed by Taylor (49).

For laminar flow, there is a velocity profile across the tube given by

$$u(r) = u_0 (1 - r^2/a^2) \qquad 3.4. - 1$$

where $u(r)$ is the fluid velocity at a distance r from the centre of the tube, u_0 is the velocity at the centre of the tube, and a is the tube radius. This equation is obviously intended to hold within the tube, i.e. $r < a$.

In the absence of molecular diffusion, or where the time taken for the solute to diffuse across the tube is long compared to the time for which the flow profile acts on a region of solute, the band will broaden due solely to the action of the flow profile. Thus solute initially at the walls of the tube will not move, and solute at the centre of the tube will move with a velocity u_0 . If solute transport across the tube by diffusion is significant, the concentration gradient across the tube produced by the action of the flow profile will be smoothed out. This may be analysed as follows:

A coordinate system is defined such that the origin moves at the mean fluid velocity $u_0/2$, and the radial coordinate varies from 0 to 1. Thus

$$z_1 = z - u_0 t/2 \quad 3.4. - 2$$

$$x = r/a \quad 3.4. - 3$$

The diffusion equation is

$$D \left(\frac{\partial^2 c}{\partial r^2} + \frac{1}{r} \frac{\partial c}{\partial r} + \frac{\partial^2 c}{\partial z^2} \right) = \frac{\partial c}{\partial t} + u_0 \left(1 - \frac{r^2}{a^2} \right) \frac{\partial c}{\partial z_1} \quad 3.4. - 4$$

This may be rewritten as

$$\frac{\partial^2 c}{\partial x^2} + \frac{1}{x} \frac{\partial c}{\partial x} = \frac{a^2}{D} \frac{\partial c}{\partial t} + \frac{a^2 u_0}{D} \left(\frac{1}{2} - x^2 \right) \frac{\partial c}{\partial z_1} \quad 3.4. - 5$$

in the above-defined coordinates. The term $\partial^2 c / \partial z^2$ has been omitted, since the rate of diffusion in the z-direction is negligible compared to the flowrate in cases of interest.

The first term on the right-hand side of 3.4. - 5 may be dropped, since there is no net flux of solute across planes of constant z_1 .

Thus equation 3.4. - 5 simplifies to

$$\frac{\partial^2 c}{\partial x^2} + \frac{1}{x} \frac{\partial c}{\partial x} = \frac{a^2 u_o}{D} \left(\frac{1}{2} - x^2\right) \frac{\partial c}{\partial z_1} \quad 3.4. - 6$$

The boundary condition required for the solution of this equation is that there be no transport of solute through the walls of the tube, i.e.

$$\frac{\partial c}{\partial x} = 0 \text{ at } z = 1 \quad 3.4. - 7$$

A solution of 3.4. - 6 which satisfied 3.4. - 7 is

$$c = c_{z_0} + A(x^2 - \frac{1}{2}x^4) \quad 3.4. - 8$$

where c_{z_0} is the value of c at $z_1=0$, and A is a constant, to be determined from the boundary condition. It may thus be shown

$$A = \frac{a^2 u_o}{8D} \frac{\partial c}{\partial z_1} \quad 3.4. - 9$$

The rate of transfer of c across the section z_1 is

$$Q = - 2\pi a^2 \int_0^1 u_o \left(\frac{1}{2} - x^2\right) c x dx \quad 3.4. - 10$$

From equations 3.4.-9 and 3.4. -10 Q may be obtained as

$$Q = - \frac{\pi a^4 u_o^2}{192D} \frac{\partial c_{z_1}}{\partial x_1} \quad 3.4. - 11$$

Since the radial variations in c are small compared to the axial variations, we may write this as

$$Q = - \frac{\pi a^4 u_o^2}{192D} \frac{\partial c_m}{\partial z_1} \quad 3.4. - 12$$

where c_m is the mean concentration at position z_1 .

Equation 3.4. - 12 has the same form as Fick's first law, with an effective diffusion coefficient given by

$$D_{\text{eff}} = \frac{a^2 u_o^2}{192D} \quad 3.4. - 13$$

This may be written in terms of the mean eluent velocity as

$$D_{\text{eff}} = \frac{a^2 u^2}{48D} \quad 3.4. - 14$$

Thus a band of solute moves along the column with a velocity u , and is dispersed by a mechanism analogous to diffusion, with an effective diffusion coefficient given by 3.4. - 14.

In accordance with the theory of diffusion, a delta-function 'spike' of solute will develop into a Gaussian peak as the solute is eluted along the tube. The variance of this peak is given by

$$\sigma^2 = 2 D_{\text{eff}} t \quad 3.4. - 15$$

Thus the molecular diffusion coefficient may be found from the peak variance after a given time:

$$\sigma^2 = a^2 u^2 t / 24D \quad 3.4. - 16$$

This may be written in terms of plate-height:

$$H = a^2 u / 24D \quad 3.4. - 17$$

There are several conditions which must be met before equations 3.4. - 16 and 3.4. - 17 may be used to calculate the molecular

diffusion from the band width. These are as follows:

The above analysis is based on the assumption of laminar flow. For this assumption to be valid, the following condition must be met:

$$\frac{2 a \rho u}{\eta} < 2000 \quad 3.4. - 18$$

where ρ is the eluent density and η is the eluent viscosity, in the appropriate units.

A second assumption is that the time required to diffuse across the column is short compared to the time for convective transport to be effective. This condition may be written as (50)

$$t \geq \frac{50a^2}{3.8^2 D} \quad 3.4. - 19$$

It is also assumed that the spreading due to axial diffusion may be ignored compared to the spreading due to the above mechanism. Otherwise the following equation must be used in place of 3.4. - 17:

$$H = \frac{2D}{u} + \frac{a^2 u}{24D} \quad 3.4. - 20$$

It may thus be shown that if the condition

$$u > \frac{70D}{a} \quad 3.4. - 21$$

is met, then axial diffusion accounts for less than 1% of the bandwidth, and equation 3.4. - 17 may be used. More stringent conditions than 3.4. - 21 have been proposed (51).

This method of measuring diffusion coefficients has been used to obtain values of D for a wide variety of systems. It was originally

used to measure values of D for the mutual diffusion of gases (52, 53), and since then has been used to measure diffusion coefficients in dense gases (54) and in gas/liquid (55) and liquid/liquid systems (56, 57).

Recently a more precise form of equation 3.4. - 20 has been published (51) which makes an allowance for the fact that the concentration pulse is observed in time, rather than in a length of column:

$$D = \frac{a^2}{24\bar{t}_{id}} \left(\frac{(1 + 4\sigma_{id}^2/\bar{t}_{id})^{\frac{1}{2}} + 3}{(1 + 4\sigma_{id}^2/\bar{t}_{id})^{\frac{1}{2}} + 2(\sigma_{id}^2/\bar{t}_{id})^{-1}} \right)^{\frac{1}{2} + \frac{1}{2}(1 - \delta_a)^{\frac{1}{2}}} \quad 3.4. - 22$$

where

$$\delta_a = 0.26666 \frac{u^2 a^2}{LD} \quad 3.4. - 23$$

\bar{t}_{id} and σ_{id}^2 are the first and second moments or the concentration/time curve. The value of D calculated from this equation differs only slightly from that calculated from equation 3.4. - 16.

A set of equations is given with equation 3.4. - 22 which allows corrections to be made for non-idealities in the experiment. Ideally, there would be no effect on the measured diffusion coefficient due to the finite volume of the detector flowcell or the connecting tubing, or due to the finite volume injected. These effects are dealt with as follows:

The values of \bar{t}_{id} and σ_{id}^2 used in equation 3.4. - 22 may be obtained from the following equations:

$$\bar{t}_{id} = \bar{t} + \delta t_1 + \delta t_2 + \delta t_3 \quad 3.4. - 24a$$

$$\sigma_{id}^2 = \sigma^2 + \delta\sigma_1^2 + \delta\sigma_2^2 + \delta\sigma_3^2 \quad 3.4. - 24b$$

where \bar{t} and σ^2 are the experimental values of the moments, and the other terms are correction terms to be added to these values. These terms are described in detail below.

To correct for the effect of the flow-cell volume, the following terms are used:

$$\delta t_1 = \left(\frac{L}{u}\right) \left(3 \zeta_0 - \frac{V_D}{\pi a^2 L}\right) \quad 3.4. - 25a$$

$$\delta \sigma_1^2 = \left(\frac{L}{u}\right)^2 \left[13 \zeta_0^2 - \left(\frac{V_D}{\pi a^2 L}\right)^2 - 2 \zeta_0 \frac{V_D}{\pi a^2 L}\right] \quad 3.4. - 25b$$

where

$$\zeta_0^2 = \frac{u^2 a^2}{48DL} \quad 3.4. - 26$$

and V_D is the volume of the detector flow-cell.

The corresponding terms for the effect of the volume of injection are:

$$\delta t_2 = - \frac{V_i}{2\pi a^2 u} \quad 3.4. - 27a$$

$$\delta \sigma_2^2 = - \frac{1}{12} \frac{V_i}{\pi a^2 u} \quad 3.4. - 27b$$

where V_i is the volume of the injection.

To correct for the effect of connecting tubing on the moments, the following terms are used:

$$\delta t_3 = \left(\frac{L}{u}\right) \left(\frac{a_c}{a}\right)^2 \left[1 + \frac{a^2}{a_c^2} \frac{L}{\ell} \zeta_o \left(1 + \frac{a_c^2}{a^2} \right) \right] \quad 3.4. - 28a$$

$$\delta \sigma_3^2 = \left(\frac{L}{u}\right)^2 \left(\frac{a_c}{a}\right)^4 \left[\frac{2a^2}{a_c^2} \frac{L}{\ell} \zeta_o + \frac{a^2}{a_c^2} \left(\frac{L}{\ell}\right)^2 \zeta_o^2 \left(\frac{3a_c^2}{a^2} + 2\right) \right] \quad 3.4. - 28b$$

where ℓ is the length of the connection tubing and a_c is the radius. ζ_o is again given by equation 3.4. - 26.

It is obviously impractical to thermostat a long length of straight tubing. Since temperature control is required, most authors have used coiled tubes. This may lead to the above equations being inaccurate, since the parabolic flow profile may be distorted. This leads to an effect known as secondary flow. An element of eluent at the centre of the column will be moving faster than an element near the wall. It will therefore have a greater momentum, so it will move to the outside of the tube (58). This is another mechanism for transfer of solute across the column.

This effect is expected to be negligible if the following conditions are met:

$$R_c/a > 100 \quad 3.4. - 29$$

$$\frac{a R_c u^2 \rho}{\eta D} < 5 \quad 3.4. - 30$$

It is expected that the above effect will cause the calculated values of D to be velocity dependent. If no such velocity dependence is found, it may be assumed that this effect is absent.

3.5. EXPERIMENTAL

The chromatographic broadening technique was used to determine the diffusion coefficients of all eluent/solute combinations used. A glass capillary tube was drawn by Shimadzu glass drawing machine GDM1-B (Shimadzu Seisa Kusho Ltd., Kyoto, Japan): the length of this tube was 10.89m and the internal radius 0.206mm. The radius was calculated from the weight of the tube when empty and when filled with water, and thus represents a mean value. Inspection showed that the radius was not precisely uniform throughout the column length. The tube was formed into a coil of radius 110mm. The ends of the tube were straightened to allow sleeving in PTFE tubing. This enabled the tube to be connected to the injector and detector flow-cell by Swagelok 1/16" unions, with minimal dead volume. The column was placed in the oven compartment of a Shandon Southern liquid chromatography U.V. detector (Shandon Southern Products Ltd., Runcorn, England). The injector had a 20 μ l loop attached. The column was connected to the 8 μ l flowcell by a 60mm length of stainless steel tubing, diameter 0.12mm. The compartment was thermostatted at a temperature of 30°C. Eluent, which had been degassed by refluxing, was fed through the column under gravity. A length of stainless-steel tubing within the oven ensured that the eluent was at the required temperature before passing through the injector.

Dilute solutions of each solute/eluent combination of interest were prepared. Details of the solutes are given in Appendix 1. The concentration of solute in these samples was less than 0.01M, except for solutions of acetone, for which a concentration of 0.08M was required in order to obtain a reasonable detector response.

The detector response was recorded on a Bryans 28000 recorder, (Bryans Southern, Surrey, England) from which the elution time and peak width could be determined. Several samples were eluted down the column at one time, with an interval between each sample and the next to ensure that the peaks did not overlap at the end of the column.

Equation 3.4. - 22 was used, with the correction factors described above, to determine the value of D. \bar{t} was measured as the time to the maximum of the peak and σ^2 was calculated from the peak-width at the baseline found by drawing tangents to the points of inflection, and also by measuring the peak width at half height.

Initial experiments showed no dependence of D on flowrate. Later experiments were carried out at a single flowrate, since no secondary flow effect was found. It was also noted that the correction terms led to a change in the value of D of only about 2%.

Eight injections of each sample were made, and the mean diffusion coefficient was calculated from this set of results. The experimental error was taken as twice the standard deviation of the set of results. This was of the order of 10%, which is taken as the experimental error. The main source ^{of} error is the determination of \bar{t} and σ^2 from the recorder trace.

The results of this experiment are shown in Table 3.5. - 1.

Since it was not possible to perform the experiment at lower temperatures, the diffusion coefficients at 20°C and 26°C were calculated from the equation

$$\frac{D\eta}{T} = \text{constant} \qquad 3.5. - 1$$

TABLE 3.5. - 1

EXPERIMENTAL CALCULATION OF DIFFUSION COEFFICIENTS

Experiment performed at 30°C

a) Methanol/water 60:40

Solute	$\bar{\tau}/s$	w_b/s	$w_{\frac{1}{2}}/s$	$D_m/10^{-9}m^2s^{-1}$
Acetone	2075	237	139	1.10 \pm 0.03
Phenol	2084	291	172	0.72 \pm 0.02
p-Cresol	2098	310	184	0.62 \pm 0.02
3,4-Xylenol	2218	335	197	0.57 \pm 0.01
Anisole	2236	280	166	0.83 \pm 0.03
Phenetole	2246	291	172	0.78 \pm 0.03

b) Methanol/water 55:45

Solute	$\bar{\tau}_s$	w_b/s	$w_{\frac{1}{2}}/s$	$D_m/10^{-9}m^2s^{-1}$
Acetone	2282	225	150	1.03 \pm 0.02
Phenol	2234	309	182	0.68 \pm 0.02
p-Cresol	2260	330	194	0.60 \pm 0.02
3,4-Xylenol	2381	370	217	0.50 \pm 0.01
Anisole	2442	297	174	0.82 \pm 0.02
Phenetole	2401	308	181	0.74 \pm 0.02

c) Methanol/water 50:50

Solute	$\bar{\tau}/s$	w_b/s	w_z/s	$D_m/10^{-9} m^2 s^{-1}$
Acetone	2342	263	156	0.99 ± 0.02
Phenol	2335	319	187	0.67 ± 0.01
p-Cresol	2364	340	199	0.60 ± 0.02
3,4-Xylenol	2361	354	208	0.54 ± 0.02
Anisole	2379	291	173	0.82 ± 0.03
Phenetole	2396	320	188	0.68 ± 0.02

d) Methanol/water 45:55

Solute	$\bar{\tau}/s$	w_b/s	w_z/s	$D_m/10^{-9} m^2 s^{-1}$
Acetone	2681	301	179	0.87 ± 0.04
Phenol	2785	358	211	0.64 ± 0.02
p-Cresol	2860	384	226	0.56 ± 0.02
3,4-Xylenol	2909	408	239	0.51 ± 0.01
Anisole	3105	354	210	0.73 ± 0.02
Phenetole	3127	376	221	0.65 ± 0.02

e) Methanol 100%

Solute	$\bar{\tau}/s$	w_b/s	$w_{\frac{1}{2}}/s$	$D_m/10^{-9} m^2 s^{-1}$
Naphthalene	951	118	71	1.92 ± 0.05
Anthracene	959	132	78	1.58 ± 0.06
Chrysene	964	136	80	1.52 ± 0.04
Benzo(L)- fluoranthene	982	142	84	1.40 ± 0.04
Naphtho (2,3-k)- fluoranthene	977	150	89	1.28 ± 0.04
Sulphanilic acid	969	167	99	0.98 ± 0.04

f) 0.005M $NaNO_3/MeOH$

Solute	$\bar{\tau}/s$	w_b/s	$w_{\frac{1}{2}}/s$	$D_m/10^{-9} m^2 s^{-1}$
Sulphanilic acid	1033	176	103	0.97 ± 0.02

In order to use this equation, the viscosities of the eluents at the required temperatures must be known. These were measured by the Ubbelohde bulb method. This involves measuring the time taken for a given volume of eluent to flow through a length of capillary tubing under gravity. From a knowledge of these times, the viscosity may be calculated as

$$\eta = \eta_{H_2O} \frac{\rho t}{\rho_{H_2O} t_{H_2O}} \quad 3.5. - 2$$

η is the viscosity of the sample and η_{H_2O} is the viscosity of water at the temperature of the experiment. ρ and ρ_{H_2O} are the densities of eluent and water respectively, and t and t_{H_2O} are the times of flow through the viscometer. Values of η_{H_2O} and of ρ and ρ_{H_2O} were taken from the International Critical Tables (60), interpolating as necessary. Table (3.5. - 2) shows the viscosities measured by this experiment. Table (3.5. - 3) shows the values of D calculated from this data at the temperatures of interest.

TABLE 3.5. - 2

VISCOSITY OF METHANOL:WATER MIXTURES AT VARIOUS TEMPERATURES

T/°C	COMPOSITION METHANOL:WATER v:v					
	100:0 η/mNsm^{-2}	60:40 η/mNsm^{-2}	55:45 η/mNsm^{-2}	50:50 η/mNsm^{-2}	45:55 η/mNsm^{-2}	0:100 η/mNsm^{-2}
20	0.60	1.70	1.76	1.79	1.81	1.00
25	0.54	1.40	1.46	1.51	1.49	0.89
30	0.52	1.28	1.32	1.33	1.33	0.80
26	0.54	1.38	1.48	1.47	1.47	0.88

26°C Data obtained by interpolation

TABLE 3.5. - 3

DIFFUSION COEFFICIENTS AT TEMPERATURES OF INTEREST

a) 30°C

Solute	COMPOSITION METHANOL:WATER v/v			
	100:0	60:40	55:45	50:50
	$D_m / 10^{-9} \text{ m}^2 \text{ s}^{-1}$	$D_m / 10^{-9} \text{ m}^2 \text{ s}^{-1}$	$D_m / 10^{-9} \text{ m}^2 \text{ s}^{-1}$	$D_m / 10^{-9} \text{ m}^2 \text{ s}^{-1}$
Acetone		1.10	1.03	0.99
Phenol		0.72	0.68	0.67
p-Cresol		0.62	0.60	0.60
3,4-Xylenol		0.57	0.50	0.54
Anisole		0.83	0.82	0.82
Phenetole		0.78	0.74	0.68
Naphthalene	1.92			
Anthracene	1.58			
Chrysene	1.52			
Benzo(L) fluoranthene	1.40			
Naphtho (2,3-k) fluoranthene	1.28			
Sulphanilic acid	0.97			

b) 26°C

Solute	COMPOSITION METHANOL:WATER v/v			
	100:0	60:40	55:45	50:50
	$D_m / 10^{-9} \text{ m}^2 \text{ s}^{-1}$	$D_m / 10^{-9} \text{ m}^2 \text{ s}^{-1}$	$D_m / 10^{-9} \text{ m}^2 \text{ s}^{-1}$	$D_m / 10^{-9} \text{ m}^2 \text{ s}^{-1}$
Acetone		1.00	0.90	0.88
Phenol		0.65	0.59	0.59
p-Cresol		0.56	0.52	0.53
4,5-Xylenol		0.52	0.44	0.48
Anisole		0.75	0.71	0.73
Phenetole		0.71	0.64	0.60
Naphthalene	1.83			
Anthracene	1.50			
Chrysene	1.45			
Benzo(L) fluoranthene	1.33			
Naphtho (2,3-k) fluoranthene	1.22			
Sulphanilic acid	0.92			

c) 20°C

Solute	COMPOSITION METHANOL:WATER v/v			
	100:0	60:40	55:45	50:50
	$D_m / 10^{-9} \text{ m}^2 \text{ s}^{-1}$	$D_m / 10^{-9} \text{ m}^2 \text{ s}^{-1}$	$D_m / 10^{-9} \text{ m}^2 \text{ s}^{-1}$	$D_m / 10^{-9} \text{ m}^2 \text{ s}^{-1}$
Acetone		0.79	0.72	0.71
Phenol		0.52	0.47	0.48
p-Cresol		0.45	0.42	0.43
3,4-Xylenol		0.41	0.35	0.39
Anisole		0.60	0.57	0.59
Phenetole		0.56	0.52	0.49
Naphthalene	1.61			
Anthracene	1.32			
Chrysene	1.27			
Benzo(L) fluoranthene	1.17			
Napthho(2,3-k) fluoranthene	1.07			
Sulphanilic acid	0.81			

CHAPTER 4

BAND BROADENING BY LONGITUDINAL DIFFUSION

The rate of diffusion is directly proportional to the square of the distance. This is followed by a description of an experimental investigation of diffusion in liquid chromatography.

4.1 THE EXPERIMENTAL METHOD

Diffusion in liquid chromatography is a complex phenomenon. This diffusion is not only a function of the distance but also of the concentration of the diffusing substance.

The rate of diffusion is directly proportional to the square of the distance.

CHAPTER 4.

BAND BROADENING BY LONGITUDINAL DIFFUSION

4.1. INTRODUCTION

The role of longitudinal diffusion in band broadening has already been discussed in section 2.2. In this chapter the subject will be explored in greater depth, and the results of a set of experiments performed to investigate this phenomenon will be presented.

There are two ways in which diffusion within a chromatographic column differs from diffusion in bulk liquid:-

- a) The rate of diffusion in the mobile phase is hindered by the presence of the solid support.
- b) Diffusion in the stationary phase must be taken into account, as well as mobile phase diffusion.

These topics form the subjects of the following two sections. This is followed by a description of an experimental investigation of diffusion in liquid chromatography.

4.2. THE OBSTRUCTIVE FACTOR

Diffusion in a porous matrix is found to proceed more slowly than diffusion in bulk solvent, even if there is no interaction between the solute and the solid support. The ratio of the diffusion coefficients for these two cases is normally written as γ :

$$\gamma = D_{\text{eff}}/D_m \quad (4.2. - 1)$$

where D_{eff} is the effective diffusion coefficient of the solute in

the porous matrix, and D_m is the diffusion coefficient in bulk eluent. This ratio is less than unity because of the obstructive nature of the solid support. The paths within the matrix over which the molecules can move are neither straight nor uniform as they would be in bulk eluent, but tortuous and constricted (20,21). This is illustrated in Figure 4.2. - 1.

The effect of tortuosity on D_{eff} may be described in terms of the tortuosity factor τ . This is defined as the ratio of the length of a tortuous path between two points to the direct distance between them:

$$\tau = l_t/l \quad (4.2. - 2)$$

where l_t and l are the tortuous and direct path lengths respectively. These terms are illustrated in Figure 4.2. -1.

From the above definition it follows that, in order to diffuse between the two points, a molecule must travel τ times the distance between them, and the diffusion coefficient is correspondingly smaller.

A further consequence of this increase in path length is a decrease in the concentration gradient, since the same change in concentration is spread over a distance τ times greater than in the absence of tortuosity. Thus the value of γ due to tortuosity alone is given by

$$\gamma = \frac{D_{eff}}{D_m} = \left(\frac{1}{\tau}\right)^2 \quad (4.2. - 3)$$

A second factor which slows the rate of diffusion in porous matrices is the constriction of the channels along which diffusion must take place. Fick's first law of diffusion (33) may be used in

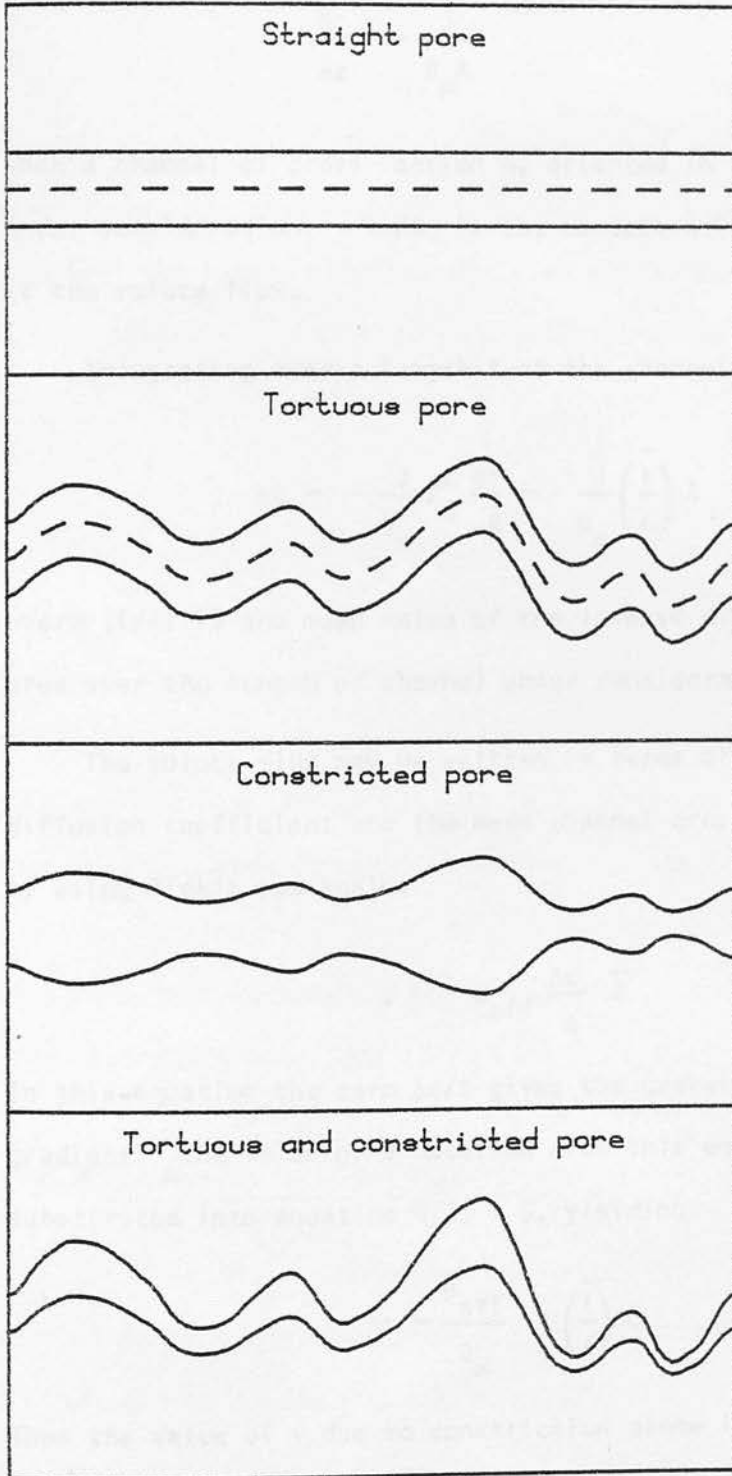


FIGURE 4.2. - 1.

Schematic Diagram of Tortuous and Constricted Pores. The length of the pore is indicated by the dashed line in the first two cases.

considering this effect. This law may be written as

$$\frac{\partial c}{\partial z} = - \frac{J}{D_m A} \quad (4.2. - 4)$$

when a channel of cross-section A , oriented in the z direction, is under consideration. $\partial c/\partial z$ is the concentration gradient, and J is the solute flux.

Integrating over a length ℓ of the channel, we obtain

$$\Delta c = - \frac{J}{D_m} \int_0^\ell \frac{dz}{A} = - \frac{J}{D_m} \left(\overline{\frac{1}{A}} \right) \ell \quad (4.2. - 5)$$

where $\overline{(1/A)}$ is the mean value of the inverse of the cross-sectional area over the length of channel under consideration.

The solute flux may be written in terms of the effective diffusion coefficient and the mean channel cross-section simply by using Fick's law again:

$$J = - D_{\text{eff}} \frac{\Delta c}{\ell} \overline{A} \quad (4.2. - 6)$$

In this equation the term $\Delta c/\ell$ gives the overall concentration gradient. The value of J obtained from this equation may be substituted into equation 4.2. - 5, yielding

$$\Delta c = \frac{D_{\text{eff}}}{D_m} \overline{A} \left(\overline{\frac{1}{A}} \right) \Delta c \quad (4.2. - 7)$$

Thus the value of γ due to constriction alone is given by

$$\gamma = \frac{D_{\text{eff}}}{D_m} = \left(\overline{A} \left(\overline{\frac{1}{A}} \right) \right)^{-1} \quad (4.2. - 8)$$

For a channel of uniform cross-section this ratio is unity, since $\bar{A} = (\bar{l}/A)^{-1}$. For constricted channels γ is less than unity.

The effects of tortuosity and constriction are assumed to be independent. For channels which are both tortuous and constricted the total obstructive factor γ is obtained by multiplying together the values obtained for each mechanism. Therefore for 'real' channels the obstructive factor is given by

$$\gamma = \tau^{-2} \left(\bar{A} \left(\frac{\bar{l}}{A} \right) \right)^{-1} \quad (4.2. - 9)$$

γ is thus shown to be a constant dependent only on the geometry of the support material. It is assumed to be independent of flow rate or diffusion coefficient, but it will vary from one type of packing material to another. In particular, materials with a high porosity and open structure will have values of γ closer to unity than will less porous materials, since in the latter both tortuosity and constriction of channels may be assumed to be greater. Values of γ have been calculated for simple models (61) and the agreement with experiment was found to be satisfactory

4.3. COMBINATION OF DIFFUSION TERMS

It is possible for molecules to diffuse in the stationary phase as well as in the mobile phase. In liquid-liquid and reversed-phase chromatography, where the solute molecules are not bound to specific sites, a concentration gradient will inevitably cause diffusion, and in many cases it is necessary for a molecule to diffuse through a static liquid film before reaching the site of adsorption. This type of diffusion has already been discussed in section 2.2.

Expressions for the plate-height contributions from mobile and stationary phase diffusion are given in equations 2.2. - 5 and 2.2.-6. These may be combined to give the following expressions for the total contribution to the plate height from longitudinal diffusion processes:

$$H = 2(\gamma D_m + k' D_s) \frac{1}{u} \quad (4.3. - 1a)$$

$$h = 2\left(\gamma + k' \frac{D_s}{D_m}\right) \frac{1}{v} \quad (4.3. - 1b)$$

where D_s is the effective stationary phase diffusion coefficient. The phase capacity ratio k' is used rather than the zone capacity ratio k'' in these equations because it is more natural to think in terms of diffusion in the eluent (whether stagnant or mobile) as being different from true stationary phase diffusion, rather than considering stationary zone diffusion which includes diffusion in true stationary phase and in stagnant mobile phase. These equations may be written in terms of k'' if required since stationary zone diffusion must take place in some form. In this case the value of γ may be slightly different from that in equation 4.3. - 1, since obstruction in the stationary zone will not be included. The diffusion coefficient D_s will also be different, since this will refer only to true stationary phase diffusion. This point will be returned to at the end of the chapter.

The plate-height contribution from diffusion processes is often referred to as the 'B term' (21) and may be written as

$$h = B/v \quad (4.3. - 2)$$

where B is a constant. From equation 4.3. - 1b the value of this constant may be found as

$$B = 2\left(\gamma + k' \frac{D_s}{D_m}\right) \quad (4.3. - 3)$$

The object of the experiments described below is to evaluate the various terms of this equation and to verify its validity.

4.4. EXPERIMENTAL

Values of the effective diffusion coefficients for a number of solute/eluent systems were obtained by the arrested elution method of Knox and McLaren (21). In this method the solute is eluted part-way down the column, and the eluent flow is halted. After a set time the flow is restarted and the solute is eluted and detected in the usual way. During the period when there is no flow the only dispersive mechanism operating is diffusion. The variance of the peak due to other factors (e.g. slow mass transfer during the time when the eluent is flowing) may be found by measuring the width of a peak allowed to elute without the flow being interrupted. When this variance is subtracted from the total variance of the arrested peak the residual variance may be related to the effective diffusion coefficient by the Einstein equation

$$\sigma_{res}^2 = 2 D_{eff} t_{int} \quad (4.4. - 1)$$

where σ_{res}^2 is the residual peak variance as described above, D_{eff} is the effective diffusion coefficient and t_{int} is the time for which the flow was halted. In order to apply this equation the

variance must have dimensions of (length)².

A stainless-steel HPLC column (250mm x 8mm) was slurry-packed with ODS Hypersil (Shandon Southern Products Ltd., Runcorn, England), a reverse-phase packing material with a C₁₈ coating and a mean particule diameter of 5µm. The column was thermostatted in a water-jacket at a temperature of 26°C. The eluent was fed through the column by a suitable pump (DMP 1515 Dosing pump, Orlita, Giessen, Germany) at a flow-rate of about 1ml/min. The column effluent was passed through a UV monitor (CE 212, Cecil Instruments, Cambridge, England) and collected in a vessel on an electronic balance (Oertling F22 TD, Liverpool, England.) The outputs from the detector and balance were recorded on a two-pen recorder (Bryans 28200, Bryan Southern Instruments Ltd., Surrey, England).

Samples were introduced onto the column by means of a Valco injection valve (Valco Instrument Co., Houston, Texas).

This method was chosen rather than syringe injection partly for simplicity of operation, and partly because it was felt that radial diffusion subsequent to syringe injection could lead to erroneous results, since the column would be an 'infinite diameter' column when the flow was not interrupted, and thus the solute would not reach the regions near the column walls where the packing structure may be different from the central region, whereas after the flow had been halted the solute would diffuse to these regions. When valve injection is used the solute is introduced over the breadth of the column, and all samples will experience both core and wall regions. The volume of injection was 20µl.

Samples normally contained up to three different solutes, chosen such that even after the longest interruption time the peaks did not overlap. Care was taken that when the flow was halted no solute was either so near the top or so near the bottom of the column that its diffusion would be disturbed by these features.

Three different eluent/solute systems were examined by this system. The eluent in the first system was a mixture of methanol and water (60:40 v:v), and the solutes were Oxy-aromatic hydrocarbons. The second system consisted of pure methanol as eluent, and a set of polycyclic aromatic hydrocarbons as solutes. Details of these solutes are given in Appendix 1. The third system consisted of sulphamic acid in a set of eluents consisting of sodium nitrate in methanol.

The experimental parameter required in order to use equation 4.4. - 1 is the peak variance. This was obtained as follows:

The peak width was measured as the distance between the points where tangents drawn to the points of inflection of the peak cross the baseline. The corresponding weight of eluent was then calculated from the balance output. This procedure is demonstrated in Figure 4.4. - 1. The peak variance in the desired units may be calculated from the formula

$$\sigma^2 = \left(\frac{W_b}{4} \frac{L}{W_c} \frac{1}{1+k'} \right)^2 \quad (4.4. - 2)$$

where W_b is the weight of eluent in which the peak is eluted, W_c is the total weight of eluent in the column which may be taken as the weight of eluent measured from time of injection to the appearance of the solvent disturbance peak with no interruption to flow, and

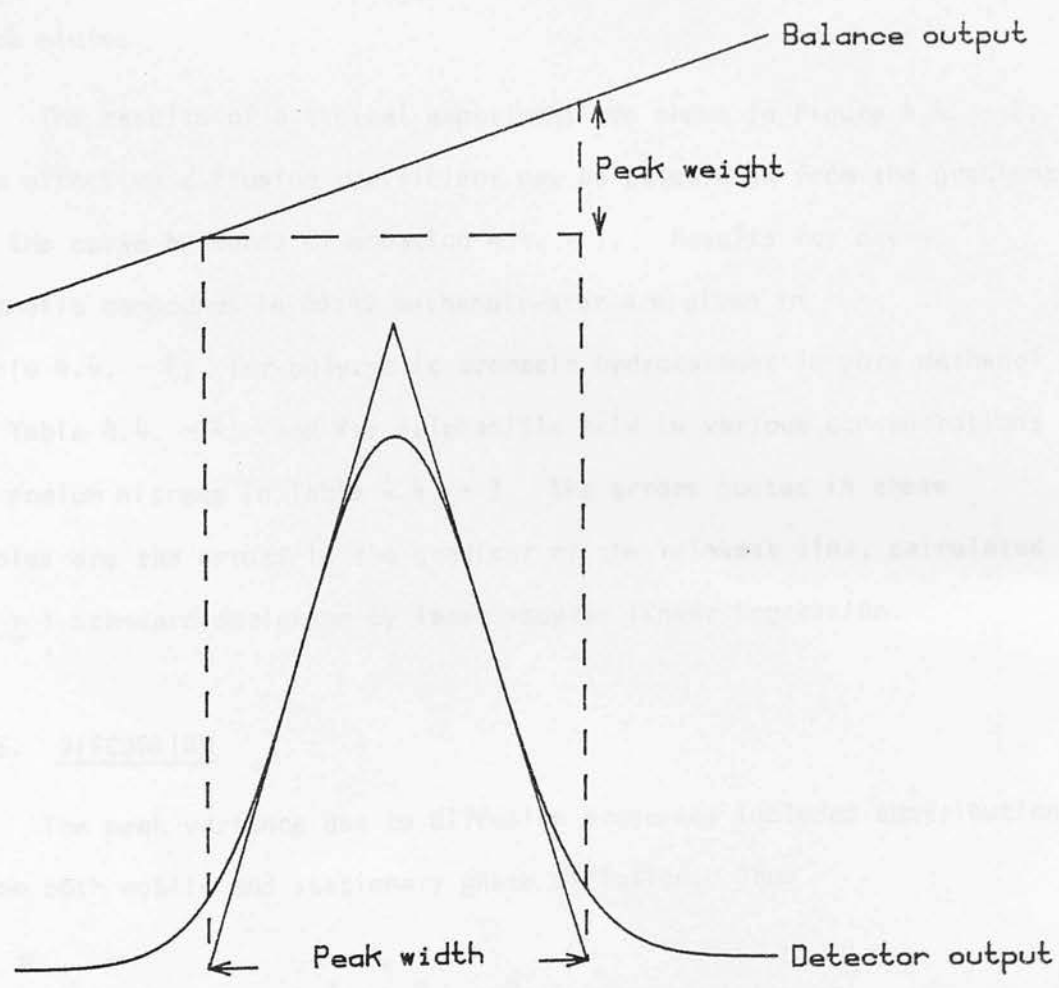


FIGURE 4.4. - 1. Schematic Diagram for Data Reduction in the B-term Experiment.

L is the column length. The factor 4 is included since the width of the peak as described above is equal to 4σ , and the factor $1/1+k'$ is required because a volume of eluent $1+k'$ times the dead volume of the column is required to elute a solute of capacity factor k' , and this increase in volume will hold during the time for which the peak elutes.

The results of a typical experiment are shown in Figure 4.4. - 2. The effective diffusion coefficient may be calculated from the gradient of the curve by means of equation 4.4. - 1. Results for oxyaromatic compounds in 60:40 methanol:water are given in Table 4.4. - 1; for polycyclic aromatic hydrocarbons in pure methanol in Table 4.4. - 2; and for sulphanilic acid in various concentrations of sodium nitrate in Table 4.4. - 3. The errors quoted in these Tables are the errors in the gradient of the relevant line, calculated as ± 1 standard deviation by least-squares linear regression.

4.5. DISCUSSION

The peak variance due to diffusion processes includes contributions from both mobile and stationary phase diffusion. Thus

$$\sigma_{\text{eff}}^2 = \sigma_m^2 + \sigma_s^2 \quad 4.5. - 1$$

Each of these variances may be related to the appropriate diffusion coefficient, yielding the equation

$$D_{\text{eff}} t_{\text{int}} = \gamma D_m t_m + D_s t_s \quad 4.5. - 2$$

by application of the Einstein equation. The mobile and stationary

FIGURE 4.4. - 2.

Typical set of results for B-term Experiment.

Solute: Phenol

Solvent: 60:40 MeOH:H₂O

$D_{\text{eff}} = 0.54 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$

For other conditions see text.

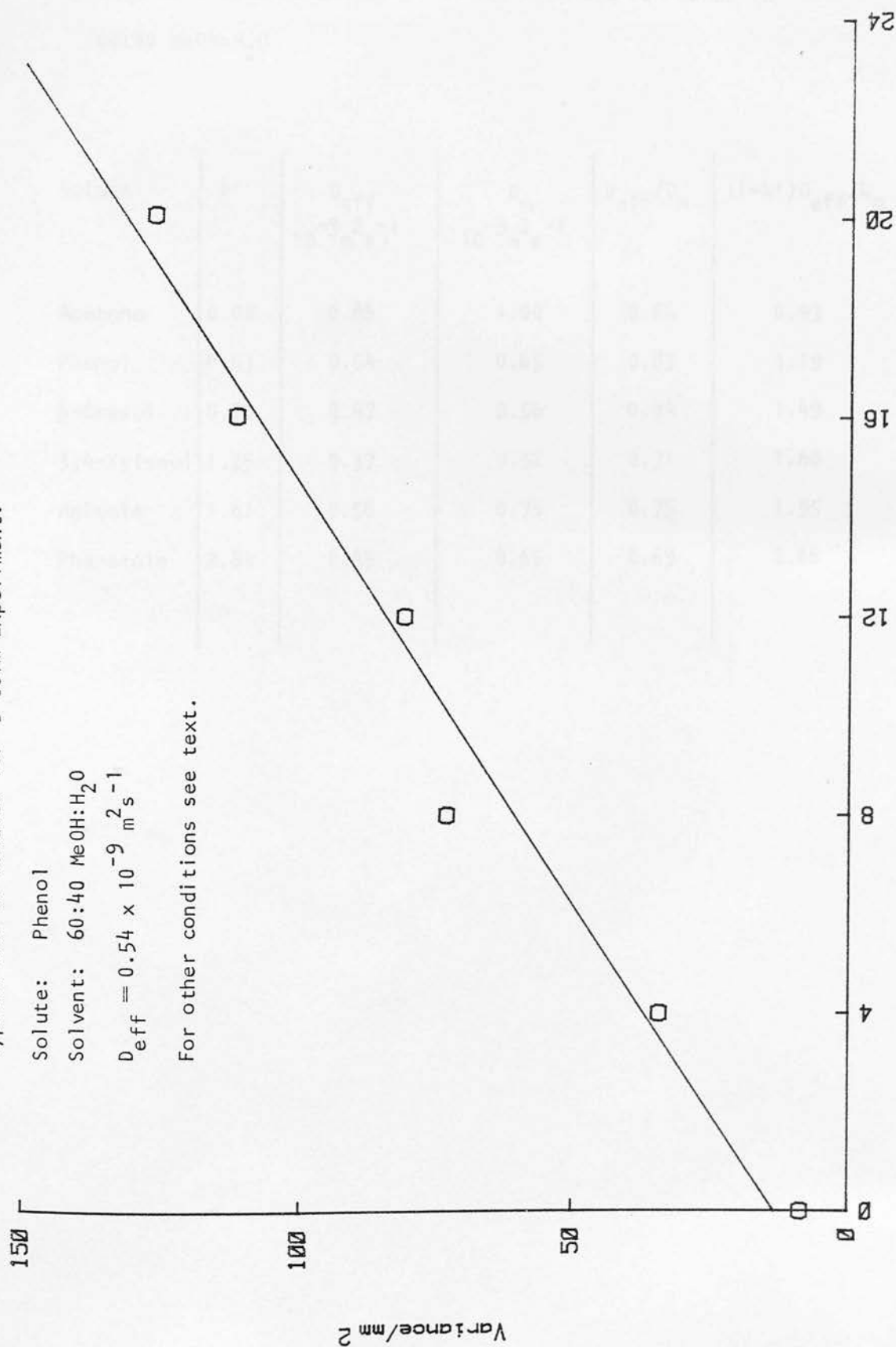


TABLE 4.4. - 1

RESULTS OF B-TERM EXPERIMENTS FOR OXY-AROMATIC COMPOUNDS IN

60:40 MeOH:H₂O

Solute	k'	D_{eff} $10^{-9} \text{ m}^2 \text{ s}^{-1}$	D_m $10^{-9} \text{ m}^2 \text{ s}^{-1}$	D_{eff}/D_m	$(1+k')D_{eff}/D_m$
Acetone	0.08	0.86	1.00	0.86	0.93
Phenol	0.43	0.54	0.65	0.83	1.19
p-Cresol	0.77	0.47	0.56	0.84	1.49
3,4-Xylenol	1.25	0.37	0.52	0.71	1.60
Anisole	1.61	0.56	0.75	0.75	1.95
Phenetole	2.84	0.45	0.65	0.69	2.66

TABLE 4.4. - 2

RESULTS OF B-TERM EXPERIMENT FOR POLYCYCLIC AROMATIC HYDROCARBONS
IN METHANOL

Solute	k'	D_{eff} $10^{-9} \text{ m}^2 \text{ s}^{-1}$	D_{m} $10^{-9} \text{ m}^2 \text{ s}^{-1}$	$D_{\text{eff}}/D_{\text{m}}$	$(1+k')D_{\text{eff}}/D_{\text{m}}$
Naphthalene	0.15	1.42	1.83	0.78	0.90
Anthracene	0.30	1.45	1.50	0.97	1.26
Chrysene	0.49	0.95	1.45	0.66	0.98
Benzo(L)- fluoranthene	0.68	0.68	1.33	0.51	0.86
Naphtho(2,3,-R) fluoranthene	1.25	0.76	1.22	0.62	1.40

TABLE 4.4. - 3

RESULTS OF B-TERM EXPERIMENT FOR SULPHANILIC ACID IN METHANOL
+ SODIUM NITRATE

NaNO ₃ concentration (Molar)	k''	D _{eff} 10 ⁻⁹ m ² s ⁻¹	D _m 10 ⁻⁹ m ² s ⁻¹	D _{eff} /D _m	(1+k'')D _{eff} /D _m
0.001	0.00	0.60	0.92	0.65	0.65
0.002	0.11	0.58	0.92	0.63	0.70
0.003	0.12	0.60	0.92	0.65	0.73
0.005	0.20	0.62	0.92	0.67	0.80
0.010	0.26	0.61	0.92	0.66	0.83

phase residence times t_s and t_m may be related to the interrupt time t_{int} by the equations

$$t_m = \frac{1}{1+k'} t_{int} \quad 4.5. 3a$$

$$t_s = \frac{k'}{1+k'} t_{int} \quad 4.5. - 3b$$

The diffusion coefficients are therefore related by

$$D_{eff} = \frac{\gamma D_m}{1+k'} + \frac{k' D_s}{1+k'} \quad 4.5. - 4$$

Since values of D_m vary from solute to solute, it is helpful to scale this equation to the value of D_m :-

$$\frac{D_{eff}}{D_m} = \left(\gamma + k' \frac{D_s}{D_m} \right) \frac{1}{1+k'} \quad 4.5. - 5$$

This equation may be rewritten as

$$(1+k') \frac{D_{eff}}{D_m} = \gamma + k' \frac{D_s}{D_m} \quad 4.5. - 6$$

which is a more useful form.

If D_s/D_m may be assumed to be a constant for a set of solutes in a given eluent/stationary phase system, then a plot of $(1+k') D_{eff}/D_m$ as a function of k' should have a gradient of D_s/D_m and an intercept of γ .

Values of $(1+k') D_{\text{eff}}/D_m$ are included in Tables 4.4. - 1 and 4.4. - 2. Graphs of this as a function of k' are shown in Figures 4.5. - 1 and 4.5. - 2. In both cases the intercept (which should be equal to the obstructive factor, γ) has a value of 0.9. Since γ is a structural parameter, it is not expected that changes in eluent or solute should affect its value.

The commonly accepted value of γ is 0.6. This value was obtained from experiments on glass beads (21), which are impermeable and have a porosity of about 40%. In silica gel it is possible for molecules to diffuse through the beads as well as around them. This, together with the lower packing density of silica gel columns, should explain the higher values of γ found in this experiment.

For oxy-aromatic compounds in 60:40 methanol:water a value of 0.61 was obtained for the value of (D_s/D_m) . Figure 4.5. - 1 indicates the approximate nature of this value, which is the gradient of the curve. Qualitatively this value indicates that stationary phase diffusion is somewhat slower than mobile phase diffusion. This may be due to an increase in the intermolecular forces which hinder diffusion, or to a higher obstructive factor in the stationary phase. It is likely that both factors play a part in producing the slower rate of diffusion.

For the polycyclic aromatic hydrocarbons in methanol the value of (D_s/D_m) is about 0.3 as shown by the gradient in Figure 4.5. - 2. Since the mobile phase diffusion coefficients for this system are greater than those for the previous system by a factor of 2-3 (see Table 3.5. - 3), this result is consistent with a similar rate of stationary phase diffusion for both systems, the difference in the

FIGURE 4.5. - 1.

Graph of $(1+k') D_{eff}/D_m$ as a function of k' for Oxy-Aromatic Compounds.

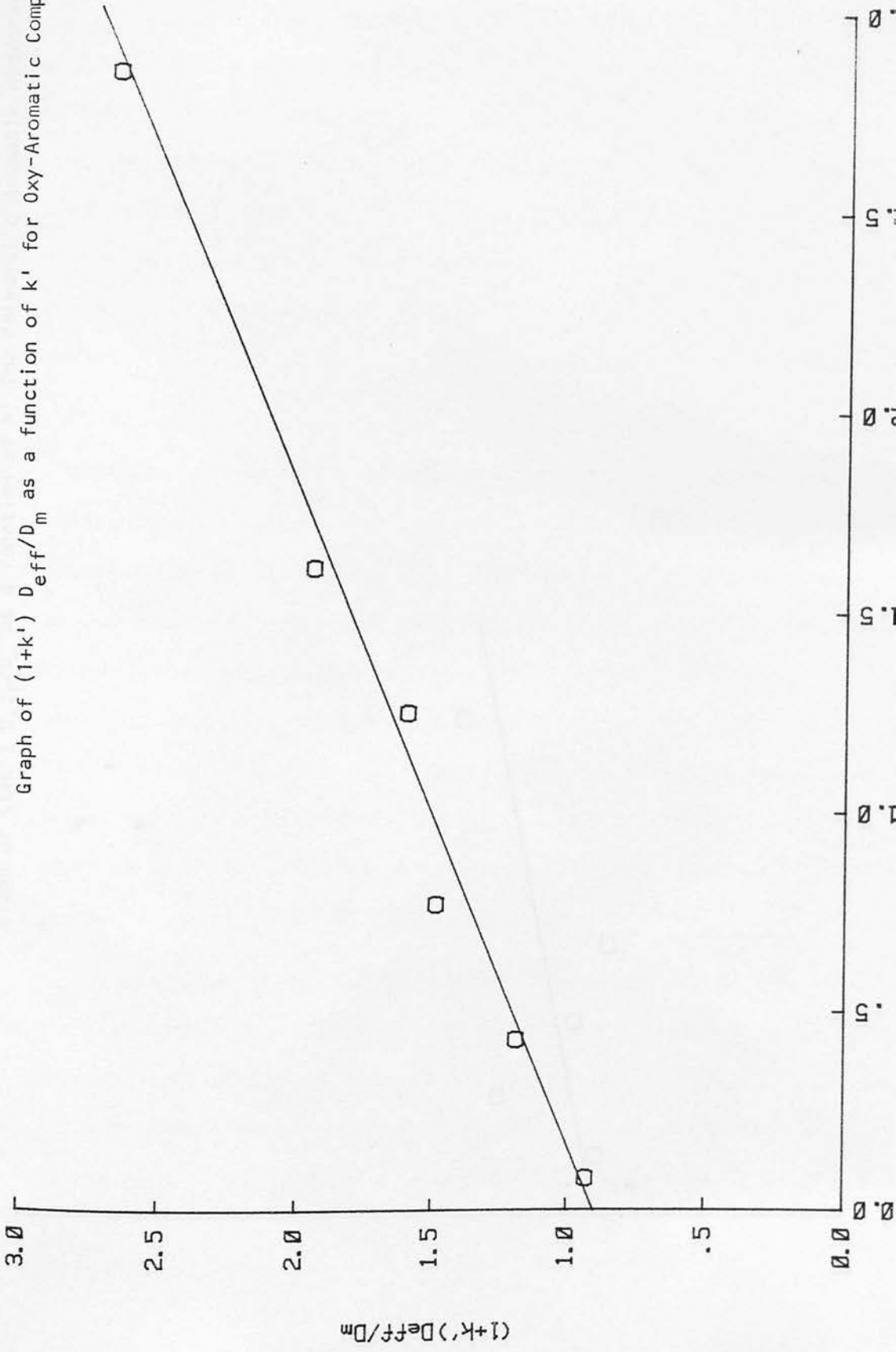
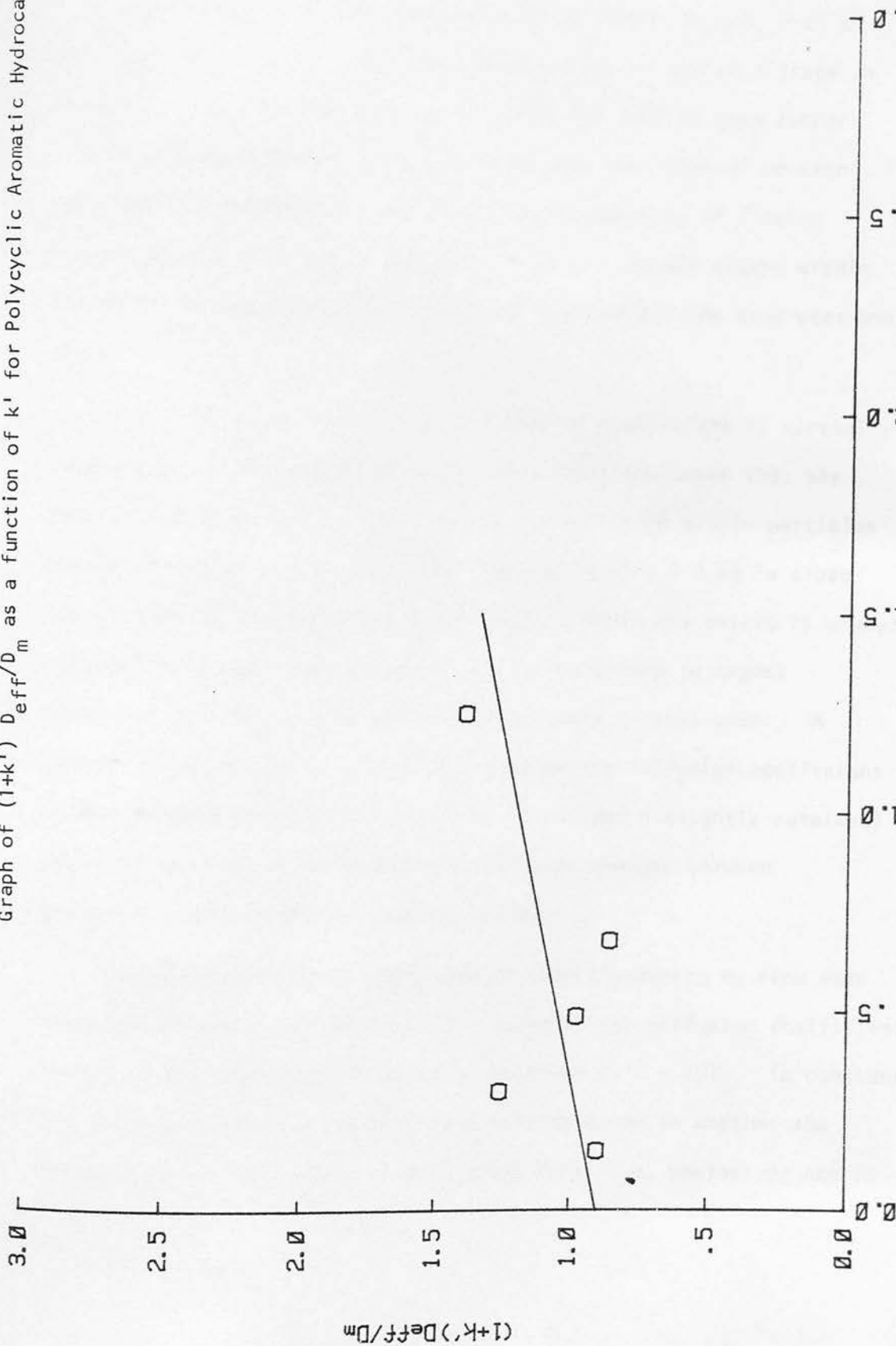


FIGURE 4.5. - 2.

Graph of $(1+k') D_{eff}/D_m$ as a function of k' for Polycyclic Aromatic Hydrocarbons.



value of (D_s/D_m) being due to mobile phase effects. This result is in accordance with the accepted retention mechanism of reverse-phase liquid chromatography outlined in chapter 1.

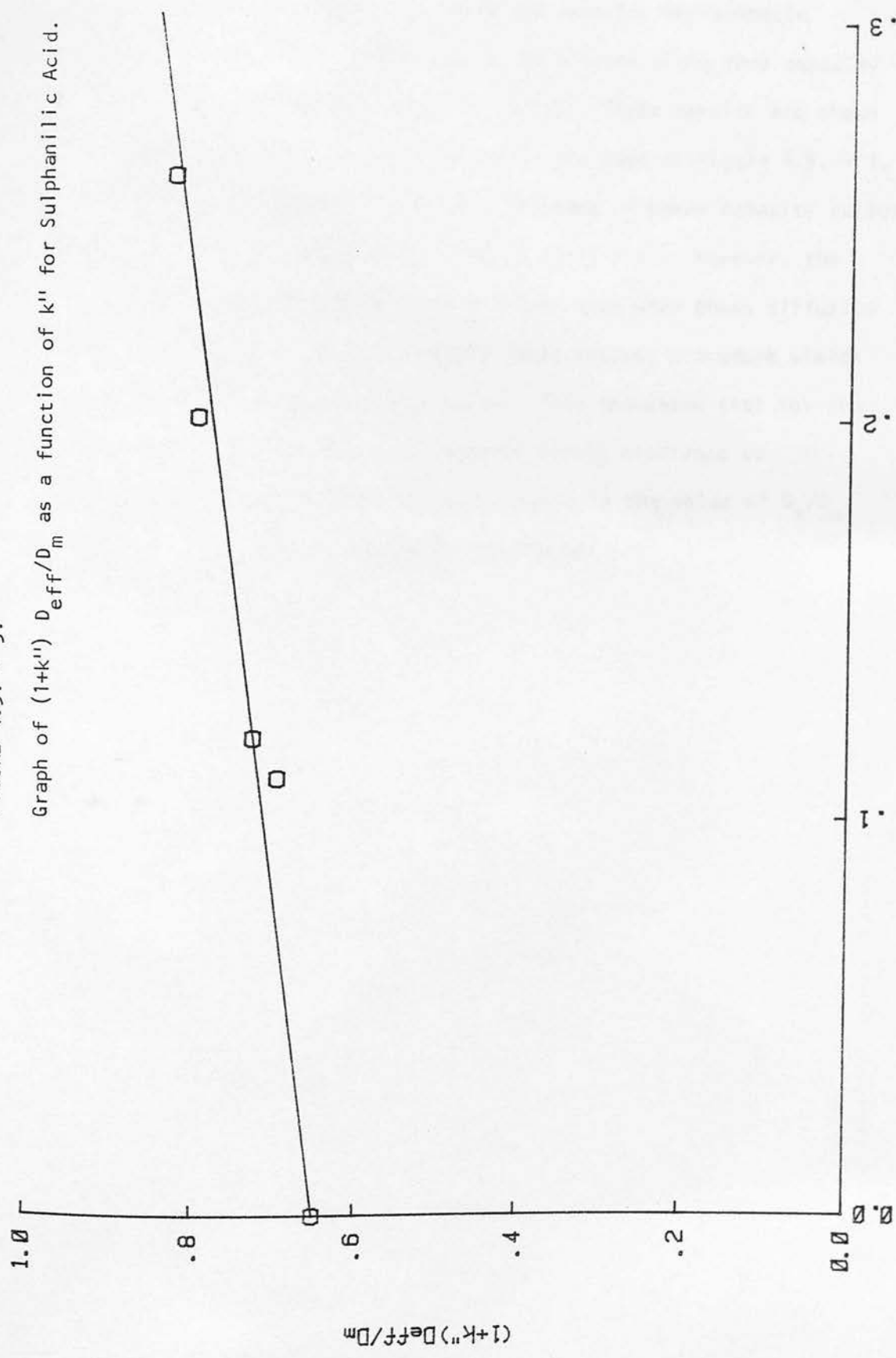
Figure 4.5. - 3 shows the variation of $(1+k'') D_{eff}/D_m$ with k'' for sulphanic acid in various concentrations of sodium nitrate in methanol. In this case the zone capacity ratio k'' is used rather than the phase capacity ratio k' , since only two types of environment need be considered: the mobile zone consisting of flowing eluent and the stationary zone consisting of stagnant eluent within the pores of the material. The solute never enters the true stationary phase.

For this system the effective diffusion coefficient is virtually independent of the degree of exclusion. This indicates that the obstructive factor γ is much the same for diffusion within particles as for diffusion around particles. The value of $\gamma = 0.65$ is close to the theoretical value for glass beads. Since the solute is always partially excluded from the pores, it is reasonable to expect behaviour similar to that for non-porous beads in this case. A careful study of the behaviour of the effective diffusion coefficient in the neighbourhood of $k' = 0$ (slightly excluded - slightly retained) would be required to determine how diffusion changes between exclusion and retention in chromatography.

Certain equations in the theory of band broadening by slow mass transfer include a term which is the ratio of the diffusion coefficient in mobile and stationary zones (e.g. equation 2.7. - 65). In considering slow mass transfer the important consideration is whether the molecule is in the flowing or stationary zone, i.e. whether or not it

FIGURE 4.5. - 3.

Graph of $(1+k'') D_{eff}/D_m$ as a function of k'' for Sulphanilic Acid.



is being moved by the flow of eluent. The ratio of diffusion coefficients used should therefore be the ratio of the coefficients in the different zones.

In order to obtain this ratio the data for oxy-aromatic compounds in methanol:water eluent was treated using zone capacity ratios rather than phase capacity ratios. These results are shown in Table 4.5. - 1. Figure 4.5. - 4 is the same as Figure 4.5. - 1, but with zone capacity ratios used in place of phase capacity ratios. The value of the ratio of D_s to D_m is still 0.61: however, the value of γ for the mobile zone is higher than when phase diffusion was considered. In fact a simple least squares procedure yields a value slightly greater than unity. This indicates that for the stationary zone in this case there is little hindrance to diffusion; however, the important result is the value of D_s/D_m which will be used in the following chapter.

FIGURE 4.5. - 4.

Graph of $(1+k'') D_{eff}/D_m$ as a function of k'' for Oxy-Aromatic Compounds.

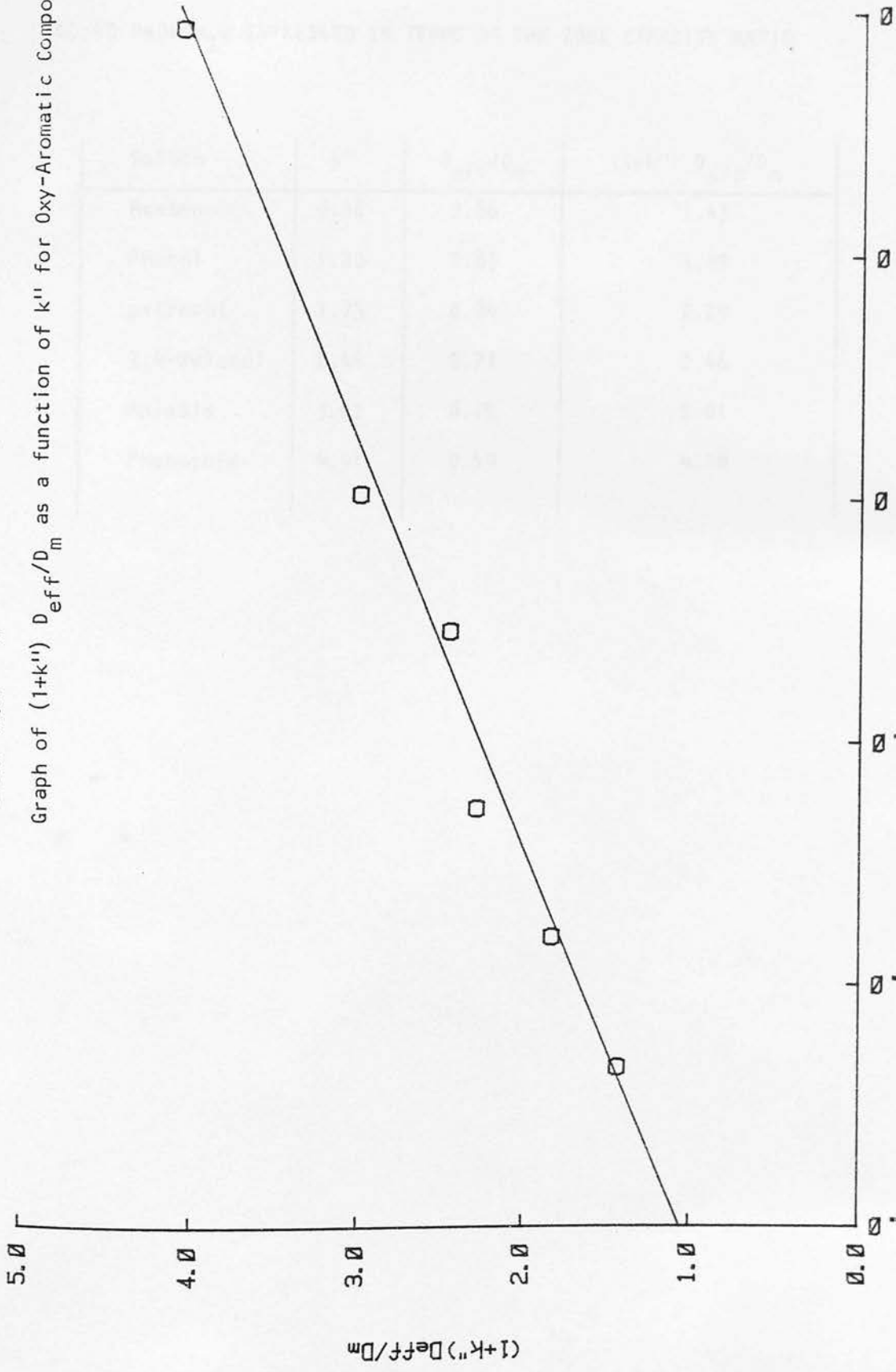


TABLE 4.5. - 1

RESULTS OF B-TERM EXPERIMENT FOR OXY-AROMATIC COMPOUNDS IN
60:40 MeOH:H₂O EXPRESSED IN TERMS OF THE ZONE CAPACITY RATIO

Solute	k''	D_{eff}/D_m	$(1+k'') D_{\text{eff}}/D_m$
Acetone	0.66	0.86	1.43
Phenol	1.20	0.83	1.83
p-Cresol	1.73	0.84	2.29
3,4-Xylenol	2.46	0.71	2.46
Anisole	3.02	0.75	3.01
Phenetole	4.91	0.69	4.08

BAND BROADENING BY SLOW MASS TRANSFER

INTRODUCTION

One of the major aspects of band broadening in chromatography is the slow mass transfer of solute between the stationary and mobile phases. This mechanism has been described in detail by Giddings and his co-workers. In this chapter, the experimental investigation of this mechanism is described, and the results are discussed.

The relationship between column diameter and plate height is also discussed. It is shown that the plate height is independent of column diameter in the region of slow mass transfer.

CHAPTER 5

BAND BROADENING BY SLOW
MASS TRANSFER

It is shown in this chapter that the slow mass transfer mechanism is the dominant factor in band broadening in the region of slow mass transfer. The experimental results are compared with the theoretical predictions of Giddings and his co-workers. It is shown that the plate height is independent of column diameter in the region of slow mass transfer.

$$H = \frac{2k}{1+k} \frac{d_p^2}{D_m} + \frac{2k}{1+k} \frac{d_p^2}{D_s} + \frac{2k}{1+k} \frac{d_p^2}{D_m} + \frac{2k}{1+k} \frac{d_p^2}{D_s}$$

In which the first two terms represent the effects of slow mass transfer, the third term represents the effects of longitudinal diffusion, and the fourth term represents the effects of eddy diffusion. It is shown that the plate height is independent of column diameter in the region of slow mass transfer.

As will be shown in this chapter, the slow mass transfer mechanism is the dominant factor in band broadening in the region of slow mass transfer. The experimental results are compared with the theoretical predictions of Giddings and his co-workers.

CHAPTER 5

BAND BROADENING BY SLOW MASS TRANSFER

5.1. INTRODUCTION

One of the major causes of band broadening in chromatography is the slow mass transfer of solute between different environments. This phenomenon has been described in detail in chapter 2. In this chapter an experimental investigation of this cause of band broadening will be described, and the results discussed.

The relationship between reduced plate-height and reduced velocity in chromatography maybe described by the equation

$$h = \frac{B}{v_o} + C_s v_o + \frac{1}{1/A + 1/C_m v_o} \quad (5.1. - 1)$$

as shown in section 2.4. This equation does not adequately fit the experimental results (61); various semi-empirical equations have been proposed which perform better in this respect (18, 62-64). One of the best of these is the equation

$$h = A v_o^{1/3} + B/v_o + C v_o \quad (5.1. - 2)$$

in which the A-term describes mobile zone flow effects, the B-term describes longitudinal diffusion effects and the C-term describes slow mass transfer effects. Typical values of these parameters in HPLC are: $A \leq 1$, $B \approx 2$, $C < 0.1$ (65).

At high reduced velocities the contribution to band broadening by longitudinal diffusion will be negligible. Since the value of A

is much greater than the value of C, very high reduced velocities would be required to make the A-term negligible and allow the direct determination of the C-term. At fairly high reduced velocities, ($v > 500$) the A-term may be approximated roughly by a term which varies directly with v . These approximations yield the equation

$$h = (A' + C)v_0 \quad (5.1.13)$$

where A' is a constant depending on the magnitude of A and the range of v under consideration. This equation will only hold for a limited and specified ranges of v .

To obtain the required range of reduced velocities with typical packing materials ($d_p = 5 - 10\mu\text{m}$), very high pumping pressures would be required. Even if these could be readily realised, thermal effects caused by friction at the high flowrates would make chromatography difficult. The simplest way to achieve the required reduced velocities is to use large particles. If the particle diameter is increased by a factor of 10, the same linear velocity of eluent will yield a reduced velocity ten times greater, while the pumping pressure required will decrease by a factor of 100 (all other experimental conditions remaining constant).

5.2. EXPERIMENTAL

The packing material chosen consisted of silica spheres of mean diameter $50\mu\text{m}$, treated to have a reversed-phase ODS coating. These particles were prepared in house. They were packed into a stainless steel column (500mm x 8mm) by mechanically rotating and bouncing the column as the particles were added. The injector and detector were

as described in chapter 4, except that for a time a Du Pont 820 single wavelength UV detector (Du Pont, Wilmington, Delaware) was used. Pumping pressure was obtained from a Haskel pressure intensifier (Haskel Energy Systems Ltd., North Hylton Road, Sunderland).

The solutes used in this experiment were the set of oxyaromatic hydrocarbons used in the B-term experiment described in Appendix 1. A set of eluents consisting of various proportions of methanol and water was used to obtain a wide range of k'' values. The experiment was carried out ambient temperature.

The eluent flow-rate was recorded by means of a balance and recorder, as described in the previous chapter. The mobile phase velocity was calculated from the length of the column and the time taken for the refractive index peak associated with the injection to appear. This method gives the mobile phase residence time t_m and the mobile phase reduced velocity v . The theory requires the use of the mobile zone residence time t_o or the mobile zone reduced velocity v_o . These figures were obtained from the knowledge that t_o is approximately $0.59 t_m$, from observation of the elution time of a totally excluded solute.

The reduced velocities used in this experiment ranged from about 500 to about 5000. The number of plates (N) ranged from about 150 (at high velocities) to about 600 (at low velocities). The plate number was calculated from the retention time t_r and the peak width at half height $W_{\frac{1}{2}}$ by the formula

$$N = 5.54 \left(\frac{t_r}{W_{\frac{1}{2}}} \right)^2 \quad 5.2. - 1$$

Even at high velocities the peaks could be adequately approximated by a Gaussian function and thus the use of this equation is justified.

The reduced plate-height h is obtained from the formula

$$h = \frac{L}{N d_p} \quad 5.2. - 2$$

A typical plot of h against v_0 is shown in Figure 5.2. - 1. The experimental data is shown with a least-squares straight line and with a curve of the form

$$h = A v_0^{1/3} + C v_0 \quad 5.2. - 3$$

The value of A which gives the best fit to this set of data is 2.5. The quasi-linear nature of the curve is evident from the graph; the slight departure from linearity gives a slightly better fit to the data than does the least-squares straight line.

By simply measuring the gradient an 'apparent' C-term, C_{ap} is obtained. This term includes a contribution from the A-term as suggested in equation 5.1. - 3. Thus

$$C_{ap} = A' + C \quad 5.2. - 4$$

Apparent C-terms for all eluent/solute combinations are shown in Table 5.2. - 1. This data is displayed as a plot of C_{ap} against k' in Figure 5.2. - 2. Also shown in this figure is a plot of the theoretical C-term:

FIGURE 5.2. - 1. Typical set of results for C-term Experiment on 50 μ m material.

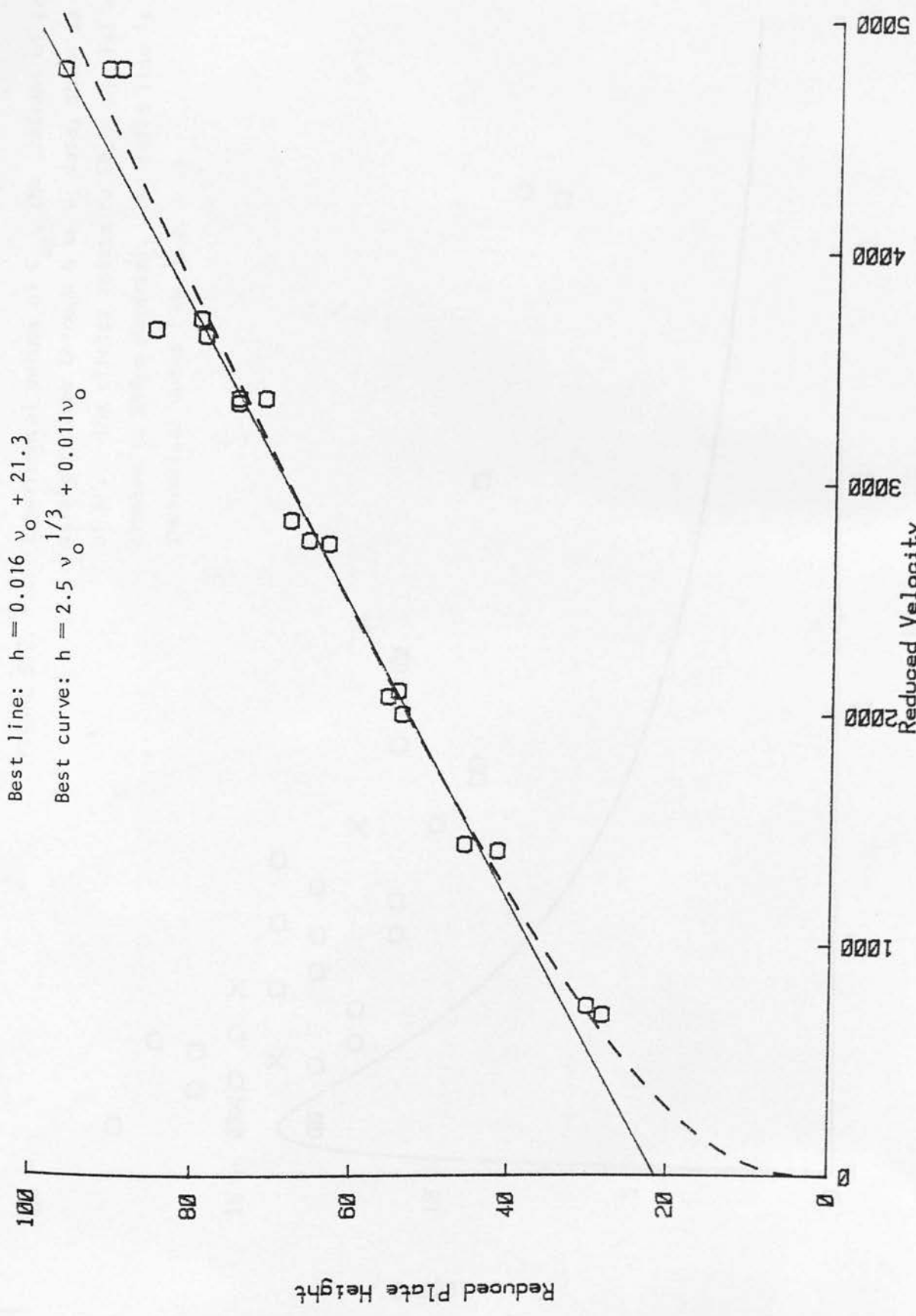


FIGURE 5.2. - 2. Experimental values of C_{ap} , the gradient of the best straight lines through h vs v_0 data, shown as a function of k'' . The circles relate to 50 μ m material and the crosses to 540 μ m material. The solid line is the theoretical curve (eq. 5.2. - 5)

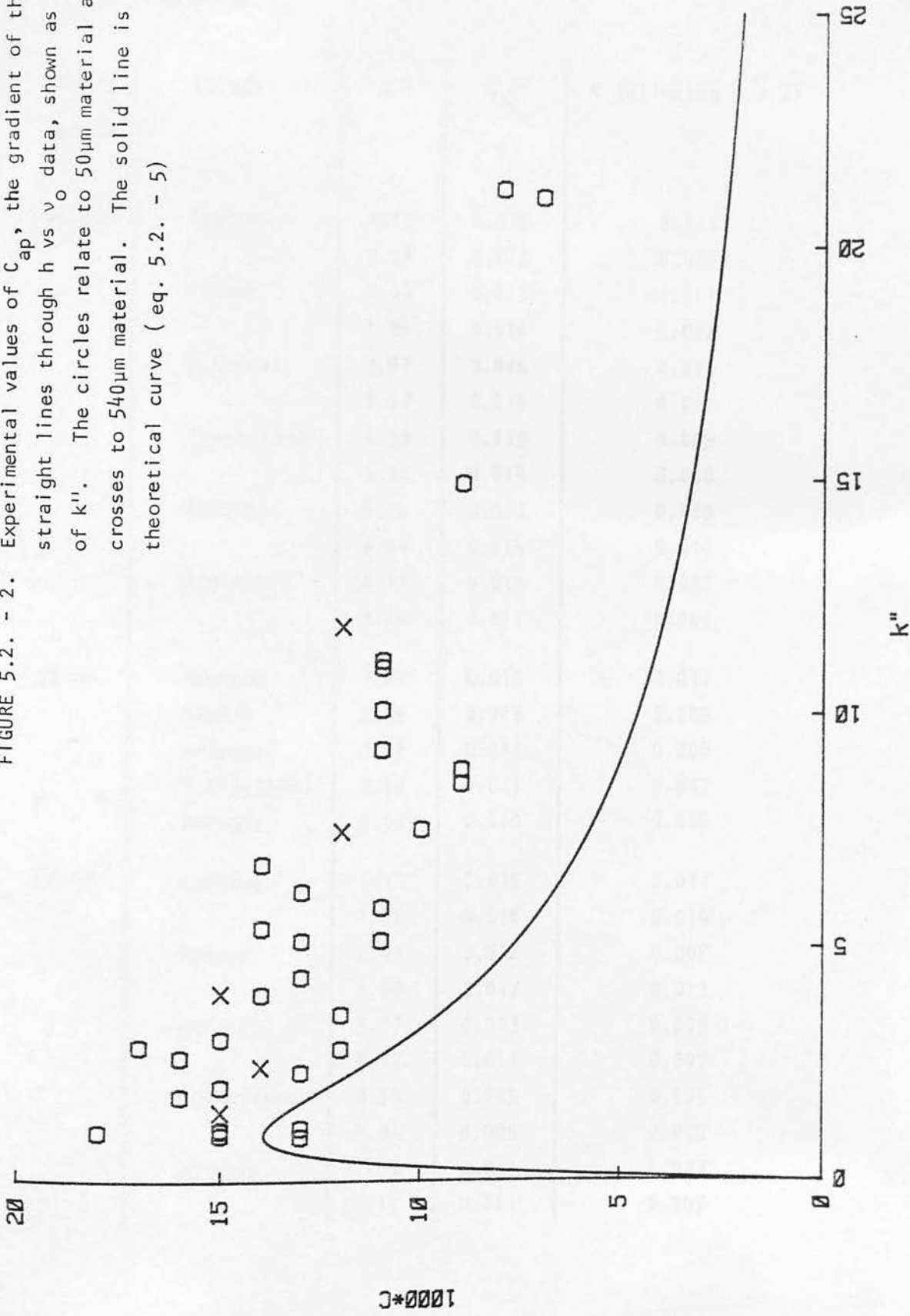


TABLE 5.2. - 1

RESULTS OF C-TERM EXPERIMENT

a) 50 μ m Material

Eluent	Solute	k''	C_{ap}	C (allowing A = 2)	
MeOH:H ₂ O					
60:40	Acetone	1.11	0.015	0.011	
		0.96	0.013	0.009	
	Phenol	2.04	0.015	0.011	
		1.84	0.016	0.012	
	p-Cresol	3.07	0.015	0.011	
		2.67	0.016	0.012	
	3,4-Xylenol	4.39	0.013	0.009	
		4.02	0.014	0.010	
	Anisole	6.22	0.013	0.009	
		5.44	0.014	0.010	
	Phenetole	10.12	0.011	0.007	
		9.26	0.011	0.007	
	55:45	Acetone	1.01	0.015	0.011
		Phenol	2.35	0.013	0.009
p-Cresol		3.58	0.012	0.008	
3,4-Xylenol		5.88	0.011	0.007	
Anisole		7.55	0.010	0.006	
50:50	Acetone	1.13	0.015	0.011	
		1.06	0.018	0.014	
	Phenol	2.84	0.012	0.008	
		2.90	0.017	0.013	
	p-Cresol	5.17	0.013	0.009	
		5.17	0.011	0.007	
	3,4-Xylenol	8.53	0.009	0.005	
		8.84	0.009	0.005	
	Anisole	11.02	0.011	0.007	
		11.17	0.011	0.007	

Eluent MeOH:H ₂ O	Solute	k''	C _{ap}	C (allowing A = 2)
	Phenetole	21.05	0.007	0.003
		21.22	0.008	0.004
45:55	Acetone	1.15	0.013	0.009
	p-Cresol	6.81	0.014	0.010
	Anisole	14.95	0.009	0.005

b) 540 μ m Material

(allowing A = 4)

60:40	Acetone	1.51	0.015	0.011
	Phenol	2.47	0.014	0.010
	p-Cresol	4.05	0.015	0.011
	Anisole	7.51	0.012	0.008
	Phenetole	11.9	0.012	0.008

$$C = \frac{1}{30} \frac{D_m}{D_s} \frac{k''}{(1+k'')^2} \quad 5.2. - 5$$

The ratio D_m/D_s has been taken as 1.67 in accordance with the results of the experiment with similar eluent/solute combinations in Chapter 4. Data reduction will be discussed further in section 5.3.

In order to confirm that the C-term is indeed independent of particle size, the experiment was repeated with particles with a mean diameter of about ten times greater than that of the particles in the experiment described above. These could not be produced by the normal procedure so a novel method of production was required. After a variety of attempts, the following method was found to produce suitable particles.

The starting material for the preparation of silica spheres is a colloidal suspension of silica. Such suspensions may be obtained commercially. Ludox HS 40 (Du Pont, Wilmington, Delaware) was found to be a suitable starting material. 25-50cm³ of this suspension was placed in a stainless-steel bomb and acidified slightly with hydrochloric acid. The bomb was topped up with hexane, and the silica was forced through a short (~ 2cm) length of stainless-steel tubing (i.d. 0.5mm) by pumping hexane into the bomb via the Haskel pressure intensifier. The resulting jet was directed into a beaker of molten wax about 1m distant. Warm water (about 70°C) was circulated in plastic tubing around the top of the beaker to keep the wax molten, but the bottom of the beaker was placed in a little cold water. When the spray from the jet reached the beaker

the wax solidified around the spherical droplets. These waxed beads fell to the bottom of the beaker, where the cold water prevented them from re-melting. After all of the silica had been pumped into the beaker in this way the wax was allowed to set, and left overnight. This allowed the silica spheres to harden. The following day the wax was melted and the silica spheres were removed into water. They were then taken out of the water and allowed to dry. Residual water was removed by repeated washing with iso-amyl alcohol. Several separate batches were prepared in this way. These batches were combined into a single batch, and fired overnight at 500°C. The beads were then fractionated by sieving. It was decided that the fraction from 420 to 710µm was most suitable for the proposed experiment. This fraction was derivatised to yield a batch of large silica spheres with an ODS surface.

Examination under an optical microscope showed that most of the particles were spherical. A small percentage of lumps and chipped or broken spheres was observed, and a few of the particles had surface defects. Apart from the size, the beads looked similar to a typical sample of commercial ODS packing material with mean diameter 5µm (ODS Hypersil, Shandon Southern, England).

A representative sample of the large beads were measured under the microscope. A mean particle diameter of 540µm was found. This figure has been used as the particle diameter for this material in all subsequent calculations.

These spheres were packed into a 1000 x 8mm column by the 'rotate and bounce' method described above. The variation of reduced plate-height with reduced velocity was determined for the same set of

solutes as in the previous study. Only a single eluent (60:40 methanol:water) was used. Several modifications to the experimental technique were required.

The column dead volume could not be determined from the position of the solvent disturbance peak, since because of the low efficiency of the column this peak could not be detected. Instead the dead volume was calculated from the weight of the column when filled with methanol and with dichloromethane. The difference in weights and the difference in densities between the two solvents allows the column dead volume to be calculated. It was assumed that the ratio of pore volume to eluent volume was the same as for the previous material.

For the range of eluent velocities used the column efficiency was very low (5-20 theoretical plates). A Gaussian peak shape could not be assumed for this reason. The mean and standard deviation of a peak are better related to physico-chemical parameters than are the peak maximum and width at half height, although for Gaussian peaks the latter parameters are closely related to the former.

Since this assumption could not be made in the current experiment the peak mean and standard deviation were calculated by digitising the peaks. The number of theoretical plates was obtained from the formula

$$N = (\bar{t} / \sigma_t)^2 \quad 5.2. - 6$$

and h was then calculated from equation 5.2. - 2.

-50-

In this experiment reduced velocities ranged from about 3000 to about 15000. Reduced plate-heights ranged from about 80 to about 400. A typical set of results is shown in Figure 5.2. - 3. The results for all solutes are given in Table 5.2. - 1 and appear on Figure 5.2. - 2. The values of C_{ap} are in good agreement with the results on the 50 μ m material, confirming that the particle size does not affect the C-term. This implies that the dispersion processes responsible for band broadening are scaled to the particle size.

5.3 DISCUSSION

The theoretical curve in Figure 5.2. - 2 shows the same form of variation with k'' as does the experimental data. As explained above, the experimental results plotted in this Figure include a contribution from the A-term, and so are positively displaced from the theoretical curve. The mean vertical displacement of the experimental data with respect to the theoretical curve is 0.004 units. Thus the value of the A' factor in equation 5.2. - 4 is about 0.004.

The A' factor is the gradient of the best-fit line to a graph of $A v_0^{1/3}$ as a function of v_0 . A family of curves of this type is shown in Figure 5.3. - 1. The corresponding values of A' for the various values of A are shown in Table 5.3. - 1. From this table it may be seen that an A-term of about 2 is required to bring the experimental results into line with the theoretical equation 5.2. - 3. This figure is high compared to the normal value of $A \leq 1$. This would indicate a very poorly packed column.

It is possible that the column is poorly packed, since no previous experience of the 'rotate and bounce' method of column packing had been

FIGURE 5.2. - 3.

Typical set of results for C-term Experiment on 540 μ m Material.

Best line: $h = 0.016 v_o + 37.2$

Best curve: $h = 3.5 v_o^{1/3} + 0.012 v_o$

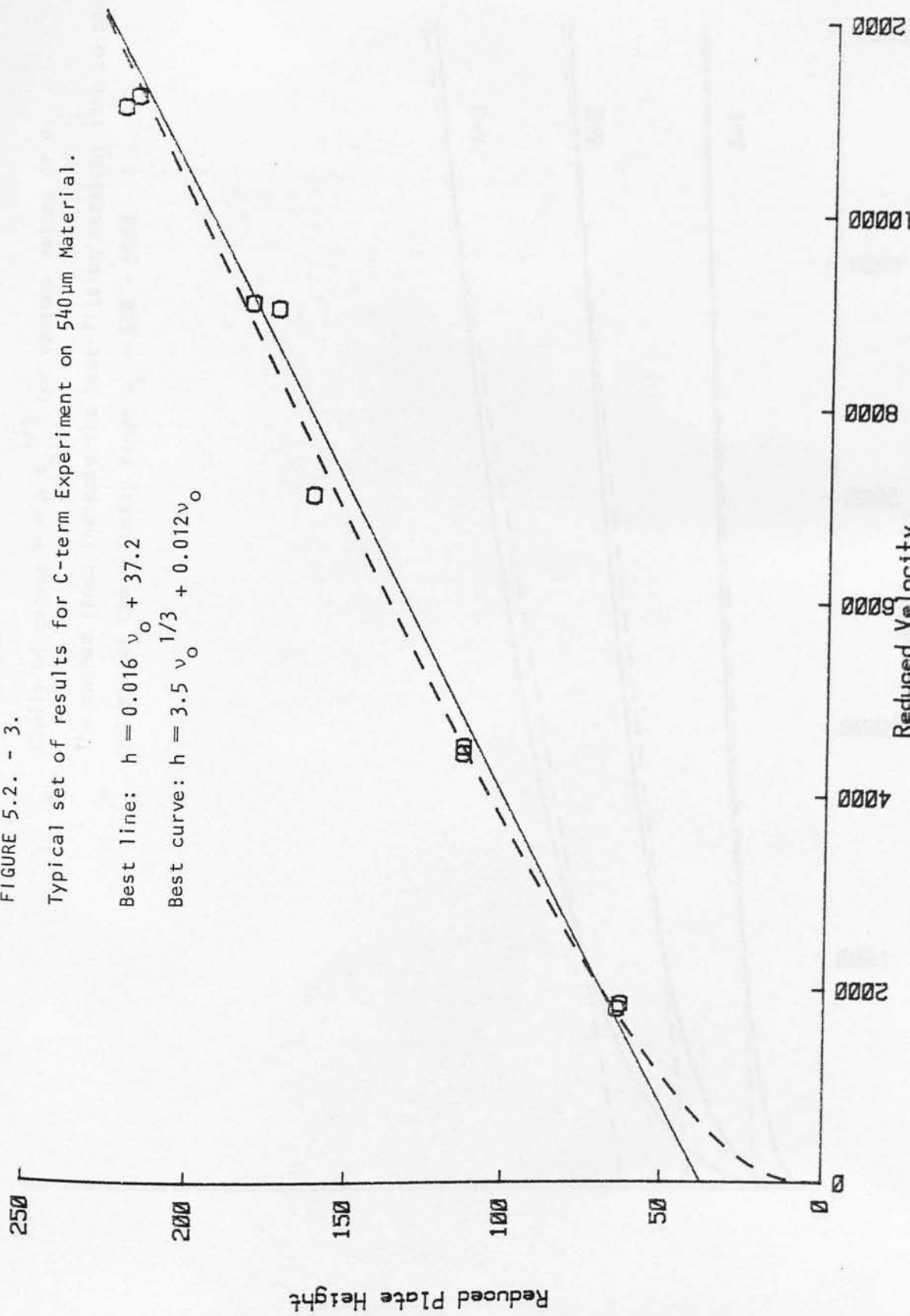


FIGURE 5.3. - 1.

Family of curves $h = A v_0^{1/3}$ for various values of A .

The dashed lines indicate the best-fitting straight line to these curves for the velocity range $v_0 = 500 - 5000$.

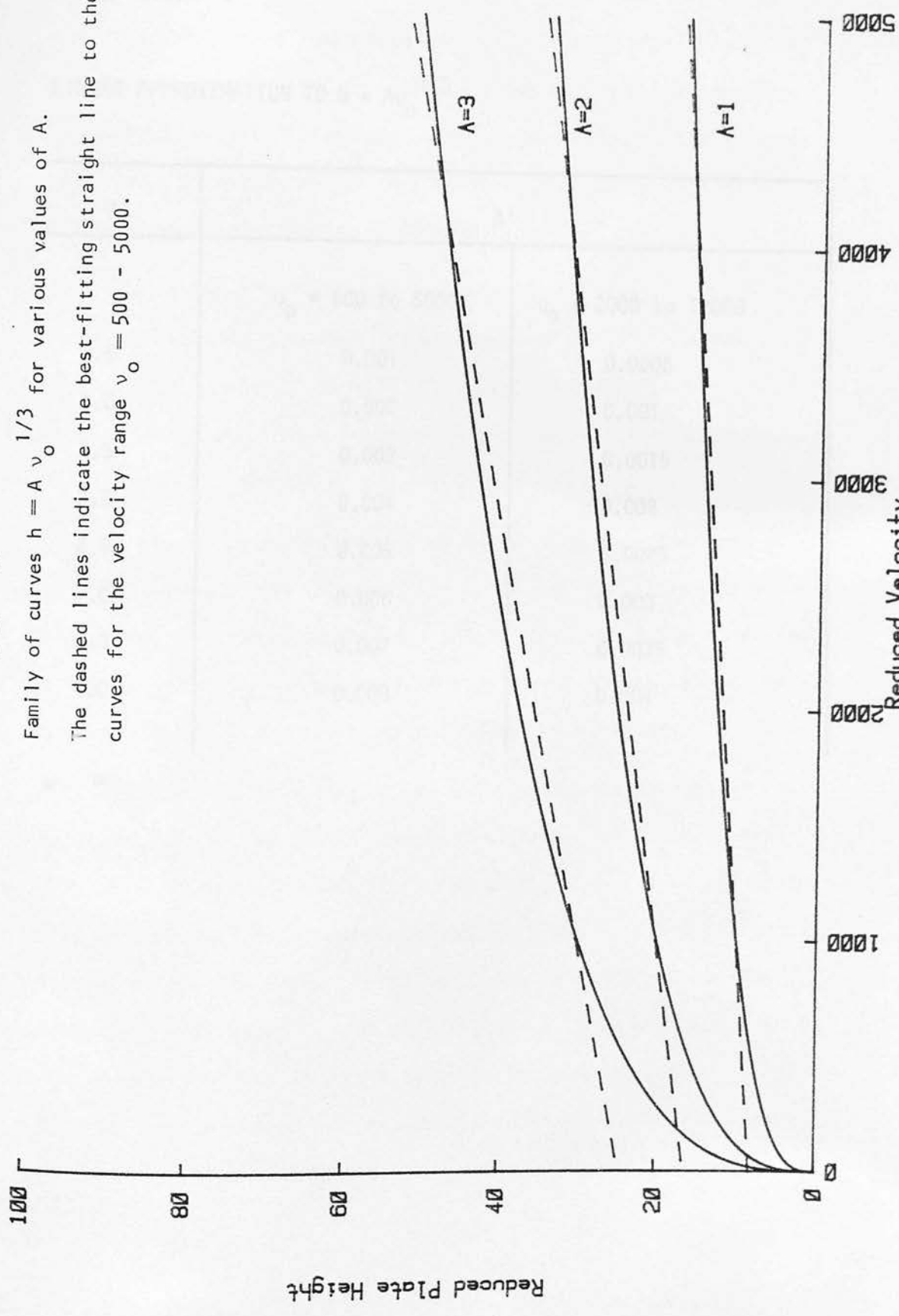


TABLE 5.3. - 1

LINEAR APPROXIMATION TO $h = Av_0^{1/3}$

A	A'	
	$v_0 = 500$ to 5000	$v_0 = 2000$ to 12000
0.5	0.001	0.0005
1.0	0.002	0.001
1.5	0.003	0.0015
2.0	0.004	0.002
2.5	0.005	0.0025
3.0	0.006	0.003
3.5	0.007	0.0035
4.0	0.008	0.004

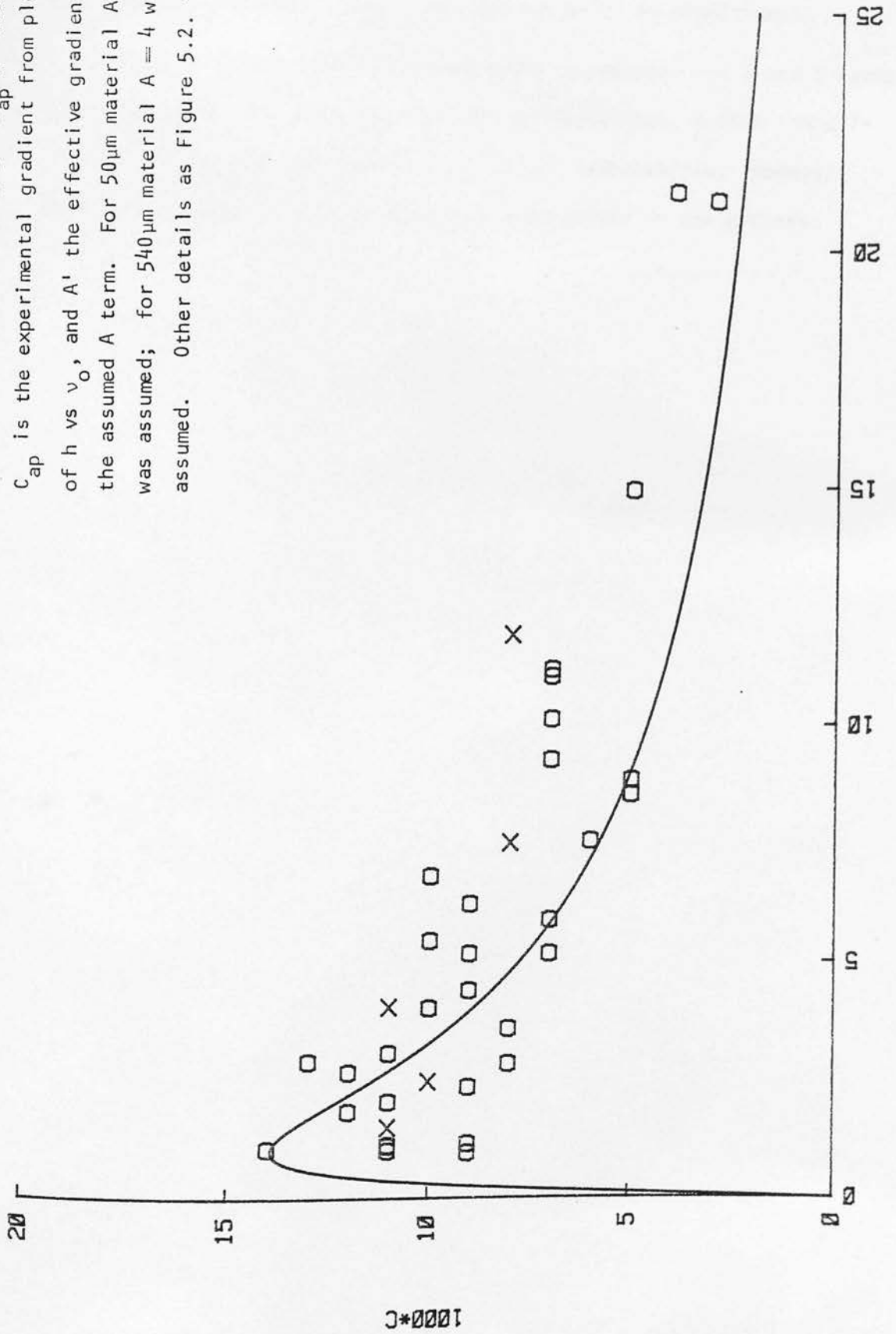
obtained. Experiments involving the measurement of h as a function of v_0 at the optimum velocity, or involving re-packing the column, perhaps by slightly different methods, would have thrown more light on this subject. It must also be borne in mind that this experiment was intended to give insight into the C-term, and that a number of assumptions are required to enable a value of the A-term to be calculated. These include the velocity dependence of the A-term, the linear approximation to this velocity dependence and the accuracy of the theoretical expression for the C-term.

Figure 5.3. - 2 shows the experimental results for the $50\mu\text{m}$ particles with an allowance for an A-term of 2. The theoretical variation of C with k'' is well borne out. The experimental uncertainty shows that a lower value of A may still give an acceptable fit, though not as good as the fit with $A = 2$.

For the $540\mu\text{m}$ particles, a much higher A-term is required as shown by the figures in the lower part of Table 5.3. - 1. This is because the values of v_0 used are higher, and so $v_0^{1/3}$ increases more slowly with v_0 . This column may well be very poorly packed, since there are only about 15 particles across the column diameter.

Apart from attributing all of the excess band broadening over equation 5.3. - 2 to mobile zone flow effects, there may be other dispersion mechanisms operating which would account for some of the band broadening. Such a mechanism may be found in the transfer of solute to and from regions of stagnant mobile phase outwith the particles. For example, some mobile phase will be held stagnant in the cusp shaped regions where neighbouring particles touch. These regions will be scaled to the particle diameter, and so will have the

FIGURE 5.3. - 2. Values of C obtained from $C = C_{ap} - A'$ where C_{ap} is the experimental gradient from plots of h vs v_0 , and A' the effective gradient of the assumed A term. For $50\mu\text{m}$ material $A = 2$ was assumed; for $540\mu\text{m}$ material $A = 4$ was assumed. Other details as Figure 5.2. - 2.



same effect on the h vs v curves for any particle size. Since band broadening by diffusion into and out of such pockets will depend on the depth of the stationary zone involved, the effect will be smaller than that of the beads themselves, but may still be significant.

Since it has proved to be impossible to separate the A and C-terms by using large particles and high reduced velocities, a firm identification of all the factors involved has not been possible. However, many useful results and insights have been gained in the process.

THEORETICAL MODELS OF SIZE-EXCLUSION CHROMATOGRAPHY

1. INTRODUCTION

The chromatography of a mixture is said to be "size-exclusion" if the components are separated on the basis of their size. The term "size-exclusion" is used to describe the separation of a mixture of components on the basis of their size. The term "size-exclusion" is used to describe the separation of a mixture of components on the basis of their size. The term "size-exclusion" is used to describe the separation of a mixture of components on the basis of their size.

CHAPTER 6

THEORETICAL MODELS OF SIZE-EXCLUSION
CHROMATOGRAPHY

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CHAPTER 6

THEORETICAL MODELS OF SIZE-EXCLUSION CHROMATOGRAPHY

6.1. INTRODUCTION

In chromatography, a solute is said to be 'excluded' if it travels down the column more quickly than the eluent. Such behaviour is possible because, whereas the eluent can fully penetrate all the pores of the support, the solute cannot, and so on average it moves more quickly than the eluent, since eluent in the pores does not move. There are several reasons why a solute may not be able to penetrate the pores completely. For example, if molecules of one component of the eluent bind more strongly to the stationary phase than the solute molecules do, the solute molecule will find it more difficult to reach the surface than will these eluent molecules, and so they will be partially excluded (18). A second possibility is that molecules may be hindered from entering the pores by a charge effect similar to the Donnan potential (66). However, only one exclusion mechanism has been exploited so far to provide a separation technique. This is the mechanism of exclusion from pores on the basis of size. The technique based on this phenomenon is known by a variety of names, such as Gel Permeation Chromatography (67) or Gel Filtration Chromatography (68). The most descriptive name, which is generally applicable to all variants of the technique, is Size Exclusion Chromatography (SEC) (16).

SEC as a technique was realised before the theory behind it was elucidated. Several theories were postulated to explain this separation of molecules on the basis of size. These included a theory based on differing diffusion rates of solutes of various

sizes (69), and one based on separation by flow, in which separation is the result of the flow profile in the channels of the packing (70, 71). The consensus opinion now is that such separations are adequately explained by steric exclusion (72). This mechanism will be described in detail in section 6.2., but the basis of it is that large molecules cannot fully permeate the pores of the support, whereas small molecules can.

Based on this theory, a simple equation relating retention volume to the volumes within the column as described in figure 1.4. - 1 may be derived. Molecules (such as eluent molecules) which can fully permeate the pores of the support material will be eluted from the column by a volume of eluent V_m . Molecules which are totally excluded from the pores will elute in a volume V_o equal to the volume of eluent outside the pores. The difference between these volumes is the pore volume V_p . Thus

$$V_m = V_o + V_p \quad 6.1. - 1$$

in agreement with figure 1.4. - 1.

In well conditioned SEC the solute does not interact with the surface of the packing material. In this case the retention volume for any molecule must lie somewhere between V_o and V_m . This fact may be represented by the equation

$$V_r = V_o + KV_p \quad 6.1. - 2$$

in which K is allowed to vary from 0 to 1. K is known as the exclusion coefficient.

The relationship of retention volume to molecular size (or molecular weight) is usually demonstrated by a calibration curve, in which the logarithm of the molecular weight is plotted as a function of V_r or K . A typical graph of this type is shown in figure 6.1. - 1. This data, which was taken from the paper by Knox and McLennan (73), is listed in Table 6.1. - 1. Such graphs enable molecular weights to be estimated from retention volumes. From a theoretical point of view, however, the retention volume is a function of the molecular size rather than vice versa. In the remainder of this chapter various models which predict the variation of K with molecular size are examined, and the predictions are compared with experimental results.

6.2. THE THEORY OF SIZE EXCLUSION CHROMATOGRAPHY

In SEC the elution volume of a solute depends on the size and shape of the solute molecules, and on the sizes and shapes of the pores in the column, as well as on the volume of eluent in the column. Although the separation mechanism is quite different from that of retentive chromatography, the mechanisms which lead to band broadening are the same as those dealt with in the earlier chapters of this thesis, namely axial diffusion, slow mass transfer and flow effects. Since the samples analysed are often artificial polymers, apparent band broadening due to the polydispersity of the polymer is almost always observed in addition (73).

The way in which the degree of exclusion will vary with molecular size is best illustrated by a simple example. The simplest type of molecule to consider is the 'hard sphere' molecule. One of the

FIGURE 6.1. - 1.

Exclusion data of Knox and McLennan (73) shown in terms of elution volume and exclusion coefficient. Mobile zone volume V_o and pore volume V_p are also indicated.

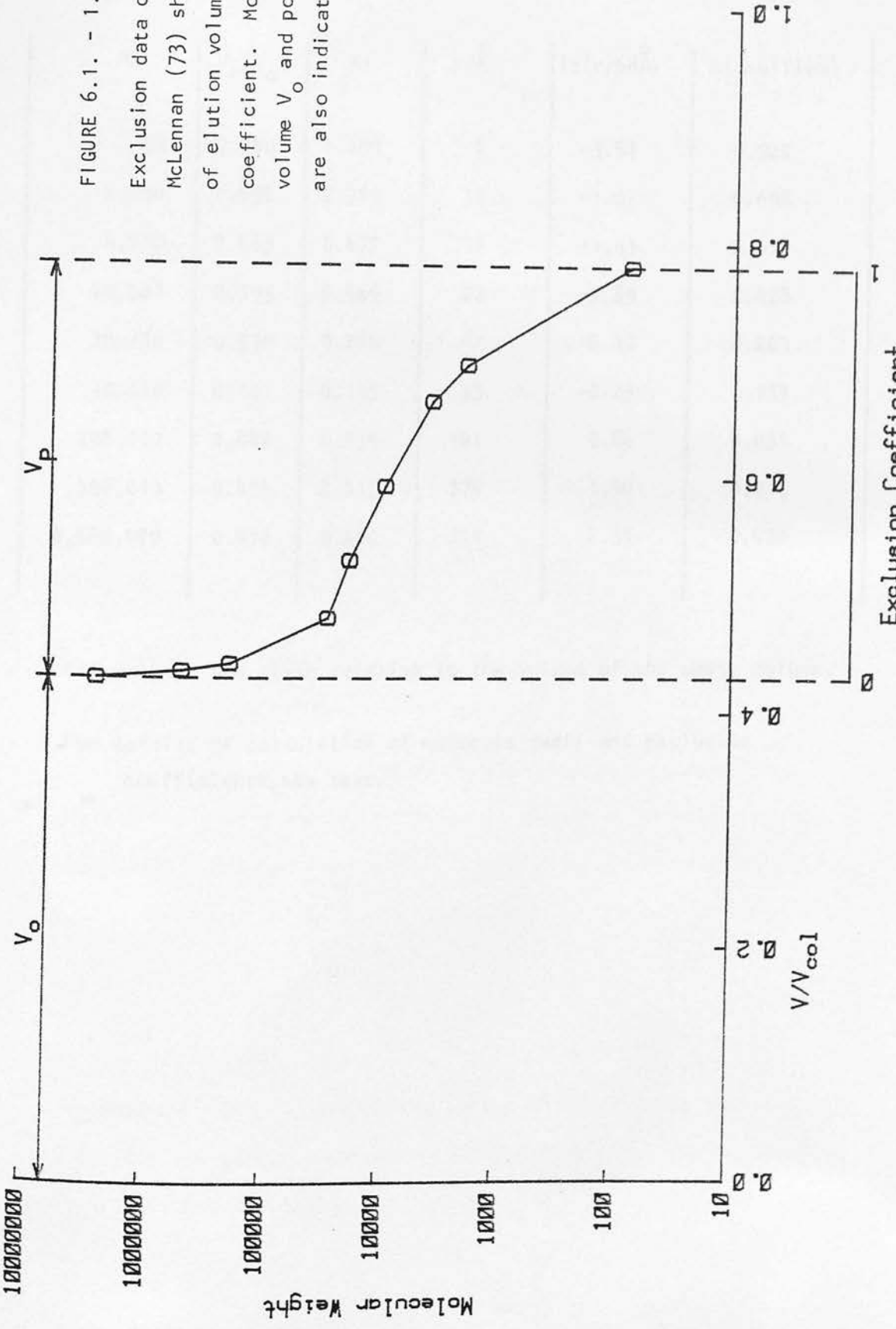


TABLE 6.1. - 1

EXPERIMENTAL EXCLUSION DATA OF KNOX AND McLENNAN (73)

MW	V_r/V_c	K	$r/\text{Å}$	$\ln(r/68\text{Å})$	K(modified)
78	0.780	1.000	2	-3.51	0.920
2,000	0.696	0.759	11	-1.83	0.698
4,000	0.665	0.672	16	-1.43	0.618
10,000	0.593	0.465	28	-0.89	0.428
20,000	0.530	0.286	42	-0.48	0.263
30,000	0.481	0.145	53	-0.25	0.133
200,000	0.442	0.034	161	0.86	0.031
500,000	0.436	0.017	276	1.40	0.016
2,500,000	0.430	0.000	711	2.35	0.000

All volumes are given relative to the volume of the empty column.

For details of calculation of molecule radii and exclusion coefficients, see text.

simplest model pores is the 'infinite cylinder'. This has a uniform circular cross section, and if of sufficient length that end effects may be neglected. This system is the three-dimensional analogue of a circular molecule in a circular pore in two dimensions, since the length element may be cancelled from all equations. This two-dimensional analogue is shown in figure 6.2. - 1. The radius of the pore is denoted by R , and that of the molecule by r .

The centre of mass of the molecule cannot come closer to the wall of the pore than a distance r . If the radius of the molecule is greater than the radius of the pore, the molecule is totally excluded from the pore. The exclusion coefficient K is defined as the function of the pore volume available to the molecule. Thus

$$K = \frac{\pi(R-r)^2}{\pi R^2} \quad r < R \quad 6.2. - 1a$$

$$K = 0 \quad r > R \quad 6.2. - 1b$$

These equations illustrate typical variation of exclusion coefficient with molecular radius for a given pore structure.

The above example is a special case of a general equation for spherical molecules which is assumed to give K as the ratio of the volume of pores accessible to the centre of mass of the molecules (V_a) to the total volume of the pores (V_p):

$$K = \frac{V_a}{V_p} \quad 6.2. - 2$$

This approach ignores problems of access to pores. For non-spherical molecules such a simple approach is not sufficient; for example the closest distance of approach to the pore wall by a rod-shaped

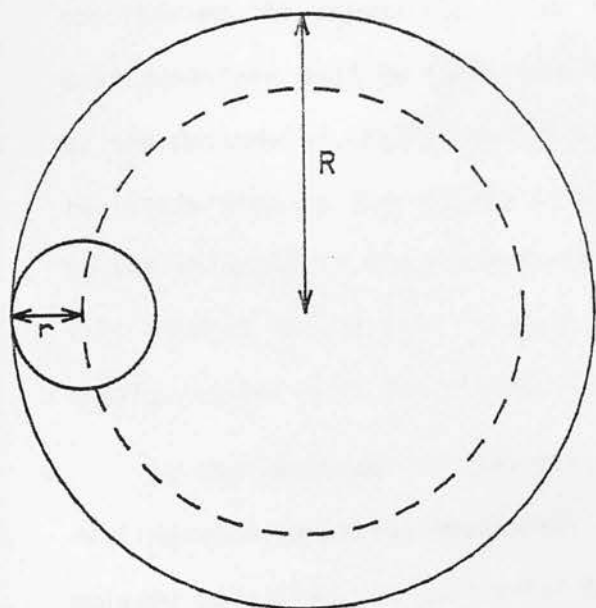


FIGURE 6.2. - 1. Exclusion of a spherical molecule from a cylindrical pore.

The centre of the molecule is excluded from the annular region between the dashed and solid lines.

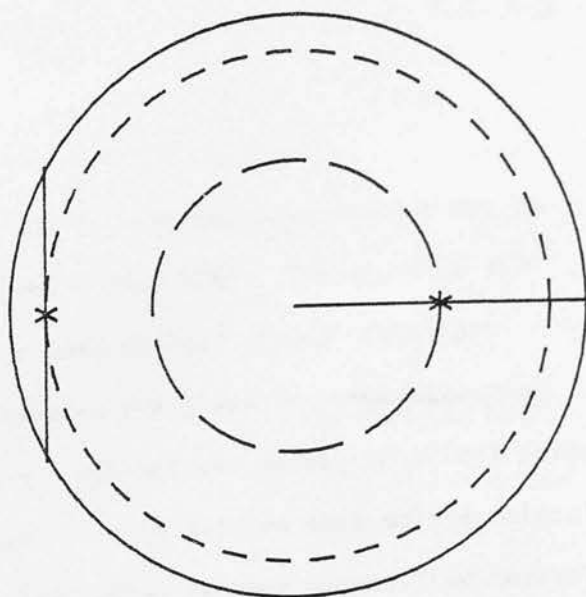


FIGURE 6.2. - 2. Exclusion of a rod-shaped molecule from a cylindrical pore.

The area from which the centre of the molecule is excluded depends on the orientation of the molecule.

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molecule depends not only on the length of the molecule but also on its orientation. This is illustrated in figure 6.2. - 2. In considering the accessible volume all possible orientations and configurations must be taken into account. This may be achieved by the methods of statistical mechanics (74). V_a and V_p may be re-interpreted as the volumes of configuration space available to the molecule in the presence and in the absence of the pore walls. This amounts to the same thing as counting the total number of configurations with and without the walls.

In the remainder of this chapter it will be assumed that we are dealing with spherical molecules. This assumption is made since many polymer molecules, in particular polystyrenes, are random chain polymers (75) which may be approximated as spheres. The effective radius of these spheres may be calculated from the radius of gyration r_g of the molecules by the equation

$$r = 0.886 r_g \qquad 6.2. - 3$$

(see reference (76)).

For spherical molecules theoretical exclusion curves may be calculated for a variety of pore shapes (74). The equation for infinite cylindrical pores has been derived above. Equations for various other simple geometries are given in *reference (74)*. The pore shapes considered are: cylindrical pores, spherical pores and inverse cylindrical pores. It is assumed that solute molecules can enter and leave the spherical pores by some unspecified mechanism. The 'inverse cylinder' pores consist of the space between touching cylinders. A diagram of such a pore is shown in figure 6.2. - 3.

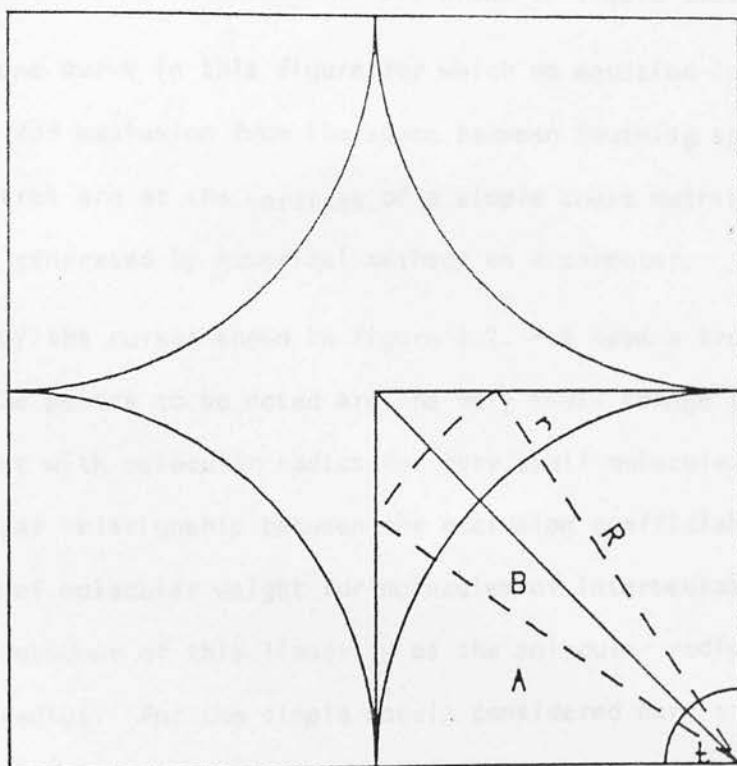


FIGURE 6.2. - 3.
INVERSE CYLINDRICAL PORE.

The pore consists of the space between four touching cylinders of radius R , as shown. By symmetry only the triangle bounded by solid lines in the bottom right-hand corner need be considered. The area of this triangle is $\frac{1}{2}R^2$.

The void area of this part of the pore is the area of the triangle outwith the solid circle. This is equal to $(\frac{1}{2} - \frac{\pi}{8})R^2$.

A molecule of radius r is excluded from all but the small area outside the dashed circle to the centre of the pore. This area is the area of the triangle less the areas of triangle A and sector B.

The area of triangle A is $\frac{1}{2} R^2 \tan \theta$, where θ is given by $\cos \theta = R/R+r$.

The area of sector B is given by $\frac{(\frac{\pi}{4} - \theta)(R+r)^2}{2}$

Thus the free area is $\frac{R^2}{2} - \frac{R^2}{2} \tan \theta - \frac{(\frac{\pi}{4} - \theta)(R+r)^2}{2}$

In combination with the void area, K is given by

$$K = \frac{1 - \tan \theta - (\frac{R+r}{R})^2 (\frac{\pi}{4} - \theta)}{1 - \frac{\pi}{4}} \quad \text{or } K = \frac{1 - \tan \theta - (\frac{1}{\cos \theta})^2 (\frac{\pi}{4} - \theta)}{1 - \frac{\pi}{4}}$$

Exclusion curves for these models are shown in figure 6.2. - 4.

The one curve in this figure for which no equation is given is the curve for exclusion from the space between touching spheres whose centres are at the vertices of a simple cubic matrix. This curve was generated by numerical methods on a computer.

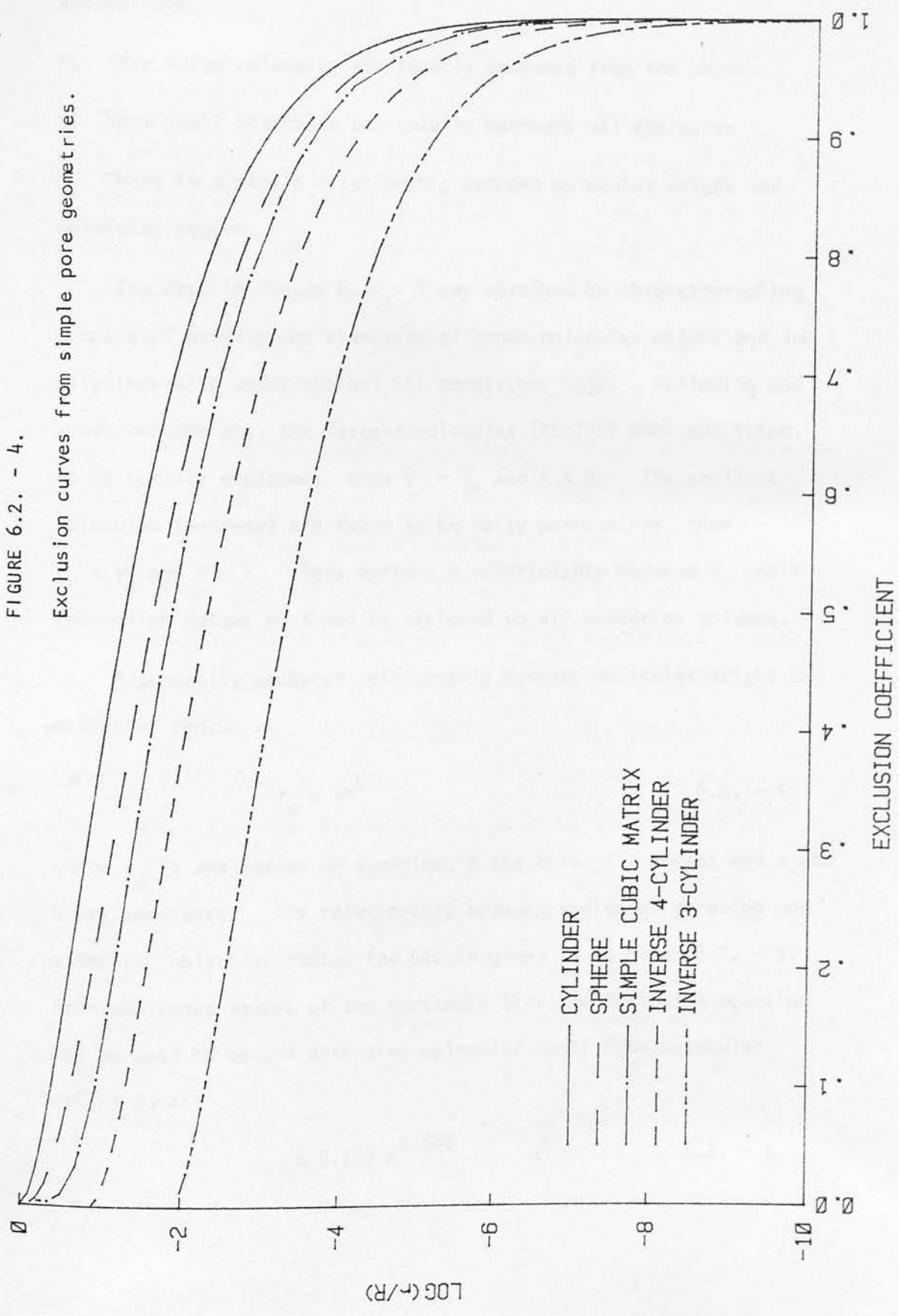
All of the curves shown in figure 6.2. - 4 have a broadly similar shape. The points to be noted are the very small change in exclusion coefficient with molecular radius for very small molecules, the quasi-linear relationship between the exclusion coefficient and the logarithm of molecular weight for molecules of intermediate size, and the breakdown of this linearity as the molecular radius approaches the pore radius. For the simple models considered here this effect is not particularly marked, but may be observed on close inspection of the figure as K tends to zero. For experimental curves and for models which have a distribution of pore sizes this breakdown of linearity is much more pronounced.

The curves for the inverse cylinders and the simple cubic matrix cut the vertical axis below the origin. For these models total exclusion occurs at a radius smaller than that of the matrix unit. For example, figure 6.2. - 3 shows that for the inverse 4-cylinder total exclusion occurs at a radius of $(\sqrt{2}-1)R$. If these curves were plotted such that (r/R) is the ratio of the molecular radius to the maximum molecular radius which can enter the pore, then the two inverse cylinder curves would be practically superimposable, and would lie between the curves for the cylinder and sphere models.

Experimental data such as that shown in figure 6.1. - 1 is obtained as a set of values of retention volume and molecular weight. For comparison with theoretical models such as those described above

FIGURE 6.2. - 4.

Exclusion curves from simple pore geometries.



or to be developed in the remainder of this chapter it is necessary to convert this data into values of exclusion coefficient and molecular radius. This may be done by making the following assumptions:

- 1) Very large molecules are totally excluded from the pores.
- 2) Very small molecules can totally permeate all the pores.
- 3) There is a simple relationship between molecular weight and molecular radius.

The data in figure 6.1. - 1 was obtained by chromatographing samples of polystyrene standards of known molecular weight and low polydispersity under typical SEC conditions (73). Following the above assumptions, the largest molecules (PS 2700 000) are taken to be totally excluded; thus $V_r = V_o$ and $K = 0$. The smallest molecules (benzene) are taken to be fully permeating; thus $V_r = V_m$ and $K = 1$. This defines a relationship between V_r and K from which values of K may be assigned to all retention volumes.

A generally accepted relationship between molecular weight and molecular radius is

$$r_g = aM^b \quad 6.2. - 4$$

where r_g is the radius of gyration, M the molecular weight and a and b are constants. The relationship between radius of gyration and effective molecular radius for SEC is given in equation 6.2. - 3. From published values of the constants (77) the following equation may be used to obtain effective molecular radii from molecular weight data:-

$$r_g = 0.123 M^{0.588} \quad 6.2. - 5$$

The experimental data of Knox and McLennan have been processed in this way, and the results are also presented in Table 6.1. - 1. This data with the curve for exclusion from an infinite cylinder is shown in figure 6.2. - 5.

6.3. COMPUTER MODELLING

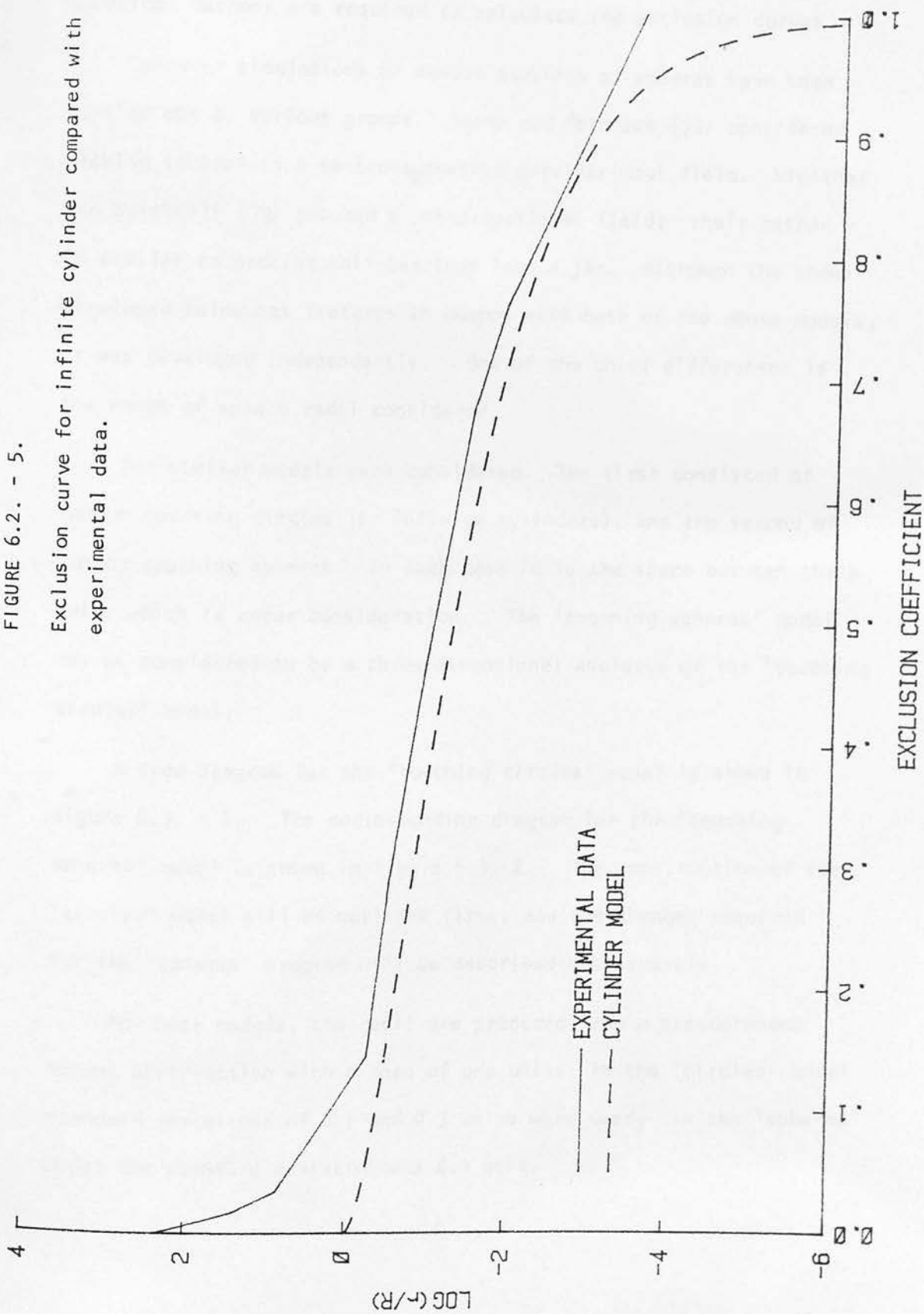
The pore models described above are all of regular geometry, and each model consists of pores of a single uniform size. They cannot therefore be very accurate models of real packing materials for SEC. While the nanometre -sized units which make up the particles of the packing material may be spherical, neither they nor the pores which they define will be of a uniform size, nor will they be positioned in a regular array.

One model which overcomes some of these shortcomings is the random sphere model (77) which was used as a model for SEC by van Krefeld and van den Hoed (76). This model consists of spheres of uniform radius positioned at random points throughout the matrix. The spheres are allowed to overlap if necessary. The void volume in such a model may be calculated from simple mathematical equations derived from the statistics of the model. The exclusion of molecules from the matrix is modelled by increasing the radius of the spheres by the molecular radius. The void volume remaining corresponds to the accessible volume for molecules of that radius. In this way a calibration curve may be calculated. Very good agreement with experiment was found in this study.

The model developed in the current work is somewhat different. In keeping with answering the criticisms of the uniform pore models, the sizes of the units are random. A further change from the random sphere model is that the units are not allowed to overlap. This may effect the

FIGURE 6.2. - 5.

Exclusion curve for infinite cylinder compared with experimental data.



exclusion from small pores, since the cusp-like regions where the spheres touch will be sharper than in the random sphere model. Because of these changes statistical methods cannot be used, and numerical methods are required to calculate the exclusion curves.

Computer simulations of random packings of spheres have been carried out by various groups. Adams and Matheson (78) considered packing spheres in a centro-symmetric gravitational field. Visscher and Bolsterli (79) assumed a uni-directional field; their method is similar to packing ball-bearings into a jar. Although the model developed below has features in common with both of the above models, it was developed independently. One of the chief differences is the range of sphere radii considered.

Two similar models were considered. The first consisted of random touching circles (or infinite cylinders), and the second of random touching spheres. In each case it is the space between these units which is under consideration. The 'touching spheres' model may be considered to be a three-dimensional analogue of the 'touching circles' model.

A flow diagram for the 'touching circles' model is shown in figure 6.3. - 1. The corresponding diagram for the 'touching spheres' model is shown in figure 6.3.-2. The construction of the 'circles' model will be outlined first, and the changes required for the 'spheres' program will be described subsequently.

For both models, the radii are produced from a pseudorandom normal distribution with a mean of one unit. In the 'circles' model standard deviations of 0.1 and 0.3 units were used; in the 'spheres' model the standard deviation was 0.1 unit.

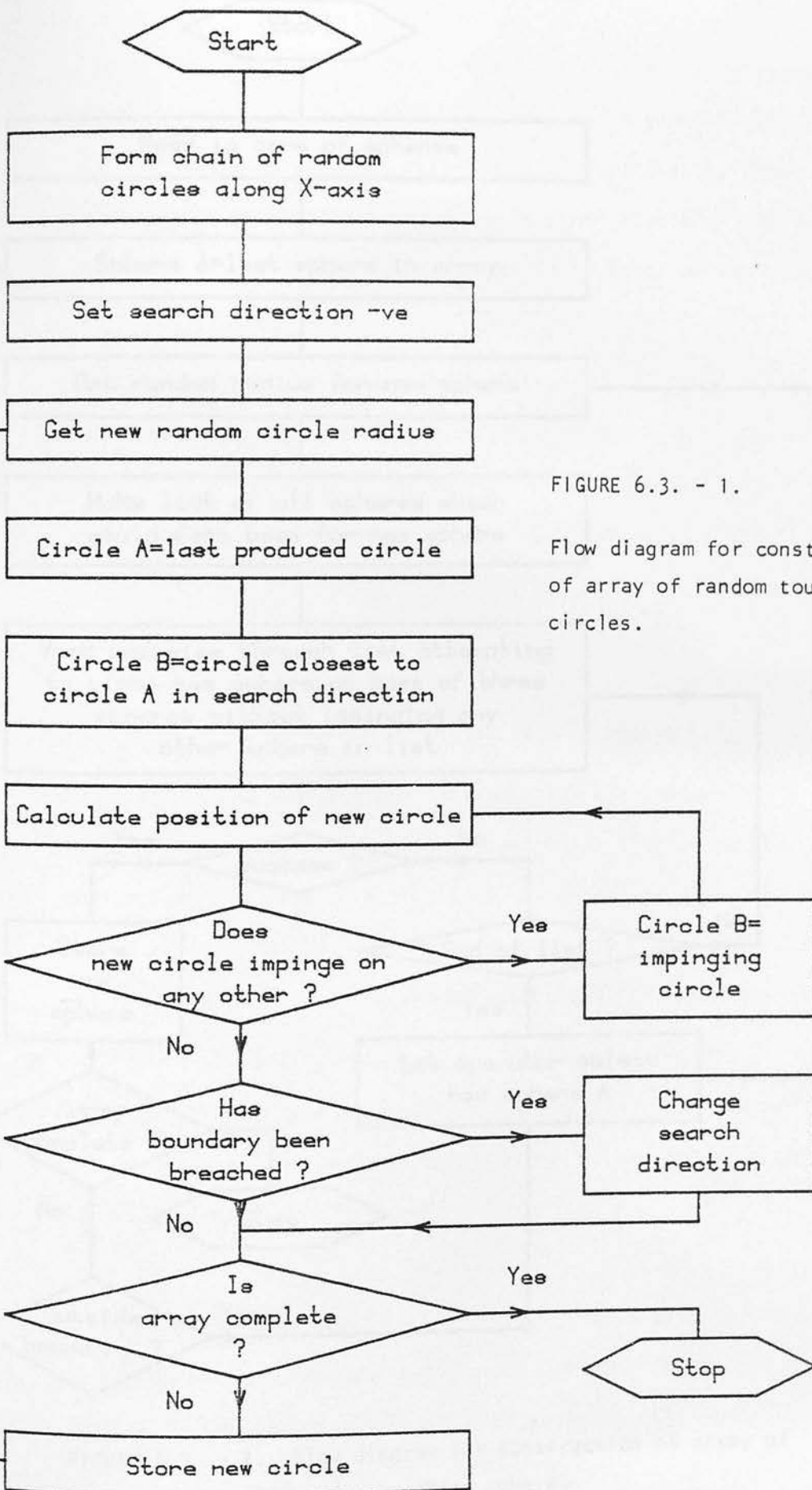


FIGURE 6.3. - 1.

Flow diagram for construction of array of random touching circles.

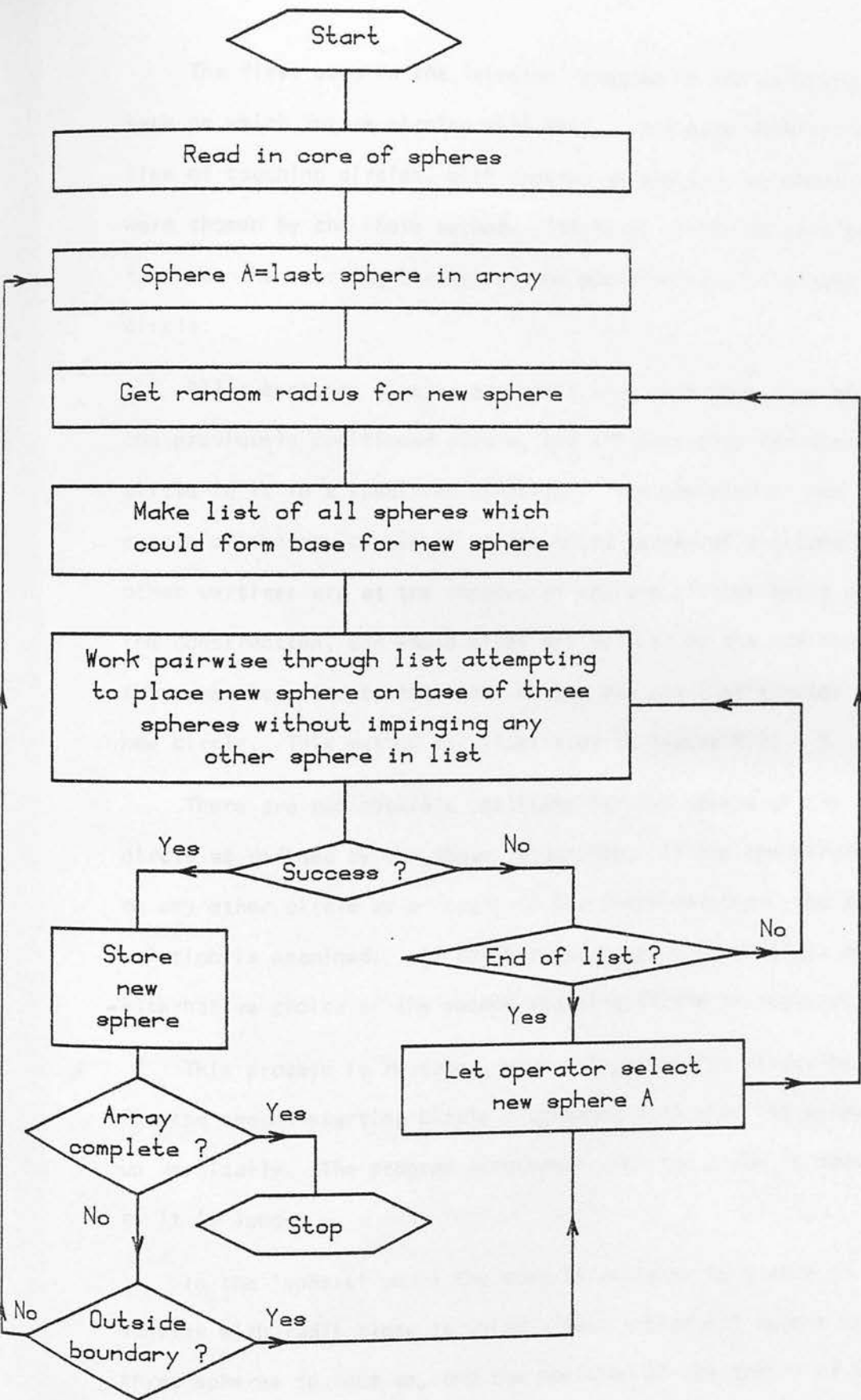


FIGURE 6.3. - 2. Flow diagram for construction of array of random touching spheres.

The first step in the 'circles' program is the defining of a base on which future circles will rest. This base consists of a line of touching circles, with centres on the x-axis, whose radii were chosen by the above method. The final circle on this base is taken as the starting circle for the positioning of the next circle.

All subsequent circles are positioned such that they touch the previously positioned circle, and (if possible) the nearest circle to it in a specified direction. The position of the centre of the new circle is at the third vertex of a triangle whose other vertices are at the centres of the two circles being used in the construction, and whose sides are defined by the positions of these vertices and by the radii of the two starting circles and the new circle. This method is illustrated in figure 6.3. - 3.

There are two possible positions for the centre of the new circle as defined by the above algorithm. If the new circle impinges on any other circle as a result of the first solution, the alternative solution is examined. If neither solution is successful, an alternative choice of the second starting circle is required.

This process is repeated, with changes in the direction of search for the second starting circle programmed such that the array is built up vertically. The program terminates when the array is about as tall as it is long.

In the 'sphere' model the base is replaced by a core of five spheres with radii close to unity. Each subsequent sphere requires three spheres to rest on, and the position of the centre of the new sphere is the fourth vertex of a tetrahedron. The other three

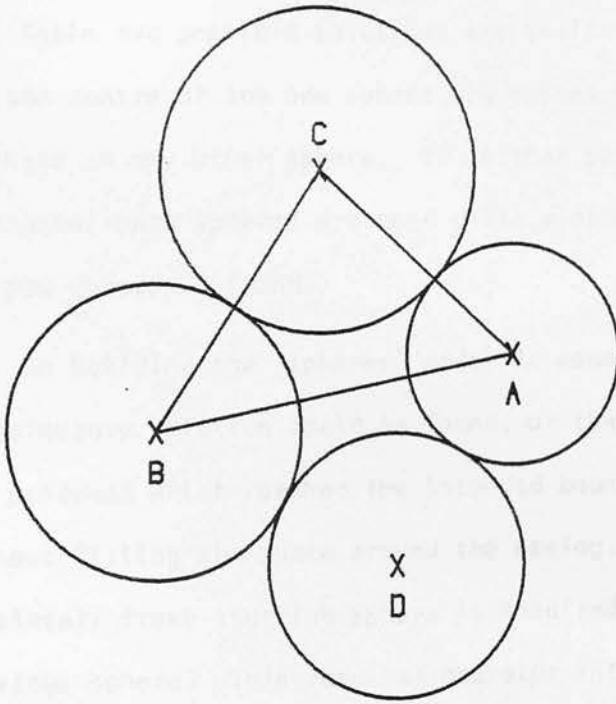


FIGURE 6.3. - 3.

Construction method for array of random touching circles.

The position for the centre of circle C is found by solving triangle ABC, where AB is the distance between the centres of circles A and B, and AC and BC are the sums of the radii of the respective circles.

Circle D is not used, since a circle based on A and D would impinge on circle B. The alternative solution of the triangle would result in circle C impinging on circle D.

vertices are the three spheres on which the new sphere rests, and the remaining edges have lengths defined by the sum of the new sphere radius and the radii of these 'base' spheres. Details of the solution of the tetrahedron are shown in figures 6.3. - 4.

Again two possible solutions are available, and both positions for the centre of the new sphere are tested to see if this sphere impinges on any other sphere. If neither position is satisfactory then other base spheres are used until a permissible position for the new sphere is found.

In building the 'spheres' model it sometimes happened that no satisfactory solution could be found, or that a 'string' of spheres was produced which reached the intended boundary of the array without filling the space around the string. In these cases a completely fresh starting sphere is required, rather than using the previous sphere. This required operator intervention, and may have taken away from the truly random nature of the array. However, the randomness should not be significantly corrupted by this process, and the array should still be a good model for the purposes for which it was constructed.

The completed arrays of circles contained about 100 circles. The central position only was used for the calculation of exclusion curves, in order to avoid edge effects and the effect of the base line of circles. The portions of the arrays used for further calculations are shown in figures 6.3. - 5 (s.d. = 0.1) and 6.3. - 6 (s.d. = 0.3). The porosities of these arrays are 18.7% and 21.1% respectively.

The completed array of spheres contained about 1800 spheres, from which the central portion of about 1600 spheres was used for exclusion coefficient calculations in order to avoid the edges of the

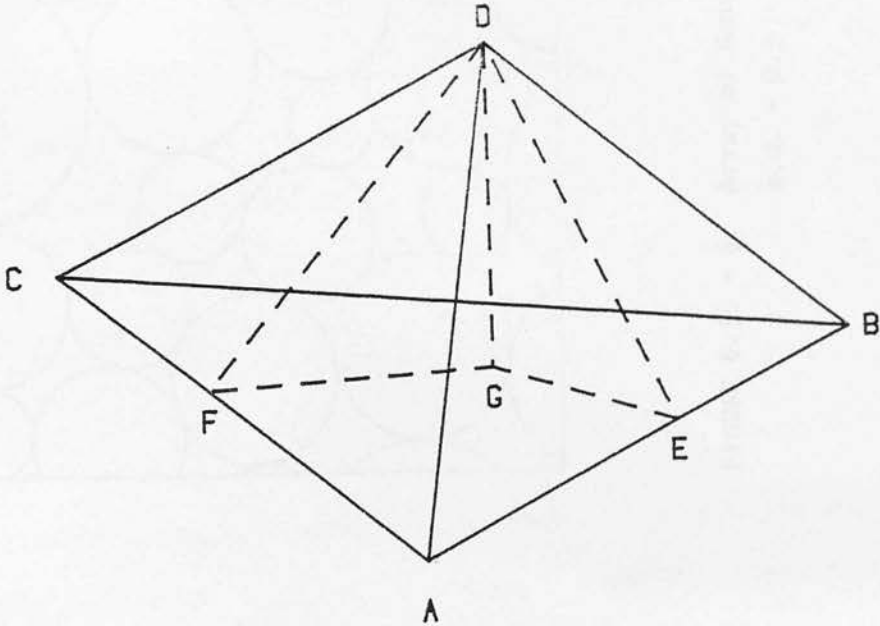


FIGURE 6.3. - 4.

CONSTRUCTION METHOD FOR ARRAY OF RANDOM TOUCHING SPHERES.

Let A, B and C be the centres of the three base spheres, and D the centre of the new sphere. The lengths of all line segments are known either as distances between sphere centres or sums of sphere radii.

For sake of simplicity let A, B and C lie in the x-y plane, with A and B on the x axis. This may be achieved by a suitable transformation of coordinates.

The x-coordinate of D is given by point E, which is the point where a line from D cuts the line AB at right angles.

Point F is obtained by a similar line which cuts AC at right angle. Point G is the intersection of lines through E and F at right angles to AB and AC respectively in the x-y plane. The y-coordinate of D is given by the length of EG.

Finally the z-coordinate of D may be found by solving either of the right-angled triangles DEG or DFG to find the length GD.

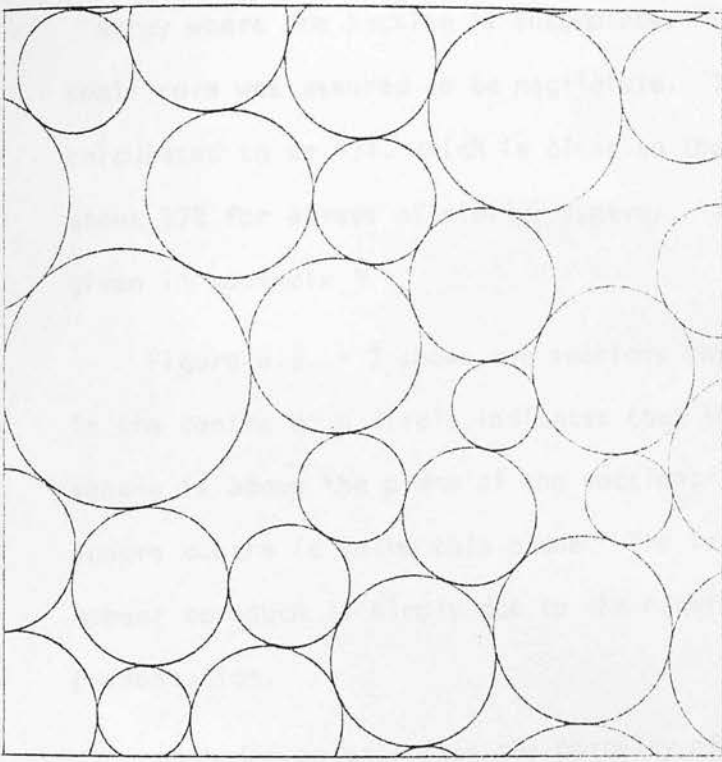


FIGURE 6.3. - 6. Array of Random Circles.
s.d. = 0.3

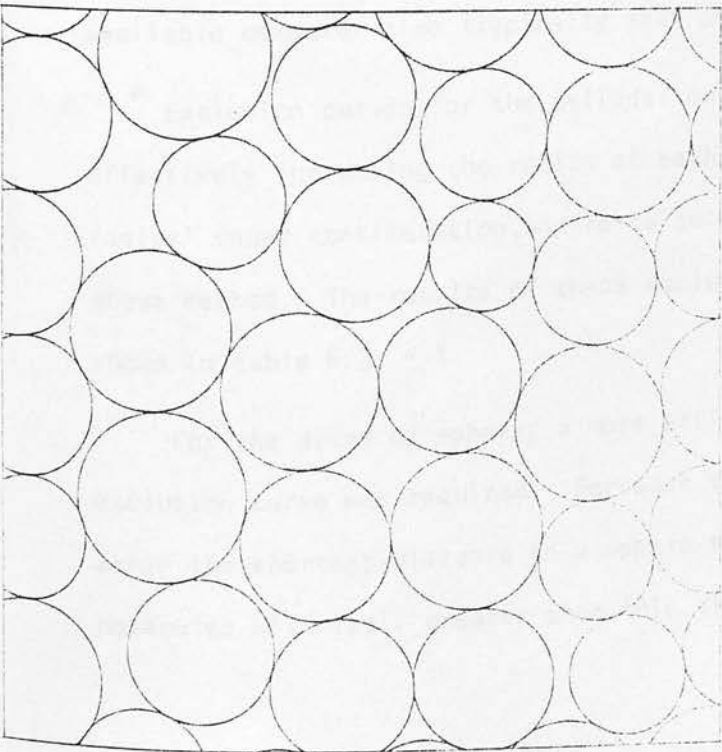


FIGURE 6.3. - 5. Array of Random Circles.
s.d. = 0.1

array where the packing is incomplete. The effect of the initial small core was assumed to be negligible. The porosity of the array was calculated to be 43%, which is close to the experimental value of about 37% for arrays of similar spheres. The data for this array is given in appendix 3.

Figure 6.3. - 7 shows two sections through the array. A '+' in the centre of a circle indicates that the centre of the corresponding sphere is above the plane of the section; A '-' indicates that the sphere centre is below this plane. The fact that the circles do not appear to touch is simply due to the nature of the sectional presentation.

In order to calculate the porosity of these arrays, or to calculate the relevant exclusion curves, it is necessary to know the void volume, i.e. the volume not occupied by circles or spheres. In each case the array was examined point by point, the fractional void volume being the ratio of the number of test points outside the circles or spheres to the total number of test points. The distance between neighbouring test points was as small as practicable within the constraints of available computer time (typically 1000 points per unit).

Exclusion curves for the cylinder models were calculated by effectively increasing the radius of each circle by the 'molecular radius' under consideration, and re-calculating the void volume by the above method. The results of these exclusion curve calculations are shown in table 6.3. - 1.

For the array of spheres a more efficient method for obtaining the exclusion curve was required. For each test point in free space in the array the shortest distance to a sphere surface was calculated. Molecules with radii greater than this distance are 'excluded' from

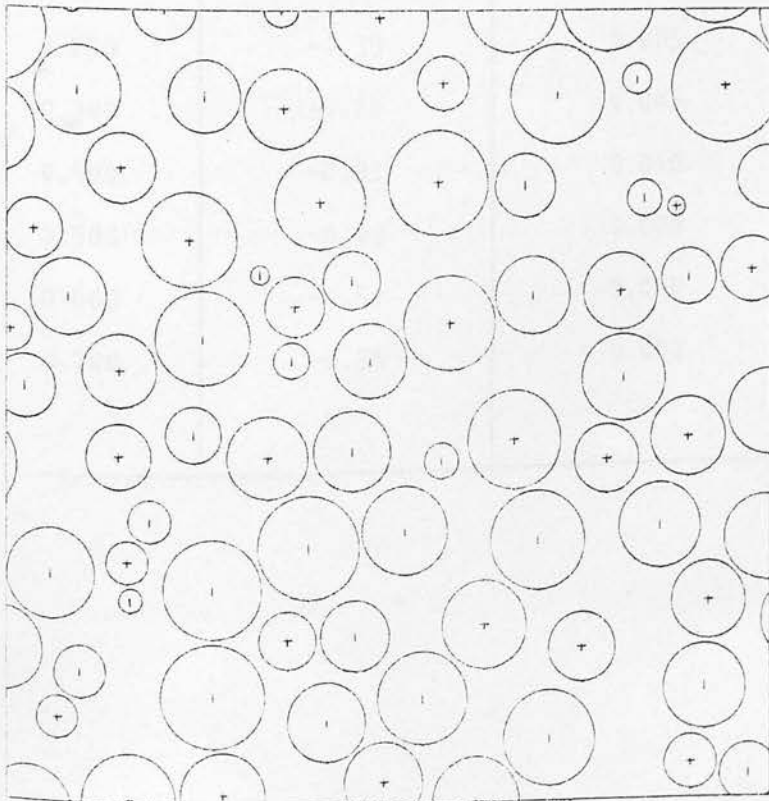
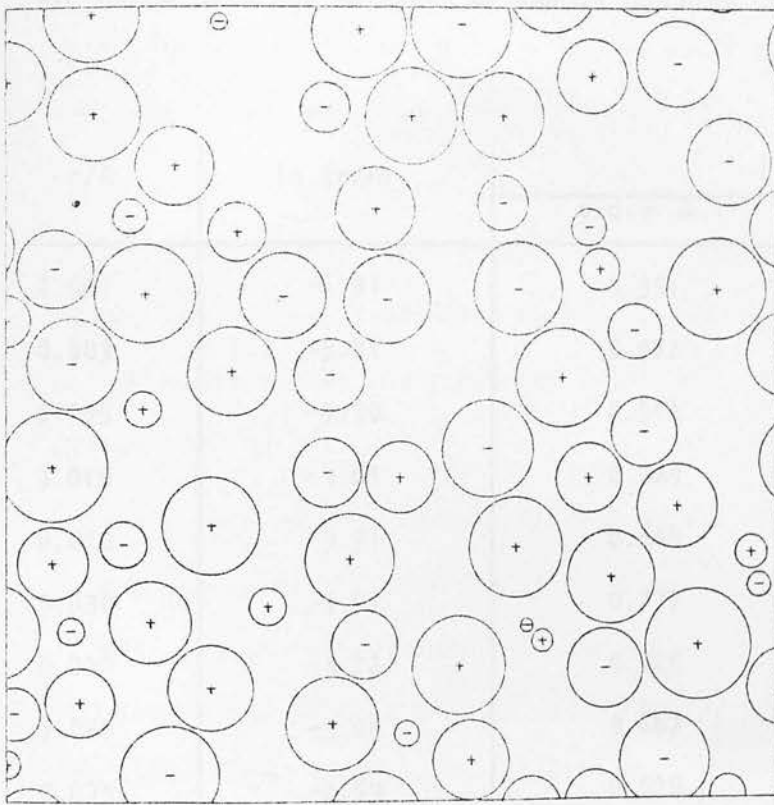


FIGURE 6.3. - 7. Sections through array of Random Spheres.

TABLE 6.3. - 1

EXCLUSION DATA FOR MATRICES OF RANDOM TOUCHING CIRCLES

r/R	$\ln (r/R)$	K	
		s.d. = 0.1	s.d. = 0.3
0.001	-6.91	0.991	0.995
0.003	-5.81	0.972	0.982
0.005	-5.30	0.954	0.968
0.010	-4.61	0.924	0.948
0.020	-3.91	0.846	0.896
0.030	-3.51	0.777	0.839
0.040	-3.22	0.725	0.791
0.050	-3.00	0.667	0.743
0.075	-2.59	0.529	0.634
0.100	-2.30	0.423	0.542
0.150	-1.90	0.264	0.400
0.200	-1.61	0.152	0.285
0.250	-1.39	0.085	0.207
0.300	-1.20	0.045	0.139
0.400	-0.92	0.010	0.058
0.500	-0.69	0.000	0.018
0.600	-0.51	0.000	0.002
0.700	-0.36	0.000	0.000

this point; molecules of smaller radius are assumed to be able to reach the point. In this way each test point need only be considered once, yet a full exclusion curve can be built up. The results of this calculation are shown in table 6.3. - 2.

The three calculated exclusion curves are shown in figure 6.3. - 8. The experimental curve is included in this figure for comparison. These calculated curves have the same general shape as the experimental curve and the curves calculated from simple geometrical pores.

The experimental curve differs from all of the model curves as K tends to unity, in that the model curves all turn sharply downwards in this region, whereas the experimental curve is relatively flat. The reason for this is that in the models values of K can be calculated for 'molecules' whose radius is practically zero. Because of this the exclusion curves approach total permeation asymptotically. For the experimental data benzene was somewhat arbitrarily assigned an exclusion coefficient of unity, since it was assumed to be totally permeating. However, any molecule with a finite radius must be slightly excluded from any pore.

For this reason the experimental values of K were re-calculated to give a reasonable exclusion coefficient for benzene. The effective radius of the benzene molecule was calculated from the density of liquid benzene, and from this and the touching sphere model a value of K for benzene of 0.92 was calculated; the other values of K were scaled accordingly. Figure 6.3. - 9 demonstrates the improvement in the match between theoretical and experimental curves which this change yields. For the best results, such a scaling should be

FIGURE 6.3. - 8. Exclusion curves from Array of Random Touching Cylinders and Spheres.

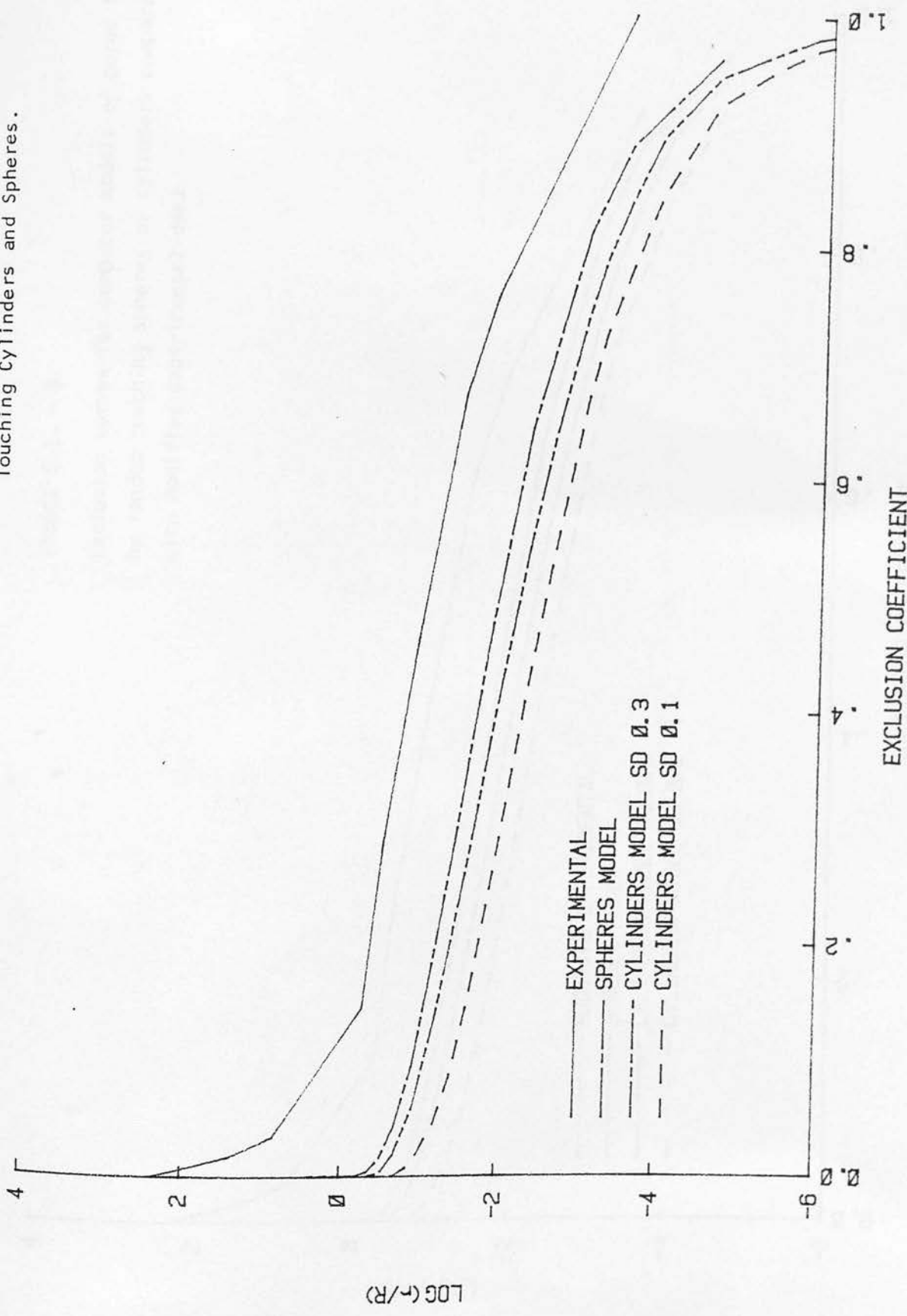


FIGURE 6.3. - 9.

Exclusion curves for computer models of pores formed by random touching spheres or cylinders compared with modified experimental data.

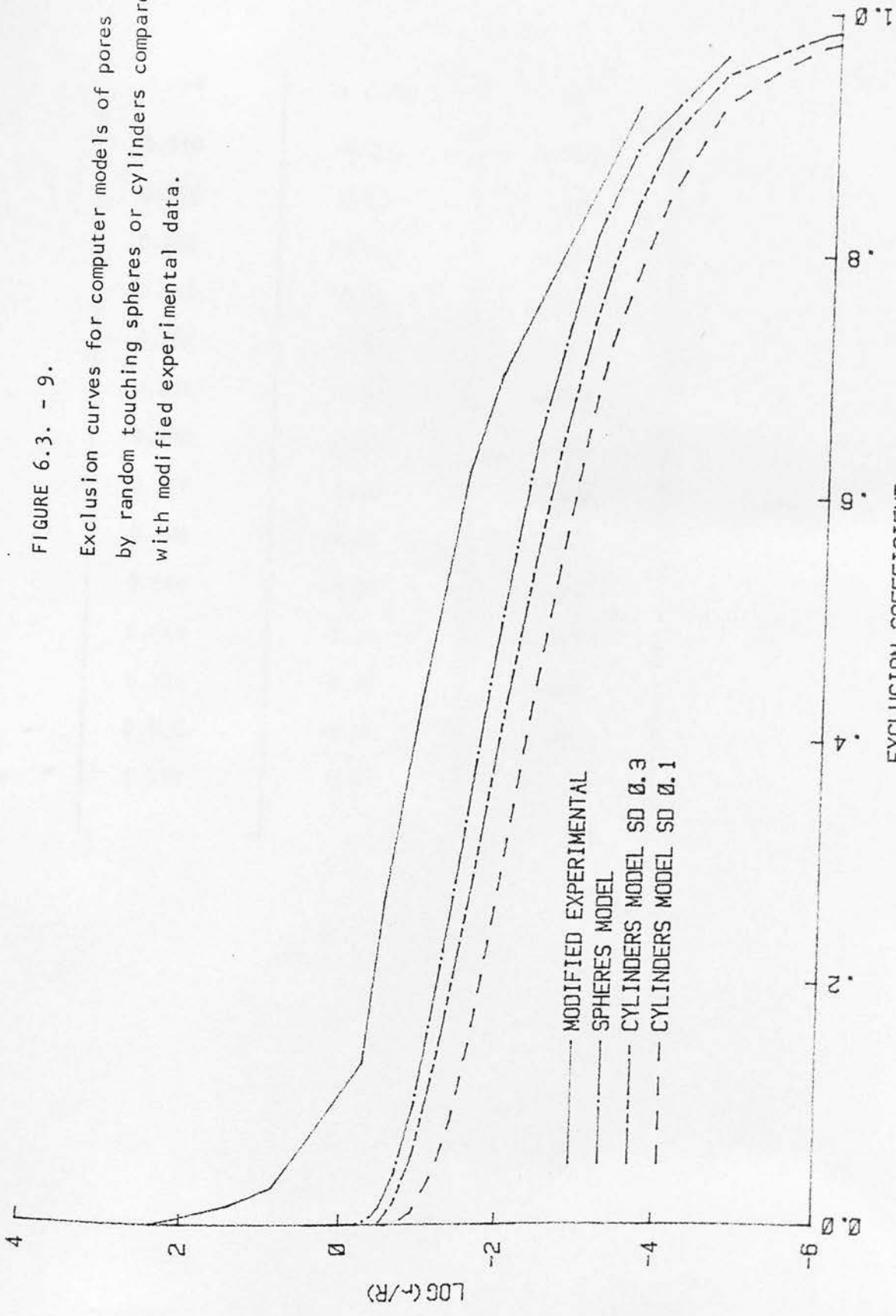


TABLE 6.3. - 2

EXCLUSION DATA FOR MATRIX OF RANDOM TOUCHING SPHERES

r/R	$\ln (r/R)$	K
0.010	-4.60	0.963
0.030	-3.51	0.888
0.050	-3.00	0.814
0.100	-2.30	0.644
0.160	-1.83	0.472
0.200	-1.61	0.378
0.240	-1.43	0.299
0.300	-1.20	0.205
0.400	-0.92	0.098
0.500	-0.69	0.042
0.600	-0.51	0.015
0.700	-0.36	0.004
0.800	-0.22	0.0005
1.500	0.40	0

performed on each model being tested to obtain the best comparison between model and experimental exclusion curves. Our interest, however, is centred on the sphere model at present, and so only that scaling has been used.

All the curves from the random models show a better fit to the experimental curve at high molecular radius than do those from the simple geometrical models. The reason for this is that with the random models a molecule may be too big to enter the majority of the pores in the packing, and yet not be totally excluded from the largest pores. With pores of regular geometry and uniform size the onset of total exclusion is sharp: a molecule can either enter all of the pores or none of them. The curvature due to the existence of large pores is in addition to the slight curvature at large molecular radius found in the simple geometrical models.

The effect of a small number of large pores on the shape of the exclusion curve may be demonstrated by a simple model. For example, consider a matrix of ten circular pores, nine of which have a radius of unity and the tenth a radius of two. It may readily be shown that the exclusion curve for this matrix is given by

$$K = \frac{9K_1 + K_2}{13} \qquad 6.3. - 1$$

where K_1 and K_2 are given by

$$\begin{aligned} K_1 &= (1-r)^2 & r < 1 & \qquad 6.3. - 2a \\ &= 0 & r \geq 1 & \end{aligned}$$

$$\begin{aligned} K_2 &= (2-r)^2 & r < 2 & \qquad 6.3. - 2b \\ &= 0 & r \geq 2 & \end{aligned}$$

The equations for K_1 and K_2 consist of the numerators of the equations for exclusion from the respective circular pores. The denominators, which refer to the areas of the pores, have been combined as the denominator of equation 6.3. - 1, which represents the total area of pores in the matrix. The exclusion curve calculated from these equations is shown in figure 6.3. - 10. Its superiority to the simple equation for cylindrical pores is obvious. The effect of outside pores on exclusion curves in the region of $K = 0$ is well demonstrated by this curve.

Although this model was derived simply to examine the effects of large pores on the shape of the exclusion curve, it does suggest a possible way of building a model for exclusion chromatography. Such a model would assume a simple geometrical shape for the pores (e.g. circular pore as discussed above), and derive an exclusion curve based on this and the pore size distribution of the material to be modelled. The pore size distribution data may be obtained, for instance, by mercury porosimetry (80). In this technique the total volume of pores with a radius greater than a given radius may be calculated from the volume of mercury forced into the pores at a given pressure. The pore size distribution of Hypersil obtained by this method is shown in table 6.3. - 3.

A computer model based on this pore size distribution data was constructed. The pores were assumed to be circular in shape, since this assumption is made in the calculation of pore volumes in mercury porosimetry. The model consisted of pores of 100 different radii, chosen such that each pore size accounted for 1% of the total pore volume. Interpolation of the pore size distribution was required to make this assignment. The equations describing the model were

FIGURE 6.3. - 10.

Exclusion curve for modified infinite cylinder model compared with standard infinite cylinder model and modified experimental data. (see text for details of modification).

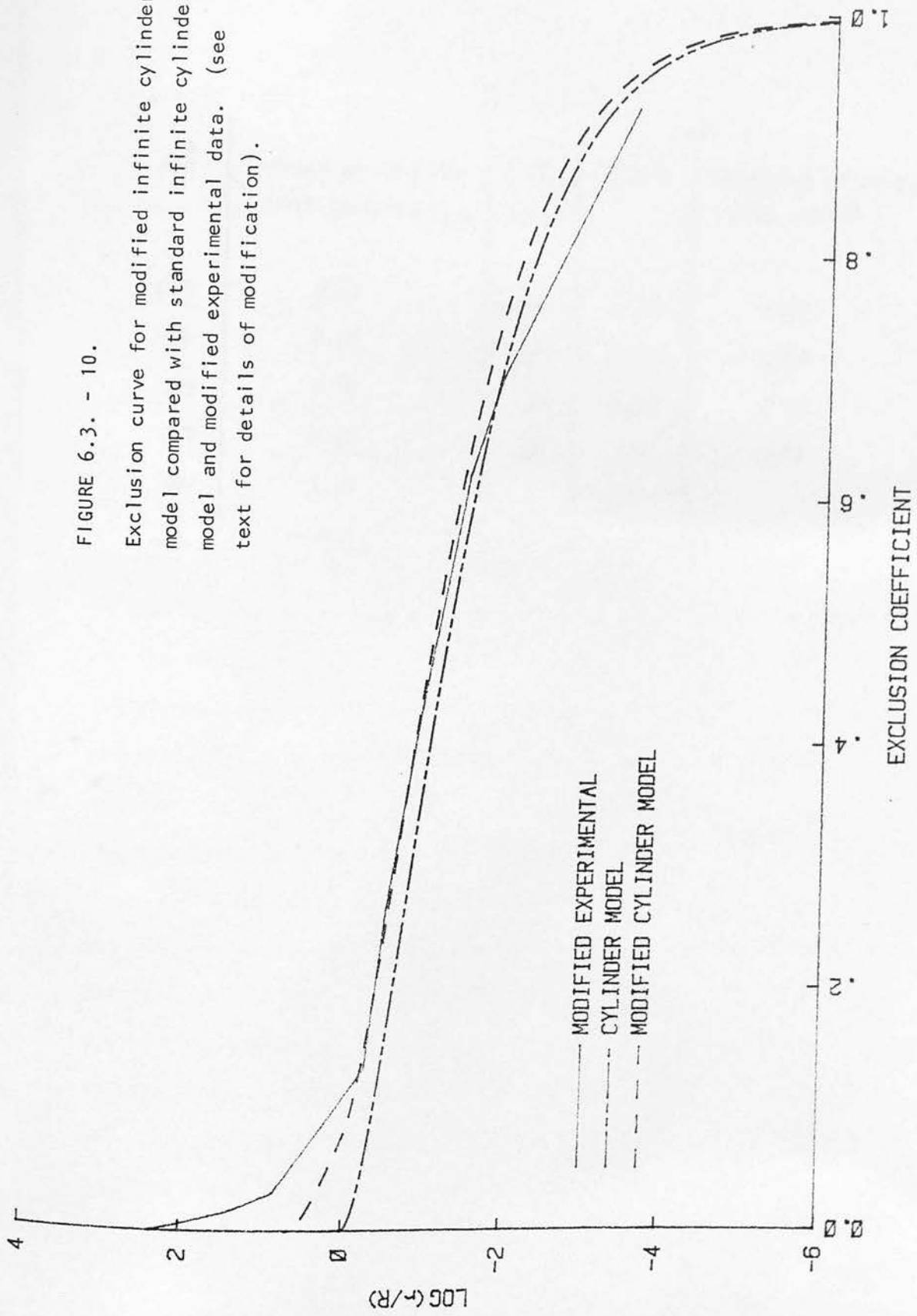


TABLE 6.3. - 3

MERCURY POROSIMMETRY DATA FOR HYPERSIL

$R/\text{Å}$	Fraction of pores with radius $\geq R$	$\bar{R}/\text{Å}$	$\bar{R}/68\text{Å}$	Fraction of pores with this \bar{R}
180	0.00	128	1.88	0.05
90	0.05	63	0.92	0.84
44	0.89	36	0.53	0.09
30	0.98	26	0.38	0.02
22	1.00			

analogous to equations 6.3. - 1 and 6.3. - 2, with a set values of radius and volume for each pore size. The model thus consists of a set of 100 equations, each of which uses pore size and volume data obtained from the porosimetry data. The exclusion curve from this model is shown in figure 6.3. - 11. This data is given in Table 6.3. - 4.

Unfortunately the match between model and experimental curves in this figure is not as good as might be expected for a model with the benefit of additional experimental information in the form of the pore size distribution of the material being modelled. More extensive pore size data (rather than only five unevenly-spaced points), and the use of more appropriate pore shapes may increase the accuracy of this type of model.

One point which is difficult to explain when the porosimetry and exclusion data are examined together is the divergence of the values for the maximum pore size. According to mercury porosimetry the maximum pore radius is 180\AA . The elution volumes of large polystyrene molecules in SEC indicate that molecules with an effective radius of 276\AA are not totally excluded. There are various possible explanations for this. For example, there may be batch-to-batch variations in the material; the material for which pore size distribution data is available may be different from that from which the exclusion data was measured. Partial uncoiling of the polymer molecules may allow partial ingress into pores from which full molecules are excluded. Accurate measurement of the relatively small volumes required for calculating the degree of exclusion at high molecular weights is difficult, and experimental errors in this may have occurred. These factors may have had a deleterious effect on

FIGURE 6.3. - 11. Exclusion curve based on Pore-size Distribution for Hypersil.

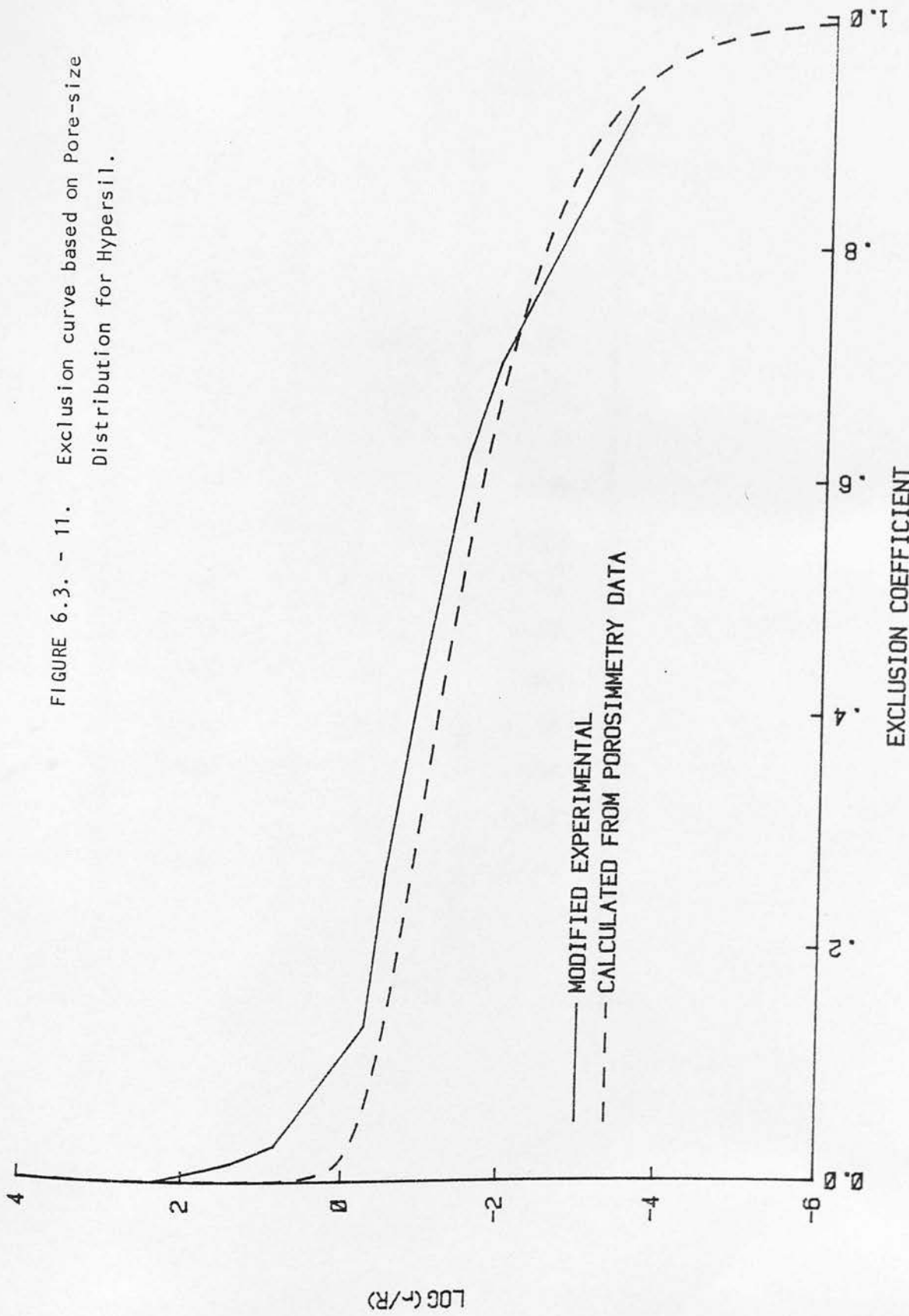


TABLE 6.3. - 4

EXCLUSION DATA BASED ON PORE SIZE DISTRIBUTION FROM MERCURY
POROSIMMETRY

r/R	$\ln (r-R)$	K
0.010	-4.61	0.977
0.030	-3.51	0.931
0.050	-3.00	0.887
0.100	-2.30	0.782
0.160	-1.83	0.665
0.200	-1.61	0.594
0.240	-1.43	0.527
0.300	-1.20	0.436
0.400	-0.92	0.308
0.500	-0.69	0.209
0.600	-0.51	0.136
0.700	-0.36	0.086
0.800	-0.22	0.053
1.500	0.41	0.004

the fit between model and experiment in this case.

A final demonstration of the effect of pore size distribution on exclusion curves involves manipulation of the array of random spheres. In order to produce large pores, spheres were eliminated from the array at random. The resulting array would possibly be unstable in real life, since floating 'islands' of spheres could be created by this process, but for present purposes the model so produced should be adequate.

The exclusion curve for this new array was calculated in the same way as before. This is shown in figure 6.3. - 12 and in Table 6.3. - 5. An improvement on the original model is apparent, but an adequate match between model and experiment has not yet been produced.

One possible reason for this may lie in the porosities of the various matrices. Hypersil has a porosity of 61%, whereas the original spheres model has a porosity of only 43%. The 'shot spheres' model described above has a porosity of 56%. It may be that if the porosity of the experimental material could be reproduced, an adequate fit between model and experiment may be found.

6.4. CONCLUSION

The assumed shape of the pores has surprisingly little effect on the shape of the theoretical exclusion curve. This applies especially to the gradient of the linear part of the curve.

The departure from linearity at high molecular weights has been shown to be due mainly to the presence of large pores into which the large molecules can penetrate, although they are excluded from the majority of the pores.

FIGURE 6.3. - 12.

Exclusion curve from 'shot spheres' model compared with modified experimental data. 'Shot spheres' model consists of array of random touching spheres with a percentage of spheres removed at random.

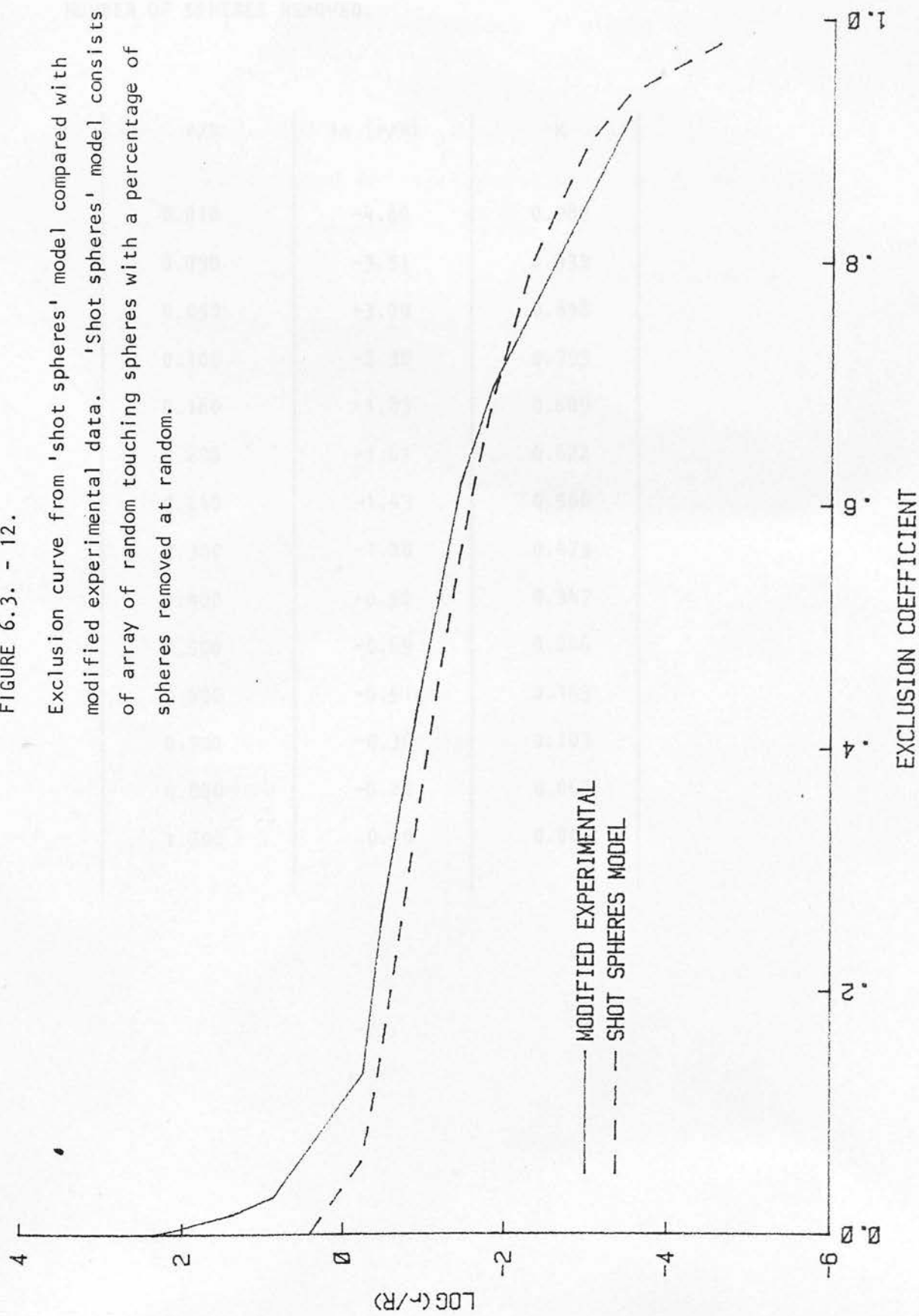


TABLE 6.3. - 5

EXCLUSION DATA FOR MATRIX OF RANDOM TOUCHING SPHERES WITH A
NUMBER OF SPHERES REMOVED.

r/R	$\ln (r/R)$	K
0.010	-4.60	0.980
0.030	-3.51	0.939
0.050	-3.00	0.898
0.100	-2.30	0.799
0.160	-1.83	0.689
0.200	-1.61	0.622
0.240	-1.43	0.560
0.300	-1.20	0.473
0.400	-0.92	0.347
0.500	-0.69	0.246
0.600	-0.51	0.165
0.700	-0.36	0.103
0.800	-0.22	0.060
1.500	0.40	0.001

If a model is to be accurate it must match the experimental material in porosity and pore size distribution. Simple models cannot be expected to achieve this; models where this data can be incorporated from independent measurements should be much more successful.

A simple factor which should be taken into account in any comparison of experimental and model exclusion curves is the small but significant degree of exclusion of small molecules. When an allowance is made for this the match at the low molecular weight end of the exclusion curve is greatly improved.

CHAPTER 7

CONCLUSIONS

7.1. INTRODUCTION

In this chapter the main conclusions of the study will be brought together, and suggestions for further work will be put forward.

7.2. DISCUSSION

All of the reported reactions of the investigated compounds are of the same type, and are of the same order. The results are in good agreement with the proposed mechanism.

CHAPTER 7
CONCLUSIONS

The results of the present study are in good agreement with the proposed mechanism. The reaction is of the same order and the rate constants are in good agreement with the proposed mechanism.

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CHAPTER 7

CONCLUSIONS

7.1. INTRODUCTION

In this chapter the most important conclusions of the thesis will be brought together, and suggestions for further work will be put forward.

7.2. DIFFUSION

All of the important equations on band broadening include diffusion coefficients of one sort or another, except in the case of slow mass transfer in adsorption chromatography. In a study of band broadening it is vital that mobile phase diffusion coefficients are measured directly for the eluents and solutes of interest, since semi-empirical equations like the Wilke-Chang equation are not sufficiently accurate. The chromatographic technique described in Chapter 3 makes this a relatively straightforward task.

It has been necessary to assume that the ratio of D_m to D_s is constant for a set of solutes in a given mobile/stationary phase combination. Direct measurement of D_s would be of great value in the study of band broadening, but it is difficult to envisage how the experiment could be carried out since it involves the measurement of a diffusion coefficient for an ODS surface at which molecules of organic modifier may be loosely bound, without the presence of a liquid phase which would provide an alternative migration route for the solute molecules.

7.3. BAND BROADENING BY AXIAL DIFFUSION

The notable conclusions to the study of band broadening by axial diffusion concern the magnitude of the obstructive factor and the magnitude of stationary phase diffusion.

The obstructive factor for totally excluded solutes in ODS Hypersil has been shown to be about 0.6, in agreement with the value obtained by experiments on impermeable particles. For retained solutes a value of about 0.9 was obtained. This implies that molecules which can fully permeate the particles are not significantly hindered from diffusing through them.

It would be of interest to find how γ varies with the degree of exclusion from fully excluded to fully permeating non retained solutes. The evidence obtained so far suggests that near the limit of total exclusion there is little change of γ with k'' .

It would also be of interest to investigate any dependence of γ with k' for retained solutes. It seems probable that for a molecule with a high degree of retention γ will be less than for an unretained molecule, since the particle will appear less permeable. This assumes no significant diffusion in the stationary phase, and the effect would most probably be found in adsorption chromatography.

For ODS material it has been shown that stationary phase diffusion is significant and proceeds at rates comparable with mobile phase diffusion. Some evidence has been found to suggest that the rate of stationary phase diffusion is independent of the mobile phase, provided that the adsorbed organic modifier remains the same.

7.4. BAND BROADENING DUE TO SLOW MASS TRANSFER

The validity of the reduced parameter approach to slow mass transfer has been confirmed by obtaining comparable values of the C term by this method for 50 μ m and 540 μ m particles.

By making reasonable estimates of the magnitude of the A term, values of the C term close to those predicted by the theory have been obtained. The C term given by the equation

$$C = \frac{1}{30} \frac{k''}{(1+k'')^2} \frac{D_m}{D_s} \quad 7.4. - 1$$

has been found to fit the experimental data adequately.

After the bulk of this thesis had been written the following equation for C was derived:-

$$C = \frac{1}{30} \left(\frac{k''}{1+k''} \right)^2 \frac{1 - \emptyset}{\emptyset \left(\gamma_{sm} - \frac{D_s}{D_m} \right) + k''(1-\emptyset) \frac{D_s}{D_m}} \quad 7.4. - 2$$

where \emptyset is the fraction of total eluent which is stagnant and γ_{sm} is the obstructive factor for the stagnant mobile phase. The obstructive factor for the stationary phase is again included in D_s . Full details of the derivation of this equation and of its fit to the experimental data of Chapter 5 are given in reference (81). A brief summary is given in appendix 2. It may be shown that equation 7.4. - 2 reduced to equation 7.4. - 1 when $D_s = D_m$.

Further work on band broadening due to slow mass transfer should begin with an increase in experimental accuracy. Close attention to temperature control and eluent composition should yield more

reproducible values of k'' . The use of a computer to digitise the data and calculate moments of the peaks for use in determining plate heights should also lend to greater accuracy. Well packed columns of large particles to reduce the A term as far as possible would also be a great advantage.

Since it has proved to be impossible to separate the A and C terms completely by using high reduced velocities, and since the way in which the A term has been treated in this work is highly approximate, a full investigation of the A term would be desirable. Such a study could be made by using pellicular materials for which the mass transfer coefficient is very small. It would be of interest to find out how flow effects are altered by retention. Accurate knowledge of the A term would lead to better values for the C term.

7.5. SIZE-EXCLUSION CHROMATOGRAPHY

In modelling the process of SEC various insights into the mechanism of exclusion were gained. These include the slight but significant exclusion of small molecules when comparing experimental results with theoretical exclusion curves, and the effect of a few large pores on the exclusion behaviour of large molecules. This latter point emphasises the need for careful control of pore-size distribution to extend the linear range of the material at the high molecular weight end of the exclusion curve.

The possibility of using pore-size distribution data to obtain exclusion curves has been demonstrated. A further investigation of this topic, including the direct measurement of pore size distribution with a large number of data points and measurement of an SEC

calibration curve on the same batch of material, would be of interest.

The inverse process of obtaining pore size data from SEC experiments is not as straightforward as it may appear from the literature (82). The development of a suitable way of transforming SEC data to yield the pore size distribution of the material would be of great practical benefit since SEC is a much more straightforward technique than mercury porosimetry.

The array of random touching spheres could be put to various uses in computer modelling, apart from simply eliminating spheres in an attempt to model the porosity of Hypersil. In chromatography it could be used for Monte-Carlo type investigations of on-column processes such as diffusion (83). It may also be useful for modelling various other processes, such as those of interest in chemical engineering.

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- α ...
- β ...
- γ ...
- δ ...
- ϵ ...
- ζ ...
- η ...
- θ ...
- ι ...
- κ ...
- λ ...
- μ ...
- ν ...
- ξ ...
- \omicron ...
- π ...
- ρ ...
- σ ...
- τ ...
- υ ...
- ϕ ...
- χ ...
- ψ ...
- ω ...

LIST OF SYMBOLS

- α ...
- β ...
- γ ...
- δ ...
- ϵ ...
- ζ ...
- η ...
- θ ...
- ι ...
- κ ...
- λ ...
- μ ...
- ν ...
- ξ ...
- \omicron ...
- π ...
- ρ ...
- σ ...
- τ ...
- υ ...
- ϕ ...
- χ ...
- ψ ...
- ω ...

LIST OF SYMBOLS USED

A	Mobile zone flow term in plate-height equation
A'	Mean gradient of curve $Av_0^{1/3}$ for a specified range of v_0
A_1, A_2	Velocity-independent mobile phase mass transfer term
A	Pore cross-sectional area
a	Tube radius
a_c	Radius of connecting tubing
a, b	Constants
B	Axial diffusion term in plate-height equation
C	Slow mass transfer term in plate-height equation
C_m	Slow mass transfer term for mobile zone
C_s	Slow mass transfer term for stationary zone
C_{ap}	Apparent C-term
C_1, C_2	Velocity-dependent mobile phase mass transfer terms
c	Overall concentration
c_m	Mobile zone concentration
c_m^*	Equilibrium mobile zone concentration
c_s	Stationary zone concentration
c_s^*	Equilibrium stationary zone concentration
c_s^i	Local stationary zone concentration
c_s^{*i}	Equilibrium local stationary zone concentration
D	Distribution coefficient
D	General diffusion coefficient
D_{eff}	Effective diffusion coefficient

List of Symbols (contd)

D_m	Mobile zone diffusion coefficient
D_s	Stationary zone diffusion coefficient
d	Depth of stationary zone
d_p	Particle diameter
F, F^\neq	Partition Functions
G_0, G_1	Constants of integration
H	Height equivalent to a theoretical plate
h	Reduced plate-height
h_d	Reduced mobile zone plate-height due to diffusion-terminated mobile zone mass transfer
h_f	Reduced mobile zone plate-height due to flow-terminated mobile zone mass transfer
h_{1d}	Reduced plate-height due to longitudinal diffusion
h_{mm}	Reduced plate-height due to mobile zone mass transfer
h_{sm}	Reduced plate-height due to stationary zone mass transfer
h_{tot}	Total reduced plate-height
K	Distribution coefficient
K	Exclusion coefficient
K_1, K_2	Partial exclusion coefficients
k	Boltzmann constant
k	Rate constant
k_a	Rate constant for adsorption
k_d	Rate constant for desorption

List of Symbols (contd)

L	Column length
l	Length of connecting tubing
l	Step length
l	Straight pore length
l_t	Tortuous pore length
M	Molecular weight
M	Mass of solute
N	Avogadro's number
N	Number of theoretical plates
N	Number of steps
n	Number of incremental volumes
n_d	Number of steps in mobile zone mass transfer due to diffusion
n_f	Number of steps in mobile zone mass transfer due to flow
p	Probability
Q	Quantity of solute
R	Gas constant
R	Particle radius
R_c	Radius of coil
R_s	Degree of resolution
r	Serial number of a plate
r	Molecular radius
r_g	Radius of gyration

List of Symbols (contd)

t	Time
t_m	Mobile phase residence time
t_o	Mobile zone residence time
t_s	Stationary zone residence time
t_a	Mean time for adsorption
t_d	Mean time for desorption
t_e	Exchange time between velocity regimes
\bar{t}	Mean time
t_{int}	Interrupt time
u	Mobile phase velocity
u_o	Mobile zone velocity
V	Column volume
V_m	Volume of mobile phase
V_o	Volume of mobile zone
V_s	Volume of stationary zone
V_D	Detector flowcell volume
V_i	Injector volume
V_s	Molar volume of solute
δV	Incremental volume
W_b	Peak width at baseline
W_b	Weight of eluent associated with peak width
W_c	Weight of eluent in column

List of Symbols (contd)

x	Distance variable
x	Amount of solute per unit length of column
z	Length variable
z	Distance along columns
z	Reduced radius variable
Δz	Distance between peaks
γ	Obstructive factor
ϵ_m	Mobile zone equilibrium departure term
ϵ_s	Stationary zone equilibrium departure term
ϵ'_s	Local stationary zone equilibrium departure term
ζ_0	Constant (equation 3.4 - 26)
η	Viscosity
κ	Specific reaction rate for diffusion
κ_{visc}	Specific reaction rate for viscous flow
λ	Equilibrium intermolecular distance
$\lambda_1, \lambda_2, \lambda_3$	Intermolecular distances
λ_i	A-term parameter
v	Reduced mobile phase velocity
v_0	Reduced mobile zone velocity
ρ	Density
σ	Standard deviation
σ^2	Variance

List of Symbols (contd)

σ_{eff}^2	Effective variance
σ_m^2	Mobile zone variance
σ_s^2	Stationary zone variance
σ_{res}^2	Residual variance
σ_z	Standard deviation in length units
σ_t	Standard deviation in time units
τ	Tortuosity factor
ψ	Association constant
ω_α	Distance between velocity regimes
ω_β	Fractional velocity change between velocity regimes
ω_i^2	Combination of ω_α and ω_β
ω_λ	Reduced persistence of velocity span
∇^2	Laplacian operator

Acetone



Phenol



p-Cresol



APPENDIX 1

LIST OF COMPOUNDS

m-Cresol



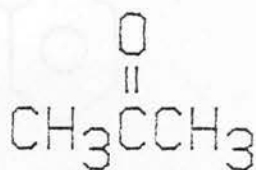
Anisole



Phenetole



Acetone



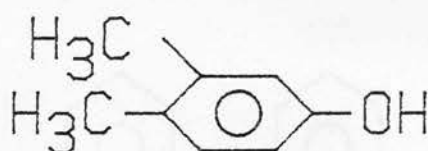
Phenol



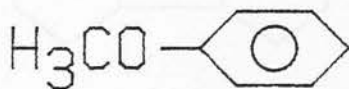
p-Cresol



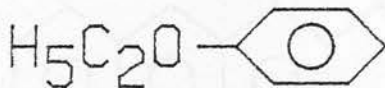
3,4-Xylenol.



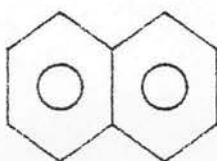
Anisole



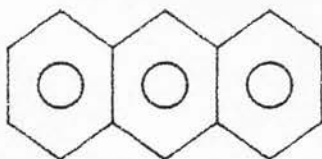
Phenetole



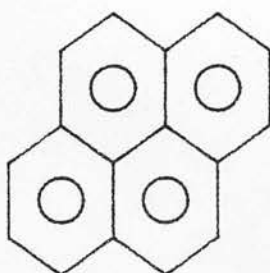
Naphthalene



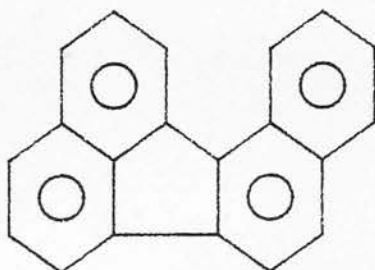
Anthracene



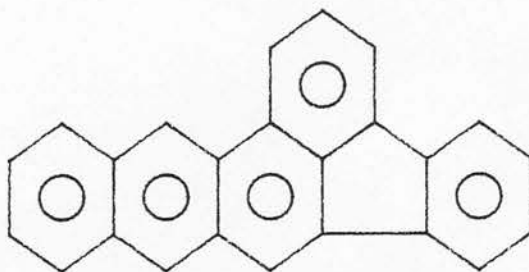
Chrysene



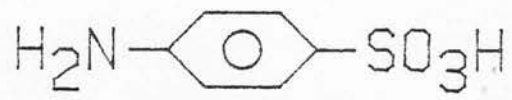
Benzo (1)
Fluoranthene



Naphtho (2, 3-k)
Fluoranthene



Sulphanilic Acid



APPENDIX 1

AN EXPRESSION FOR THE C-TERM OF THE CROWTHER EQUATION

In this appendix a further derivation of equation (4) is given. The equation is applied to the experimental data in Chapter 5.

The basic equation for the column for stationary phase mass transfer may be written as:

$$\frac{d^2C}{dz^2} = \frac{C}{L^2} \left(\frac{1}{\beta} + \frac{1}{\alpha} \right) \quad (A1)$$

where C is the concentration of the solute and L is the length of the column.

APPENDIX 2
AN EXPRESSION FOR THE C-TERM
IN LIQUID CHROMATOGRAPHY

The basic equation for the column for stationary phase mass transfer and the stationary phase may be written as:

$$\frac{d^2C}{dz^2} = \frac{C}{L^2} \left(\frac{1}{\beta} + \frac{1}{\alpha} + \frac{1}{\gamma} \right) \quad (A2)$$

where γ is the distribution factor for the stationary phase and α and β are the mass transfer coefficients in the stationary phase and the mobile phase respectively. The distribution factor for the stationary phase is denoted by γ .

The fraction of total volume which is stationary, β , may be expressed in terms of porosity, ϵ , as

APPENDIX 2

AN EXPRESSION FOR THE C TERM IN LIQUID CHROMATOGRAPHY

In this appendix a brief derivation of equation 7.4. -2 is given. This equation is applied to the experimental data of Chapter 5.

The basic equation for the C term for stationary zone mass transfer may be written as

$$C = \frac{1}{30} \frac{k''}{(1+k'')^2} \frac{D_m}{D_{sz}} \quad A3 - 1$$

where D_m is the diffusion coefficient in bulk eluent and D_{sz} is the diffusion coefficient in the stationary zone. D_{sz} may be expressed in terms of the diffusion coefficient and times of residence in the two parts of the stationary zone, namely the stagnant mobile zone and the stationary phase. Thus

$$D_{sz} = \frac{\gamma_{sm} d_m t_{sm} + D_s t_s}{t_{sm} + t_s} \quad A3 - 2$$

where γ_{sm} is the obstructive factor for the stagnant mobile zone, and t_s and t_{sm} are the residence times in the stationary phase and stagnant mobile zone. The obstructive factor for the stationary zone is included in D_s .

The fraction of total eluent which is stagnant, \emptyset , may be expressed in terms of residence times as

$$\emptyset = t_{sm}/t_m \quad A3 - 3$$

by equating column volumes and residence times, assuming no exclusion. D_{sz} may therefore be written as

$$D_{sz} = \frac{\gamma_{sm} D_m \emptyset + k' D_s}{\emptyset + k'} \quad \text{A3 - 4}$$

The ratio of D_{sz} to D_m is given by

$$\frac{D_{sz}}{D_m} = \frac{(\gamma_{sm} \emptyset + k' D_s/D_m)}{\emptyset + k'} \quad \text{A3 - 5}$$

k' appears in this equation in order to substitute for t_s . However, equation A3 - 1 is expressed in terms of k'' . The two capacity ratios are related by the equation

$$k' = k''(1 - \emptyset) - \emptyset \quad \text{A3 - 6}$$

Thus

$$\frac{D_{sz}}{D_m} = \frac{\gamma_{sm} \emptyset + (k''(1-\emptyset) - \emptyset) D_s/D_m}{k''(1-\emptyset)} \quad \text{A3 - 7}$$

From this and equation A3-1, C is obtained as

$$C = \frac{1}{30} \left(\frac{k''}{1+k''} \right)^2 \frac{1-\emptyset}{\emptyset \left(\gamma_{sm} \frac{D_s}{D_m} \right) + k''(1-\emptyset) \frac{D_s}{D_m}} \quad \text{A3- 8}$$

This is the required equation. In order to test this equation on

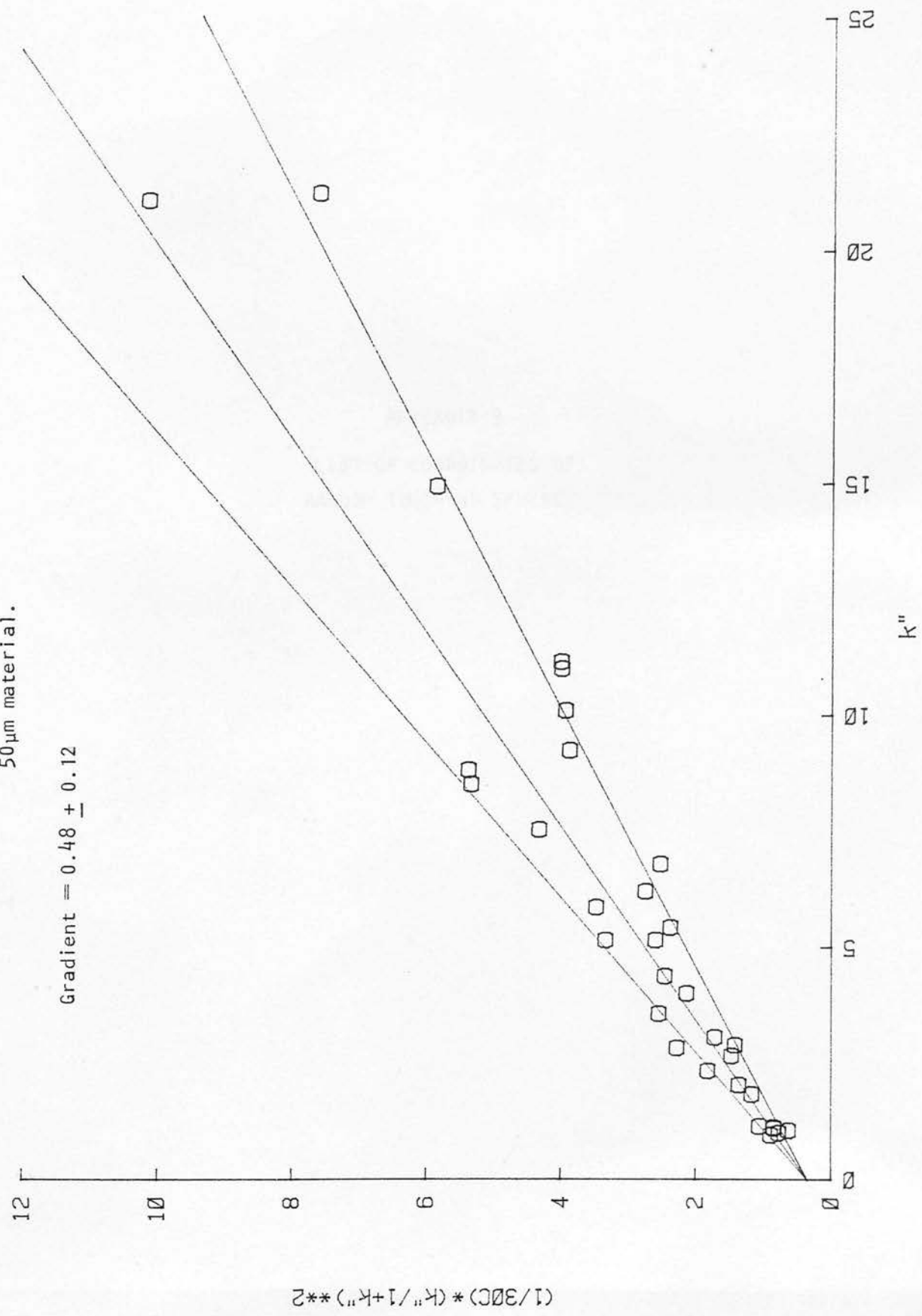
experimental data, it may be rewritten as

$$\frac{1}{30C} \left(\frac{k''}{1+k''} \right)^2 = \left(\frac{\emptyset}{1-\emptyset} \right) \left(\gamma_{sm} - \frac{D_s}{D_m} \right) + k'' \frac{D_s}{D_m} \quad \text{A3 - 9}$$

A plot of $(k''/1+k'')^2/30C$ against k'' should give a straight line with gradient D_s/D_m and intercept $(\emptyset/1-\emptyset)(\gamma_{sm} - D_s/D_m)$. The experimental data of Chapter 5 is plotted in this way in Figure A3 - 1. The value of D_s/D_m is 0.48 ± 0.12 , which is in good agreement with the value for the same ratio obtained independently by the interrupted flow experiment.

FIGURE A3. - 1. Graph of $(k''/1+k'')^2$ against k'' for 50 μ m material.

Gradient = 0.48 ± 0.12



APPENDIX 3

LIST OF COORDINATES OF
RANDOM TOUCHING SPHERES

I	X	Y	Z	R
1	0.000	0.000	0.000	1.000
2	2.000	0.000	0.000	1.000
3	1.000	1.732	0.000	1.000
4	1.000	0.577	1.649	1.013
5	1.000	-1.389	1.092	1.030
6	1.000	-1.392	-0.852	0.914
7	1.000	0.326	-1.708	1.006
8	-0.528	1.459	-1.202	0.963
9	-0.961	1.710	0.830	1.130
10	-0.977	-0.323	1.917	1.176
11	-0.837	-1.796	0.314	1.006
12	-0.707	-1.042	-1.470	0.935
13	-1.945	0.200	-0.569	1.036
14	0.863	-0.910	3.217	1.152
15	2.836	-0.570	2.015	1.182
16	2.712	1.363	1.168	0.931
17	2.806	1.620	-0.673	0.930
18	3.096	-0.063	-1.824	1.129
19	2.893	-1.779	-0.495	1.051
20	1.336	-3.210	-0.002	1.121
21	0.001	-3.040	1.589	0.963
22	1.693	-2.688	2.432	0.961
23	2.798	-1.808	3.926	1.095
24	2.474	0.204	3.962	0.944
25	1.087	0.974	3.365	0.751
26	1.618	2.301	2.408	0.970
27	0.445	3.372	1.131	1.068
28	0.059	3.186	-0.796	0.906
29	1.763	3.426	-0.306	0.883
30	3.479	3.062	0.503	1.049
31	4.315	1.324	0.303	0.891
32	4.441	1.135	2.367	1.185
33	4.572	-0.836	3.333	1.014
34	3.962	-2.492	2.249	1.058
35	4.530	-1.185	0.690	1.055
36	4.734	-1.387	-1.371	1.026
37	3.210	-2.056	-2.470	0.969
38	1.356	-1.377	-2.760	1.027
39	0.146	-2.631	-1.897	0.919
40	-1.475	-2.623	-1.210	0.841
41	-2.796	-1.671	-0.266	1.041
42	-2.274	-1.988	1.633	0.954
43	-0.959	-2.174	3.078	1.010
44	-0.946	-0.543	3.895	0.815
45	-2.586	-0.933	3.239	0.994
46	-3.017	-0.289	1.506	0.905
47	-2.383	1.301	2.396	1.025
48	-3.154	1.633	0.448	1.097
49	-1.884	2.567	-0.707	0.857
50	-2.258	1.452	-2.165	1.017
51	-1.015	2.947	-2.264	0.929
52	0.024	1.465	-3.198	1.108
53	2.178	1.807	-2.698	1.130
54	4.186	1.750	-2.007	0.994
55	3.327	3.099	-1.296	0.757
56	3.124	4.875	-0.667	1.137
57	1.119	5.203	0.228	1.082
58	-0.995	4.644	0.110	1.108
59	-1.506	3.521	1.834	1.011
60	-0.278	2.343	2.568	0.842

I	X	Y	Z	R
61	0.686	2.526	4.186	1.050
62	1.075	0.799	5.010	0.903
63	1.435	-1.016	5.251	0.964
64	0.994	-2.657	4.375	0.948
65	0.550	-4.162	3.155	1.040
66	0.642	-4.944	1.178	1.088
67	2.625	-4.183	1.455	1.054
68	3.244	-3.632	-0.260	0.850
69	2.250	-3.846	-1.955	1.127
70	0.346	-4.403	-1.364	0.942
71	-0.848	-3.975	0.097	0.992
72	-1.767	-3.873	1.876	1.013
73	-3.269	-3.338	0.723	0.954
74	-3.136	-3.451	-1.238	1.015
75	-2.697	-1.821	-2.254	0.955
76	-1.042	-1.905	-3.018	0.869
77	-1.749	-0.382	-2.737	0.834
78	-1.831	0.896	-4.081	1.023
79	-2.433	2.885	-3.700	1.090
80	-3.439	3.184	-1.757	1.119
81	-2.995	3.656	0.204	0.946
82	-3.398	3.012	1.943	0.951
83	-4.400	1.334	2.122	1.012
84	-4.390	-0.521	2.705	0.933
85	-4.665	-1.269	0.789	1.142
86	-3.904	-0.088	-0.767	0.954
87	-4.359	-1.767	-1.422	0.905
88	-4.852	-3.176	-0.322	0.950
89	-4.004	-4.967	-0.159	1.039
90	-2.131	-5.481	0.626	1.056
91	-0.961	-5.685	2.330	1.022
92	0.845	-6.024	2.806	0.877
93	2.304	-5.046	3.207	0.925
94	3.648	-3.774	3.889	1.047
95	4.656	-2.237	4.754	0.984
96	3.835	-0.488	5.165	0.991
97	2.916	-2.007	5.964	0.956
98	1.105	-2.624	6.308	0.989
99	-0.254	-1.753	5.362	0.883
100	-2.055	-2.112	4.805	1.037
101	-2.703	-2.827	3.177	0.856
102	-1.407	-3.981	3.864	1.011
103	0.114	-4.195	5.123	0.976
104	1.999	-3.965	5.270	0.928
105	3.138	-5.499	4.903	1.018
106	3.889	-5.428	3.347	0.711
107	4.792	-4.557	1.916	1.192
108	5.860	-2.670	1.357	1.047
109	6.181	-0.630	1.969	1.106
110	6.267	1.373	0.920	1.156
111	5.271	2.760	-0.273	0.928
112	5.014	4.628	0.590	1.146
113	3.096	4.797	1.419	0.950
114	1.578	4.334	2.685	1.082
115	2.707	3.035	3.941	1.048
116	2.646	1.556	5.119	0.844
117	4.389	1.898	4.466	1.049
118	5.858	0.586	3.928	0.993
119	6.207	1.908	2.788	0.787
120	4.972	3.084	2.993	0.930

I	X	Y	Z	R
121	6.509	3.425	1.755	1.072
122	7.035	2.967	-0.050	0.862
123	6.561	1.474	-1.464	1.248
124	5.220	0.089	-2.910	1.162
125	3.448	-0.672	-3.788	0.957
126	2.412	-2.199	-4.189	0.931
127	3.347	-3.489	-3.502	0.802
128	4.214	-3.539	-2.079	0.865
129	5.177	-2.248	-3.386	1.209
130	6.657	-1.288	-2.037	1.011
131	5.827	-2.822	-1.584	0.790
132	4.999	-3.869	-0.492	0.935
133	3.712	-5.316	-1.133	1.104
134	2.296	-5.833	0.308	0.982
135	0.509	-6.173	-0.530	1.020
136	1.330	-5.842	-2.497	1.136
137	0.538	-3.952	-3.350	1.083
138	0.288	-2.125	-4.298	0.990
139	1.477	-0.593	-4.594	0.971
140	1.524	1.376	-4.679	1.001
141	1.065	3.113	-3.892	0.961
142	0.403	4.602	-2.392	1.254
143	2.732	4.051	-2.885	1.189
144	3.870	2.431	-3.886	1.029
145	5.556	2.954	-2.852	1.017
146	4.743	4.727	-2.143	1.059
147	4.759	6.382	-0.773	1.089
148	3.122	6.683	0.637	1.092
149	1.865	6.747	-1.279	1.201
150	-0.206	6.261	-0.908	0.959
151	-0.321	6.310	0.902	0.856
152	-1.848	6.490	-0.005	0.929
153	-2.694	5.107	-1.106	1.032
154	-3.005	4.766	-3.061	0.977
155	-4.473	3.500	-3.627	1.042
156	-4.000	1.499	-3.269	1.046
157	-3.434	-0.529	-3.668	1.097
158	-2.484	-2.347	-4.131	1.006
159	-1.735	-3.710	-2.759	1.069
160	-1.433	-5.232	-1.361	1.020
161	-3.299	-5.258	-1.998	0.952
162	-4.796	-4.164	-1.891	0.905
163	-4.240	-2.748	-3.082	1.027
164	-5.312	-1.042	-2.898	0.996
165	-5.807	-0.570	-1.000	1.022
166	-5.306	1.503	-0.359	1.205
167	-4.854	3.635	-0.281	0.975
168	-4.688	5.002	-1.818	1.088
169	-3.401	6.561	-2.336	0.999
170	-1.537	6.198	-2.209	0.904
171	-1.223	5.249	-3.878	1.042
172	-2.670	4.457	-4.860	0.878
173	-3.652	2.911	-5.456	1.048
174	-1.633	2.301	-5.616	1.067
175	-3.185	0.900	-5.615	1.023
176	-1.270	0.273	-5.942	1.018
177	0.255	-1.176	-6.157	1.097
178	1.458	-2.887	-5.761	1.032
179	3.469	-3.431	-5.384	1.084
180	3.456	-1.424	-5.517	0.928

I	X	Y	Z	R
181	4.878	-0.124	-5.082	1.047
182	6.625	-0.832	-4.276	1.002
183	7.018	1.244	-3.817	1.160
184	6.087	-0.086	-2.347	0.996
185	7.477	-0.311	-0.524	0.967
186	7.513	-1.688	0.812	0.951
187	7.263	-2.161	2.534	0.852
188	6.314	-1.427	3.782	0.879
189	5.859	-0.760	5.583	1.094
190	4.780	-2.215	6.855	1.120
191	4.655	-4.023	5.692	1.033
192	5.800	-3.765	3.905	1.105
193	6.027	-2.925	5.524	0.733
194	7.489	-2.034	5.146	1.021
195	7.555	-0.230	4.373	0.943
196	8.282	-0.533	2.522	1.069
197	7.963	1.562	2.333	1.059
198	8.427	1.560	0.191	1.131
199	8.439	3.509	1.030	0.990
200	7.127	4.601	0.389	0.833
201	6.511	5.510	1.878	1.017
202	5.087	6.587	1.179	0.901
203	3.813	6.233	2.619	1.054
204	1.768	6.391	2.246	1.030
205	2.447	5.649	3.913	0.917
206	1.089	4.505	4.749	1.046
207	-0.541	4.860	3.276	1.180
208	-1.805	5.370	1.754	0.863
209	-2.776	4.451	2.811	0.841
210	-2.246	2.904	3.545	0.952
211	-1.693	1.133	4.381	1.083
212	-2.838	-0.354	5.024	0.900
213	-4.159	-1.597	4.340	1.039
214	-5.150	-2.377	2.645	1.074
215	-4.592	-4.540	2.011	1.249
216	-6.073	-4.704	0.221	1.081
217	-6.488	-2.622	0.835	1.129
218	-6.688	-0.469	0.855	1.034
219	-6.223	0.419	2.771	1.129
220	-4.549	0.875	4.191	1.113
221	-4.472	2.731	3.422	0.898
222	-5.456	3.143	1.703	1.125
223	-4.092	4.805	1.405	1.045
224	-3.846	6.423	0.063	1.072
225	-2.789	7.571	-0.975	0.802
226	-1.110	7.893	-0.932	0.907
227	0.120	7.548	-2.331	0.988
228	1.416	6.410	-3.514	1.103
229	0.758	4.819	-4.471	0.867
230	2.363	4.037	-5.053	1.010
231	0.892	2.893	-5.849	1.016
232	2.645	2.358	-5.726	0.821
233	1.609	1.208	-6.656	0.985
234	2.723	0.295	-5.548	0.832
235	2.225	-0.850	-6.966	1.058
236	1.043	-2.380	-7.692	1.007
237	-0.525	-3.217	-6.526	1.118
238	0.470	-4.526	-5.288	0.939
239	-0.219	-5.798	-3.982	1.010
240	-0.567	-6.828	-2.275	1.013

I	X	Y	Z	R
241	-1.307	-7.047	-0.417	0.999
242	-3.260	-6.816	-0.394	0.968
243	-4.776	-6.282	-1.416	0.937
244	-5.136	-6.504	0.433	0.959
245	-3.593	-6.435	1.327	0.825
246	-2.040	-7.161	1.667	0.923
247	-0.318	-7.041	1.172	0.874
248	-0.359	-7.608	3.098	1.134
249	-2.213	-6.703	3.492	0.966
250	-2.596	-5.135	2.835	0.776
251	-3.397	-4.254	4.183	1.022
252	-2.171	-3.866	5.607	0.895
253	-0.866	-2.966	6.750	1.059
254	0.039	-1.184	7.101	0.970
255	1.343	0.187	6.744	0.955
256	1.412	2.068	6.345	0.969
257	-0.341	3.325	6.035	1.211
258	-0.256	1.205	6.181	0.915
259	-0.994	-0.335	5.923	0.812
260	-2.407	-1.279	6.686	1.050
261	-4.303	-0.718	6.168	0.994
262	-3.482	1.198	6.093	1.092
263	-3.526	3.169	5.130	1.102
264	-2.303	4.784	4.561	1.002
265	-0.598	5.580	5.390	1.054
266	0.665	6.245	4.134	0.847
267	-0.144	6.964	2.634	1.004
268	-1.259	7.951	1.207	1.058
269	0.738	7.874	0.384	1.102
270	2.699	8.634	-0.264	1.098
271	4.490	8.023	0.248	0.863
272	6.303	7.557	0.052	1.020
273	5.189	8.324	-1.474	1.020
274	6.451	6.865	-1.765	0.931
275	5.009	6.494	-2.637	0.795
276	3.872	5.680	-3.690	0.956
277	5.076	4.360	-3.993	0.856
278	5.607	2.809	-4.862	0.999
279	7.031	3.187	-3.858	0.784
280	7.371	3.471	-2.124	1.006
281	8.738	1.978	-1.906	1.030
282	9.197	1.093	-3.705	1.027
283	8.240	0.042	-5.264	1.083
284	6.503	-0.511	-6.226	0.978
285	5.509	-1.954	-5.477	0.928
286	5.473	-3.808	-4.929	1.005
287	4.592	-4.807	-3.412	1.013
288	6.552	-4.087	-2.859	1.147
289	6.976	-2.765	-4.373	0.907
290	8.000	-1.722	-3.318	0.895
291	8.536	-1.644	-1.538	0.966
292	7.520	-3.319	-1.032	1.059
293	6.630	-4.489	0.538	1.092
294	6.854	-5.047	2.569	1.026
295	5.347	-6.461	3.062	1.099
296	3.388	-7.070	3.618	1.027
297	2.390	-6.473	2.113	0.875
298	4.155	-6.690	1.052	1.196
299	3.147	-7.118	-0.666	0.841
300	3.161	-6.803	-2.413	0.934

I	X	Y	Z	R
301	5.189	-6.514	-2.283	1.119
302	6.186	-5.839	-3.993	0.972
303	4.554	-5.742	-5.405	1.188
304	4.850	-4.081	-6.849	1.032
305	3.158	-4.886	-6.627	0.855
306	1.391	-4.497	-6.982	0.990
307	0.034	-4.045	-8.514	1.107
308	-0.878	-2.110	-8.344	1.039
309	-0.607	-0.190	-7.771	0.982
310	-0.474	1.631	-7.129	0.953
311	-2.436	1.564	-7.407	1.029
312	-2.508	3.492	-6.983	0.946
313	-3.701	4.653	-6.257	0.870
314	-4.352	5.332	-4.600	1.036
315	-6.317	4.872	-3.504	1.260
316	-5.761	3.216	-2.056	1.009
317	-5.875	2.079	-3.654	0.955
318	-5.652	3.169	-5.236	0.980
319	-5.023	1.572	-6.356	1.069
320	-5.175	0.575	-4.600	0.955
321	-4.990	-1.482	-4.966	1.143
322	-4.002	-3.596	-5.123	1.196
323	-2.155	-4.073	-4.542	0.798
324	-1.364	-5.139	-5.870	1.079
325	-1.904	-4.318	-7.712	1.009
326	-2.395	-2.509	-7.101	0.963
327	-2.436	-0.599	-7.275	0.956
328	-1.972	-0.568	-9.221	1.045
329	0.033	-0.752	-9.557	0.996
330	1.812	-1.073	-8.897	0.929
331	2.514	0.664	-8.361	1.020
332	2.879	2.444	-7.514	0.984
333	2.003	4.033	-6.947	0.917
334	1.586	5.773	-5.993	1.111
335	-0.119	6.614	-4.992	1.037
336	-1.478	7.168	-3.728	0.899
337	-2.516	8.391	-2.616	1.053
338	-4.214	8.146	-1.694	0.895
339	-5.392	6.869	-2.506	1.023
340	-5.748	6.300	-0.641	0.960
341	-5.944	4.915	0.663	0.953
342	-6.700	3.291	0.068	0.935
343	-6.560	1.964	0.943	0.660
344	-7.472	1.115	-0.161	1.004
345	-6.689	0.971	-2.039	1.035
346	-7.186	-0.927	-2.370	0.955
347	-6.703	-2.336	-3.791	1.103
348	-6.136	-2.498	-1.730	1.041
349	-6.561	-4.378	-1.658	0.888
350	-5.692	-5.471	-2.847	0.945
351	-4.087	-6.619	-3.274	1.073
352	-3.973	-7.948	-1.761	0.945
353	-2.512	-8.574	-0.700	0.967
354	-0.854	-8.511	-1.484	0.868
355	0.991	-7.952	-1.494	1.060
356	1.539	-7.514	0.295	0.862
357	0.576	-8.774	1.443	1.096
358	-1.302	-9.045	1.911	1.052
359	-3.490	-8.318	2.746	1.224
360	-5.008	-6.711	2.455	1.006

I	X	Y	Z	R
361	-6.682	-5.537	2.107	1.069
362	-6.281	-3.952	3.367	0.995
363	-6.039	-2.345	4.509	0.991
364	-4.759	-2.606	5.957	0.959
365	-3.944	-4.427	6.173	1.048
366	-2.635	-5.542	5.333	0.865
367	-1.147	-5.143	5.845	0.759
368	-0.714	-5.661	4.360	0.873
369	1.018	-5.674	4.504	0.865
370	1.172	-5.343	6.329	0.997
371	2.199	-3.946	7.080	0.893
372	2.334	-2.110	8.003	1.167
373	3.509	-0.641	7.102	0.918
374	4.715	0.991	6.513	1.195
375	3.562	2.847	6.116	1.027
376	4.876	3.989	4.842	1.131
377	6.056	2.377	5.514	0.977
378	6.724	3.289	3.853	1.032
379	7.988	1.728	4.449	1.064
380	7.461	0.615	6.254	1.121
381	7.253	-1.335	7.005	0.979
382	6.611	-3.153	7.087	0.951
383	4.976	-4.268	7.863	1.175
384	3.862	-5.463	6.641	0.865
385	4.897	-5.838	5.354	0.829
386	3.841	-7.155	5.450	0.862
387	2.010	-7.162	5.103	1.001
388	3.177	-8.692	4.740	0.957
389	1.228	-8.832	4.090	1.102
390	-0.843	-9.683	3.906	1.144
391	-1.518	-7.914	4.805	0.952
392	-3.568	-7.434	4.959	1.160
393	-4.828	-5.642	4.719	1.042
394	-6.375	-6.525	3.836	0.946
395	-5.547	-8.273	3.598	1.003
396	-5.346	-8.439	1.704	0.908
397	-4.449	-8.262	0.073	0.961
398	-6.148	-7.674	-0.903	1.084
399	-7.496	-5.950	-0.872	1.104
400	-7.816	-4.020	-0.433	0.902
401	-7.804	-2.198	-0.705	0.940
402	-8.292	-1.616	1.021	0.945
403	-7.097	-1.518	2.510	0.967
404	-7.725	-3.278	2.320	0.912
405	-8.026	-4.762	3.365	0.929
406	-8.130	-6.643	3.047	0.982
407	-8.755	-5.334	1.584	1.079
408	-7.712	-6.955	1.265	0.876
409	-7.182	-8.431	2.374	1.045
410	-7.530	-8.189	4.597	1.218
411	-5.501	-8.326	5.692	1.091
412	-4.784	-6.609	6.405	0.902
413	-3.033	-6.076	7.157	1.077
414	-2.420	-4.258	7.286	0.846
415	-2.292	-2.651	8.238	1.025
416	-0.361	-2.333	8.606	0.966
417	-1.000	-4.047	8.355	0.880
418	-2.456	-5.128	8.944	1.027
419	-4.248	-4.753	8.143	0.972
420	-5.708	-3.903	7.101	1.013

I	X	Y	Z	R
421	-6.857	-4.046	5.358	1.079
422	-6.736	-2.184	6.461	1.089
423	-6.019	-0.683	5.390	0.890
424	-6.538	0.974	4.758	0.958
425	-7.854	-0.372	4.011	1.067
426	-8.342	0.342	1.991	1.130
427	-7.351	2.194	2.393	1.008
428	-6.304	3.420	3.722	1.082
429	-5.419	2.403	5.185	0.907
430	-4.627	2.777	6.900	1.019
431	-2.689	2.842	6.942	0.921
432	-3.553	1.694	7.956	0.837
433	-2.322	0.482	7.585	0.929
434	-0.368	0.580	8.082	1.089
435	1.610	1.479	8.314	1.096
436	1.001	3.332	7.719	0.943
437	0.962	4.899	6.685	0.934
438	2.703	5.289	5.826	1.046
439	1.979	7.025	5.111	0.966
440	2.711	7.897	3.400	1.089
441	3.967	6.690	4.707	1.089
442	5.720	6.605	3.461	1.063
443	4.735	8.088	2.350	1.035
444	3.876	9.422	1.189	0.931
445	5.680	9.271	0.747	0.932
446	6.805	9.296	-0.802	0.982
447	7.970	7.741	-1.024	0.973
448	7.805	8.599	0.629	0.897
449	6.585	8.146	1.825	0.870
450	7.532	6.886	1.235	0.813
451	8.445	5.411	1.217	0.921
452	8.033	4.371	2.756	0.982
453	9.395	2.969	2.660	0.975
454	9.944	1.282	1.713	1.036
455	9.309	-0.430	0.627	1.089
456	9.889	-0.157	-1.412	1.049
457	9.539	-0.735	-3.232	0.893
458	10.387	-0.161	-4.941	1.099
459	9.567	1.777	-5.742	1.153
460	7.484	1.839	-5.766	0.930
461	5.651	1.374	-6.303	1.035
462	4.607	-0.344	-7.320	1.218
463	3.415	-2.054	-8.242	1.062
464	2.231	-3.728	-8.648	1.028
465	0.961	-2.633	-9.562	0.882
466	-0.896	-3.053	-10.353	1.180
467	-1.520	-4.889	-9.523	0.929
468	-0.751	-6.066	-8.044	1.112
469	0.481	-6.129	-6.350	0.984
470	1.558	-5.965	-4.765	0.939
471	0.856	-7.531	-3.782	1.039
472	-1.274	-7.643	-4.116	1.120
473	-1.233	-7.238	-6.257	1.059
474	-2.711	-6.094	-7.162	1.017
475	-2.686	-6.429	-5.330	0.845
476	-2.171	-5.809	-3.714	0.961
477	-3.226	-8.435	-3.812	1.008
478	-5.115	-8.383	-3.233	0.969
479	-5.899	-6.877	-4.039	0.910
480	-4.476	-7.548	-5.026	0.948

I	X	Y	Z	R
481	-3.232	-8.152	-6.632	1.171
482	-1.591	-8.105	-8.201	1.100
483	-1.567	-6.800	-9.863	1.013
484	0.304	-5.741	-10.009	1.141
485	1.733	-5.622	-8.507	0.936
486	3.615	-5.187	-8.397	0.998
487	5.307	-6.031	-7.529	1.083
488	6.609	-5.100	-6.088	1.070
489	7.011	-2.986	-6.369	1.101
490	8.264	-1.293	-6.979	1.091
491	9.138	-1.838	-5.155	1.003
492	8.666	-3.248	-3.975	0.895
493	8.666	-4.385	-2.419	1.033
494	7.288	-5.322	-1.299	0.975
495	8.554	-4.686	-0.033	0.925
496	9.081	-2.812	0.289	1.048
497	9.046	-2.403	2.262	0.967
498	10.218	-0.931	2.342	0.916
499	9.751	0.455	3.527	0.967
500	9.139	-1.210	4.194	0.929
501	9.190	-1.346	6.227	1.109
502	8.469	-3.372	6.382	1.047
503	6.978	-4.275	5.470	0.920
504	7.797	-3.556	4.006	0.905
505	7.938	-5.470	4.264	1.030
506	7.200	-6.704	3.177	0.772
507	6.702	-6.767	1.436	1.039
508	8.646	-6.126	2.110	1.115
509	9.042	-4.446	3.098	0.874
510	9.927	-4.411	1.376	1.062
511	10.351	-4.176	-0.683	1.053
512	9.916	-2.852	-2.142	0.964
513	10.939	-1.379	-2.417	0.850
514	10.958	0.248	-2.939	0.858
515	10.677	2.120	-2.661	1.056
516	10.317	2.549	-0.551	1.127
517	10.354	3.691	1.169	0.939
518	9.442	4.656	-0.199	0.968
519	9.334	3.925	-2.034	1.011
520	8.219	4.564	-3.642	1.048
521	6.649	4.539	-4.936	0.987
522	5.014	5.712	-5.364	1.070
523	4.688	7.445	-4.185	1.051
524	3.172	7.258	-2.912	0.938
525	1.788	8.432	-2.553	0.913
526	0.496	9.322	-1.273	1.112
527	-1.049	9.428	-0.026	0.877
528	-2.916	8.948	0.299	1.078
529	-3.190	7.466	1.730	1.001
530	-2.589	6.246	3.208	1.007
531	-4.191	5.288	3.245	0.860
532	-5.488	4.814	2.482	0.718
533	-7.009	4.392	2.095	0.906
534	-7.716	4.675	0.521	0.841
535	-7.189	6.466	0.732	1.037
536	-5.430	7.634	0.866	1.078
537	-6.059	8.239	-1.077	1.052
538	-5.415	8.644	-2.729	0.766
539	-4.047	9.627	-2.727	0.918
540	-4.145	8.062	-3.528	0.843

I	X	Y	Z	R
541	-5.612	7.212	-4.721	1.230
542	-6.014	5.310	-5.601	0.904
543	-5.394	4.108	-7.049	1.077
544	-4.060	2.741	-8.028	1.070
545	-2.167	2.985	-8.831	1.001
546	-1.165	1.436	-8.778	0.845
547	0.486	1.066	-8.560	0.860
548	0.015	2.951	-8.600	1.083
549	0.033	4.434	-7.006	1.094
550	-1.839	5.275	-6.393	1.048
551	-3.164	5.124	-8.179	1.181
552	-4.696	6.037	-6.965	0.977
553	-3.573	6.935	-5.599	1.007
554	-2.760	8.162	-4.481	0.841
555	-1.120	8.294	-5.115	0.922
556	0.537	8.347	-4.117	1.012
557	2.144	7.608	-5.092	1.008
558	0.806	7.507	-6.334	0.821
559	0.610	6.382	-7.922	1.135
560	2.630	5.631	-7.846	1.021
561	2.241	3.838	-8.972	1.131
562	3.371	2.131	-9.362	0.953
563	4.250	0.245	-9.575	1.139
564	2.237	0.178	-10.305	1.003
565	0.500	0.910	-10.295	0.882
566	-1.095	0.171	-10.878	0.970
567	-2.365	-1.470	-11.154	1.123
568	-2.640	-2.418	-9.357	0.926
569	-3.731	-3.533	-8.173	1.033
570	-4.037	-1.622	-7.631	0.977
571	-4.054	0.377	-7.935	1.046
572	-5.847	1.516	-8.392	1.128
573	-6.994	2.475	-6.704	1.126
574	-7.596	3.680	-5.003	1.044
575	-7.431	3.019	-3.149	0.932
576	-7.381	4.201	-1.732	0.914
577	-8.396	4.544	-3.162	0.873
578	-8.154	5.743	-4.724	1.111
579	-7.882	6.266	-2.721	0.977
580	-6.953	7.912	-3.139	0.959
581	-6.844	9.864	-2.343	1.152
582	-7.923	8.293	-1.614	0.889
583	-7.633	8.452	0.292	1.045
584	-7.030	7.905	2.175	1.007
585	-6.020	6.393	2.162	0.811
586	-4.824	7.523	2.802	0.954
587	-4.172	9.266	2.009	1.069
588	-2.079	9.681	2.135	1.070
589	-0.262	8.932	2.673	0.968
590	0.188	7.975	4.276	0.953
591	0.313	7.246	6.029	0.949
592	-0.597	6.070	7.619	1.228
593	-2.010	4.676	6.590	1.007
594	-1.257	3.507	8.041	1.002
595	-3.166	3.347	8.793	1.056
596	-5.022	2.394	8.876	1.032
597	-5.196	0.831	7.459	1.085
598	-3.839	-0.694	8.195	1.085
599	-1.938	-0.829	8.843	0.928
600	-1.612	-2.218	9.986	0.900

I	X	Y	Z	R
601	-0.425	-3.601	10.004	0.922
602	1.202	-2.486	9.877	1.054
603	2.137	-4.023	8.943	0.973
604	2.286	-5.575	7.871	0.920
605	1.556	-7.173	7.066	1.013
606	0.195	-6.885	5.708	0.931
607	-0.473	-5.946	7.217	0.967
608	-1.258	-7.599	6.658	0.946
609	0.047	-8.821	5.849	1.016
610	1.891	-9.363	5.911	0.907
611	3.345	-8.446	6.629	0.956
612	4.293	-7.023	7.048	0.804
613	5.745	-6.025	6.920	0.962
614	7.186	-4.943	8.003	1.140
615	6.481	-3.171	9.086	1.052
616	5.887	-1.337	8.419	0.988
617	3.964	-0.975	8.986	1.049
618	2.063	-0.694	9.565	0.958
619	0.169	-0.802	9.609	0.940
620	0.298	1.227	10.130	1.159
621	-1.702	0.871	9.588	0.943
622	-3.702	0.868	9.656	1.059
623	-3.965	2.725	10.660	1.068
624	-4.838	4.336	9.601	1.049
625	-4.264	4.726	7.588	1.080
626	-5.036	4.307	5.806	0.906
627	-4.015	5.972	5.136	1.159
628	-2.224	6.413	5.948	0.856
629	-2.724	7.587	4.648	0.965
630	-4.594	7.921	4.634	0.935
631	-6.457	7.017	4.113	1.200
632	-7.679	6.038	2.637	0.951
633	-6.599	5.124	3.431	0.671
634	-8.150	4.481	3.799	1.048
635	-8.111	2.441	4.289	1.050
636	-8.290	0.880	5.556	0.969
637	-7.144	-0.134	6.858	1.039
638	-6.765	1.857	6.510	1.017
639	-7.081	3.541	5.578	0.934
640	-6.446	3.709	7.521	1.117
641	-6.525	3.404	9.494	0.881
642	-5.912	2.084	10.655	0.982
643	-6.596	1.047	9.052	1.047
644	-5.738	-0.619	8.653	0.870
645	-7.321	-1.507	8.321	0.975
646	-7.401	-3.398	7.961	0.952
647	-7.316	-5.201	7.090	1.053
648	-6.016	-5.119	8.505	0.870
649	-4.954	-6.455	8.162	0.870
650	-4.640	-8.059	7.472	0.904
651	-3.676	-9.138	6.257	0.985
652	-4.302	-9.830	4.358	1.131
653	-4.743	-9.976	2.399	0.883
654	-2.928	-10.425	2.381	0.987
655	-3.122	-9.289	0.958	0.844
656	-1.731	-9.893	0.243	0.833
657	-1.764	-10.247	-1.656	1.099
658	-3.934	-9.960	-1.323	1.114
659	-5.777	-9.645	-0.337	1.000
660	-5.823	-10.013	-2.328	1.025

I	X	Y	Z	R
661	-7.207	-8.364	-2.773	1.173
662	-7.274	-6.339	-3.044	0.871
663	-8.410	-4.688	-2.558	1.191
664	-6.970	-4.913	-4.096	0.927
665	-8.222	-3.566	-4.346	0.928
666	-8.538	-2.501	-2.759	1.009
667	-8.223	-1.000	-3.938	0.926
668	-7.060	-1.120	-5.501	1.026
669	-6.607	0.696	-5.702	0.856
670	-5.826	-0.120	-7.111	0.950
671	-5.432	-0.884	-8.951	1.081
672	-3.846	0.194	-10.140	1.177
673	-4.362	2.141	-9.855	0.917
674	-5.465	3.958	-9.305	1.186
675	-6.071	5.594	-8.163	0.899
676	-6.749	6.808	-6.720	1.105
677	-5.024	7.907	-6.692	0.941
678	-6.590	8.740	-5.972	0.974
679	-5.522	9.511	-4.374	1.097
680	-5.248	10.964	-3.027	0.904
681	-5.012	10.389	-1.095	1.126
682	-2.968	10.167	-1.311	0.942
683	-2.414	10.253	-3.019	0.855
684	-1.350	9.661	-1.847	0.835
685	-0.647	11.099	-0.872	1.039
686	0.420	10.257	0.493	0.888
687	1.728	9.349	1.360	0.925
688	2.682	10.570	0.340	0.930
689	2.446	10.319	-1.676	1.115
690	4.224	9.994	-0.623	0.977
691	5.566	10.171	-1.961	0.927
692	6.694	8.816	-2.711	0.989
693	7.972	7.239	-2.993	1.059
694	6.474	5.981	-3.416	0.942
695	7.810	6.212	-4.875	1.050
696	8.617	4.362	-5.825	1.180
697	8.347	2.724	-7.305	1.044
698	10.219	3.424	-6.942	0.986
699	10.421	3.560	-5.019	0.952
700	8.984	2.883	-4.190	0.840
701	6.678	4.045	-6.945	1.082
702	4.725	3.214	-6.676	1.058
703	4.727	1.719	-8.054	0.975
704	6.600	0.671	-8.171	1.175
705	8.347	0.660	-7.112	0.869
706	10.095	0.130	-6.963	0.964
707	11.530	1.089	-6.112	0.960
708	11.723	-0.847	-6.421	1.009
709	11.158	-2.173	-4.942	1.056
710	11.180	-1.206	-0.107	1.065
711	11.608	0.097	1.467	1.022
712	11.459	1.316	3.072	0.999
713	10.161	2.171	4.200	0.922
714	8.883	3.653	4.506	1.059
715	7.184	4.116	5.777	1.112
716	5.405	3.674	6.947	1.062
717	4.190	2.469	7.889	0.892
718	5.998	1.999	7.947	0.977
719	4.651	0.933	8.549	0.843
720	3.330	1.840	9.442	0.992

I	X	Y	Z	R
721	1.738	2.529	9.885	0.798
722	0.179	3.243	9.447	0.973
723	1.947	4.301	9.246	1.098
724	2.275	5.993	7.838	1.128
725	4.167	6.421	6.828	1.059
726	5.464	5.760	5.563	0.870
727	5.782	7.614	5.294	1.030
728	4.374	8.583	4.342	0.977
729	2.577	8.956	5.267	1.061
730	3.081	10.011	3.497	1.060
731	4.794	9.962	2.630	0.861
732	6.194	9.019	3.452	1.017
733	7.505	7.545	3.291	0.962
734	7.265	6.313	4.802	1.003
735	9.064	5.709	3.940	1.081
736	9.167	7.009	2.204	1.091
737	9.435	7.728	0.275	0.985
738	8.917	9.341	-0.617	0.930
739	7.785	10.432	0.413	0.949
740	6.115	10.768	-0.039	0.814
741	4.609	11.213	0.915	1.023
742	6.364	10.728	1.884	1.039
743	7.983	9.450	2.419	1.093
744	7.917	9.041	4.438	0.968
745	6.223	9.730	5.488	1.140
746	4.562	8.795	6.202	0.895
747	5.592	7.720	7.221	0.909
748	6.033	5.961	7.231	0.904
749	7.119	4.470	7.926	1.066
750	7.777	5.868	6.654	0.935
751	9.140	4.602	6.274	0.964
752	8.644	2.760	6.283	0.943
753	9.466	1.084	5.740	1.001
754	9.134	0.315	7.627	1.064
755	8.777	-1.679	8.361	1.090
756	10.161	-2.817	7.337	0.973
757	10.145	-2.953	5.455	0.913
758	10.723	-1.346	5.001	0.854
759	10.563	-2.406	3.500	0.991
760	11.923	-2.012	1.891	1.152
761	11.999	-4.000	0.753	1.140
762	10.979	-5.774	0.342	0.947
763	9.172	-6.547	0.065	1.038
764	8.854	-6.222	-1.826	0.906
765	7.189	-7.317	-1.811	1.087
766	6.233	-7.836	-3.655	1.054
767	5.586	-8.436	-1.943	0.872
768	3.700	-8.560	-1.722	1.030
769	3.957	-8.288	-0.003	0.729
770	2.815	-8.472	1.355	1.054
771	4.289	-8.130	2.431	0.802
772	6.143	-8.447	2.614	1.088
773	4.941	-8.088	4.168	0.910
774	6.278	-6.931	4.878	0.996
775	8.239	-7.574	4.477	1.106
776	9.788	-6.252	3.899	1.011
777	9.485	-4.689	4.633	0.741
778	8.815	-5.364	6.087	0.996
779	9.206	-4.550	7.788	0.929
780	8.684	-6.305	7.731	0.903

I	X	Y	Z	R
781	7.015	-6.978	7.902	0.905
782	5.779	-7.927	6.914	0.940
783	4.430	-8.380	8.361	1.089
784	3.711	-6.507	8.600	0.932
785	2.304	-5.836	9.823	1.050
786	3.928	-4.456	9.854	1.083
787	5.345	-5.825	9.288	0.967
788	6.901	-4.912	10.161	1.037
789	5.528	-3.427	11.104	1.194
790	6.103	-1.686	10.207	0.847
791	8.058	-2.297	10.466	1.218
792	7.406	-0.438	9.390	1.027
793	6.087	1.098	9.734	1.026
794	4.278	0.386	10.651	1.124
795	3.305	-1.557	10.907	1.064
796	1.368	-1.077	11.329	0.976
797	-0.180	-1.862	10.945	0.801
798	-1.281	-3.084	11.616	0.975
799	-1.779	-4.613	10.665	0.893
800	-3.275	-3.429	9.992	1.131
801	-4.251	-2.841	8.258	0.944
802	-4.745	-1.719	9.675	0.930
803	-3.163	-1.454	10.688	0.966
804	-1.464	-0.742	11.014	0.904
805	-1.399	1.137	11.537	1.048
806	-1.243	2.411	10.227	0.787
807	-1.644	4.015	9.843	0.910
808	-2.218	5.056	8.506	0.879
809	-2.722	6.646	7.694	0.975
810	-1.516	7.931	6.776	1.012
811	-0.342	8.863	5.719	0.822
812	0.322	10.030	4.334	1.107
813	-1.490	9.382	3.945	0.857
814	-3.261	9.637	3.762	0.941
815	-3.478	11.101	2.556	0.968
816	-3.834	10.789	0.639	1.008
817	-1.937	10.843	0.559	0.893
818	-1.748	11.602	2.149	0.879
819	0.955	11.961	-0.020	0.970
820	-3.697	11.869	-1.759	0.963
821	-6.923	11.754	-3.304	0.969
822	-7.551	10.185	-4.394	1.041
823	-8.777	8.821	-3.316	1.087
824	-9.314	6.965	-3.669	0.877
825	-9.817	5.313	-3.771	0.853
826	-9.528	3.539	-4.296	1.018
827	-8.776	1.964	-5.484	1.093
828	-8.581	1.178	-3.292	1.244
829	-9.095	-0.681	-2.189	0.978
830	-9.227	1.002	-1.141	1.009
831	-7.938	2.343	-1.526	0.890
832	-8.459	2.728	0.159	0.914
833	-9.379	2.297	1.882	1.086
834	-9.206	4.059	1.068	0.862
835	-9.181	5.880	0.465	1.057
836	-9.375	7.697	-0.524	1.022
837	-9.695	6.321	-1.958	0.991
838	-9.367	4.323	-1.467	1.093
839	-11.206	4.034	-2.870	1.238
840	-10.951	4.634	-4.872	0.867

I	X	Y	Z	R
841	-10.205	6.445	-5.439	1.172
842	-8.572	7.659	-5.113	0.889
843	-8.233	8.226	-6.830	0.951
844	-6.604	8.579	-8.059	1.120
845	-4.901	7.204	-8.768	1.181
846	-3.243	7.097	-7.512	0.903
847	-1.624	6.461	-7.896	0.878
848	-0.912	4.919	-8.949	1.120
849	-0.957	3.338	-10.555	1.134
850	-2.745	4.339	-10.125	0.959
851	-2.316	6.313	-9.897	1.074
852	-0.720	7.579	-9.217	1.074
853	0.339	8.412	-7.746	0.920
854	0.157	9.027	-6.098	0.849
855	-0.383	9.863	-4.711	0.858
856	0.791	10.203	-3.235	1.059
857	2.811	9.671	-3.756	1.094
858	3.704	8.740	-5.321	0.933
859	5.028	7.620	-6.223	1.022
860	5.500	9.046	-4.994	0.918
861	6.233	10.497	-3.828	1.082
862	7.513	10.862	-2.048	1.141
863	4.161	11.288	-1.936	0.868
864	1.083	11.003	2.090	0.995
865	1.740	11.509	3.892	0.989
866	3.055	10.999	5.349	1.039
867	3.270	9.830	6.867	0.889
868	3.776	8.232	7.988	1.127
869	2.123	7.810	6.834	0.933
870	0.539	7.865	7.758	0.901
871	0.817	6.692	9.270	1.033
872	-0.785	7.704	8.951	0.888
873	-0.846	6.211	9.546	0.720
874	-0.091	4.936	10.345	0.963
875	1.154	3.700	10.984	0.905
876	1.704	1.973	11.607	1.012
877	0.893	-2.870	11.945	0.979
878	-0.010	-4.597	11.596	1.001
879	-0.314	-5.558	9.813	1.047
880	-1.435	-6.893	8.687	1.028
881	-2.478	-6.582	10.626	1.196
882	-4.541	-5.730	9.978	1.127
883	-6.302	-6.707	9.391	0.971
884	-7.834	-5.374	9.146	1.074
885	-8.904	-4.418	7.837	0.869
886	-8.458	-3.426	6.403	0.930
887	-8.760	-4.872	5.151	1.005
888	-9.619	-5.636	3.624	0.906
889	-9.712	-3.836	2.801	1.075
890	-9.123	-3.407	0.837	1.020
891	-9.310	-3.214	-1.079	0.914
892	-9.459	-5.102	-0.500	1.067
893	-9.036	-7.027	-0.010	0.964
894	-7.776	-8.734	0.248	1.174
895	-9.152	-8.066	1.835	1.030
896	-9.384	-7.835	3.706	0.869
897	-8.712	-9.572	3.241	1.051
898	-6.709	-10.043	3.607	1.039
899	-6.353	-10.021	1.747	0.854
900	-5.066	-11.196	0.876	1.094

I	X	Y	Z	R
901	-4.339	-11.801	2.770	1.023
902	-2.854	-11.419	4.134	1.030
903	-1.335	-11.433	2.854	0.956
904	-0.349	-10.643	1.372	0.991
905	-0.053	-9.674	-0.264	0.934
906	0.350	-9.896	-2.277	1.131
907	1.904	-9.721	-0.719	1.076
908	1.934	-10.183	1.179	0.878
909	2.779	-9.923	2.957	1.107
910	2.821	-10.505	4.872	0.895
911	4.255	-9.838	5.762	0.919
912	3.009	-10.260	7.082	0.943
913	1.598	-9.080	7.809	1.034
914	-0.479	-9.004	7.833	1.044
915	0.633	-7.611	8.758	0.964
916	2.520	-7.871	9.386	1.042
917	3.087	-10.006	9.184	1.176
918	4.852	-10.436	7.867	1.068
919	6.441	-9.121	8.368	1.054
920	7.556	-8.402	6.934	0.900
921	6.712	-8.911	5.238	1.062
922	5.572	-9.691	3.951	0.826
923	7.256	-9.795	3.569	0.904
924	8.138	-8.483	2.682	0.909
925	7.386	-8.694	0.842	1.090
926	5.739	-7.746	-0.132	1.046
927	5.024	-9.602	-0.596	0.996
928	4.827	-10.071	-2.559	1.032
929	6.826	-9.619	-2.762	1.027
930	8.010	-8.280	-3.243	0.824
931	7.968	-6.501	-3.545	0.981
932	7.170	-6.933	-5.306	1.000
933	5.414	-7.736	-6.155	1.110
934	3.438	-7.055	-6.949	1.126
935	3.702	-7.685	-4.957	0.980
936	4.987	-9.308	-4.642	1.113
937	7.040	-9.333	-4.697	0.941
938	8.675	-10.009	-3.709	1.086
939	8.545	-9.225	-1.801	0.981
940	9.550	-7.770	-2.550	0.939
941	10.839	-7.242	-1.069	1.094
942	11.981	-5.392	-1.409	1.106
943	11.719	-3.428	-1.926	0.941
944	10.572	-4.412	-3.171	1.016
945	10.551	-2.458	-6.937	1.049
946	8.712	-3.356	-7.881	1.205
947	6.441	-3.906	-8.382	1.185
948	5.273	-5.380	-9.523	1.015
949	4.231	-6.904	-8.966	0.913
950	2.860	-6.104	-9.943	0.951
951	2.015	-4.257	-10.897	1.292
952	2.507	-1.952	-10.573	1.087
953	0.630	-1.732	-11.005	0.852
954	-2.798	-3.685	-10.572	0.837
955	-3.422	-5.010	-9.501	0.977
956	-2.404	-5.547	-10.815	0.769
957	-1.075	-4.783	-11.550	0.932
958	-0.626	-6.812	-11.827	1.164
959	0.160	-7.947	-10.070	1.070
960	0.418	-7.755	-8.083	0.943

I	X	Y	Z	R
961	0.652	-8.123	-6.163	1.026
962	0.063	-9.419	-4.530	1.141
963	2.173	-9.305	-3.569	1.180
964	2.214	-11.417	-2.318	1.275
965	0.350	-11.617	-0.817	1.126
966	-1.665	-11.691	-0.130	1.004
967	-1.720	-8.767	-10.173	0.984
968	-3.466	-7.767	-9.422	1.163
969	-4.192	-6.910	-7.817	0.796
970	-5.213	-7.625	-6.697	0.880
971	-4.396	-6.096	-6.202	0.922
972	-6.378	-6.258	-5.882	1.092
973	-5.414	-4.547	-6.421	0.944
974	-7.289	-4.369	-5.922	1.005
975	-8.790	-5.472	-4.877	1.131
976	-8.669	-7.223	-3.782	0.938
977	-9.124	-6.674	-1.984	0.997
978	-9.355	-8.562	-2.570	0.994
979	-8.551	-9.057	-4.290	0.968
980	-6.830	-8.308	-4.926	1.014
981	-5.148	-9.314	-4.906	0.946
982	-3.276	-9.891	-5.259	1.045
983	-4.712	-9.818	-6.801	1.063
984	-3.145	-9.702	-8.291	1.102
985	-1.564	-9.772	-6.683	1.154
986	-0.119	-9.635	-8.310	1.026
987	1.534	-8.908	-9.013	0.912
988	0.494	-9.940	-10.168	0.953
989	1.634	-7.378	-11.660	1.172
990	3.224	-5.938	-11.832	0.980
991	4.674	-6.792	-10.812	0.988
992	6.192	-7.329	-9.415	1.143
993	6.991	-7.233	-7.443	0.988
994	8.368	-6.044	-6.640	1.001
995	8.468	-4.312	-5.650	0.996
996	10.653	-4.258	-5.609	1.190
997	11.897	-5.358	-4.266	0.946
998	11.057	-9.005	-2.240	1.034
999	9.901	-10.828	-2.050	1.133
1000	7.790	-11.456	-2.348	1.088
1001	5.906	-11.359	-1.274	1.083
1002	6.354	-10.529	0.615	1.028
1003	4.718	-9.825	1.739	1.078
1004	3.324	-11.105	1.528	0.826
1005	1.741	-11.717	2.340	1.056
1006	1.032	-10.995	4.213	1.073
1007	-0.474	-10.870	5.774	1.100
1008	-1.750	-9.394	6.362	0.938
1009	-2.632	-8.406	7.785	1.005
1010	-3.289	-7.020	8.798	0.833
1011	-4.773	-7.852	9.300	0.941
1012	-6.323	-8.295	8.269	0.973
1013	-6.480	-7.041	6.872	0.910
1014	-8.050	-6.895	7.843	0.941
1015	-8.213	-7.420	9.823	1.114
1016	-7.172	-5.990	11.066	1.048
1017	-6.186	-4.380	10.232	1.016
1018	-5.870	-3.308	8.803	0.798
1019	-7.214	-2.777	9.696	0.900
1020	-6.761	-1.031	10.098	0.948

I	X	Y	Z	R
1021	-5.126	-0.155	10.808	1.039
1022	-3.829	1.020	11.578	0.873
1023	-5.084	4.189	11.657	1.027
1024	-3.310	4.635	10.874	0.962
1025	-1.248	3.136	11.838	0.980
1026	-7.421	0.687	11.006	1.104
1027	-7.825	2.825	10.840	1.078
1028	-7.904	2.464	8.911	0.887
1029	-8.852	2.319	7.067	1.192
1030	-9.267	3.772	5.419	1.044
1031	-9.700	3.321	3.577	0.901
1032	-10.090	5.126	4.099	1.019
1033	-8.751	6.776	4.235	1.110
1034	-7.917	8.873	3.912	1.170
1035	-6.054	8.798	3.366	0.773
1036	-6.142	9.605	1.863	0.936
1037	-5.221	10.498	3.382	1.053
1038	-4.563	9.834	5.316	1.096
1039	-6.096	8.323	6.078	1.187
1040	-4.121	7.624	6.419	0.936
1041	-5.164	6.219	6.763	0.848
1042	-5.930	5.488	8.033	0.805
1043	-4.581	6.270	8.955	1.006
1044	-5.455	5.925	10.710	0.985
1045	-5.134	11.723	1.786	0.961
1046	-6.541	11.019	0.425	1.120
1047	-7.568	11.539	-1.297	0.951
1048	-8.459	10.966	-2.748	0.845
1049	-9.287	9.790	-1.364	1.151
1050	-9.480	9.306	0.769	1.045
1051	-8.881	7.582	1.596	0.959
1052	-9.496	6.029	2.375	0.884
1053	-10.560	5.023	1.455	0.845
1054	-10.990	3.251	0.692	1.132
1055	-11.043	2.873	2.604	0.817
1056	-10.321	1.498	3.593	1.024
1057	-9.878	-0.540	3.313	1.080
1058	-9.934	-0.773	1.323	0.925
1059	-10.557	-2.450	1.679	0.899
1060	-11.092	-4.381	1.088	1.191
1061	-10.531	-6.200	2.095	0.962
1062	-11.522	-4.930	3.434	1.133
1063	-10.643	-5.668	5.283	1.044
1064	-10.145	-3.987	6.071	0.879
1065	-9.977	-2.700	7.650	1.166
1066	-8.666	-1.573	6.456	0.936
1067	-8.019	-2.400	4.877	0.961
1068	-10.090	-1.999	4.989	1.152
1069	-10.057	-0.061	6.202	1.134
1070	-8.822	-0.329	7.772	0.882
1071	-8.794	-0.907	9.697	1.128
1072	-7.944	-2.042	11.341	1.044
1073	-6.011	-2.607	11.133	0.981
1074	-4.158	-2.727	11.785	0.987
1075	-9.916	-1.870	8.113	1.039
1076	-11.643	-1.349	7.024	1.068
1077	-11.748	-3.338	6.677	0.954
1078	-11.172	-5.058	7.048	0.898
1079	-10.494	-4.454	8.588	0.890
1080	-9.091	-3.968	9.476	0.840

I	X	Y	Z	R
1081	-9.695	-5.562	9.714	0.881
1082	-9.896	-7.060	8.533	1.037
1083	-8.712	-8.758	8.178	1.064
1084	-9.165	-7.720	6.262	1.162
1085	-9.054	-9.704	5.304	1.044
1086	-6.971	-10.035	6.038	1.189
1087	-5.177	-9.939	7.380	1.054
1088	-4.114	-11.016	6.062	0.953
1089	-5.404	-11.772	4.628	1.119
1090	-6.165	-11.645	2.858	0.812
1091	-7.987	-11.473	2.861	1.018
1092	-9.440	-11.179	4.088	0.906
1093	-10.441	-9.738	4.014	0.850
1094	-11.077	-8.336	2.773	1.128
1095	-10.053	-9.869	1.733	0.988
1096	-9.925	-8.758	0.115	0.979
1097	-10.796	-7.273	-0.962	1.052
1098	-10.710	-5.576	-1.986	0.933
1099	-10.436	-3.706	-2.626	1.062
1100	-9.738	-2.782	-4.082	0.800
1101	-9.647	-3.674	-5.650	1.007
1102	-9.333	-1.609	-5.503	1.087
1103	-9.906	-0.301	-4.114	0.905
1104	-10.582	1.528	-4.388	1.064
1105	-10.631	2.958	-5.966	1.066
1106	-9.118	4.088	-6.004	0.823
1107	-7.596	4.461	-6.842	0.954
1108	-8.938	3.096	-7.687	1.139
1109	-7.984	1.180	-8.144	1.049
1110	-7.027	0.322	-9.915	1.139
1111	-7.323	2.435	-9.641	1.013
1112	-5.935	2.777	-11.274	1.158
1113	-4.028	1.762	-11.705	1.046
1114	-2.319	1.690	-10.575	1.004
1115	-0.911	1.770	-11.934	0.955
1116	1.700	1.653	-11.404	0.913
1117	2.611	3.018	-10.743	0.856
1118	4.028	3.775	-9.981	0.922
1119	3.269	5.535	-9.767	1.006
1120	2.181	7.074	-9.153	0.977
1121	2.383	7.544	-7.243	1.000
1122	2.144	9.167	-6.236	0.925
1123	0.929	10.125	-7.292	0.948
1124	-0.678	9.634	-7.296	0.733
1125	-1.281	8.148	-6.935	0.911
1126	-2.991	8.793	-6.242	1.044
1127	-4.125	9.148	-7.975	1.058
1128	-5.620	10.045	-6.990	0.944
1129	-7.469	10.480	-6.444	1.032
1130	-5.758	11.560	-5.442	1.225
1131	-4.038	10.866	-4.379	0.913
1132	-2.185	10.745	-4.780	0.987
1133	-0.480	11.677	-4.645	0.960
1134	1.202	10.768	-5.207	1.033
1135	-0.396	10.728	-6.153	0.824
1136	-0.583	11.348	-7.981	1.115
1137	0.214	9.768	-8.975	0.914
1138	-1.739	9.421	-8.753	1.082
1139	-3.370	8.492	-9.915	1.125
1140	-5.586	9.044	-10.049	1.163

I	X	Y	Z	R
1141	-6.814	7.343	-9.719	0.960
1142	-6.873	5.456	-9.800	0.929
1143	-7.562	6.339	-8.485	0.798
1144	-8.751	6.085	-7.087	1.054
1145	-10.106	7.684	-7.282	1.051
1146	-10.220	8.698	-5.410	1.081
1147	-9.517	10.318	-4.447	0.930
1148	-8.531	11.954	-4.390	0.981
1149	-7.812	9.117	-9.813	1.077
1150	-6.618	10.661	-8.841	1.104
1151	-4.703	11.012	-8.292	0.919
1152	-3.013	10.859	-7.061	1.178
1153	-10.719	9.655	-3.025	1.047
1154	-10.985	7.774	-3.733	0.980
1155	-10.671	7.919	-1.902	0.883
1156	-11.208	6.584	-0.505	1.123
1157	-11.057	8.588	-0.151	0.918
1158	-10.821	10.396	-0.135	0.906
1159	-9.152	11.243	0.213	0.998
1160	-11.725	7.653	-6.181	0.907
1161	-11.361	6.144	-7.179	0.939
1162	-10.870	6.742	1.485	0.902
1163	-10.858	6.860	3.389	1.006
1164	-10.529	6.640	5.346	0.991
1165	-10.656	4.917	5.814	0.799
1166	-10.988	3.741	4.814	0.780
1167	-10.802	2.784	6.265	0.968
1168	8.300	11.619	1.996	1.095
1169	9.419	10.155	1.231	0.900
1170	10.145	8.628	1.722	0.861
1171	11.006	7.069	1.254	0.980
1172	11.364	5.246	0.307	1.105
1173	11.015	4.029	-2.945	0.903
1174	11.793	2.871	-4.039	0.870
1175	9.140	11.252	-0.717	0.997
1176	9.411	10.105	-2.216	0.910
1177	9.766	8.098	-2.068	1.134
1178	9.866	6.004	-3.106	1.205
1179	8.297	5.783	-1.482	1.064
1180	9.318	7.629	-4.560	1.043
1181	7.489	8.541	-4.515	1.001
1182	8.132	7.710	-6.156	0.948
1183	9.685	6.350	-6.265	1.120
1184	7.828	5.972	-6.745	0.836
1185	6.096	5.956	-7.013	0.917
1186	5.046	4.726	-7.999	0.977
1187	4.390	6.632	-8.396	1.078
1188	4.009	8.363	-7.617	0.858
1189	4.731	9.798	-6.620	1.032
1190	3.250	10.550	-5.544	0.947
1191	2.635	11.750	-4.109	1.022
1192	8.962	4.484	-7.879	0.907
1193	9.629	3.072	-8.609	0.817
1194	7.948	3.495	-9.155	1.000
1195	6.062	2.496	-9.541	1.169
1196	4.482	1.906	-11.136	1.152
1197	3.736	-0.083	-11.664	1.037
1198	4.441	-1.516	-10.576	0.896
1199	5.143	-1.541	-8.951	0.875
1200	4.453	-3.092	-9.617	0.949

I	X	Y	Z	R
1201	6.061	-3.789	-10.585	1.054
1202	7.133	-5.205	-9.826	0.877
1203	7.926	-3.587	-9.848	0.926
1204	7.324	-1.925	-8.949	1.057
1205	8.463	-0.245	-8.757	0.982
1206	6.999	-0.239	-10.147	1.037
1207	7.719	1.400	-9.653	0.820
1208	9.187	2.327	-10.210	1.003
1209	9.550	4.177	-9.926	0.904
1210	10.802	4.579	-8.498	1.037
1211	11.498	5.047	-6.504	1.126
1212	11.529	2.482	-8.393	1.184
1213	11.703	0.134	-8.367	1.171
1214	4.968	9.048	-3.132	0.802
1215	7.310	5.407	-8.501	1.080
1216	8.470	7.183	-8.086	1.081
1217	9.774	8.335	-6.968	0.988
1218	9.027	9.473	-5.472	1.036
1219	8.081	10.188	-4.062	0.806
1220	7.705	11.875	-3.849	0.934
1221	7.351	10.965	-5.501	0.984
1222	6.891	9.212	-6.298	0.996
1223	5.764	8.614	-7.559	0.798
1224	5.007	8.452	-9.178	0.997
1225	3.739	7.415	-10.352	1.018
1226	1.971	6.496	-11.177	1.138
1227	0.955	8.097	-10.262	0.967
1228	-0.607	9.115	-10.464	0.908
1229	-1.253	7.497	-11.204	0.985
1230	-3.280	7.124	-11.716	1.139
1231	-4.277	5.840	-10.339	0.991
1232	-4.301	4.154	-10.874	0.777
1233	8.437	4.490	-5.596	0.920
1234	11.501	3.553	3.016	1.239
1235	11.121	0.432	4.894	0.969
1236	11.060	-0.511	6.588	0.971
1237	10.820	1.326	7.140	0.961
1238	9.385	2.297	7.979	0.965
1239	7.752	1.567	7.925	0.824
1240	7.319	2.826	9.255	1.057
1241	5.689	3.527	8.764	0.783
1242	4.094	4.296	8.437	1.018
1243	3.605	3.597	10.200	0.941
1244	2.587	4.983	11.153	1.026
1245	2.805	6.584	9.859	1.044
1246	4.469	6.468	8.797	0.934
1247	6.177	6.935	8.672	0.841
1248	5.546	8.478	8.785	0.829
1249	5.607	9.467	7.429	0.851
1250	7.107	8.680	6.995	0.899
1251	8.092	9.740	6.072	0.818
1252	7.804	10.963	4.775	0.989
1253	9.146	-1.798	-5.187	0.970
1254	-2.663	-7.107	-11.439	0.931
1255	-4.109	-5.869	-11.139	0.996
1256	-4.491	-1.637	-11.146	1.010
1257	-8.520	-7.454	-5.754	1.053
1258	-7.007	-7.843	-7.014	0.955
1259	-6.012	-6.504	-7.824	0.900
1260	-5.254	-7.821	-8.598	0.806

I	X	Y	Z	R
1261	-4.912	-9.412	-9.443	1.027
1262	-3.491	-9.360	-10.931	1.031
1263	-2.191	-10.848	-9.989	1.158
1264	-2.151	-11.654	-7.844	1.134
1265	-3.663	-11.508	-6.393	0.968
1266	-4.759	-11.220	-4.779	1.004
1267	-3.812	-10.251	-3.371	0.951
1268	-3.173	-11.804	-2.232	1.078
1269	-1.919	-10.890	-3.732	1.079
1270	0.321	-11.596	-3.508	0.968
1271	1.493	-10.865	-4.778	0.908
1272	3.504	-10.986	-4.442	1.135
1273	5.527	-11.190	-4.024	0.941
1274	3.995	-11.066	-1.086	0.859
1275	-8.882	-4.987	-6.927	0.977
1276	-8.360	-3.205	-6.947	0.879
1277	-6.488	-2.665	-6.803	1.074
1278	-5.752	-3.974	-8.289	1.039
1279	-4.653	-3.732	-9.764	0.816
1280	-6.203	-2.582	-10.199	1.163
1281	-7.320	-1.423	-8.937	0.883
1282	-7.918	-1.342	-7.236	0.922
1283	-9.727	-2.012	-7.545	1.031
1284	-11.140	-2.429	-6.148	1.000
1285	-11.747	-2.345	-3.974	1.259
1286	-10.965	-0.751	-2.658	0.951
1287	-11.135	-2.209	-1.125	1.170
1288	-11.011	0.000	-0.783	1.068
1289	-11.842	0.212	3.054	1.039
1290	-11.956	5.575	4.291	0.910
1291	6.328	10.892	-7.180	0.983
1292	5.453	11.312	-5.513	0.946
1293	11.003	7.781	-5.592	0.938
1294	11.102	7.608	-3.746	0.919
1295	11.064	9.522	-3.164	1.083
1296	10.776	9.582	-1.277	0.827
1297	11.140	8.035	-0.533	0.927
1298	10.857	10.240	2.686	1.147
1299	10.286	11.837	1.373	0.997
1300	5.151	5.206	-9.815	0.905
1301	4.211	5.006	-11.577	1.102
1302	-11.459	2.092	-1.162	1.104
1303	-11.576	0.855	4.770	0.813
1304	-11.462	-1.672	3.059	0.883
1305	-10.423	-3.902	-7.470	0.984
1306	-10.729	-5.429	-6.085	1.100
1307	-10.431	-6.801	-4.505	1.013
1308	-10.552	-8.835	-4.201	1.048
1309	-9.579	-10.224	-3.343	0.852
1310	-8.241	-10.150	-2.040	1.017
1311	-6.822	-11.412	-0.989	1.154
1312	-7.081	-11.429	-3.179	1.052
1313	-5.374	-11.879	-2.361	0.894
1314	9.081	-6.876	-5.051	0.929
1315	9.816	-6.247	-3.517	0.884
1316	10.818	-4.393	-7.933	1.143
1317	10.426	-2.607	-8.903	0.927
1318	9.016	-2.068	-10.022	0.952
1319	7.384	-2.232	-11.243	1.092
1320	5.445	-2.376	-11.864	0.950

I	X	Y	Z	R
1321	-7.561	6.610	6.051	1.068
1322	-8.011	5.445	8.035	1.276
1323	-8.765	3.958	9.477	0.928
1324	-7.316	4.873	10.488	1.061
1325	-2.691	9.245	5.436	0.871
1326	-1.468	9.913	6.646	0.975
1327	-2.134	9.091	8.445	1.112
1328	-2.469	7.364	9.391	0.885
1329	-3.851	7.946	8.429	0.897
1330	-4.256	9.297	7.254	0.938
1331	-5.875	10.374	6.909	1.037
1332	-6.510	10.107	5.120	0.880
1333	-7.300	11.029	3.556	1.100
1334	-8.375	10.504	5.330	1.039
1335	-9.181	10.443	3.600	0.870
1336	-2.197	10.783	4.724	0.895
1337	-0.638	11.402	5.629	1.010
1338	0.784	10.163	6.423	1.037
1339	4.282	11.681	3.836	1.025
1340	8.276	7.603	5.955	1.001
1341	9.674	8.999	5.422	1.046
1342	10.181	6.909	5.386	1.105
1343	11.019	6.555	3.344	1.131
1344	10.878	4.896	4.817	1.092
1345	10.600	3.197	6.122	1.068
1346	9.954	3.957	7.712	0.810
1347	9.091	5.500	7.998	0.980
1348	8.316	4.635	9.661	1.048
1349	6.314	4.823	9.756	0.965
1350	5.638	3.057	10.587	1.100
1351	7.037	10.715	7.271	1.053
1352	5.078	11.114	6.699	1.026
1353	3.290	11.569	7.161	0.875
1354	1.968	10.555	8.055	1.017
1355	4.099	10.437	8.727	1.220
1356	4.230	8.660	9.972	0.953
1357	2.329	8.547	9.520	1.004
1358	1.470	7.538	10.846	0.871
1359	-0.518	7.395	10.920	1.123
1360	-0.524	9.425	9.959	1.123
1361	-0.843	10.792	8.331	1.026
1362	9.491	8.571	3.464	0.942
1363	11.365	8.690	4.187	1.070
1364	11.668	10.984	-0.073	1.178
1365	10.732	11.665	-2.080	1.139
1366	-6.860	5.228	4.851	0.776
1367	10.867	5.496	6.905	1.080
1368	11.668	8.127	6.098	0.945
1369	10.071	8.017	7.116	0.952
1370	8.759	9.406	7.825	1.087
1371	7.995	7.413	8.241	1.087
1372	6.921	8.071	9.861	0.964
1373	5.560	6.685	10.463	1.069
1374	3.875	6.421	11.520	0.939
1375	4.528	4.599	11.580	0.998
1376	10.437	1.041	9.013	0.972
1377	10.576	-0.772	8.466	0.927
1378	10.480	-2.554	9.318	1.051
1379	10.971	-4.236	8.351	0.950
1380	11.306	-4.157	6.416	1.016

I	X	Y	Z	R
1381	9.948	-5.803	9.220	1.114
1382	8.043	-6.116	9.318	0.819
1383	8.480	-7.929	8.880	1.097
1384	9.348	-7.895	7.011	0.964
1385	10.424	-7.994	5.123	1.211
1386	9.229	-9.191	3.903	0.875
1387	8.613	-9.210	5.556	0.889
1388	7.452	-10.263	6.598	0.994
1389	5.673	-11.431	5.972	1.224
1390	3.519	-11.763	6.038	0.957
1391	1.709	-11.225	6.133	0.934
1392	10.264	-5.826	7.137	0.852
1393	11.973	-5.587	7.537	0.919
1394	11.645	-3.962	10.086	0.931
1395	11.352	-1.043	10.019	0.830
1396	10.195	-1.877	11.390	1.148
1397	8.864	-0.213	10.851	1.050
1398	7.067	-0.658	11.208	0.836
1399	5.622	-1.441	11.931	0.959
1400	6.665	-7.120	9.694	0.926
1401	4.799	-7.509	10.310	1.077
1402	5.033	-9.524	9.974	0.979
1403	4.665	-11.529	9.704	1.078
1404	3.467	-11.749	8.187	0.867
1405	3.509	-10.673	11.242	1.029
1406	2.088	-9.154	11.001	1.065
1407	1.439	-7.251	10.936	0.947
1408	-0.401	-6.674	11.661	1.113
1409	-8.973	8.291	5.876	1.135
1410	-9.988	8.962	4.214	0.925
1411	-10.506	9.692	2.503	1.007
1412	-4.006	11.164	6.828	0.993
1413	-5.371	11.757	5.504	0.998
1414	-5.786	7.777	8.231	1.055
1415	-7.287	9.134	7.754	1.023
1416	-7.732	7.543	9.020	1.058
1417	-7.208	6.926	10.966	1.050
1418	-9.510	4.159	11.398	1.142
1419	-9.735	2.449	10.149	0.988
1420	-9.129	1.563	11.781	0.966
1421	-10.481	0.442	10.534	1.188
1422	-8.983	-0.315	11.808	0.918
1423	9.499	10.620	6.458	0.886
1424	9.585	10.731	4.771	0.807
1425	6.071	11.400	3.521	0.755
1426	8.798	11.513	8.053	1.032
1427	7.625	10.406	9.093	0.886
1428	5.978	9.870	10.006	1.073
1429	6.029	11.783	8.898	1.139
1430	-7.815	11.093	7.268	1.063
1431	-5.166	11.541	8.193	0.838
1432	-5.288	9.850	8.721	0.939
1433	-3.766	9.296	9.666	0.936
1434	-4.025	7.580	10.186	0.876
1435	-5.131	7.653	11.802	1.084
1436	10.637	10.468	8.058	1.083
1437	11.050	9.927	6.279	0.822
1438	9.662	8.081	8.817	0.799
1439	9.138	6.627	9.969	1.129
1440	10.243	4.984	9.424	0.925

I	X	Y	Z	R
1441	10.499	3.216	9.136	0.885
1442	11.731	2.898	7.788	0.968
1443	7.609	3.472	11.182	0.995
1444	7.636	1.615	10.769	0.907
1445	6.048	1.386	11.673	0.934
1446	10.871	0.562	10.988	1.106
1447	8.686	0.878	9.356	0.809
1448	9.091	2.414	9.742	0.826
1449	9.741	3.737	10.713	0.938
1450	10.667	5.582	11.493	1.270
1451	8.336	5.425	11.627	1.071
1452	7.228	7.185	11.597	1.009
1453	7.218	9.134	11.422	0.948
1454	5.239	8.814	11.732	1.081
1455	3.988	10.363	10.987	1.044
1456	4.505	11.869	10.077	0.790
1457	-7.057	-7.648	-9.011	1.052
1458	-7.800	-6.349	-7.764	0.896
1459	-8.735	-7.854	-7.634	0.881
1460	-8.217	-9.336	-6.565	1.019
1461	-7.209	-10.308	-5.074	1.027
1462	-8.963	-10.691	-4.791	0.790
1463	-10.736	-10.817	-4.653	0.993
1464	-11.311	-10.156	-2.945	0.926
1465	-10.127	-9.990	-1.496	0.953
1466	-9.002	-10.445	-0.031	0.949
1467	-9.385	-11.729	1.406	1.016
1468	2.445	9.303	-8.080	0.949
1469	1.685	10.802	-8.825	0.890
1470	1.249	11.869	-7.532	0.842
1471	8.197	9.103	-7.671	0.902
1472	9.562	8.709	-8.855	0.947
1473	10.549	7.095	-8.336	1.014
1474	10.100	5.882	-9.742	0.896
1475	11.350	4.617	-10.496	1.035
1476	10.131	3.428	-11.526	0.955
1477	8.262	3.747	-11.212	0.966
1478	7.252	2.027	-11.333	1.033
1479	6.191	3.893	-11.342	1.114
1480	11.030	9.733	-6.104	1.080
1481	9.728	10.180	-7.625	0.971
1482	9.271	11.495	-6.099	1.095
1483	9.597	11.269	-3.983	1.057
1484	9.002	-5.526	-8.561	1.087
1485	8.317	-7.500	-8.952	1.039
1486	7.035	-9.243	-8.334	1.211
1487	4.825	-8.682	-8.322	1.069
1488	3.879	-9.054	-6.571	0.955
1489	5.690	-9.782	-6.573	0.996
1490	6.750	-11.053	-5.497	0.978
1491	8.296	-10.183	-5.545	0.797
1492	8.657	-8.626	-5.087	0.866
1493	9.939	-9.843	-5.242	0.908
1494	10.201	-11.507	-4.154	1.098
1495	-0.603	3.968	-5.185	0.890
1496	7.677	8.582	-9.467	1.039
1497	7.786	10.858	-8.936	1.300
1498	6.132	9.806	-10.368	1.128
1499	11.247	8.883	-8.059	0.925
1500	10.367	7.652	-10.217	0.956

I	X	Y	Z	R
1501	8.594	6.915	-10.113	0.968
1502	9.821	6.270	-11.795	1.212
1503	9.975	10.484	-9.566	1.009
1504	11.354	11.345	-7.439	1.038
1505	9.173	9.038	-10.658	0.927
1506	7.545	8.818	-11.339	0.851
1507	6.211	7.632	-10.641	1.065
1508	-8.828	-3.951	-8.529	0.931
1509	-9.943	-5.523	-8.502	0.996
1510	-11.741	-5.249	-7.844	0.937
1511	0.745	4.663	-10.306	1.037
1512	-9.081	8.440	7.909	0.906
1513	-9.975	7.003	7.163	0.943
1514	-11.697	6.003	6.912	1.063
1515	-6.860	9.286	9.749	1.022
1516	-8.347	10.216	8.948	0.906
1517	-9.304	8.730	9.868	1.086
1518	-9.003	6.155	10.072	1.098
1519	-10.027	5.061	8.753	0.898
1520	-11.231	3.933	9.932	1.131
1521	-11.778	-2.364	8.846	1.022
1522	-10.523	-0.041	8.170	0.889
1523	-11.119	1.738	8.780	1.084
1524	-11.829	0.776	7.065	1.007
1525	-8.090	0.184	-6.362	0.845
1526	-9.513	0.929	-6.994	0.880
1527	-11.478	0.909	-6.356	1.186
1528	-1.927	11.699	7.015	0.906
1529	-2.849	11.093	8.592	1.019
1530	-1.623	11.100	10.045	0.882
1531	8.741	-7.855	-7.091	0.902
1532	10.089	-6.811	-6.557	0.885
1533	10.913	-6.609	-8.371	1.118
1534	-6.249	-9.160	-8.078	0.900
1535	-5.619	-10.983	-8.300	1.041
1536	-6.912	-10.062	-9.730	1.096
1537	10.426	-7.234	1.582	1.047
1538	11.462	-5.549	2.260	1.044
1539	10.492	-7.683	8.541	0.958
1540	9.825	-7.485	10.190	0.832
1541	8.792	-6.110	11.099	1.113
1542	8.388	-10.150	8.528	1.154
1543	6.683	-11.414	8.059	1.018
1544	6.665	-10.498	9.781	0.932
1545	-0.802	-8.481	9.788	1.005
1546	-1.568	-8.197	11.421	0.821
1547	-4.401	-7.484	11.165	0.996
1548	-3.134	-8.647	10.077	1.039
1549	-2.004	-10.091	8.926	1.125
1550	-6.321	-8.440	10.162	0.925
1551	-4.891	-9.799	9.818	1.077
1552	-6.528	-10.076	8.768	0.888
1553	0.469	-10.029	-6.445	0.909
1554	2.064	-9.417	-5.758	0.931
1555	2.114	-10.295	-7.626	1.133
1556	-0.679	-11.254	-5.375	1.012
1557	9.192	-9.166	-8.315	0.948
1558	8.349	-9.096	-9.818	0.777
1559	7.703	-7.803	-10.907	1.033
1560	10.149	-8.119	-9.700	1.034

I	X	Y	Z	R
1561	9.275	-6.459	-10.354	0.953
1562	8.417	-4.906	-11.150	0.991
1563	8.434	-10.776	-9.170	1.026
1564	8.394	-10.477	-7.242	0.925
1565	6.599	-11.417	-7.583	1.130
1566	9.817	-8.284	0.166	0.818
1567	9.156	-9.084	1.427	0.815
1568	8.814	-10.616	2.564	1.124
1569	10.939	-8.766	3.002	1.104
1570	11.441	-7.018	3.651	0.827
1571	11.386	-5.913	5.209	1.083
1572	10.136	-9.655	6.586	1.018
1573	10.673	-10.180	4.669	1.035
1574	3.088	-7.053	10.836	0.717
1575	3.418	-5.583	11.388	0.888
1576	5.476	-5.749	11.661	1.194
1577	-8.830	-0.376	-9.095	0.961
1578	-8.797	1.281	-9.884	0.874
1579	-9.186	3.069	-9.770	0.959
1580	-7.868	-1.409	-10.660	0.925
1581	-8.546	0.249	-11.486	1.048
1582	-6.581	-0.511	-11.957	1.111
1583	-9.129	7.504	11.861	1.146
1584	-10.826	5.728	10.925	0.960
1585	-10.461	6.772	8.898	0.873
1586	-11.080	8.457	8.079	1.100
1587	-11.611	7.832	6.285	0.873
1588	-10.473	-3.902	-9.372	0.918
1589	-11.583	-2.492	-8.461	1.094
1590	-9.860	-2.120	-9.543	0.974
1591	-10.902	-6.956	-7.508	0.995
1592	-8.516	-6.396	-9.427	0.915
1593	-6.688	-5.591	-9.450	1.083
1594	-7.398	-6.961	-11.114	1.186
1595	-11.533	-5.529	-9.821	1.069
1596	-10.415	-7.240	-9.437	1.015
1597	-8.865	-8.476	-9.371	0.969
1598	9.834	1.089	-8.586	0.939
1599	9.539	0.308	-10.396	1.055
1600	8.193	-0.377	-11.800	1.007
1601	10.121	-0.812	-9.045	0.793
1602	11.571	-1.420	-9.816	0.958
1603	11.517	0.354	-10.442	0.924
1604	0.738	11.085	9.563	1.000