A COMPARISON OF BATCH & CONTINUOUS

CHROMATOGRAPHY EQUIPMENT FOR THE SEPARATION

OF ORGANIC MIXTURES

A thesis submitted by Kelvin England B.Sc., for the Degree of Doctor of Philosophy to the Faculty of Engineering, University of Aston in Birmingham.

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SUMMARY

A review of general chromatography theory with particular reference to gel permeation chromatography (g.p.c.) has been made. Equipment for the preparative and production scale applications of chromatography are described.

Large-scale g.p.c. fractionations of dextran, a poly-glucose have been investigated. Two methods of operation, by repetitive batch and semi-continuous chromatography have been evaluated. Both chromatographs consisted of ten columns of 5.1 cm id by 70 cm long, packed with Sperosil XOB075. Glass columns were used for the repetitive batch chromatograph and stainless steel for the semi-continuous chromatograph. The maximum operating pressures for the semi-continuous and repetitive batch chromatographs were 1550 and 480 kNm^{-2}, respectively. For equivalent operating pressures the maximum hourly feed rates for the semi-continuous and repetitive batch chromatography were 175 and 63 g h^{-1} of dextran. Comparison of the operating methods was principally made on the basis of a hypothetically ideal product and on the concentration of dextran in the final product streams.

Modification to the semi-continuous chromatograph enabled dextran fractionations to be performed at temperatures of up to 65°C. An increase in the dextran feedrate from 690 to 960 grams per cycle was possible when the operating temperature was increased to 62°C.

A description of the concentration and temperature interactions for the gel permeation process in the semi-continuous chromatograph are discussed.

A simple linear exclusion model attempting to describe the phenomena of gel permeation has been formulated and its application to describe the fractionation of dextran in the semi-continuous chromatograph has been made.

Only partial agreement between the experimental results and computer simulations was achieved because the model could not describe the concentration dependent interactions of the gel permeation process.

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Key words: Chromatography, semi-continuous, batch, dextran, gel permeation.

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1.0 INTRODUCTION

From about 1910 in the chemical industries where many chemical or certain physical changes of materials are involved the term "unit operations" has been used. This was as a result of a classification instituted by a group of engineering professors at Massachusetts Institute of Technology headed by H. W. Walker. The term unit operations was suggested by A. D. Little who had been an industrial partner of Walker. In this classification of unit operations are the classic separation processes such as distillation, solvent extraction and crystallisation. However in addition to these commonly used separation processes comparatively new processes such as electrodialysis, electrophoresis and ultrafiltration are increasing in importance. Although they are infrequently used they offer the engineer greater licence in the design of a process.

Research has also been focused on the development of novel separation processes. One such process is chromatography. The rapid growth of gas-liquid chromatography originates from the work of Martin and Synge (1), for which they were awarded the Nobel Prize in 1952, and subsequent work by James and Martin (2). This technique is a powerful means of resolving chemical species and is common place in most analytical and quality control laboratories. The universal acceptance of gas chromatography had led to the emergence of liquid chromatography and with new packing materials allowing faster analysis times, the previous drawbacks associated with liquid chromatography have now been overcome, and it appears that liquid chromatography will prove to be an equally powerful analytical technique.

Chromatography is the partitioning of different solutes between two phases, a mobile phase and a stationary phase by a variety of different mechanisms. The degree of retardation of a solute molecule by the stationary phase governs the rate at which the solute migrates. Therefore different solute species can be separated by a suitable choice of mobile

and stationary phases.

The growth of gas chromatography as an analytical tool was meteoric in the 1950's but the development of this technique into a large-scale unit operation has not been as rapid, although industrial scale examples are available in the literature (3,4). Direct scale-up of the analytical technique has taken place although several research workers consider that this method of operation has a limited potential for development and consequently a number of novel designs have been proposed, where the mobile and stationary phase appear to move in cross and counter flow directions. Cross-current schemes offer the advantage of the continuous separation of a multicomponent feed mixture but proposed designs for this equipment have not proved feasible on a large scale. In counter-current schemes the mobile and stationary phases move in opposite directions, where the feed mixture is usually introduced at the mid-point of the separating section. The solute having least affinity for the stationary phase migrates with the mobile solvent to emerge from one end of the separating section. Whilst the solute having a greater affinity for the stationary phase emerges from the other end of the separating section. The advantage of counter-current operations is that only partial resolution of components is required to achieve pure products at the product outlets, but the disadvantage as with continuous distillation, is that only two products are readily obtained.

Barker and co-workers (5-8) have demonstrated the viability of continuous counter-current chromatographs in the field of gas-liquid chromatography for the separation of a variety of volatile organic compounds. Prompted by the emergence of new packings for liquid chromatography Barker et al. (9-11) have investigated the use of large scale liquid chromatography to separate non-volatile organic mixtures.

Two types of liquid chromatography have been used, gel permeation and ion-exchange; this research programme is concerned with the former.

Gel permeation chromatography relies on the difference in molecular size of the solute molecules in solution between the mobile phase and the liquid contained within the pores of the porous material. The liquid in the pores and the mobile phase is the same. The permeation of the solute molecule into the porous material is governed by the variation of pore sizes and the size of the solute molecule. In gel permeation chromatography the terms separation and fractionation are often used interchangeably, but in this thesis the term separation is confined to the division of a mixture containing species of different chemical and physical properties; and the term fractionation is confined to the division of a mixture where the components are chemically similar but vary in molecular weight, such as the 'fractionation' of a polymer.

The present experimental programme investigated the fractionation of dextran, manufactured by Fisons Ltd. Pharmaceutical Division, Holmes Chapel. Dextran is a polymer of glucose (poly-α-1, 6-D-gluco-pyranose), and has pharmaceutical applications as a blood viscosity stabiliser, blood volume expander and in solubilizing iron for the treatment of anemia. For such uses the mean molecular weight and the molecular weight distribution are important. The method of manufacture involves fermentation of sucrose to produce a native dextran, of several million daltons, and its subsequent hydrolysis and fractionation under controlled conditions (12-14). At present the industrial fractionation of dextran is mainly by stagewise precipitation and redissolution of dextran from aqueous and organic solutions. This process reduces the distribution of the molecular weights of the dextran

molecules in the final product as indicated by Fig. 1.1.

Column gel permeation chromatography offers an attractive alternative to the present industrial fractionation technique. Earlier studies have reported work on the continuous and semi-continuous gel permeation fractionation of dextran with an aqueous eluent (9,10,15). This project was a continuation of this research, and was devoted to the maximisation of feed throughput. In the earlier studies of dextran fractionations by Ellison and Williams an arbitrary graphical method was used to represent the products. In this programme, use of molecular weight averages and the molecular weight distribution will be made to quantify the fractionation of the products. Consequently an improved analytical system was necessary and so the feasibility of using a high pressure liquid chromatograph was investigated. Specifications for ideal hypothetical product fractions are contained in Figure 1.2.

A semi-continuous chromatograph that has been used for removal of high molecular weight material at ambient temperatures was modified to operate at elevated temperatures of up to 65°C. The effects of elevated temperatures on the fractionating performance of the semi-continuous chromatograph will be discussed. The removal of low molecular weight material from a dextran feed was also attempted.

Fractionations performed on essentially idential columns but used in a repetitive batch chromatographic manner will enable a preliminary comparison of the two modes of operation to be discussed. A mathematical description of the semi-continuous chromatograph was attempted. In common with many other mass transfer processes occurring in a continuous contacting column the description could be simplified to a compartments-in-series model where the resulting state equations could be solved simultaneously using a high speed digital computer.

Fig 1.1 Reduction of dextran hydrolysate to a clinical dextran fraction.

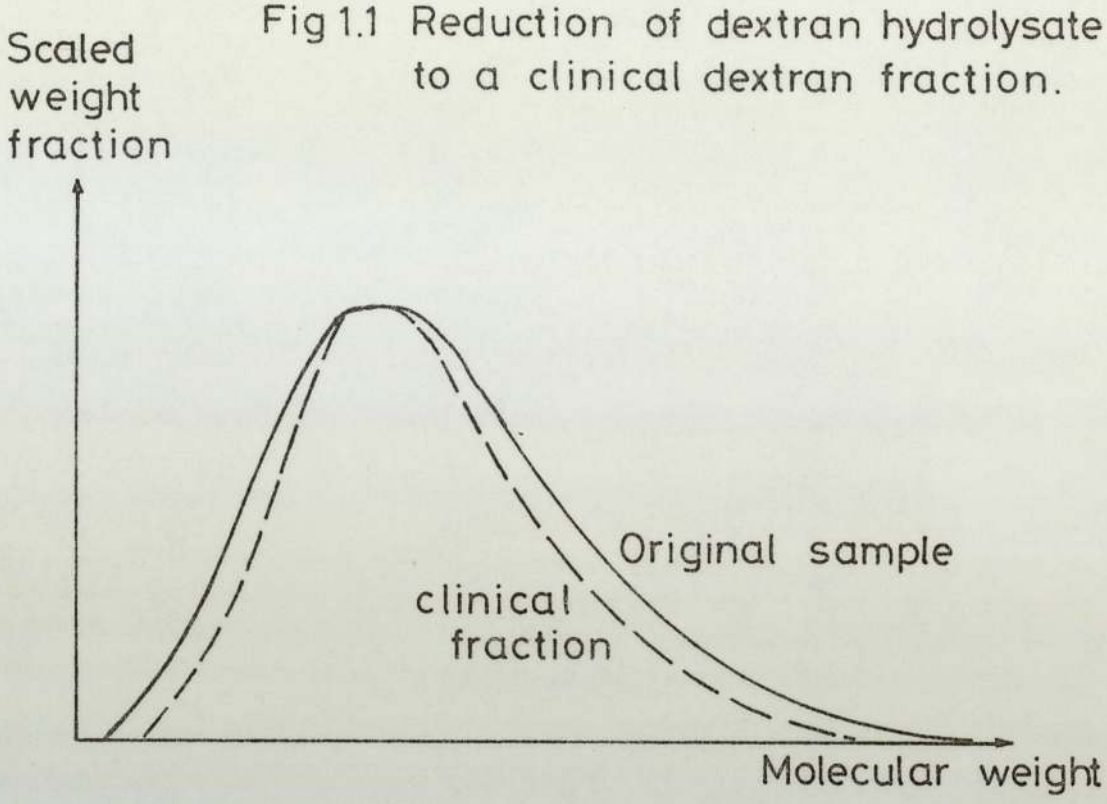


Fig 1.2 Specifications of desirable products.

Stream	$\bar{M}_w$	$\bar{M}_n$	D	% Removal
Repetitive batch feed	47,500	20,800	2·28	
Repetitive batch product	<40,000	>22,000	<1·80	10% top + bottom
SCCR5 feed batch BT161D	64,300	27,000	2·39	
High Mol. Wt. Removal	<50,000	<25,000	≈ 2·00	15% top
Low Mol. Wt. Removal	> 74,000	> 30,000	> 2·40	10% bottom
SCCR5 feed batch BT216G	32900	22 000	1·49	
Low Mol. Wt. Removal	>37,000	>23,800	≈ 1·55	10% bottom

2.0 GENERAL THEORIES IN CHROMATOGRAPHY AND GEL PERMEATION CHROMATOGRAPHY

2.1 SCOPE

Tswet (16) originally used the term chromatography to describe the separation of plant chromophores. Today chromatography is a much-used analytical technique and a preparative tool. The volume of publications in the general field of chromatography has made it necessary to be selective in the scope of the relevant literature. After the introduction of the various forms of liquid chromatography and of the basic terminology used, this review then discusses the principle theoretical models used to describe column elution chromatography. Practical details of gel permeation chromatography (g.p.c.) is then described, followed by the theories of the retention mechanisms that cause separation/fractionation and zone broadening. A review dealing with the commercial usage of the chromatographic processes in batch and continuous modes for preparative and production scale applications are contained in Chapter 3.

2.2 TYPES OF LIQUID CHROMATOGRAPHY

Classification of the various types of liquid chromatography are usually made by the retention mechanisms. It is usually considered that there are four main categories:

1) Adsorption Chromatography
2) Exclusion Chromatography
3) Ion-exchange Chromatography
4) Partition Chromatography

The mechanism of retention in adsorption chromatography depends on the solute associating with active sites on the column packing. These associations may be either physical or chemical. In recent times, appropriately charged ion-exchange resins have increasingly been used as a stationary phase although the mechanism of retention does not

involve the exchange of ions. Such separations are known as ligand exchange. Exclusion chromatography is the separation of molecules by virtue of their molecular size in solution; very large molecules cannot enter the pores of the packing and are eluted first in a volume equal to the voids in the column; very small molecules can permeate all the pore in the packing and are the last to be eluted; molecules between these two sizes are eluted at volumes between these two limits. Ion-exchange chromatography relies on reversible exchanges of ions between the stationary phase and electrolytes, separation being achieved by the different affinity of the solute ions for the resin. Finally, partition chromatography relies on the adsorption of solutes by an inert solid support coated with a liquid stationary phase.

2.3 SAMPLE APPLICATIONS

Further to the retention mechanisms (Section 2.2) the way in which the sample is introduced into a packed-bed and its mode of migration through the bed may be used as a means of classification. Generally three modes of operation are used, namely elution, frontal analysis, and displacement. In displacement chromatography the mobile phase is much more strongly retained by the stationary phase than the sample. The sample is therefore 'pushed' through the bed by advancing mobile phase. Displacement provides poorer separations than elution chromatography, but greater sample loads can be applied to the bed, therefore, it has certain advantages in preparative or production scale chromatography. In frontal analysis, the sample is introduced as a step-change, being fed continuously on to the column and so the sample constitutes the mobile phase. The components are selectively retarded with the formulation of fronts, see Fig. 2.1. The least retained component, A, is eluted first alone, and then followed by the mixture of A and B.

Fig 2.1 Example of frontal analysis for a binary system.

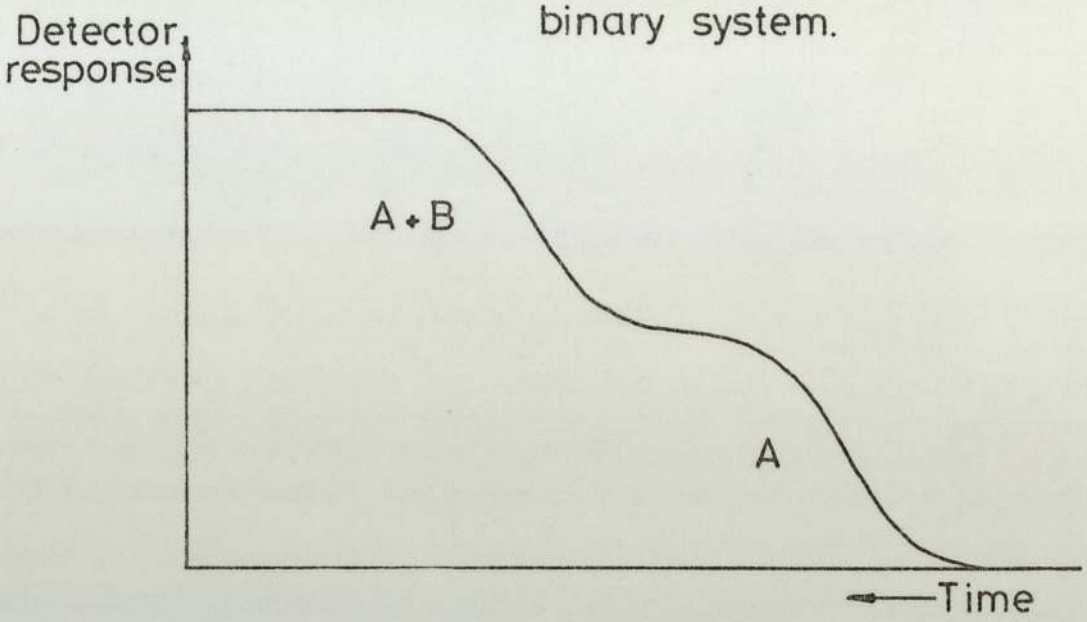
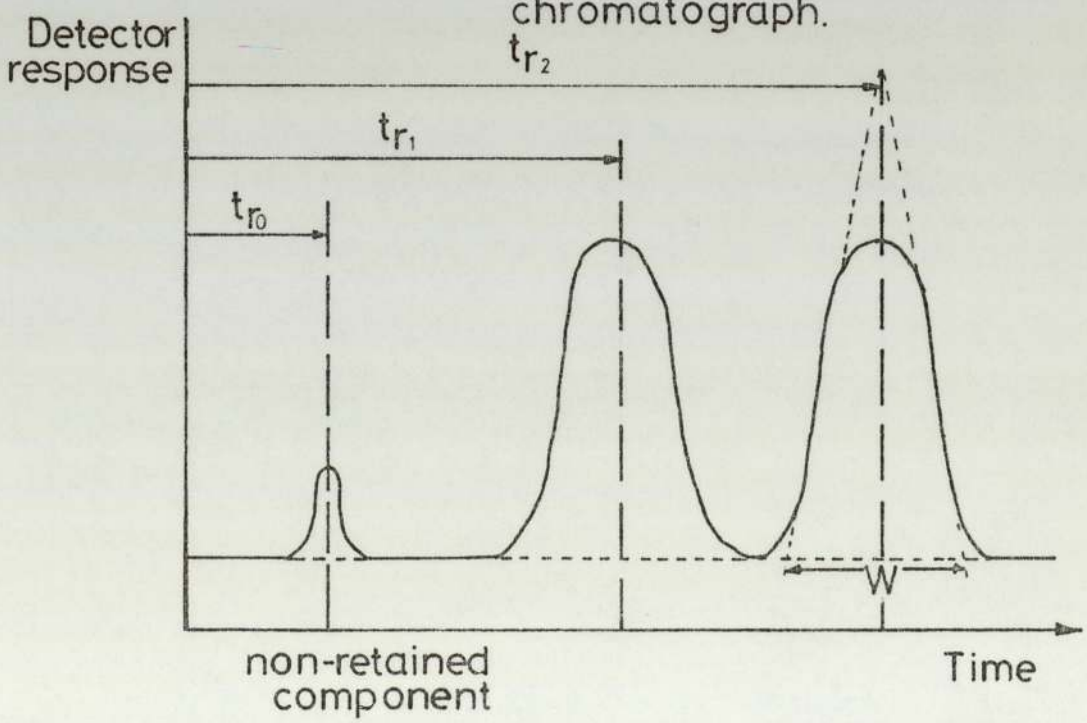


Fig 2.2 Example of elution chromatography chromatograph.



Frontal analysis cannot achieve the complete recovery of pure sample components from a mixture, but is a useful technique for measuring the distribution of equilibrium isotherms.

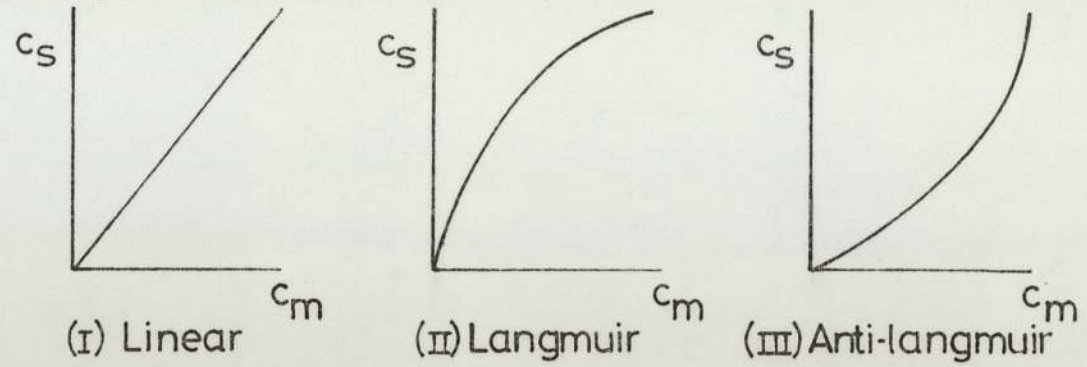
Elution chromatography is where a small pulse of material is injected on to a column. The sample components migrate through the bed at different rates and result in a chromatogram as indicated by Fig. 2.2. Elution chromatography is the only mode that can result in a quantative and qualitative separation of a mixture. Today elution chromatography is used almost exclusively in analytical separations.

2.3.1 Equilibrium

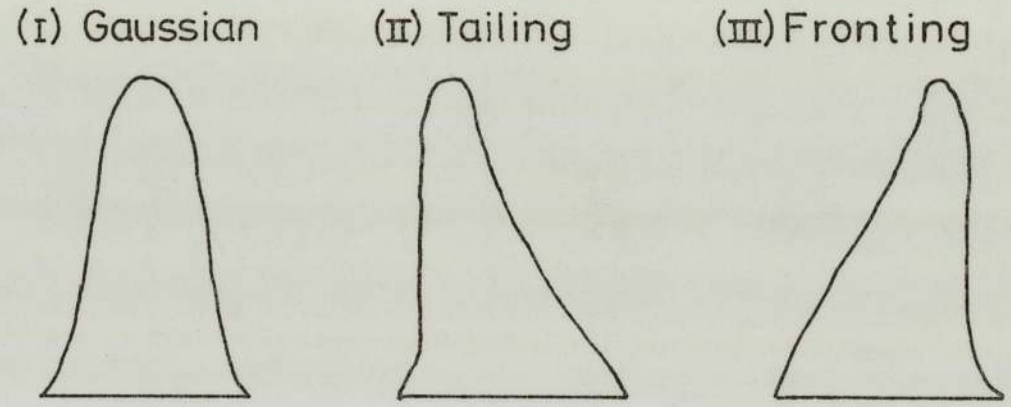
Elution chromatography separations can be divided into two forms; linear and non-linear, based on the shape of the isotherm under the conditions of the separation. In linear elution chromatography the distribution coefficient, K, which is the ratio of the solute concentration in the stationary and mobile phases, is independent of the solute concentrations. Therefore the eluted bands are symmetrical and Gaussian. Non-linear isotherms are often encountered in practice where there are high sample concentrations. Conder (17) reports the solute boundary shape for two of the commonest forms of non-linear isotherms; Langmuir and Anti-Langmuir. Fig. 2.3a shows the variations in the distribution coefficients K, and the resulting chromatographs for the linear, Langmuir and anti-Langmuir isotherms. A Langmuir isotherm results in tailing of the eluted band and since the average distribution coefficient, K, decreases with an increase with sample concentration so 'fronting' of the eluted band is experienced and the retention volume increases with an increase in sample concentration.

Fig2.3 Relationship between distribution coefficients and concentration profiles.

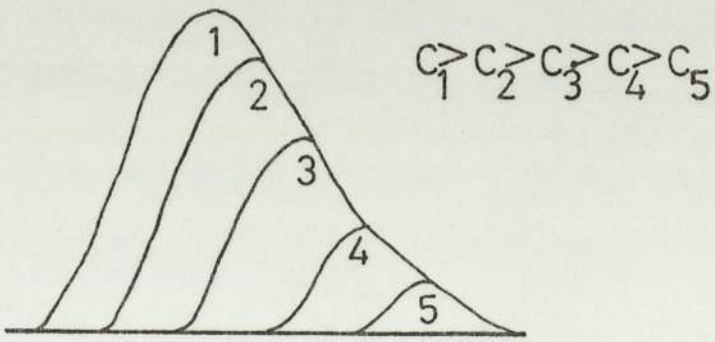
(a) Types of distribution profile



chromatograph elution profile



(b) Variation of elution profile with concentration for Langmuir isotherm



In conventional analytical applications the concentrations of the sample are sufficiently low so that the isotherm type tends towards linearity, as suggested by Fig. 2.3b.

In operations where one of the fundamental requirements is to maximise mass throughput operation in the non-linear region often results (18,19). A balance must be made between the gain in throughput and the necessary degree of separation, Pretorious and de Clerk (22) suggest a fair amount of skewing can be tolerated.

2.3.2 Retention

A fundamental parameter in column elution chromatography is the retention volume, V_R, defined as the volume of mobile phase that must pass through the column for the elution of a given component. Each component will be eluted from the column according to its equilibrium distribution between the stationary and mobile phase. If we confine the subsequent discussion to linear elution chromatography, it is possible to relate directly the time of elution of the peak maximum to the equilibrium distribution coefficient. The retention time, t_r, can be related to the elution volume by the volumetric flowrate, Q from the expression:-

$$t_r = \frac{V_R}{Q} \qquad (2.1)$$

Fig. 2.2 shows a typical chromatogram for a two component injection, note how the retention time for each component is measured at the band centre or maximum. Also indicated by Fig. 2.2 is the volume V_o corresponding to a non-retained component at $t_o Q$, this is also the total volume of the mobile phase in the column and is often termed the column "dead volume", or in g.p.c. the "void volume".

In elution column chromatography, under co-current conditions, a solute molecule can only migrate along the column when it is in the mobile phase, therefore the probability that a molecule can be found in the mobile phase can be defined by the retention parameter , R as:-

$$R = \frac{n_m}{n} = \frac{n_m}{n_m + n_s} \qquad (2.2)$$

where n_m - number of molecules in the mobile phase

n_s - number of molecules in the stationary phase

n - total number of molecules

Therefore,

$$R = \frac{1}{1 + \frac{n_s}{n_m}} \qquad (2.3)$$

or

$$= \frac{1}{1 + \frac{kV_s}{V_o}} \qquad (2.4)$$

where K is the distribution coefficient, and V_s and V_o are the volumes of the stationary and mobile phases, respectively. As we are confining this discussion to linear elution chromatography, K is constant and therefore so is R.

The average linear velocity of a component, v_s is equal to the mobile phase velocity times the fraction of the time, R, that the component spends in the mobile phase. Thus,

$$v_s = vR \qquad (2.5)$$

As the average linear velocity is inversely proportional to the time the component spends in the column,

$$v_s \propto \frac{1}{t_r} \tag{2.6}$$

and when the component is non-retained

$$v \propto \frac{1}{t_o} \tag{2.7}$$

Substituting (2.6) and (2.7) into (2.5) we get:-

$$\frac{1}{t_r} = \frac{R}{t_o}$$

or
$$t_r = \frac{t_o}{R} \tag{2.8}$$

Using equation (2.1) we get,

$$V_R = \frac{V_o}{R} \tag{2.9}$$

and from (2.4)

$$V_R = V_o \left(1 + \frac{KV_s}{V_o}\right)$$

$$= V_o + KV_s \tag{2.10}$$

In gel permeation chromatography it is more usual to represent the retention volume by the empirical expression

$$V_R = V_o + K_d V_i \tag{2.11}$$

because V_i the volume of the liquid in the pores of the packing is a conveniently measured parameter. The total volume of the stationary phase V_s equals V_i plus the volume of the packing V_p, therefore the general chromatographic partition coefficient K can be related to the more usual g.p.c. coefficient K_d by

$$\frac{K_d}{K} = 1 + \frac{V_p}{V_i} \qquad (2.12)$$

Care must be taken not to confuse these coefficients.

2.4 ZONE BROADENING THEORIES

As Figure 2.2 suggests there is a finite zone on either side of the average migration rate of a species which is caused by column dispersion effects. It is obviously desirable to keep this dispersion zone as small as possible. In common with most unit operations there are two theories that account for these effects, the plate theory and the rate theory.

2.4.1 Theoretical Plate

Martin and Synge (1) introduced the theoretical plate model into chromatography because of its common use in distillation processes. By describing the chromatographic column as a number of identical elements or plates where the mobile phase leaving was in equilibrium with the stationary phase throughout the plate and making further simplifying assumptions a description of the spreading of a single solute band was made. The degree of spreading of the solute band was quantified by the variance of the outlet profile. The height of a theoretical plate (H.E.T.P.), the characterising parameter was defined by:

$$H = \frac{d\sigma_z^2}{dz} \qquad (2.13)$$

where σ_z and z are the length based second moment and the distance along the column.

The plate model concept was reduced to infinitely small

dimensions by Glueckauf (18) who showed that the elution profile was a Poisson distribution. Van Deemter, Zuiderweg and Klinkenberg (19) subsequently showed that if the total number of plates for a column was large, more than 100, the solute elution curve could be approximated to a Gaussian distribution. Although the plate height is a useful index having almost universal acceptance for the comparison of the efficiencies of chromatographic columns, it fails to account for the kinematic processes occurring in the bed.

2.4.2 Rate Theories

Rate theories attempt to account for the kinematic processes occurring in the spreading of a solute band. Lapidus and Amundson (20) developed a model incorporating mass transfer and longitudinal diffusion terms. This model was extended by Van Deemter, Zuiderweg and Klinkenberg (19) to include contributions from axial diffusion and finite rates of mass transfer. The form of their equation is as follows:-

$$H = A + \frac{B}{v} + C_m'v + C_s'v \qquad (2.14)$$

where A = Eddy diffusion term

B = longitudinal diffusion term

C_m', C_s' = resistance to mass transfer in the mobile and stationary phase, respectively.

In equation 2.14 it is implied that the contributions to the plate height, H are independent of one another. Giddings (21) recognising the close relationship between the resistance to mass transfer in the mobile phase and the eddy diffusion terms proposed a "Coupling Theory" linking these terms. The simplified form of the equation is:

$$H = \frac{B}{v} + C_s'v + \frac{1}{\frac{1}{A} + \frac{1}{C_m'v}} \qquad (2.15)$$

Giddings (21) also developed a more vigorous theory, "the generalised non-equilibrium theory", and claimed that true equilibrium between the two phases only exists at the centre of the zone. The stationary phase concentration lags behind its equilibrium value, whilst the mobile phase concentration is ahead of its equilibrium value as indicated by Figure 2.4. Slow mass transfer rates between the two phases are accountable for the non-equilibrium situation. Giddings (21) discusses quantitatively the mass transfer processes in a variety of systems.

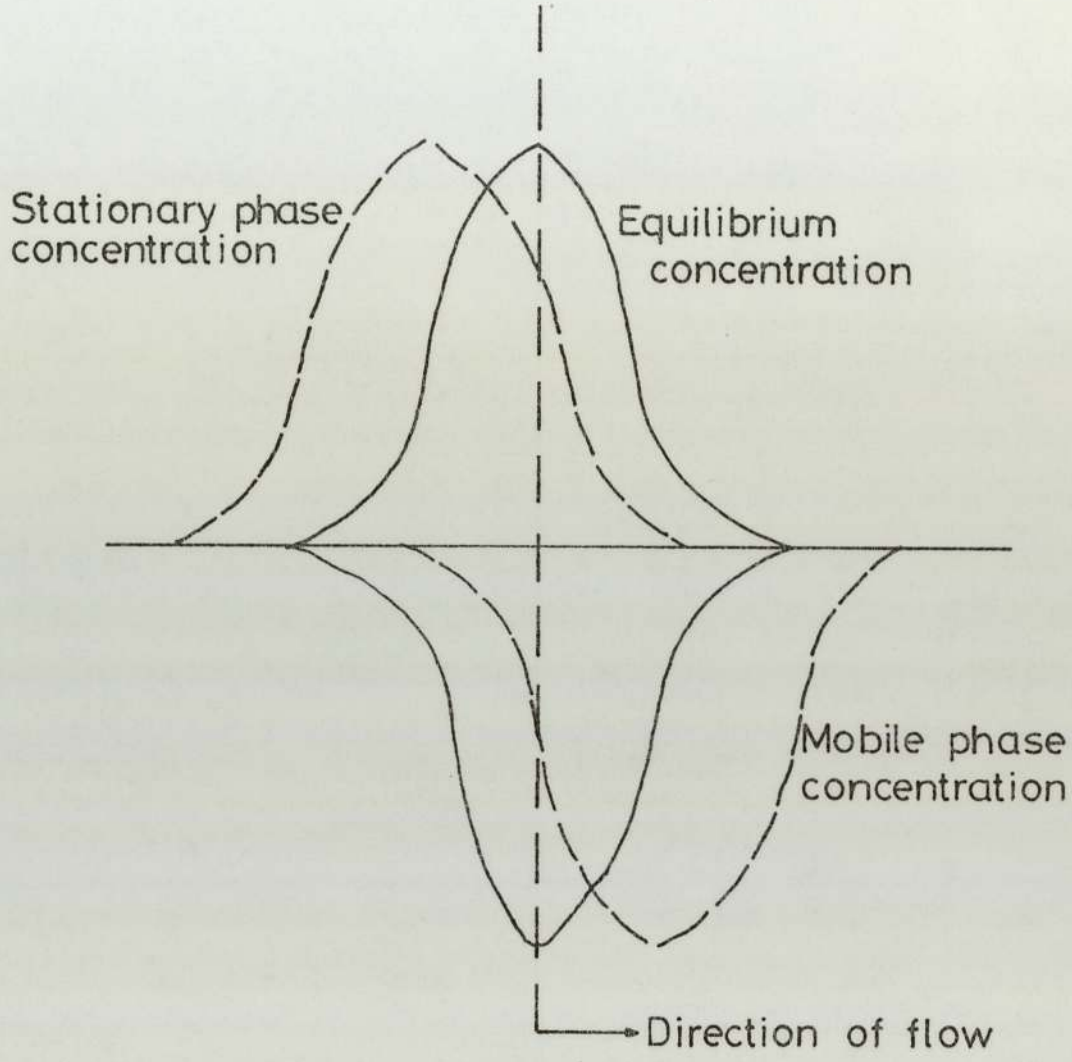
2.5 RESOLUTION AND SEPARATION EFFECTIVENESS

As Section 2.4 indicates, in all chromatographic systems axial spreading of a sample results in identical molecules eluting at different times from the column. This spreading results in a loss of the separating capabilities of the chromatographic system. A term quantifying the degree of separation in a chromatographic system is the resolution, R_s. For the separation of a binary mixture resolution can be characterised by the distance between the peak maxima and the width of the eluted bands, and is defined by

$$R_s = \frac{2(V_{R2} - V_{R1})}{W_1 + W_2} \qquad (2.16)$$

where V_{R1} and V_{R2} are the retention volumes of components 1 and 2, and W_1 and W_2 are the width of the peaks as defined in Figure 2.2.

Fig2.4 Comparison between actual and equilibrium column concentration profiles.



Purnell (22) derived a more practical relationship between resolution and three experimental variables, selectivity factor, capacity factor and the number of theoretical plates:

$$R_s = \frac{1}{4}(\alpha-1)\left(\frac{k'}{k'+1}\right)N^{\frac{1}{2}} \qquad (2.17)$$

where, N = number of theoretical plates

$\alpha = \frac{K_1}{K_2}$, ratio of distribution coefficients

$k' = \frac{n_s}{n_m}$, $\left(\frac{\text{number of moles in stationary phase}}{\text{number of moles in mobile phase}}\right)$.

Giddings (24) developed an expression to give the approximate number of peaks, ϕ, that may be resolved on a column as a function of the total number of theoretical plates, N:

$$\phi = 1 + 0.2\,N^{\frac{1}{2}} \qquad (2.18)$$

Thus on a column of 1000 theoretical plates, approximately 7 components could be separated.

Bly (25) modified equation (2.16) for gel permeation chromatography to give a resolution that was independent of the samples, specific resolution, R_{sp}:

$$R_{sp} = \frac{2(V_{R1} - V_{R2})}{W_1 + W_2}\left(\frac{1}{\log\left(\frac{M_1}{M_2}\right)}\right) \qquad (2.19)$$

where M_1 and M_2 are the molecular weights of samples 1 and 2. This equation is only valid if there is a linear relationship between the elution volume and the logarithm of the molecular weight of the sample, and if polymer samples are used they have a very narrow distribution. For broader polymer samples, equation (2.19) can be modified to allow for the polydispersity of the sample:

$$R_{sp} = \frac{2(V_{R2}-V_{R1})}{\left(\frac{W_1}{D_1} + \frac{W_2}{D_2}\right)} \left(\frac{1}{\log\left(\frac{\bar{M}_{W1}}{\bar{M}_{W2}}\right)} \right) \qquad (2.20)$$

where D is the polydispersity of the appropriate sample, and $\bar{M}_W$ its weight average molecular weight.

Yau et al. (26) have described a resolution factor, R'_{sp}, that can be used to determine the performance of a column and is given by:

$$R'_{sp} = \frac{0.576}{\sigma g_m (L)^{\frac{1}{2}}} \qquad (2.22)$$

where σ is the standard deviation of a sample, g_m is the gradient of the log(molecular weight)/elution volume relationship, and L is the length of the column.

Christopher (27) suggested a suitable measure for the performance of a gel permeation column was its ability to give molecular weight averages that agreed with their theoretical values, the percentage difference between the measured and theoretical values was a measure of the column efficiency. Cooper (28) proposed that column efficiencies could be compared by measuring the polydispersity value for a narrow polymer distribution eluted from a column. Both

Christopher's and Cooper's methods have the advantage of using only one sample. Knox and McLean (29) have shown that as the dispersion of a polymer sample arises from the polydispersity of the sample and the kinetic process within the column, the true plate height cannot generally be obtained from the elution peak of a polymer whose polydispersity is much larger than 1.01.

Other measurements of separation effectiveness have concentrated on the impurity of a component on a mass or mole basis. Metzger et al. (30) defined a purity index for both components in a binary system as:

$$I_1 = \frac{M_{21}}{M_{t1}} = \frac{M_{21}}{M_{11} + M_{21}} \text{ , for region 1} \qquad (2.23)$$

and

$$I_2 = \frac{M_{12}}{M_{t2}} = \frac{M_{12}}{M_{12} + M_{22}} \qquad (2.24)$$

where M_{ij} = number of moles of component i in region j

M_{tj} = total number of mole in region j

M_{ti} = total number of moles in region i

and a total percentage impurity (T.P.I.) for the system

$$T.P.I. = 100 \left(\frac{M_{21}}{M_{t1}} + \frac{M_{12}}{M_{t2}} \right) \qquad (2.25)$$

They also defined a quantity factor, Q.F. that represents the total amount of solutes partitioned into the correct region:

$$Q.F. = M_{11} + M_{22} \qquad (2.26)$$

for most separations it is the unstated goal that the objectives are to maximise the quantity factor and minimise the total percentage impurity. Rietena (31) and Rong (32) have also similar quantitative indices.

2.6 INTRODUCTION TO GEL PERMEATION CHROMATOGRAPHY

Porath and Flodin (33) demonstrated that particles of cross-linked dextran gel could be used to separate substances of having different molecular weights. The term "gel filtration" was used, to describe the chromatographic packing rather than the mechanism, this term is still commonly used in the bio-chemical field. Many other terms have been used to describe the process of separating or fractionating molecules by virtue of their molecular size as reviewed by Ellison (34). In this thesis the term gel permeation chromatography (g.p.c.) will be used.

G.p.c. is a form of liquid-liquid chromatography where solute molecules are retarded by their ability to permeate into the solvent filled pores of the packing material. This form of chromatography is unique in that separation takes place in a volume which is smaller than the total column volume and that the solvent in the mobile and stationary phases is the same.

It is generally accepted that the separation in g.p.c. is based on the difference in available pore volume between molecules of different sizes. The smaller the molecule a greater pore volume is available and so they are retarded to a greater extent. Molecules that are totally excluded from the pores are eluted in the column interstitial volume, V_o, and for molecules that are completely included are eluted in a volume $V_o + V_i$, where V_i is the internal or pore volume. For molecules that can only partially permeate the pore volume, its

accessible volume V_{iACC} can be related to the total pore volume to give a distribution coefficient,

$$K_d = \frac{V_{iACC}}{V_i} \qquad (2.27)$$

and so the elution volume of any molecule can be given by:

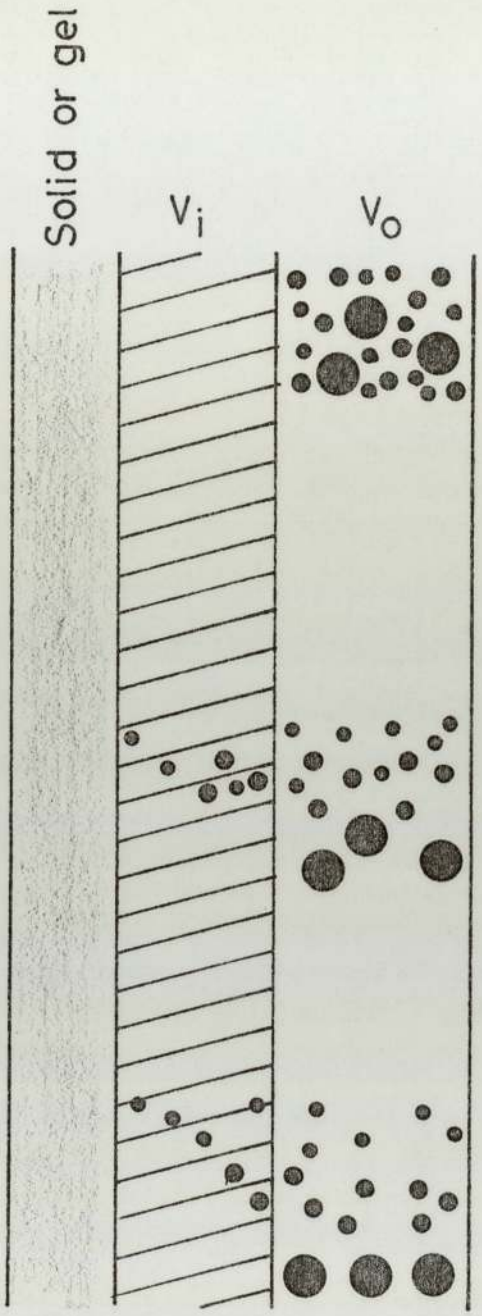
$$V_e = V_o + K_d V_i \qquad (2.28)$$

It is apparent from equation 2.28 that K_d is restricted to values equal to or between 0 and 1; in other forms of liquid chromatography the partition coefficient may have a value very much greater than 1 and so take several column volumes of mobile phase to elute. Figure 2.5 illustrates the idealised mechanism of g.p.c.

2.6.1 Column Packings

Column packings used for g.p.c. can in general be classified by the rigidity of the material, rigid, semi-rigid or soft. Rigid packings can withstand high pressures and flowrates and can be used with a variety of solvents, both aqueous and non-aqueous. However, problems may be encountered when fractionating polar molecules because of adsorption. Semi-rigid packings, usually cross-linked organic polymers have the ability to fractionate a wide variety of polymers, oligomers as well as low molecular weight samples. These materials usually have larger pore volumes than rigid materials and can in general fractionate smaller molecules. Semi-rigid gels may shrink or swell with changes in solvent and may be compressed by high flowrates. The original packing materials used for g.p.c. (33,35) were soft gels.

Fig 2.5 Idealised illustration of g.p.c.



- totally exclude ($K_d = 0$), $V_e = V_o$
- partially included ($0 < K_d \leq 1$), $V_e = V_o + K_d V_i$
- totally included ($K_d = 1$), $V_e = V_o + V_i$

These gels can only be used at very low pressures and so only very low flowrates can be used. Consequently the uses of these materials are limited and have been superseded by the rigid and semi-rigid materials. Nevertheless some fine separations of biological molecules have been reported to have been developed (41).

Cooper has recently reviewed the packings used in aqueous g.p.c. (36) and includes their typical applications. A more general review describing the developments in commercially available packing and columns particularly in microparticulate packings has been reported by Majors (37). Other lists have also been compiled (38,39).

It is important in analytical g.p.c. where accurate molecular weight distributions are required that the sample polymers should not contain material with molecular weights outside the fractionation range of the column. Haller, Basedow and Koenig (40) have proposed a useful technique for the selection of suitable packings and for the design of composite systems in the analysis of mixtures spanning a wide molecular weight distribution.

2.6.2 Sample Loading

The manner in which a sample is loaded on to a column can influence the peak shape and the column efficiency. For most chromatographic systems the injection method is either by syringe or valve injection. Bristow (41) summarised the advantages and disadvantages of each injection method to be:

(a) Syringe Injection Systems

Advantages	Disadvantages
(i) Maximum use of available sample.	(i) fragile
(ii) precise deposition on to column	(ii) restricted use > 100 bar
(iii) easily variable injection volume	(iii) wear out quickly
	(iv) variation in sample size when used for routine analysis
	(v) expensive running costs.

(b) Valve Injection Systems

Advantages	Disadvantages
(i) Precision for routine analysis	(i) inflexible
(ii) can be automated	(ii) dilute sample before column
(iii) cheap running cost	(iii) expensive capital cost
(iv) can be used at > 100 bar	(iv) reduces column efficiency.

With the increasing use of high pressure liquid chromatography (H.P.L.C.) where microparticulate materials are used in routine sample analysis and preparation, higher column resolutions are often accompanied by increased peak skewing. This is because the normal valve sample injection system commonly used in H.P.L.C. cannot deposit the sample very precisely on to the column (42) and so the column cannot exhibit the "infinite diameter effect" as proposed by Knox and Parcher (43). Kirkland et al. (42) have suggested how a sample injection valve can be modified to combine the advantages of the point injection of a syringe with the precision of a valve injection.

2.6.3 Sample Detection

Continuous monitoring of the eluent is generally achieved by measuring a suitable physical property of the column eluate. The properties of the column eluate that are most commonly monitored are refractive index, dielectric constant, and electrical conductivity although other less common detectors have been used and have been reviewed by Wise and May (44). Unfortunately there has been no detector developed as yet that can be used with a liquid chromatograph and fulfil the following requirements:

(i) high sensitivity
(ii) wide linear dynamic range
(iii) good linearity
(iv) predictable response
(v) universally compatible with solvents.

Therefore for comprehensive detection equipment at least two of the different detector types are required or the full versatility of the chromatographic technique will not be realised. Scott (45) and Bristow (41) discuss the more common detectors used with liquid chromatographic systems.

In g.p.c. the most widely used detector is the refractometer. This detector will respond to any substances that have a significantly different refractive index from that of the mobile phase. It is however one of the least sensitive detectors, and subject to baseline fluctuations caused by changes in temperature, pressure and flowrate.

2.6.4 Other Equipment

The widespread use of liquid chromatography has resulted in a large array of complete chromatographic systems being commercially

available. McNair (46) reviewed requirements for the basic parts of a chromatograph, and listed features and specifications of the instruments supplied by the various manufactures.

2.7 ANALYTICAL APPLICATIONS OF G.P.C.

Polymer systems have, almost always, a mixture of various molecular-chain lengths and therefore have a molecular weight distribution. Since many of the physical properties of the polymer system are affected by the molecular weight distribution, a knowledge of this data is essential in explaining or predicting the behaviour of the polymeric system. One method of obtaining this data is by g.p.c.

2.7.1 Characterisation of Polymer Systems

One method of communicating the molecular weight distribution of a polymeric system is in the form of a histogram which would truly represent the discrete distribution of the system, see Fig. 2.6. However, as a polymer system usually contains a great many molecular weights and it is often more convenient to treat the distribution as continuous distribution and represent it as either a differential or integral distribution curve as shown in Figs. 2.7 and 2.8 respectively.

To relay information in a simple and applied manner it is often practical to reduce the characterisation of a polymeric system by various average molecular weights, although no single average molecular weight can completely describe the distribution. Of the various averages, the most common are:-

$$\text{Number average, } \overline{M}_n = \frac{\Sigma n_i M_i}{\Sigma n_i} \qquad (2.29)$$

Molecular Weight Distribution Curves

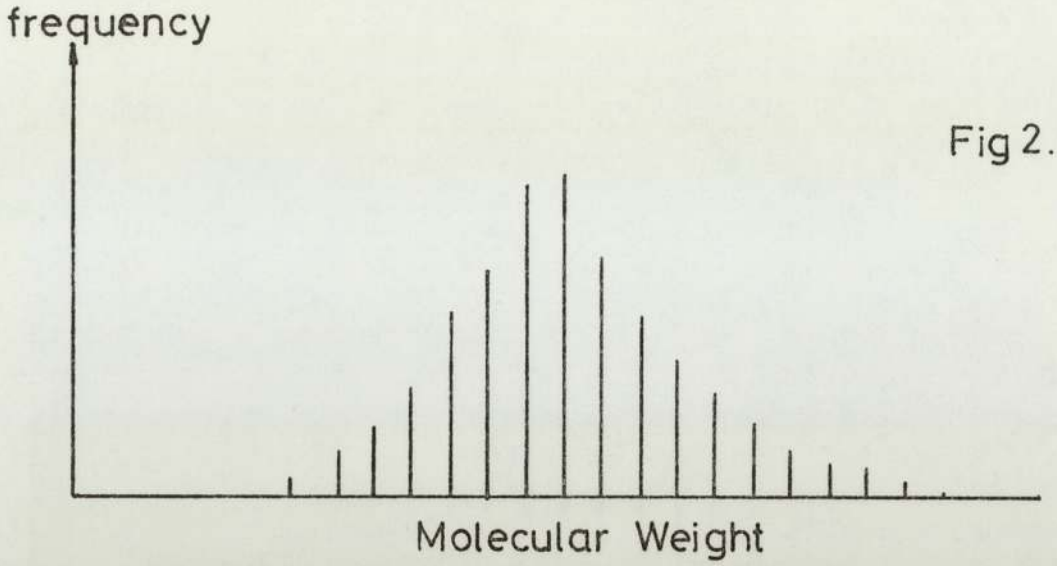


Fig 2.6

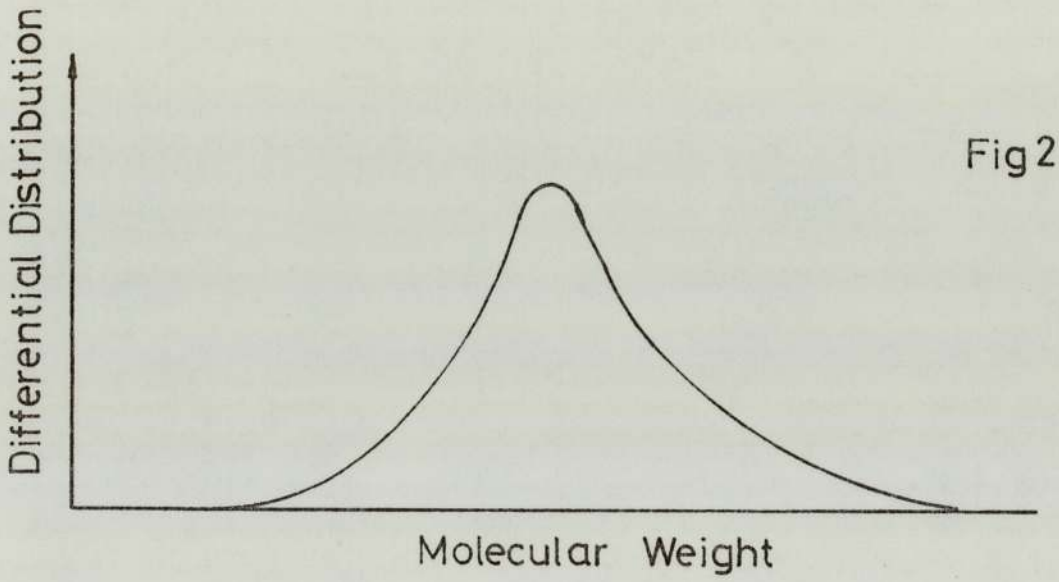


Fig 2.7

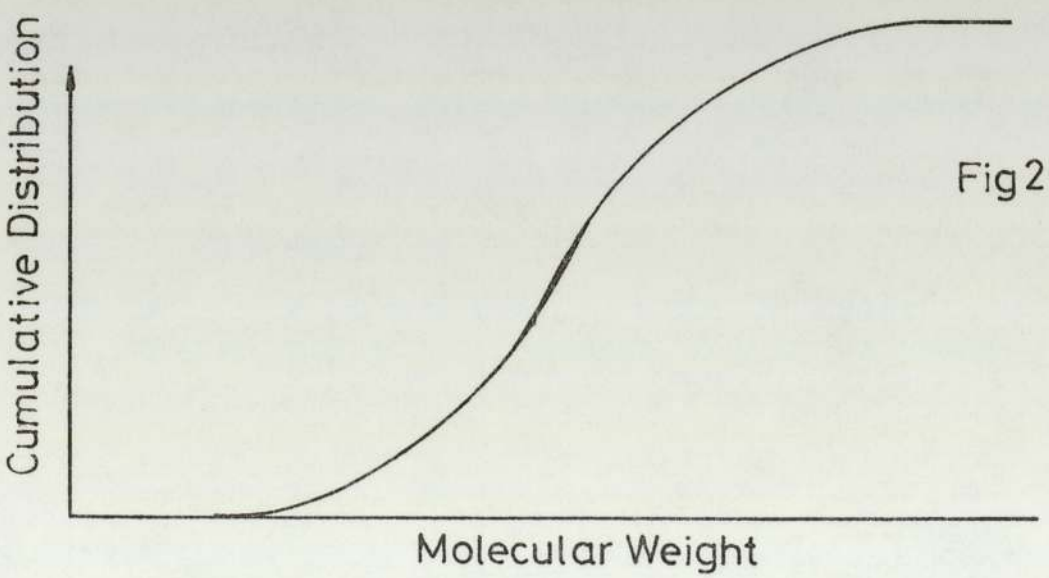


Fig 2.8

$$\text{Weight average, } \bar{M}_w = \frac{\Sigma n_i M_i^2}{\Sigma n_i M_i} \qquad (2.30)$$

$$\text{z average, } \bar{M}_z = \frac{\Sigma n_i M_i^3}{\Sigma n_i M_i^2} \qquad (2.31)$$

$$\text{Viscosity average, } \bar{M}_\eta = \left[\frac{\Sigma n_i M_i^{1+a}}{\Sigma n_i M_i} \right]^{1/a} \qquad (2.32)$$

where, n_i ~ number of molecules of molecular weight M_i

a ~ exponent in the Mark-Houwink equation relating the limiting viscosity number to molecular weight

The term polydispersity or hetrogeneity (the ratio of $\bar{M}_w$ to $\bar{M}_n$) is often used to describe the width of a molecular weight distribution. This ratio is unity for a monodisperse sample. In a polymeric system $\bar{M}_w$ is always greater than $\bar{M}_n$; the 'sharpness' of a polymeric system may be indicated by how closely this ratio approaches unity.

An alternative way of describing the molecular weight distribution in a convenient numerical form is to give the molecular weight of the polymeric system at selected points on the integral molecular weight distribution such as 0.05, 0.1, 0.5, 0.9 and 0.95. In fact, this type of classification is often used in the grinding and milling, or filtration industries to describe a particle size distribution.

2.7.2 Calibration Curves in G.P.C.

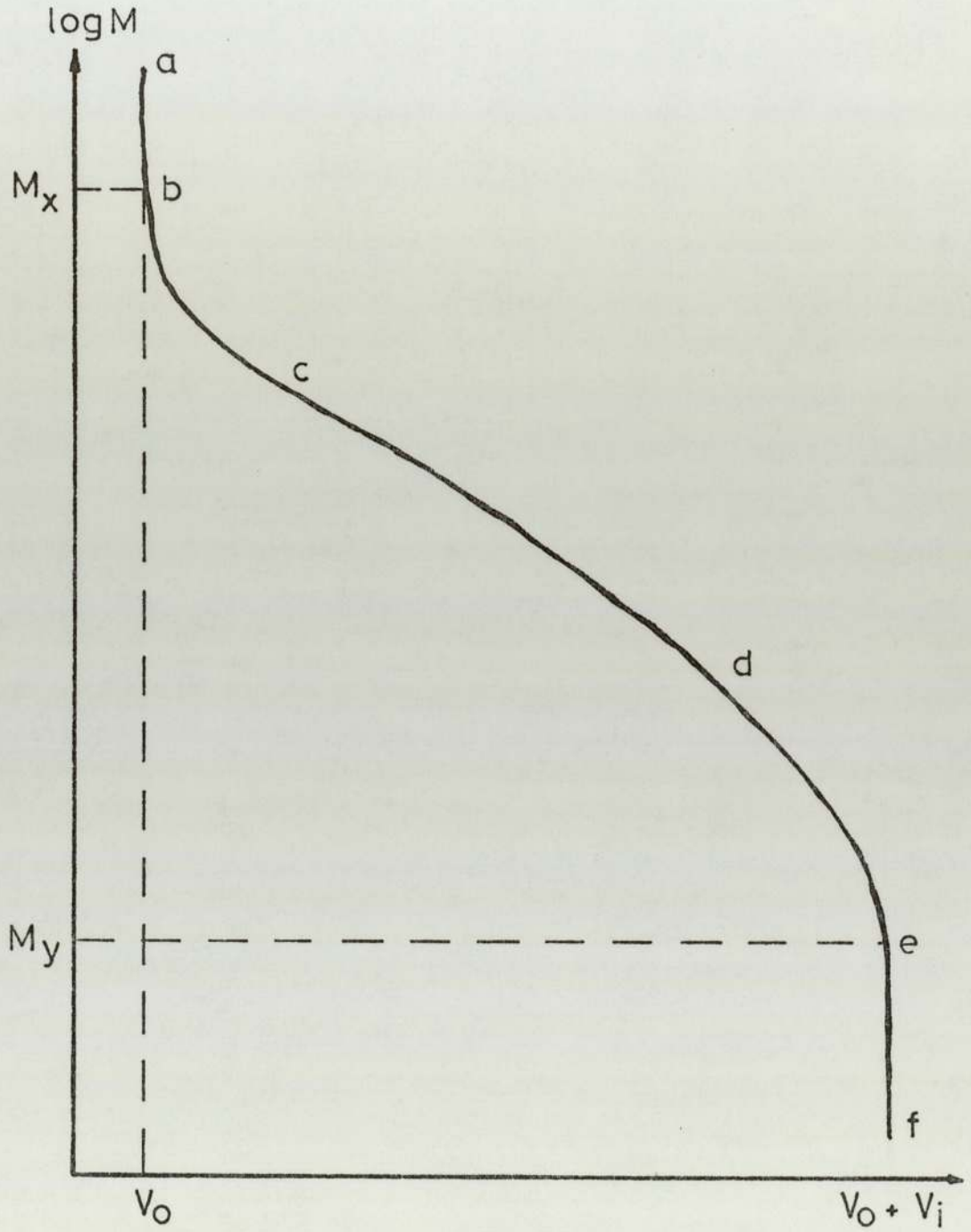
Having fractionated a polymeric sample on a g.p.c. column, a chromatogram can be obtained that represents the raw data from which a molecular weight distribution can be established. To calculate a molecular weight distribution and/or molecular weight averages it is first necessary to relate the size of a molecule in solution to an elution volume by calibrating the column. In practice this is performed as

near to infinitely dilute conditions as possible. If a precise and comprehensive theory of g.p.c. existed and if the exact nature of the pore structure was known for the relevent packing, then it should be possible to calculate the data required without further experimentation. However neither of these requirements are available and hence g.p.c. is not, at present, an absolute method; the g.p.c. packing must be calibrated with samples of known molecular weight distribution.

Figure 2.9 shows the relationship between molecular weight, or some function thereof relating to the size of the molecule in solution and its retention volume, V_R on a semi-logarithmic scale. This is a typical S-shaped calibration curve. On this curve five regions may be identified. In section a-b, no fractionation occurs as all the molecules having a molecular weight of M_x or greater were too large to penetrate any of the pores of the packing and so they were all eluted at a void volume, V_o. Similarly, in the region e-f molecules having a molecular weight of M_y or less can penetrate all the pores and they were the last to be eluted at a retention volume of the void plus the interstitial pore volumes, $V_o + V_i$. Molecules between these two molecular weight limits were fractionated because they can partially penetrate the pore to varying degrees. With many column packings there is a region that can be approximated to a straight line (section c-d) and one normally chooses a column packing which is able to fractionate completely the whole of the unknown sample in this region; this is because the resolving power of the packing is greatest here, and also it simplifies the numerical calculations. In regions b-e and d-e, fractionation is still occurring but, in practice the range of sizes of the pores is not linear and so the relationship between log M and V_R is non-linear. However, it is necessary to calibrate over the

Fig 2.9

A typical calibration curve for a g.p.c. column



whole region that is likely to be covered by the unknown samples because extrapolation of calibration curves will give erroneous results.

It is important that the molecular weight range of the polymer sample be covered by the range c to d in Fig. 2.10 because chromatographs of samples that extend into the inclusion or exclusion limits of the column packing will frequently produce an apparent bi-modal distribution. One peak is due to the fractionation of part of the polymer, and the second is an artifact caused by the grouping together of molecules at one of the extremes of the elution limits of the column packing, either by eluting a group of molecules at the void volume or at the void plus interstital volumes. Fig. 2.10 illustrates this effect.

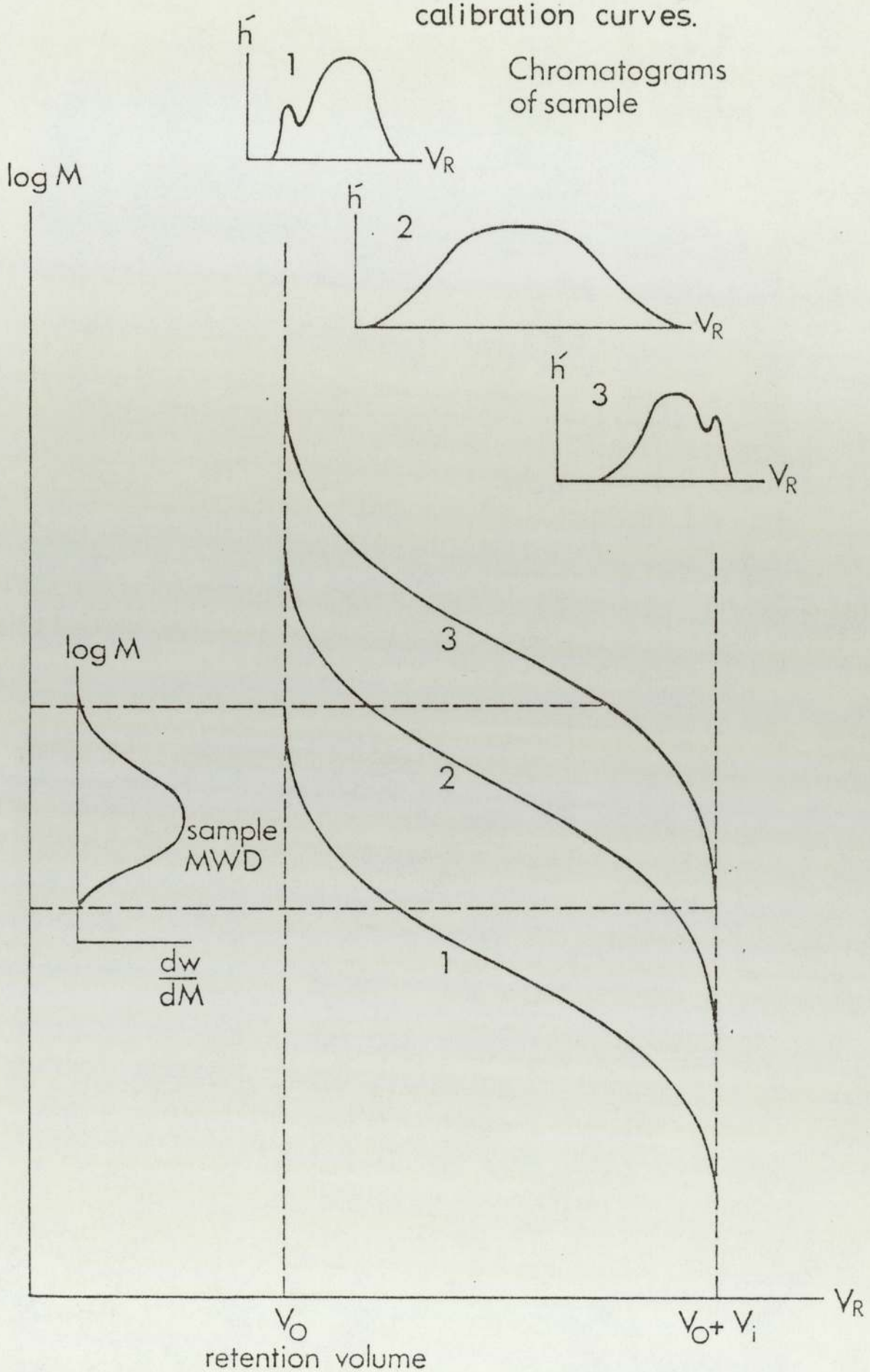
2.7.3 Linear Calibration

As shown above, the calibration curve can be considered linear over an appreciable molecular weight range. Using this simple form of relationship of molecular weight to elution volume, that is:-

$$\ln M = a - bV_R \qquad (2.33)$$

Frank et al. (47) were able to allow the values of a and b to vary from sample to sample provided an $\overline{M}_n$ and $\overline{M}_w$ that were known for each standard. This technique of linear calibration was used by Blake and Hamielec (48), where the molecular weight averages were computed from the g.p.c. curves and compared with the 'true' values obtained by absolute methods such as, osmometry, light scattering. The linear calibration equation was obtained by using a Rosenbrock search (49) to calculate the optimum

Fig 2.10 Fractionation of polymer on packings with different calibration curves.



values for the coefficients a and b. They also suggested that this technique be extended and used to modify non-linear calibration curves. Loy (50) used a simplified method to obtain a linear calibration curve. From the definitions of the weight and number average molecular weight,

$$\overline{M_w} = \Sigma w_i M_i \tag{2.34}$$

and

$$\overline{M_n} = \frac{1}{\Sigma(w_i/M_i)} \tag{2.35}$$

where w_i is the weight fraction, combining these definitions gives:-

$$\frac{\overline{M_w}}{\overline{M_n}} = (\Sigma w_i \, 10^{-v_i/b})[\Sigma w_i 10^{v_i/b}] \tag{2.36}$$

Loy then calculated equation (2.36) thirty-six times to find a unique value for b to the nearest 1/720. The fact that this expression is independent of a allows simpler and faster calculation of the constants a and b for equation (2.33). Then from an arbitrary value for a, a final value can be assessed by using a 'round-robin' technique by iterating the expression

$$a_{new} - a_{old} + b \log [\overline{M_w}/\Sigma w_i \, 10^{\frac{(a_{old} - v_i)}{b}}] \tag{2.37}$$

until

$$|10| \geq \overline{M_w} - \left(\Sigma \, w_i \left(\frac{a - v_i}{b} \right) \right) \tag{2.38}$$

Loy claims that this method is useful for polymer systems where narrow fraction standards are not available. Pitz (51) used standard

dextran T fractions to calibrate two 1 metre-long columns packed with Spheron P-300, and obtained a linear range from 5 to 500 x 10^3 daltons. Using a linear calibration technique Pitz considered that the calibration was completed when the weight average molecular weight of a standard T40 or T70 dextran sample could be reproduced to ± 3%, and the number average molecular weight to ± 5% of the $\overline{M_w}$ and $\overline{M_n}$ values when determined by absolute methods. Hamielec and Abdel-Alim (52) used polyacrylamide as a standard to test the accuracy of aqueous g.p.c. and suggested that as the Mark-Houwink constants for polyacrylamide in water are available (53) a universal calibration curve for other water-soluble polymers could be constructed. More recently Atkinson and Dietz (54) have reported the use of linear chromatography for the determination of molecular weight distributions of broad polyvinylchloride samples in 1,2-dichlorobenzene which gave a reproducability of ± 5%.

2.7.4 Non-Linear Calibration

Many examples of a linearly calibrated column exist; however in cases where this condition is not fulfilled Tung and Runyon (55) have suggested that a considerable error can be introduced. Therefore polynomial equations are often used to represent the way in which calibration lines are convex in a graphical representation at their higher limit of molecular weight; concave at the lower molecular weight range, and approximately linear in the central portion. Many people (56-61) have used non-linear calibration polynomials, generally of the type:-

$$\log M = a_o + a_1 V_e + a_2 V_e^2 + \ldots\ldots a_n V_e^n \qquad (2.39)$$

or its inverse function

$$V_e = C_o + C_1 \log M + C_2 \log^2 M + \ldots\ldots C_n \log^n M \qquad (2.40)$$

where a_i and C_i are experimentally determined constants.

Basedow et al. (62) uses the same form as expression (2.39) but reduces the elution volume to a canonical form by using the Wheaton-Bauman (63) characteristic parameter constant K_d:-

$$K_d = \frac{V_e - V_o}{V_i} \qquad (2.41)$$

where V_e is the elution volume of the substance concerned, V_o is the exclusion volume of the column and V_i is the total pore volume. Basedow (64) reported that the use of the K_d was advantageous when compared with V_e because displacements in the elution volume could easily be corrected in due course without repeating the whole calibration, and that the void volumes of the chromatographic system are avoided.

Janca et al. (65) proposed that any artificial oscillations that are introduced from the measured calibration points can be minimised by using a rational function of the form:-

$$\log M = \frac{P(V_e)}{Q(V_e)} \qquad (2.42)$$

where $P(V_e)$ and $Q(V_e)$ take the form of polynomials. The resulting calibration was expressed by:-

$$\log M = a_o V_e^{-3} + a_1 V_e^{-2} + a_2 V_e^{-1} + a_3 + a_4 V_e + a_5 V_e^2 + a_6 V_e^3 \qquad (2.42)$$

where the constants, a_i, were calculated by a non-linear regression analysis described by Rowan and Sorrell (66). Janca describes equation (2.42) as a simplified version of the rational function because in the experimental verification of this non-linear calibration technique, the final polynomial of the third degree $Q(V_e)$, both the linear and the quadratic terms could be neglected.

One of the more rigorous attempts to correlate a calibration curve was proposed by Yau and Malone (67) who from a theoretical viewpoint suggested that the diffusion of molecules into and out of the gel, together with the exclusion effects that operate in g.p.c. columns should be included. Using a one-dimensional solution to Fick's diffusion equation, an expression for the most probable distribution for molecules between the stationary and mobile phases was derived. Then using an average velocity for the flow of eluent through the column an expression of the form

$$V = \theta_o + \theta_1 \left\{ \frac{1}{\sqrt{\pi}\ \psi} \left[1 - \exp(-\psi^2) \right] + \operatorname{erfc}(\psi) \right\} \qquad (2.43)$$

$$\text{where} \quad \psi = \frac{M^{\theta_3}}{\theta_2} \qquad (2.44)$$

was obtained which describes the calibration curve. The parameters θ_i in equations (2.43) and (2.44) are related to the characteristics of the fractionating column and the solvent/solute interactions. In practice, these parameters are obtained by fitting equation (2.43) to real data. Yau and Malone use a polystyrene/toluene system to verify their theoretical interpretation. A more recent experimental

verification of the calibration equation (2.43) was reported by Cardenas and O'Driscol (68) who analysed both polystyrene and poly(methyl methacrylate) in tetrahydrofuran on styragel columns. They reported a significant improvement when this non-linear technique was applied compared with linear chromatography or peak elution volume, a technique where the calibration line was determined by plotting the peak molecular weight against their corresponding peak elution volume.

2.7.5 Methods of Calibration

As the present understanding of the mechanism of g.p.c. is not sufficiently developed to allow direct calculation of calibration curves, it is evident that these curves must be obtained by experimental methods. There are many proposed methods, some of which are outlined below:-

(a) Calibration with Monodispersed Polymers

One of the simpler methods for the production of a calibration curve is to inject into the column a series of monodispersed standards of the polymers under test. A polymer is usually considered to be monodispersed if the M_w/M_n ratio is less than 1.1. The following approximation can then be made:-

$$M_{peak} = \overline{M}_N = \overline{M}_w.$$

The calibration curve is then produced by plotting log Molecular weight against the peak's elution volume. This method is reliable because it makes no assumptions about the molecular weight distribution of the standards except they are monodispersed, nor about the separation mechanism. It is also extremely easy to apply because there is no

complicated regression analysis required. Unfortunately it is limited to systems where monodispersed standards can be obtained. The only polymer which is readily available in the form of near monodispersed samples covering a wide range of molecular weights, is polystyrene. However, polystyrene standards are not suitable for aqueous g.p.c. and for the purpose of this research project dextran is the only satisfactory molecule for calibration. Monodispersed dextran fractions are not readily available and have to be specially produced either by fractional precipitation or preparative g.p.c. in the laboratory requiring them. Basedow et al. (62) reported the use of very narrow dextran fractions (many less than 1.05) for the determinations of molecular weight distribution on controlled pore glass.

(b) Calibration with Broad Polymers

Except in cases where we have a monodispersed standard the calibration of a column is tedious because a large number of samples are required to obtain the necessary information. A limited but comparatively simple method was reviewed by Dawkins (69) who showed how a calibration curve could be obtained using the peak value of a chromatograph of a broad polymer sample. This method depended upon the assumptions that the calibration curve was linear over the molecular weight range of the sample and that the chromatograph approximated a log-normal distribution. Another method is to fractionate a 'whole' polymer of the same type as the samples under test which is sufficiently broad enough to completely cover the molecular weight range of interest and has a well characterised distribution. This technique is of particular use to laboratories dealing exclusively with one polymer type as there is a considerable effort required in characterising the molecular weight distribution. This broad polymer calibration technique

was used by Nilsson and Nilsson (59) who assumed that the molecular weight could be related to the elution volume by the equation:-

$$M = b_o + \exp [b_1 + b_2 (K_d) + b_3 (K_d)^2 + b_4 (K_d)^3] \qquad (2.45)$$

The values of the constants b_i were obtained by optimising the equation to give the best agreement between the actual values of a molecular weight average as measured by an absolute method and the calculated values obtained from the elution profile. The optimisation was carried out using Hartley's modification of a Gaussian-Newton method (70).

(c) On-line Methods

'On-line' calibration is where the molecular weight of a polymer sample in a column's eluate is monitored by an absolute method. This offers an attractive approach. In theory, a classic method for measuring $\overline{M}_w$, $\overline{M}_n$ or $\overline{M}_\eta$ could be used because at any instant the molecular weight distribution of the eluate from a high resolution column is very narrow and so the approximation that any of the above averages are equal can be made. Hatt (38) reviews many of on-line viscometric techniques that have been proposed and used. Cantow et al. (71) discussed an 'on line' light scattering technique for the measurement of the weight average of a sample. More recently the use of low-angle laser light scattering techniques have been reported which have the ability to analyse both linear or branched polymers or a mixture thereof. Ouano and Kaye (72) report that low angle laser light

scattering provided a true and direct method of obtaining a molecular weight distribution and showed this by characterising polystyrene and poly(methyl methacrylate) samples. They also indicated that a very high sensitivity could be obtained by low-angle laser light scattering when compared with other light scattering techniques. Onano (73) showed that the low-angle laser could be coupled with a computer which could then provide a fast and comprehensive characterisation of the polymer. Dextran samples have been analysed using a combination of g.p.c. and a low angle laser (64, 74). However an important point to note is that a low-angle laser light scatter does not compensate for difficiencies in the resolution properties of the gel permeation column. Automated continuous chemical analysis of the eluent from a gel permeation column has also been applied to the characterisation of dextrans (75). The limiting factor of this method is of the spreading of the automated analytical system due to the length of time necessary for development of chromophores.

2.7.6 Universal Calibration

Frequently well-characterised standards of the polymer under test are not available to construct a calibration curve. A procedure using well-characterised polymer fractions such as polystyrene or dextran to construct a calibration curve for the column and by making assumption that size exclusion of the molecules is the only factor in the separation we can use the obtained calibration curve for the interpretation of the chromatogram of the polymer under test. Molecular weight units cannot be used directly to relate the retention volumes of various polymeric solutions. For example, polystyrene and polyethylene fractions having the same molecular weights will elute at different retention volumes. However it is possible to relate the molecular weights by

the expressions such as:-

$$M_{pol} = C\, M_{aux} \tag{2.46}$$

or

$$M_{pol} = C\, (M_{aux})^{b} \tag{2.47}$$

where M_{pol} is the polymer of interest and M_{aux} is the auxiliary polymer.

Many attempts have been made to find a universal size parameter, commonly given the symbol, U, that will relate all polymers to a single calibration. It is inherent in this procedure that the relationship between the universal parameter and the retention volume will be independent. One of the more successful attempts to describe a universal parameter has been to use the intrinsic viscosity, η. Benoit et al. (76, 77) used this approach and showed that the relationship:-

$$\log M_{pol} - \log M_{aux} = \log [(\eta)_{aux}/(\eta)_{pol}] \tag{2.48}$$

was valid for many polymers. However, by using the relationship between the intrinsic viscosity and the molecular weight of a sample as given by the Mark-Houwink equation, that is:-

$$\eta = BM^{a} \tag{2.49}$$

where B and a are approximately constants for a given polymer-solvent type. By combining equations (2.48) and (2.49) we can obtain the equation

$$\log M_{pol} = \left(\frac{1 + a_{aux}}{1 + a_{pol}} \right) \log M_{aux} + \left(\frac{1}{1 + a_{pol}} \right) \log \frac{B_{aux}}{B_{pol}} \qquad (2.50)$$

which links the molecular weight of the required polymer to the auxiliary calibration polymer by their Mark-Houwink constants. It must be made clear that the coefficients B and a should only be considered truly constant over a limited molecular weight range and deviations may occur in very high and low molecular weight regions. A more comprehensive review of the calibration of a column with an auxilary polymer system is given by Hatt (38).

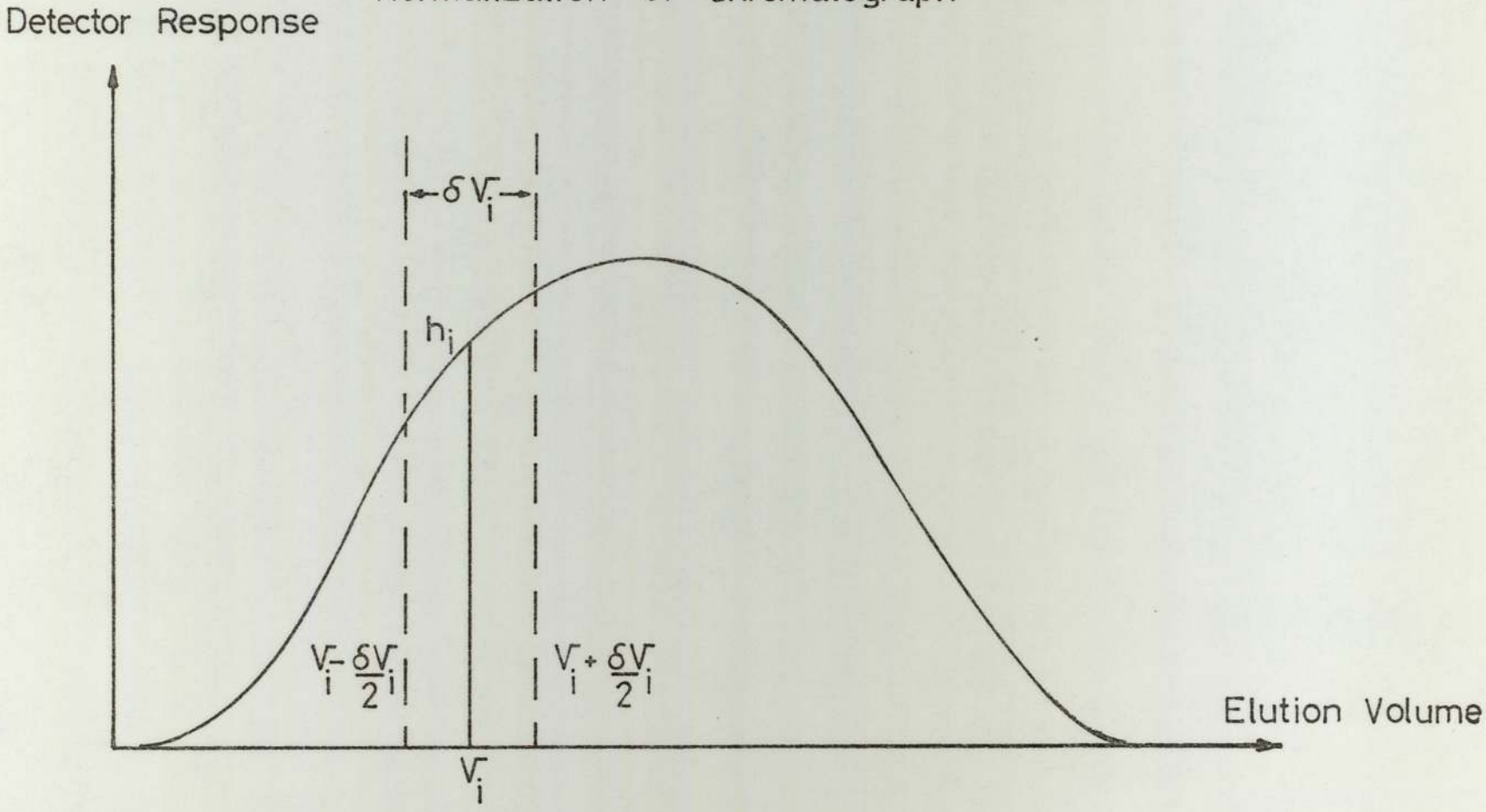
2.7.7 Data Treatment of G.P.C. Chromatographs

(a) Normalisation

One of the simplest but most rigorous methods for reducing g.p.c. data into a differential molecular weight distribution curve has been described by Yau and Fleming (78). Their description of the interpretation procedure begins where the ordinate for the curve dw_f/dV_R has already been normalised. The literature that has covered the normalisation procedure has either been ambiguous or a misunderstanding of the method has been relayed. Therefore before the interpretation procedure can be described the method of normalising a chromatograph is explained.

Most gel permeation chromatograms are produced by measuring a change of an extensive property of the eluate, where the detection response is directly proportional to the concentration of polymer. With reference to Fig. 2.11 if an element of the chromatogram of width δV_R (where V_R is the retention volume) that has an average height h_i, the weight of sample eluted between $V_R - \frac{\delta V_R}{2}$ and $V_R + \frac{\delta V_R}{2}$ is

Fig 2.11
Normalization of Chromatograph



proportional to the area of the element, therefore:-

$$\delta W_{si} = a h_i \delta V_R \tag{2.51}$$

where a is a proportionality constant.

If we consider the chromatograph to be a series of n elements of width δv then the total amount of sample injected is

$$W_S = a \sum_{i=1}^{n} h_i \, \delta V_R \tag{2.52}$$

Expressing the weight of the element as a fraction of the whole chromatogram we get:-

$$(\delta w_f)_i = \frac{\delta W_{si}}{W_s} \tag{2.53}$$

so that the weight fraction of polymer eluted in volume δV_R is:

$$\frac{(\delta w_f)_i}{\delta V_R} = \frac{h_i}{\sum_{i=1}^{n} h_i \delta V_R} \tag{2.54}$$

Note that a change in the measured incremental elution volume will change the dimensions of the ordinate in the normalised chromatogram and that if a normalisation step is to be performed the dimensions of the ordinate should be obtained in a convenient form.

(b) Data Interpretation

In g.p.c. analysis the elution volume can be related to the molecular weight of a sample by calibrating the system with one of the

methods described in Section 2.7.5. Replacement of the elution volume scale under the g.p.c. chromatogram with the logarithmic molecular weight scale does not, in general, represent the true molecular weight distribution. This is true even when the non-linearaties in the abscissa have been corrected for because the ordinate values must also be adjusted.

A differential molecular weight distribution curve may be represented by a plot of $d(w_f)/d$ (log M) versus (log M). To convert the g.p.c. chromatogram into the correct molecular weight distribution the following manipulation of the ordinate has to be made:

$$\frac{d(w_f)}{d(\log M)} = \frac{d(w_f)}{dV_R} \cdot \frac{dV_R}{d(\log M)} \qquad (2.55)$$

where the term $d(w_f)/dV_R$ is the ordinate of the normalised elution curve and dV_R/d (log M) is the reciprocal of the gradient of the conventional calibration curve. This procedure is illustrated by Fig. 2.12.

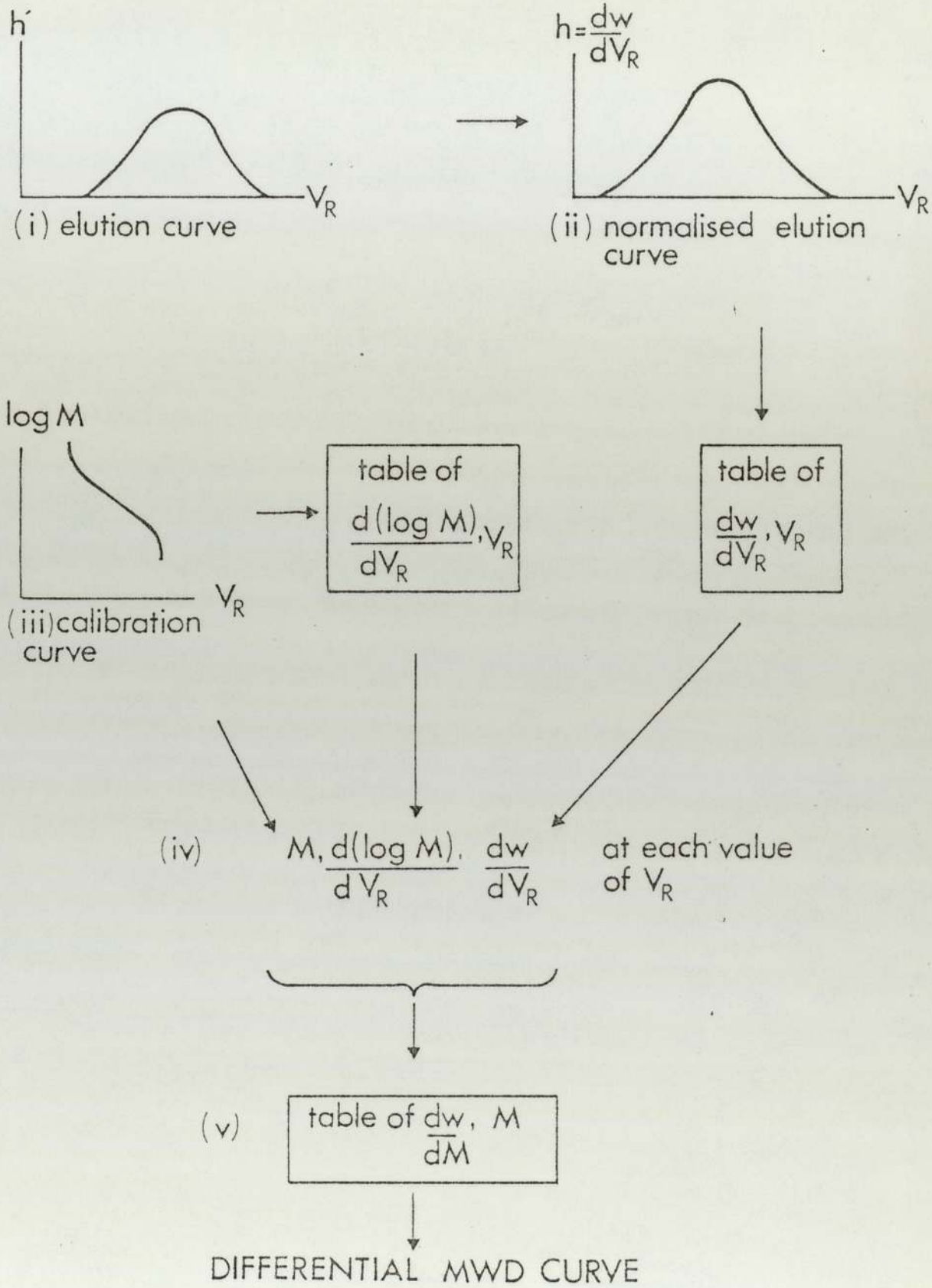
An alternative differential calibration curve could be obtained by plotting $d(w_f)/dM$ versus dM where the ordinate is now obtained by:

$$\frac{d(w_f)}{dM} = \frac{d(w_f)}{dV_R} \cdot \frac{dV_R}{d(\log M)} \cdot \frac{d \log M}{d M} \qquad (2.56)$$

$$= \frac{d(w_f)}{dV_R} \cdot \frac{dV_R}{d(\log M)} \cdot \frac{1}{M} \qquad (2.57)$$

A simple graphical description of this conversion procedure is presented by Yau and Fleming (78) who illustrated by example the

Fig 2.12 Procedure for converting a g.p.c. chromatograph into a molecular weight distribution curve.



errors that might be introduced by failure to correct the ordinates of a normalised chromatograms. With the availability of cheaper electronic hardware, it is anticipated the programmable data treatment devices such as the Dupont MWD-1 with suitable interfaces and software for on-line analysis will provide the necessary molecular weight distribution data rather than the remote computer systems that have been previously reported (60, 79-81).

2.8 RETENTION MECHANISMS IN G.P.C.

Literature dealing with the descriptions of the mechanisms occurring in chromatographic columns can be divided into two categories, equilibrium and transport models. In equilibrium models it is assumed that the time constant for transport in the mobile and stationary phases is very small compared to the minimum residence time in the column and so almost instantaneous equilibrium between the two phases is achieved. In g.p.c. where the mobility of the macromolecules in solution is very low, non equilibrium is more likely to occur.

Models for the g.p.c. separation mechanisms based on the equilibrium partitioning of the solute molecules between the mobile and stationary phases, have taken three forms:-

(a) Steric exclusion model

(b) Stochastic model

(c) Thermodynamic model

Models based on mechanisms other than equilibrium partitioning have been based on:

(d) Separation by flow model

(e) Diffusion-controlled model.

More recently, suggestions have been made that the chromatographic conditions occurring in practical g.p.c. are neither under equilibrium nor in diffusion controlled regions but rather within a 'transition region' where molecular diffusion or transport is important but not controlling. Therefore the final class of model to be discussed will be:

(f) Phenomenological model.

2.8.1 Equilibrium Models

In an equilibrium model it is assumed that as the sample band percolates through the column the rate of exchange of solute molecules between the mobile and stationary phases is greater than the interstitial velocity. Then at equilibrium the concentration between the two phase is related by a distribution coefficient, K_d;

$$C_s = K_d C_m$$

where C_s and C_m are the concentrations in the stationary and mobile phases respectively. In g.p.c. unlike other forms of chromatography the mobile and stationary phases contain the same solvent. As described in Section 2.3 the general equation for the retention volume of a solute molecule is:

$$V_R = V_o + K_d V_i \qquad (2.11)$$

This equation can relate the distribution coefficient K_d to an accessible pore volume but it provides no information about the molecular size of the molecule or the pore structure. This is why g.p.c. is only as yet an empirical science. A review of some of

the attempts to correlate the distribution coefficient is contained below.

(a) Steric Exclusion Models

Steric exclusion may be considered as a mechanism where the solute is excluded from a certain part of the gel matrix due to the configuration of the gel alone. This effect was observed as early as 1956 by Lathe and Ruthven (82) who concluded that the retardation of small molecules on columns containing starch in water was either due to adsorption or due to penetration of the starch granules. Some years later Porath (35) described the distribution coefficient to be proportional to the following relationship:

$$K_d \propto \left(1 - \frac{r_m}{r_p} \right)^3 \qquad (2.58)$$

where r_m and r_p are the mean radii of the molecule and the pore. An important assumption of this model was that the structure of the pores was considered to be conical. This restriction was alleviated by Squire (83) who extended the steric exclusion model to include pore shapes such as cylinders and 'crevices' by the expression:

$$M_{CALC}^{1/3} = \left(\frac{c}{g} \right)^{1/3} \left[1 + g - \left(\frac{V}{V_o} \right)^{1/3} \right] \qquad (2.59)$$

where c and g are constants. However these two descriptions for steric exclusion have been applied to both flexible (dextran) and very dense (protein) molecules (83,84) and are considered to be insensitive to molecular shape and so have only a limited use.

A more realistic approach was attempted by Laurent and Killander (85,86) who used a mathematical description for a distribution of spaces in a random network of straight fibres by Ogston (87) to model their gel matrix. By varying the concentration of the rods in the gel, good agreement between theoretical and experimental results was obtained.

(b) Stochastic Models

A probabilistic approach was used by Giddings and Eyring (88) originally to describe an elution density function for gas-liquid chromatography. This method was used by Carmichael (89) in an attempt to generate elution density functions for g.p.c. The model assumed that the transitions from the mobile to the stationary phases and vice-versa followed a Poisson process. Where,

$$\text{Prob (mobile phase} \rightarrow \text{stationary phase)} = \lambda_1 \Delta t + O(\Delta t)$$

$$\text{Prob (stationary phase} \rightarrow \text{mobile phase)} = \lambda_2 \Delta t + O(\Delta t)$$

and where λ_1 and λ_2 were the rate constants for entrapment multiplied by the concentration of pores, where entrapment is possible and the rate constant out of the pores, respectively. Migration through the column could only occur when the molecule was in the stationary phase.

Carmichael developed the solute/time elution profile to be:

$$P(t) \simeq \exp(-\lambda_2 t - \lambda_1 t_o) \left(\frac{\lambda_1 \lambda_2 t_o}{t} \right)^{\frac{1}{2}} \left[\frac{\exp(p)^{\frac{1}{2}}}{(2\pi)^{\frac{1}{2}} p^{\frac{1}{4}}} \right] \qquad (2.60)$$

where $p = 4 \lambda_1 \lambda_2 t\, t_o$ and t_o is the elution time of a non retained solute molecule. In the derivation of equation (2.60) the following

assumptions were made:

(i) homogeneously packed bed

(ii) dilute solute concentrations (no interactions)

(iii) no molecular or eddy diffusion

(iv) large number of transfers between mobile and stationary phases.

Assumptions (ii) and (iii) limit the application of this model to dilute sample applications and where the rate of the transport processes is small compared to the mean residence time in the column. Since this survey is seeking a form of model that could be used to simulate the semi-continuous chromatographic process where high flowrates and concentrations are used the applications of this form of model are limited.

(c) Thermodynamic Models

Because of the inflexibility of steric exclusion models over the last decade more rigorous models have been proposed to describe the equilibrium distribution of solute molecules between the stationary and the mobile phases. A statistical theory was proposed by Giddings, Kucera, Russel and Meyers (90) for the equilibrium distribution of rigid molecules in inert porous networks. The theory was applied to a model in which the solute molecules varied in shape from spherical to thin-rods and where the geometry and distribution of the pore altered. A simplification of this highly mathematical work is given in a review by Ouano (91). The characterisation parameter used to determine the partioning of all the rigid molecules was the mean external length, $\bar{L}$. This was a new parameter. In the systems that were examined the mean external length was found to characterise the partioning better than other previously suggested parameters such as the radius of

gyration (35).

Casassa (92), assuming that a dilute solution had no interactions with the porous network, considered that a flexible polymer chain was described by random flight statistics and that the distribution of the polymer was governed entirely by the loss of conformational entropy on the transfer of a chain from the mobile phase to the limited space within the pore. To obtain a solution to the change in conformational entropy, a solution to a first order partial differential equation was sought together with the appropriate boundary conditions. Explicit relations were found for the distribution coefficient in terms of the mean square radius for simple geometric pore structures. In a more recent paper Casassa (93) discusses this theoretical treatment of the partial exclusion of flexible-chain molecules in solution from cavities of macromolecular size and its applications to g.p.c.

Another simple thermodynamic model was presented by Ogston and Silpananta (94) who studied the interactions of Sephadex with solutes of bovine serum albumin, dextran and poly-ethylene glycol. Their model required no description of the pore structure or solute molecule. Relating the equilibrium of the solute for the mobile and stationary phases by means of their chemical potentials, from the osmotic pressure relationships and from expressing the activity coefficient in terms of a simple virial expansion (95,96), they obtained

$$\ln \left(\frac{C_s}{C_m}\right) = \ln K_{av} = M_2 \left(2 A_2(C_m - C_s) + \frac{3A_3}{2} [C_m^2 - C_s^2] + vPf - A^+ C_s' C\right) \quad (2.61)$$

where C_s is stationary phase concentration; C_m is mobile phase concentration; A_2 and A_3 are constants; P is constant depending on

Sephadex structure; f is function of partial specific volume, C_s' is internal concentration of Sephadex and A^+ is an interaction coefficient.

A theoretical study of chromatographic separations performed on crosslinked organic polymers by LeCourtier, Audebert and Quivoron (97) where the equilibrium of the solute in the mobile phase and in the gel phase was related by a constant, $K_{eq.}$ This constant was related to the free energy change in the passage of a solute molecule from the mobile phase to the stationary phase by the expression:-

$$\Delta F = -kT \log K_{eq.}$$

and that the total free energy change could be attributed to several factors (98). In the case of non-rigid gels two terms were considered important;

$$\Delta F = \Delta F_s + \Delta F_d$$

where ΔF_s depends upon the entropic variation in moving a molecule between the two phases (this is the steric exclusion effect) and ΔF_d is the 'dissolution' in the gel phase. The space accessible to the solute molecules is no longer limited by the rigid walls of the pores but by molecules that can penetrate the stationary phase as solvent molecules. Developing the model LeCoutier, Andelbert and Quivoron conclude that steric exclusion and 'dissolution' occur simultaneously in a macroporous gel and leads to a double equilibrium:-

Solute in the mobile phase $\rightleftharpoons$ Solute in the pores $\rightleftharpoons$ Solute in the gel phase

so consequently the elution volume can take the form:

$$V_e = V_o + K_1 V_p + K_1 K_2 V_g \qquad (2.62)$$

where K_1 and K_2 characterise fractionation by the steric exclusion effect and the 'dissolution' effect, respectively, and V_p and V_g are the pore volume and volume of the swollen gel, respectively.

A similar description is advocated by Dawkins (99-101) who considers that

$$V_e = V_o + K_{g.p.c.} V_i \qquad (2.63)$$

where $K_{g.p.c.} = K_d . K_p$ and K_d is the steric exclusion term and K_p is the solute-gel interaction term that can be regarded as free energy changes and so,

$$K_{g.p.c.} = \exp \left(\frac{\Delta S_o}{k} \right) . \exp \left(- \frac{\Delta H_o}{kT} \right) \qquad (2.64)$$

where ΔS_o and ΔH_o are the standard entropy and enthalpy changes; k is the Boltzmann constant and T is the absolute temperature. Instead of separating the distribution coefficients and relating them to the pore volume or the volume of the swollen gel, Dawkins has related both the entropic and enthalpic terms to the total interstitial volume of the bed.

2.8.2 Non-equilibrium Models

Non-equilibrium models seek to introduce terms that account for departure from equilibrium partitioning under certain conditions. These models should essentially reduce to the equilibrium cases where high residence times and low solute concentrations occur. An early attempt to apply a non-equilibrium model to chromatography was made by Giddings (102) who postulated that if the deviation from equilibrium

was not very large then the solute concentration could be described by the equilibrium concentration and an equilibrium departure term:-

$$C = C^{+} (1 - \varepsilon) \qquad (2.65)$$

where C is the solute concentration, C^{+} is the equilibrium solute concentration and ε is the "equilibrium departure" term.

Other attempts to describe the mechanism of g.p.c. under non-equilibrium conditions has led to the following classes:-

(d) Separation by Flow

Since Pederson (103) observed the separation of large and small protein molecules on columns packed with small, 20-35 μm, glass ballatini beads he proposed that size separation in g.p.c. may be related to capillary action. In this model an isolated solute molecule undergoing Brownian motion inside a thin capillary will have an average velocity greater than that of the solvent, because the larger solute molecule cannot get any closer to the capillary wall than its mean hydrodynamic radius. It was further assumed that the fluid velocity profile was laminar and therefore had a lower value near the wall. As the larger solute molecules experience only the higher velocities they migrate through the bed quicker than the small solute molecules. A good description of this mechanism of separation by flow was given by Dimarzio and Guttman (104). To relate the flow model concept to a g.p.c. column Dimarzio and Guttman concluded that a column was made up of banks of tubes having two diameters, large and small, with a number of short equal length sectionswith mixing chambers between. Separation by flow occurred in the small diameter tubes but not in the large.

As the molecules moved down the column the choice of tube through which they could travel was characterised by a set probability distribution. A similar theory was proposed by Verhoff and Sylvester (105) who characterised a column by two flow regions; rapid flow of liquid through wide channels taken to be the interstitial spaces between the packed beads; and slow flow through narrow channels inside the porous beads. Casassa (106) in a review of theoretical models used in g.p.c. pointed out that Verhoff and Sylvester's model only differed from an equilibrium model by the fact that mass flow was occurring in the "stationary phase".

(e) Diffusion-controlled Model

The theory behind the diffusion controlled model often required the solution to a partial differential equation which has complex initial and boundary condition such that no closed-form general solution can be obtained. Hermans (107) obtained a solution to the general second order partial differential equation;

$$\frac{\partial C_s}{\partial t} = D_s \left(\frac{\partial^2 C_s}{\partial r^2} + \frac{2}{r} \frac{\partial C_s}{\partial r} \right) \qquad (2.66)$$

where r is the distance from the particle centre, D_s is the solute diffusion coefficient and C_s is the solute concentration, for two limiting cases. The first, equivalent to equilibrium chromatography, where the rate of diffusion was very small compared to the mean residence time, and secondly where the rate of solute diffusion controlled the chromatographic process. The shape of the calculated

chromatograms under equilibrium conditions were symmetrical and Gaussian, but under diffusion-controlled conditions long tails on the trailing edge of the chromatogram were observed. This mechanism was also suggested by Yau and Malone (67) who used a one-dimensional solution to Fick's diffusion equation to obtain the probable distribution of molecules between the mobile and stationary phases. They concluded that both steric exclusion and pore diffusion effects operated in g.p.c. separations/fractionations and that a theoretical model including both of these mechanisms was necessary. In a later paper Yau (108) attributes peak separations to these mechanisms and shows the advantage of using $K_{g.p.c.}$

(f) Phenomenological Model

Ouano and Baker (109) proposed a phenomenological model based on a linear g.p.c. process. The small ratio of particle to column radii which is present in most chromatographic applications allowed them to represent the packed column by a lump parameter model. The geometry of the column was idealised so that the solute molecules diffused across a film from the mobile phase to the stationary phase. An analytical solution representing a wide range of operating conditions could not be obtained, but a numerical solution was incorporated into a simulator computer program.

Kubin (110) using the concept of an impermeable boundary inside the particle of the packing obtained a solution which included the effects of longitudinal and internal diffusion, and steric exclusion. In a similar manner Suzuki (111) used solutions to the general transport equations for packed beds to describe the g.p.c. process and reported the

interpore diffusion coefficient of sodium chloride and the axial diffusion coefficient of blue dextran on Sephadex.

2.9 ZONE BROADENING IN G.P.C.

A Section 2.4 mentioned, zone broadening of a solute species occurs when it migrates through a chromatographic column. One of the more rigorous theories, "the generalised non-equilibrium theory" has been applied by Giddings and Mallik (112) specifically to g.p.c. where they obtained the following expression:

$$H = \frac{4D_m}{3Rv} + \frac{R(1-R)}{20}\,\frac{d_p^2 v}{D_m} + \left[\, 2g_1 d_p + \frac{D_m}{g_2 d_p^2 v}\,\right]^{-1} \qquad (2.67)$$

Giddings eliminated the stationary phase diffusivity when deriving equation (2.67) from the generalised non-equilibrium by assuming that the value of the diffusivity in the stationary phase was two thirds its value in the mobile phase. This assumption was based on the experimental results of Horowitz and Fenichel (113) who observed the diffusion of aliphatic organic non-electrolytes in swollen dextran gels. This assumption should be revised when applied to a polymer system because as Van Kreveld (114) and others (109,110,115,116) have indicated the ratio of the stationary and mobile phase diffusivities vary with molecular weight. Giddings and Mallik (112) presented equation (2.68) in a canonical form, concluding that the use of reduced parameters allows easy comparison between experiments and also provide a 'criterion of excellence' for a chromatographic system. This view is endorsed by Knox et al. (117-119). The canonical form of equation (2.67) is:-

$$h = \frac{4}{3R} \cdot \frac{1}{v_R} + \frac{1}{20} R(1-R) v_R + [2g_1 + g_2 v_R] \qquad (2.68)$$

In this form of equation Giddings and Mallik calculated that the stationary phase term makes no significant contribution to the plate height until the reduced velocity v is in excess of 100 and they imply that relatively high velocities can be used in g.p.c. without producing tailing of the sample peak. Some experimenters have provided evidence that agreed with this theoretical prediction (120,121).

Conversely, Yau's (67,108) interpretation of g.p.c. that the retardation of a solute molecule is the combination of steric exclusion and restricted diffusion has led other researchers to formulate equations for the plate height. Billmeyer, Johnson and Kelley (122-124) considered that two independent contributions caused zone broadening of solute molecules, the free-stream dispersion in the mobile phase and the permeation process in the stationary phase. Thus they developed a diffusion model based on an effective dispersion coefficient, which also included a velocity profile effect. The resulting equation was:

$$H = \left[2\gamma' \frac{D_m}{v} + 2\lambda_e d_p + \frac{2v' R_c^2}{\left(\frac{\gamma' D_m}{v} + \lambda_e d_p\right)} \right] + P_c v \qquad (2.69)$$

where γ' - tortuosity factor = 2/3

λ_e - eddy diffusion constant = 1/11

v' - velocity profile constant

P_c - permeation constant.

If the first three terms only in equation (2.69) are used a series of curves similar in form to the curves produced by Giddings coupling theory are obtained.

Conflicting evidence exists concerning the relative importance of the mobile phase effects, as in the above equation (2.68), and elsewhere (125,126) where the velocity profile effect is assigned great importance. Whilst the data of Ouano and Biesenberger (127) and Hendrickson (128) indicate that plug flow is valid under most operating conditions.

Vink (129) defined a reduced dispersion term which is analogous to plate height. Vink (129) concluded that the reduced dispersion equation was essentially similar to the theory of Giddings and Mallik (112) despite being derived from a diffusion model. More recently Vink (130) has applied a detailed treatment for solute transport in packed columns and in particular the effects that arose from the variations in the properties in the packing material.

Kubin (110) developed a model that included the effects of diffusion within the stationary phase and longitudinal diffusion in the mobile phase and of steric exclusion by using a shrinking core model which in an elementary manner took into account the complicated geometry of the porous packing. The derived equation for the mechanism was:

$$H = \frac{2D_p}{v} + \frac{d_a^2}{15D_i} \quad \frac{2v\, F_c K\, (1 - \rho^3)[1 - G(\rho)]}{[1 + F_c K\, (1 - \rho^3)]^2} \qquad (2.70)$$

where, D_p - longitudinal dispersion

v - linear velocity

$F_c = \frac{(1-\alpha)}{\alpha}$ where α is the fractional free cross section of the column

K - partition coefficient

ρ - dimensionless parameter

$= \frac{d_a}{d_p}$

where d_a diameter of impermeable core

d_p diameter of particle

$$G(\rho) = \rho^3 . \frac{4 + 4\rho + 5\rho^2}{1 + \rho + \rho^2}$$

D_i - diffusion coefficient in particle

A similar derivation was performed by Van Kreveld and Van Den Hoed (114) who considered that as a result of previous work the inclusion of the dimensionless parameter ρ and the function $G(\rho)$ can be neglected from equation (2.70) and as a result the equation is simplified. However, having introduced one simplification Van Kreveld and Van Der Hoed like Vink (130) separate the longitudinal diffusion term into a molecular diffusion and eddy dispersion and that inter-particle diffusion into a hindered dispersion and an eddy dispersion term thus forming

$$H = 2A + \frac{2D_m}{v} + \frac{2vE_pK}{(1 + E_pK)^2} \cdot \frac{r_p^2}{15(D_i + vD_e)} \qquad (2.71)$$

As with equation (2.69), equation (2.71) is also mathematically equivalent to that of Giddings coupling theory but it must be stressed that the coupling theory considers transport processes only in the mobile phase. The separation of the diffusion terms lead Van Kreveld and Van Den Hoed to question the assumption of Kubin about the molecular weight dependence of the coefficient of diffusion in the packing being the product of the bulk phase diffusivity and a fixed tortuosity factor. They considered that the larger the molecule relative to the pore size the more the diffusion will be hampered by the tortuosity of irregually shaped pores.

Van Kreveld and Van Den Hoed justify their separation of the diffusion terms to include an eddy dispersion by stating that they observed enhancement in the mass transfer between the mobile and stationary phase with an increase in velocity. This is contrary to the effect suggested by the restricted diffusion theory.

Horvath and Lin (131) examined the effect of unsorbed solutes in liquid chromatography by the application of an interstitial stagnent film model, and obtained plate height equations for solutes that could and could not permeate the column packing. Discussing the theoretically predicted plate heights with those obtained experimentally Horvath and Lin compared the theoretical models of Giddings (132) and Huber (133) and concluded that their model described the velocity dependence of plate height more accurately. They also showed that their theoretical model reduced to a form that is in agreement with the empirical equation of Done and Knox (134) at low reduced velocities, and in a later publication (135) how at high reduced velocities predicts the linear dependence of plate height on velocity as observed by Edele, Halaszi and Unger (136). Other semi-theoretical descriptions of zone broadening have been reviewed by Grushka, Knox and Snyder (119).

2.10 FACTORS EFFECTING G.P.C.

(a) Concentration Effects

Concentration effects in g.p.c. have been observed by many researchers, but there is still confusion and opposing views on the effects of concentration. An early attempt to describe the concentration dependence of the migration rate of a solute was by Winzor and Nichol (137) who adapted an equation proposed by Johnston and Ogston (138) for the sedimentation velocities of macromolecular species in an ultracentrifuge, and found reasonable agreement the theoretically predicted and

experimentally observed migration rates for several proteins on Sephadex G-100. The same authors did not observe any concentration dependence on the elution volume of dextran-500, although Laurent and Granath (139) observed an increase in retention volume of dextran with increase in concentration.

Waters (140) attributed the increase in elution volume with increased concentration to the higher viscosity of the injected solution. This hypothesis was supported to a limited extent by Goetze, Porter and Johnson (141) who injected polymer samples that had constant polymer concentration but variable viscosity, and observed a slight increase in the elution volume with viscosity. The experiments also showed a reduction in column efficiency to about 41% of the originally observed efficiency when the viscosity of the sample was approximately doubled but then remained constant for viscosities up to 37 times the relative kinematic viscosity of the original sample. Similar observations on column efficiency were made when the polymer concentration in the sample was increased but this had a great effect on the elution volume of the solute peak. Flodin (142) in a study using g.p.c. for desalting operations observed that the eluted peaks of haemoglobin and sodium chloride changed from symmetrical and completely separate peaks to distorted profiles by the addition of dextran to the sample which varied the relative sample viscosity from 1 to 11.8. Flodin cited compression of the column packing as a possible explanation of the observed phenomena, and suggested that the viscosity of the sample rather than the concentration was a limiting factor. The concept of a sample behaving as a high-viscosity "slug" flowing through a packed bed has been discussed by Moore (143).

Goetze, Porter and Johnson (141) concluded the "viscous fingering" effect cannot alone be wholely responsible for the change in elution volume with increased concentration. Rubin (144,145) has shown that the effective hydrodynamic volume of the macromolecule decreases with increasing concentration and therefore as a result enhanced the penetrability of the macromolecule. This has been shown experimentally by Vander Linden and Van Leemput (146). In the case of compressible organic gels, Baghurst et al. (147) have proposed that osmotic shrinkage of the gel particles occurs as the concentration of the solute increases which leads to changes both in the pore and total bed volumes. In non-compressible gels finite concentration effects are commonly attributed to equilibrium distribution coefficients in addition to the conventional steric exclusion coefficient and as a result are commonly referred to as "secondary exclusion" effects. These effects have been proposed and discussed by several researchers (99,120,148-150), but recently in an extended research programme Janca (151-155) has examined and discussed concentration dependent factors effecting g.p.c.

Some observations of concentration-dependent effects have been reported where the increase in concentration has aided the separation or fractionation of a system. Algelt (120) reports several examples. An example of the concentration effects in the fractionation of dextran has been reported by Hatt et al. (10, 75, 148) where they observed a reduction in the elution volume of "large" molecules and an increase in the elution volume of "small" molecules. This effect they attributed to influence that a molecular weight species exerts on molecules of both greater and lesser molecular weight. Under concentrated conditions a molecule is influenced by lower molecular weight molecules

causing it to be excluded from the stationary phase to a greater extent than under dilute conditions, while the higher molecular weight material than the reference species causes lower molecular weight molecules to be included in the stationary phase to a greater extent. The net effect of this mechanism causes an increase in the separation of the large and small species. Using fractions of Dextran T-10, T-80, T-150 and T-500 in concentrated conditions, on a Porasil-D column a series of calibration plots were recorded exhibiting an increase in the gradient of the calibration curve compared to the curve obtained under dilute conditions. Verification of these results has recently been confirmed by Holding (156) who used a labelled dextran samples in a dextran eluent having concentrations of up to 200 $g.l^{-1}$.

When using g.p.c. as an analytical method to determine molecular weight averages, the concentration dependence of the eluted profile can be a source of error. This effect has been discussed and correction methods proposed by several authors (157-159).

(b) Temperature Effects

Relatively very few papers have considered the effect of temperature upon g.p.c.. Giddings (160) considers in the limiting case where the variations in temperature do not cause precipitation, adsorption or changes in the pore or molecular dimensions that temperature affects only the diffusivity of the solute which has an effect on the efficiency of the column. This has been demonstrated experimentally by Unger and Kern (161) who observed an increase in the number of theoretical plates for a column as the temperature increased.

The relationship was approximately linear. The proportionality factor was also observed to increase with increasing molecular weight. Giddings considered that because the mobile and stationary phase in g.p.c. are the same, changes in the diffusivity of the solute in the mobile phase determine the temperature dependence of the plate height. However this conclusion is not universal as discussed in Section 2.8 and is particularly relevant when considering dextran fractionation because Weber (162) reported that dextran molecules exhibit new rotational degrees of freedom at higher temperatures which may aid intra-pore diffusion.

The effect of temperature upon elution volume remains inconclusive. Unger and Kern (161) reported that the elution volumes of polystyrene in tetrahydrofuran (T.H.F.) were independent of the variation in temperature, whilst Cantow, Porter and Johnson (163) report a pronounced reduction in elution volume polystyrene and polyisobutene in 1,2,4-trichlorbenzene with an increase in temperature. In aqueous systems the effect of temperature upon g.p.c. has been confined to separations performed on soft gels (164,165) where the effect of temperature has caused structural changes in the packing materials. Therefore these studies could not confirm the findings of Unger and Kern who concluded that it was the effective hydrodynamic size of a polymer molecule that largely determined the elution volume and that the temperature affects the hydrodynamic volume of the polymer.

3.0

PRODUCTION SCALE CHROMATOGRAPHY

3.1 CLASSIFICATION OF EQUIPMENT

The superiority of chromatography for separating complex mixtures has been demonstrated (166) compared to other more conventional separation methods. However, the usual chromatographic technique normally operates in a small batchwise manner handling micrograms of material, whilst other separation processes, such as distillation or solvent extraction are capable of handling tonnage quantities per day. If the utilisation of the chromatographic method of separation or fractionation were to be developed into a unit operation it could become a useful separation process. For the last quarter of a century attempts have been made to increase the throughputs of chromatographs.

Two principal approaches have been used:

(1) direct scale-up of analytical equipment by automated repetitive operation of large diameter columns,

(2) the development of novel designed equipment that permit continuous introduction of feed. For this thesis for a chromatograph to be considered continuous it must exhibit two features:-

(i) separation or fractionation of the components should occur by a recognised form of chromatography (see Section 2.2 and Liodakis (224));

(ii) a continuous supply of feed is supplied to the equipment. This category of equipment can be further itemised into:

(a) counter-current systems

(b) cross-current systems

(c) co-current systems.

This section surveys the equipment that uses chromatography as a preparative or production process.

3.2 BATCH CHROMATOGRAPHY

Various factors effecting the scale up of batch chromatography have been discussed by Ellison (34). Conder (17) has for many years investigated the commercial application of repetitive pulse systems for large-scale gas chromatography. In the field of liquid chromatography there are already preparative systems commercially available. A small preparative system marketed by Waters Assoc. Massachusetts, U.S.A. is the PrepLC/System 500 (167) is designed specifically for the production of gram quantities of material in a laboratory. The PrepLC/System 500 is a completely self-contained unit comprising variable solvent inputs, pump, sample introduction, columns, detection and recording systems. The columns are prepacked silica cartridges that are radially compressed, and are 5.7 cm diameter by 30 cm long. Recycling of the eluate through the columns and detector more than once during a single separation can effectively increase the column length.

An exchange of information (168) between Abcor, Inc., an American scientific development company specialising in chemical separation technology and C. F. Boehringer and Sohne a major German pharmaceutical company has developed a baffled column system of 51 cm diameter for the commercial preparation of a pharmaceutical intermediate. In 1967, Boehringer Mannhein Co., patented a process for the simultaneous hydrolysis of sucrose or sucrose containing invert sugars and the separation of the resulting products. This process has been discussed in detail by Ching (169).

Another notable contribution to the design of large scale commercial chromatographic equipment has been by the Pharmacia Fine Chemicals Co., Uppsala, Sweden, where two types of equipment are commercially available. The two systems are the Sephmatic system which is designed for high capacities and for use with more rigid gels and

a stacked system that is designed for use with the softer cross-linked dextran gels. Numerous examples of the applications of these systems are available in group separations, desalting and purification of low molecular weight compounds (170-181). One of the largest diameter columns as yet reported for use with g.p.c. has been the Sephmatic G.F. 18-10, of dimensions 180 cm diameter by 100 cm long which Linquist and Williams (182) have described for the processing of whey, to produce a 75% protein powder at 28 kg h^{-1}. Delaney, Donnelly and co-worker (183-186) in a major pilot-plant study have examined the effects of some of the process conditions using a Sephmatic system. Horton (173) has examined in detail the cost effectiveness of using g.p.c. as a unit operation utilising data obtained for the desalting of bovine plasma albumin with a Sephmatic GF 04-10 gel filter.

Other examples of the use of g.p.c. as a gram-scale preparative technique are available using batch chromatography in both aqueous (187-194) and non aqueous (195-199) systems.

3.3 CONTINUOUS CHROMATOGRAPHY

3.3.1 Counter-current Systems

Counter-current chromatography has been developed in three stages:

(i) Moving bed

(ii) Moving column

(iii) Simulated moving bed.

Each category is described below.

(i) Moving bed

In moving bed chromatography, continuous movement of the stationary phase in the opposing direction to the mobile phase achieves a separation. A typical small scale moving bed chromatograph has been used by Barker and co-workers (200,201) for the separation of a binary mixture of volatile organics using gas chromatography. This equipment was later modified for use with ternary hydrocarbon mixtures (202,203) by the introduction of a side arm. Details of other successful laboratory scale units functioning on the same principle have also been reported (204-207). Buhl and Yeagle (208) patented a counter-current process where the bed was moved by a helical screw, Frost (209) suggested that this type of equipment could be used with a molecular sieve slurry.

Industrial applications of a moving-bed system have been used to recover ethylene from a mixture of hydrocarbon gases (210) but has subsequently proved uneconomic with the development of low temperature distillation (166). The future for moving-bed chromatographic columns is thought to be limited because of the following inherent disadvantages:-

(i) Movement of large quantities of solid packing is difficult and expensive.

(ii) Attrition of packing.

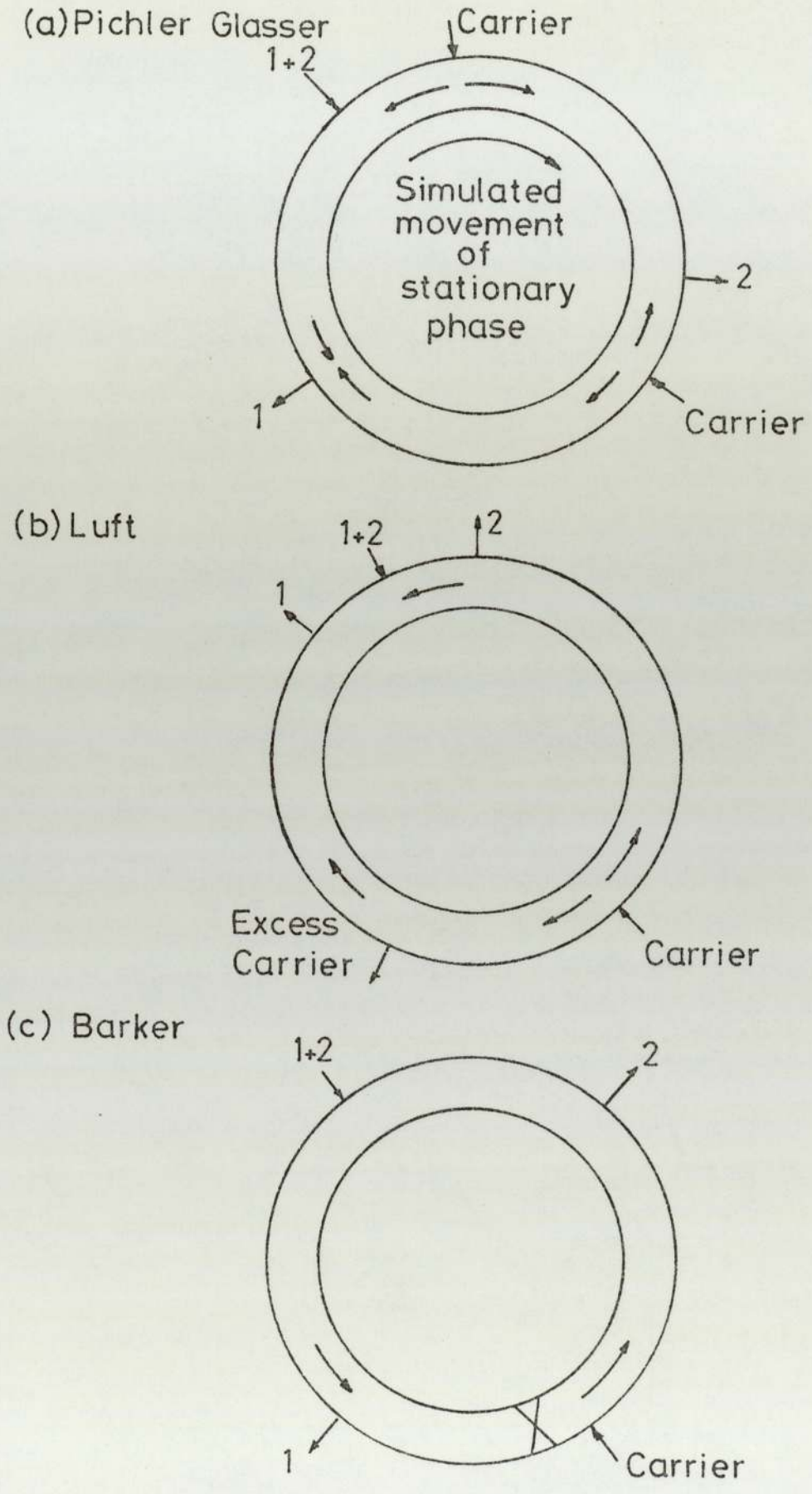
(iii) Unevenly packed columns results in low efficiencies.

(iv) Mobile phase velocity limited by fluidisation velocity of the bed.

(ii) Moving Column

To overcome the above problems various designs of equipment have been proposed based on the rotation of a closed circular column past fixed inlet and outlet parts in the opposite direction to the mobile phase flow. Three basic schemes have been proposed (211-214) and are illustrated diagrammatically in Fig. 3.1. The schemes illustrated

Fig 3.1 Proposed schemes for moving column counter-current chromatography.



in Figs. 3.1a and 3.1b propose that the mobile phase flowrates and relative port positions be selected such that the direction of flow was achieved by balancing the pressure drops across the packed columns. This led to excessive mobile phase being used to ensure that a proportion passed counter-currently against the rotation of the column. The inclusion by Barker (214) of a cam-operated valve between the mobile phase inlet port and the Product 1 outlet port ensured that the mobile phase was unidirectional. Further developments of the design ensured (9,15,148,215,216) and the improved equipment has demonstrated dextran polymer fractionations (15,216). The feasibility of having a large scale production equipment with moving columns is thought unlikely and that the main application of a moving column system would be in laboratory sized, gram scale production of fine chemicals.

(iii) Simulated Moving Bed

The inherent problems of moving bed designs and the limited size of the moving columns has activated research into achieving a counter-current scheme through simulated bed movement of all the continuous chromatographic designs available this category of design has the most industrial interest. Universal Oil Products have already developed a group of continuous chromatographic processes with industrial applications. Generally known as Sorbex (217-219) individual processes include Molex for the recovery of n-paraffins from light napthas, Parex to separate p-xylene from other C-8 hydrocarbons and Olex which separates n-olefins from an olefin/paraffin mixture. A report by de Rosset et al. (220) lists other aromatic isomer separations. There are already 20 Molex units in operation producing 0.446×10^6 tons per year of n-paraffin, and four Olex units producing 0.268×10^6 tons per year (221).

Sussman (166) reports that the planned or installed capacity for the Parex units is 1.339×10^6 tons per year. The Sorbex process has a generalised flow scheme where the solid phase is packed into the compartments of a vertical column, and each compartment is connected to a specially designed rotary valve operating on a multiport stopcock principle. The simulated movement of the packing is achieved by the indexing of the input and output ports.

Szepesy et al. (222) constructed a chromatograph similar to the Sorbex process. The equipment consists of twelve glass columns interconnected by means of a switching valve which was centrally mounted on a rotary P.T.F.E. disc. Rotation of the valve alters the inlet and outlet ports and in such a manner that the counter-current column movement is simulated.

Barker and co-workers have also investigated an alternative means of scaling up the continuous counter-current process. A design by Barker and Deeble (5,6,223-226) was based on a moving port system where the inlet and outlet port functions are controlled by an automatic timing device actuating the appropriate solenoid valves in the chromatograph. Several development stages of this design have followed (34,169,223,224). Although this type of equipment fulfills both the conditions for continuous chromatography as outlined in Section 3.1, the concentration and solute composition at any point within the chromatography cycles from one set of port functions to the next. This is because of the discontinuity of the movement of the port functions relative to the mobile phase. Therefore only a pseudo-equilibrium occurs and so it is considered that the term "semi-continuous chromatography" be applied to this type of equipment.

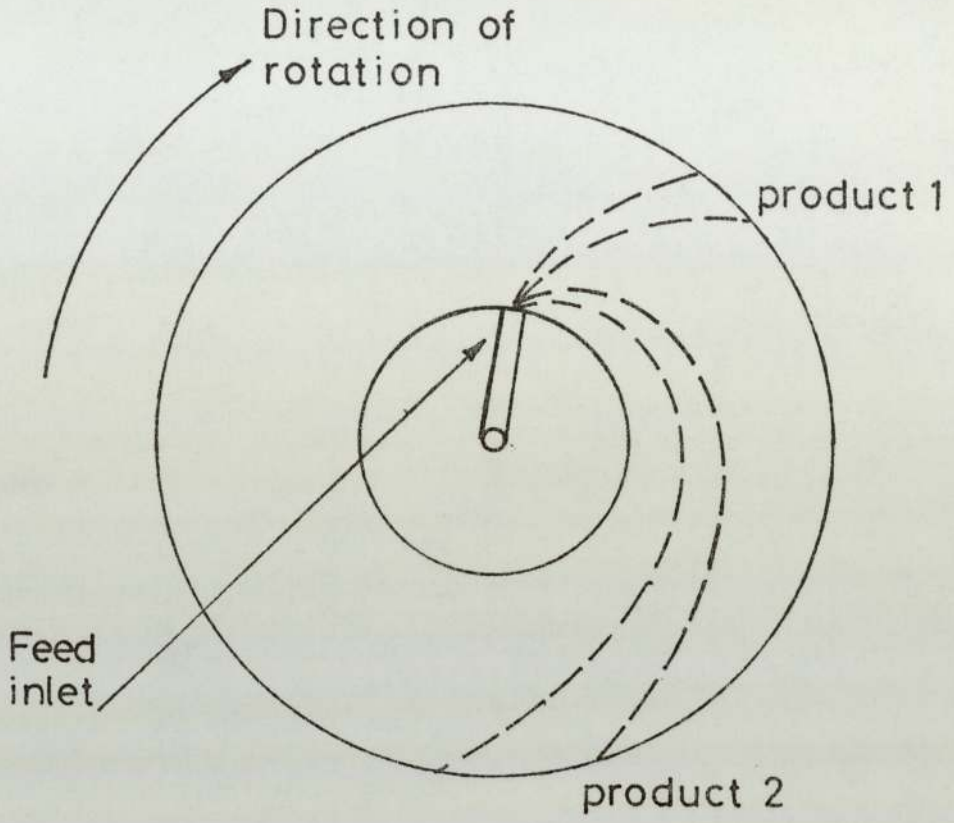
3.3.2 Cross-current Systems

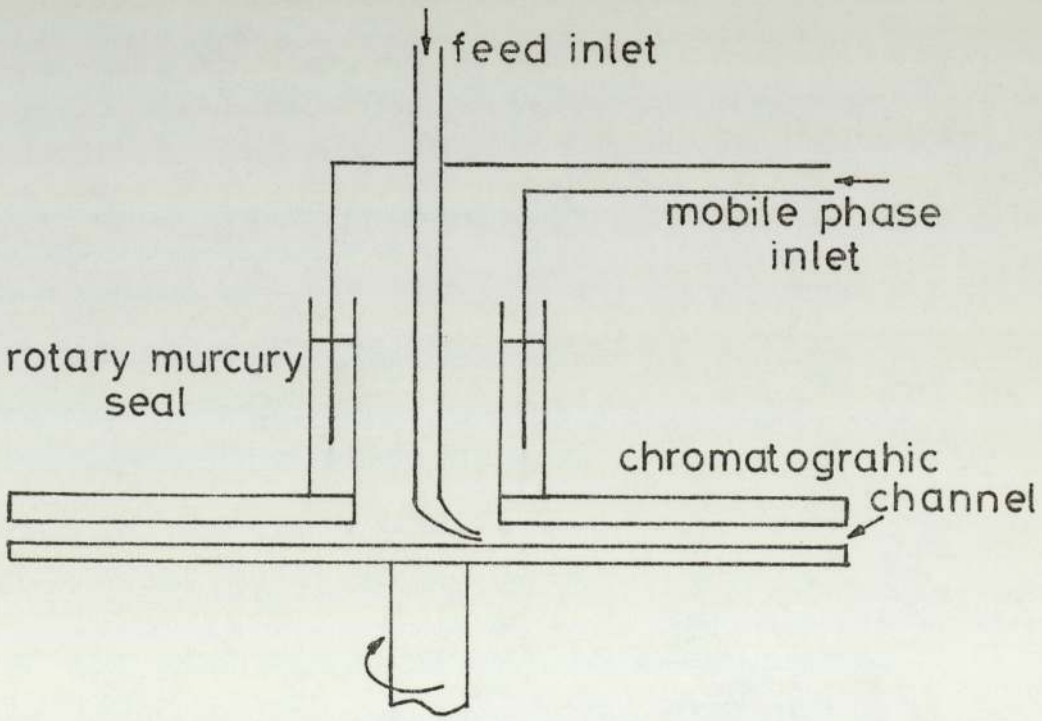
In a cross-current system the chromatographic bed moves perpendicularly to the mobile phase. Martin (225) as early as 1949 suggested this type of equipment and provided a theoretical analysis. Giddings (226), Sussman (227,228) and more recently Wankat (229,230) have also provided theoretical treatment of this type of equipment. Several researchers have built cross-current units based on the principle of a helical flow scheme, where the rotating annulus has been constructed in the form of a packed bed (231, 232), a series of packed columns arranged in a circle (233-239) and a paper cylinder (240-242). Fox (237-239), and Dunhill and Lilley (243) have reported the use of such a device for g.p.c. separations. Sussman and co-workers (227,228,244,245) have essentially reduced this principle to a radial thin layer gas/liquid chromatography form where the stationary phase is coated on to two rotating discs as shown in Figure 3.2. The unique characteristic exhibited by cross-current systems when compared to other forms of continuous/semi-continuous chromatographic equipment is the ability to continuously resolve a multi-component mixture in a single-stage operation. However, the degree of precision necessary in the construction of this type of equipment is likely to restrict the large scale uses of the proposed chromatographs to laboratory scale applications.

3.3.3 Co-current Systems

Considerable interest in cyclic separation processes, or operation under unsteady state conditions to enhance the separation process have been increasing in recent years. Process variables such as feed flow (246,247), temperature (248-250), pH (251), and pressure (252,253) have been varied. However extensive development work is necessary before any of these schemes could be applied to a commercial process. Reviews covering the cyclic co-current schemes in greater

Fig3.2 Radial flow scheme for continuous-chromatography.





detail have been presented by Barker (254) and Sussman (166);
these reviews also cover the above types of continuous chromatographs.

4.0

ANALYTICAL EQUIPMENT AND PROCEDURE

4.1 ANALYTICAL EQUIPMENT

A relatively simple chromatographic system was used for the analysis of the products, consisting of a pump, sample introduction device, column(s), detector and recorder. All results were obtained by manual measurement of the chromatograms. An illustration of the arrangement for the chromatographic system is shown in Fig. 4.1, and sketched in Fig. 4.2.

4.1.1 Eluent and Sample Delivery Systems

Throughout the analytical programme, the eluent was either distilled or deionised water. Occasional checks on the pH found the water to be slightly acidic. No buffers, electrolytes or bacteriostats were added to the eluent although the samples to be injected were often saturated with chloroform or had sodium azide present at 0.02% w/w as bacteriostats. Two different pumps were used to pump the eluent, both positive displacement pumps; for low pressure chromatography (less than 20 bar) a Metering Pumps Ltd. Series II model and for high pressure chromatography a Bran and Luebbe Ltd. Normados model. Samples were applied using sample injection valves supplied by Spectroscopic Accessory Co., types 30.100 and 30-501 and fitted with constant volume (20 µl) sample loops. All the samples were filtered before being injected on to the column using a syringe filter (supplied by Millipore, London) that was fitted with a 0.46 µm cellulose acetate and nitrate mixed disposable filter.

4.1.2 Fractionating Columns

Three types of fractionating column system were used during the research project. The initial fractionating system was a low pressure (below 20 bar) arrangement performed on a glass H.P.L.C.

Fig 4.1 Photograph of analytical system

Key

C Fractinating column

Fr Flat-bed recorder

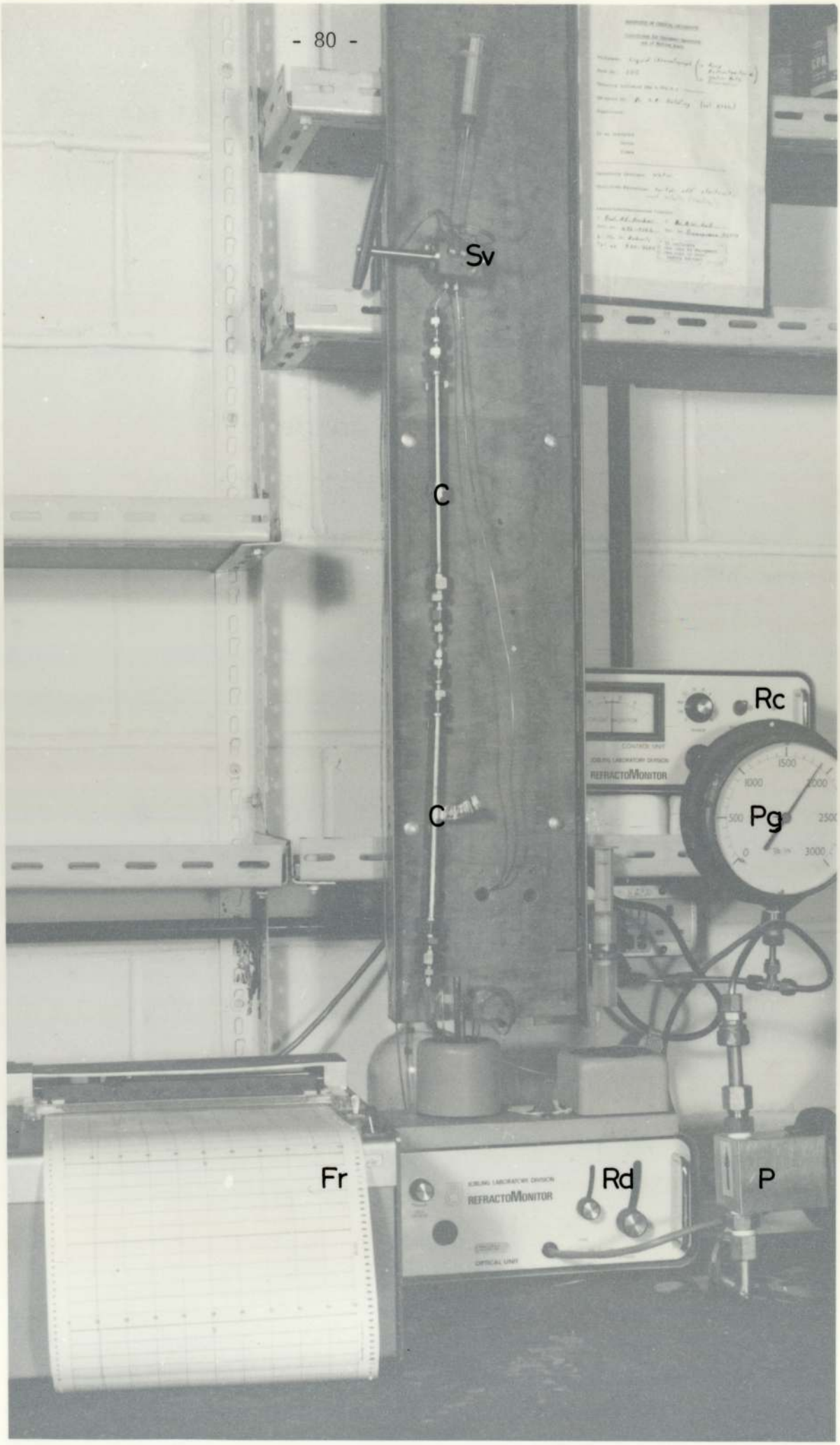
P Eluent pump

Pg Pressure gauge

Rc Refractometer control unit

Rd Refractometer detector unit

Sv Sample valve


Sv
C
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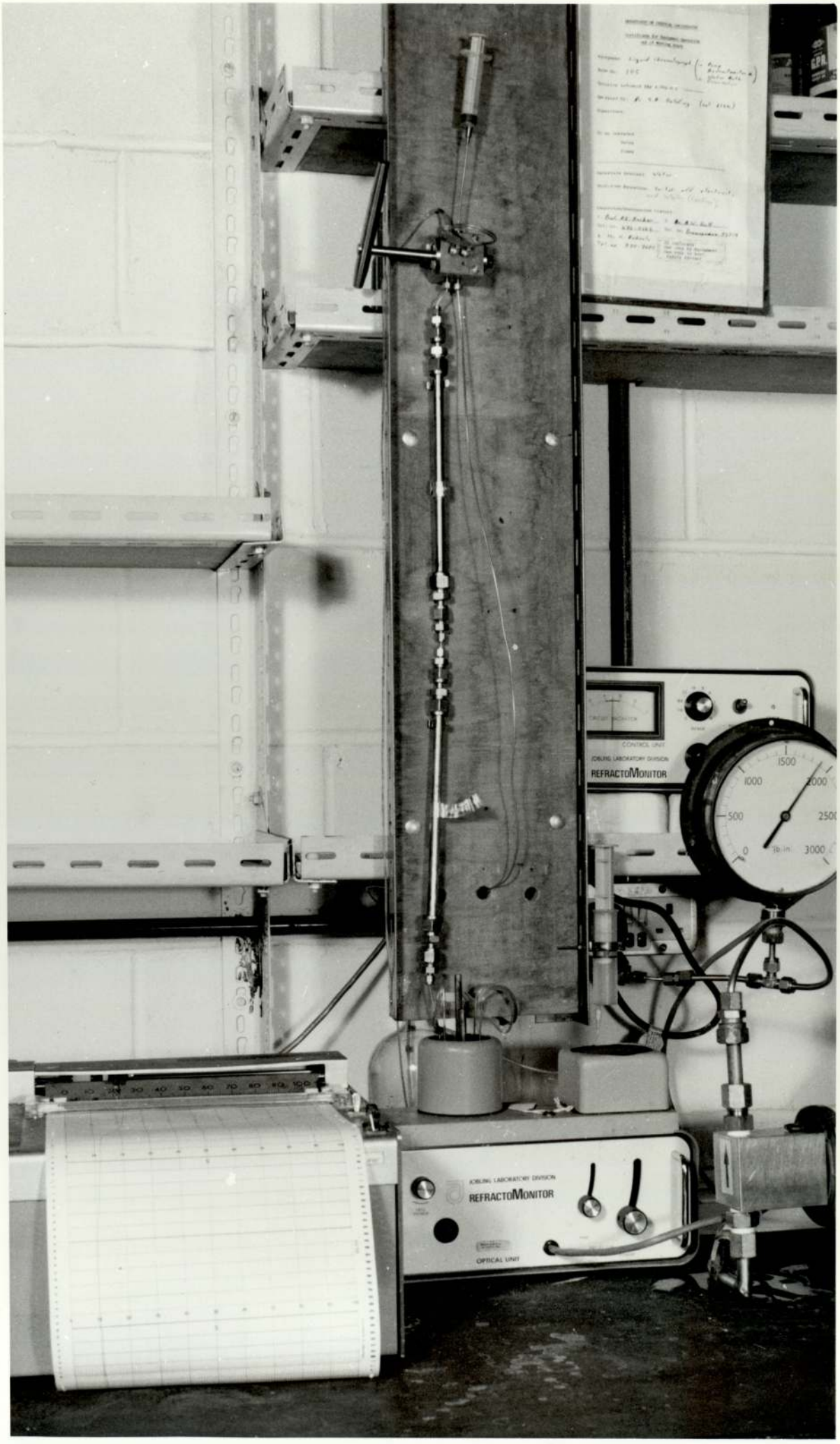
REFRACTOMONITOR
REFRACTOMONITOR


CONTROL UNIT
JOBLING LABORATORY DIVISION
REFRACTOMONITOR
1500
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lb-in
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REFRACTOMONITOR
OPTICAL UNIT

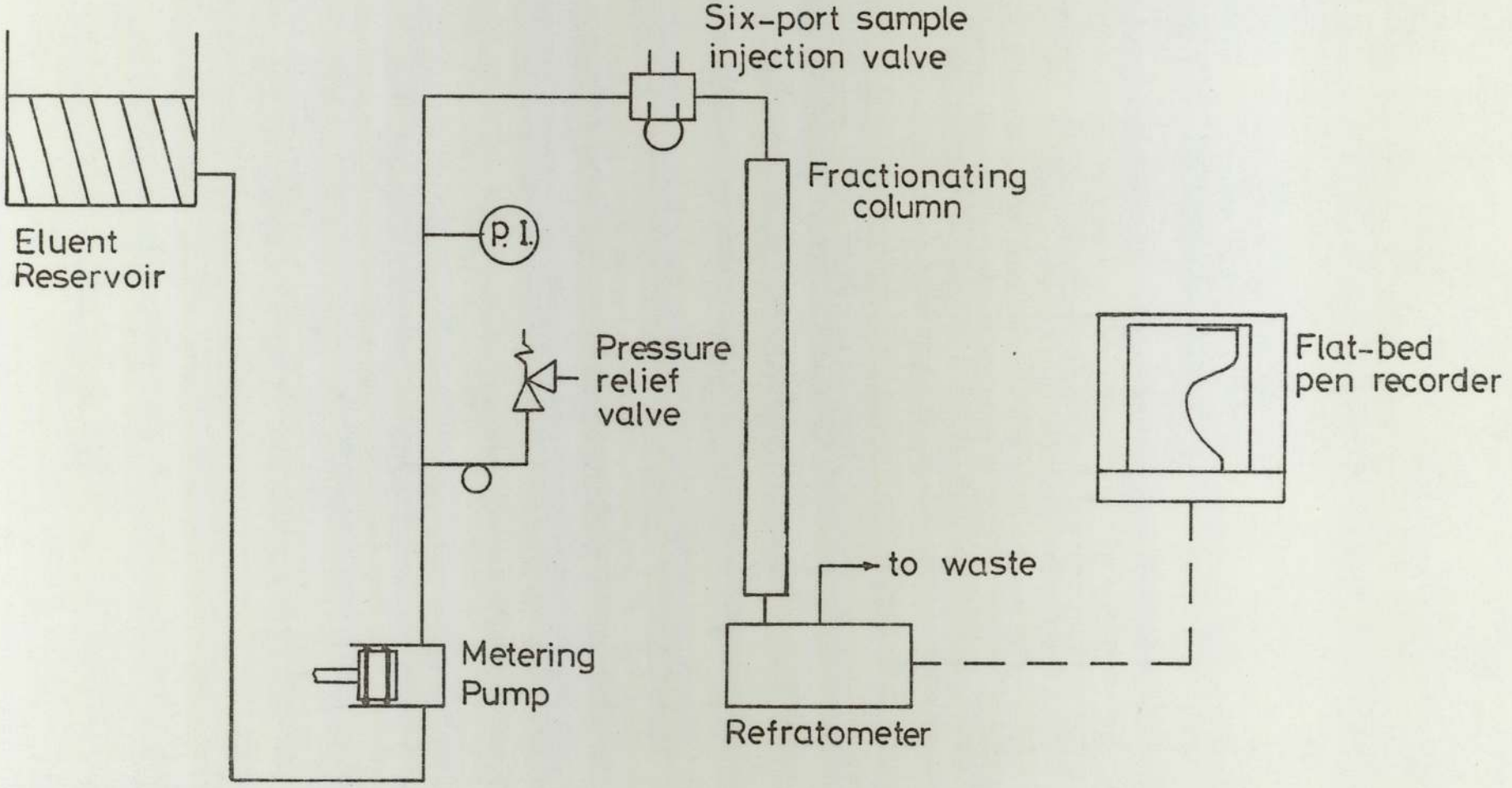


Fig 4.2 Analytical Equipment

column 1M long by 4MM i.d. and packed with Porasil C. Subsequent analytical chromatography was performed at higher pressures (greater than 20 bar) on either a pair of pre-packed Du Pont Size Exclusion Chromatography (S.E.C.) columns, or on a stainless steel H.P.L.C. column 250MM long by 8MM i.d. and packed with a research batch of Spheron P 1000. The problems associated with each set of fractionating columns were:-

Porasil C

Porasil is a trade name under which Spherosil is marketed by Waters Associates (255). The 'XOB075' and the 'C' grades are of equivalent porosity. Therefore this analytical packing was equivalent to the material used in the preparative columns that are described in the following chapters. However the particle size range for the analytical column was much smaller at 35-75 μm.

The column efficiency was approximately 1000 plates when the eluent flowrate was about 0.25 $cm^3\ min^{-1}$. The analysis time was approximately 40 minutes. The column was in use for several months during which time the column did not show any signs of deterioration. However, inconsistencies were observed when some dextran peaks were eluted with extensions beyond the total liquid volume of the column. This column did not have sufficient resolution to enable the conversion procedure into a molecular weight distribution form to be used.

This system was greatly affected by base-line noise at the most sensitive settings and so higher sample concentrations had to be used. The base-line instability appeared to be an inherent feature of the glass column; subsequent experience with metal columns showed that base-line noise was minimal even at the most sensitive range of the refractometer.

The minimising of the baseline noise was thought to be due to the greater thermal capacity of the metal columns.

Du Pont S.E.C. Columns

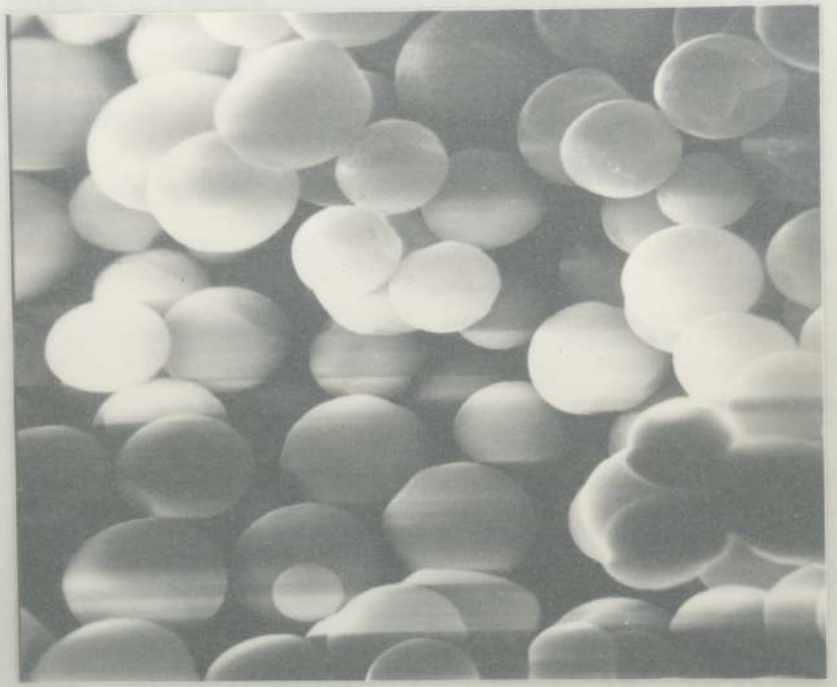
A pair of pre-packed Du Pont S.E.C. columns, SI 500 and SI 100, were arranged in series with the higher porosity column (SI 500) first. These columns initially had a very high efficiency, approximately 13,000 plates, and a very wide fractionating range, from 10^3 to 10^6 daltons. Unfortunately there was a marked drop in efficiency with use. The first part in the drop of efficiency was accompanied by the appearance of a shoulder on the trailing side of the glucose peaks, until this developed into a large tail as the efficiency continued to drop to about 1000 plates after about 500 hours of use. When the columns were opened, the SI 500 column bed had dropped by approximately 5 mm and the SI 100 by 1 mm. Scanning electron photomicrographs showed that both column packings still consisted of spherical unbroken beads, as shown in Fig. 4.3(a) and (b).

The Du Pont S.E.C. material was a silica gel and therefore thought to be similar to the Spherosil XOB075. If silica dissolution was the cause of the loss of efficiency in the analytical columns there was concern that silica dissolution may be occurring in the preparative chromatograph as well. Therefore advice from Pilkington Brothers Limited, Glass Manufacturers Research and Development Laboratories was sought. From the resulting discussion the following conclusions were made.

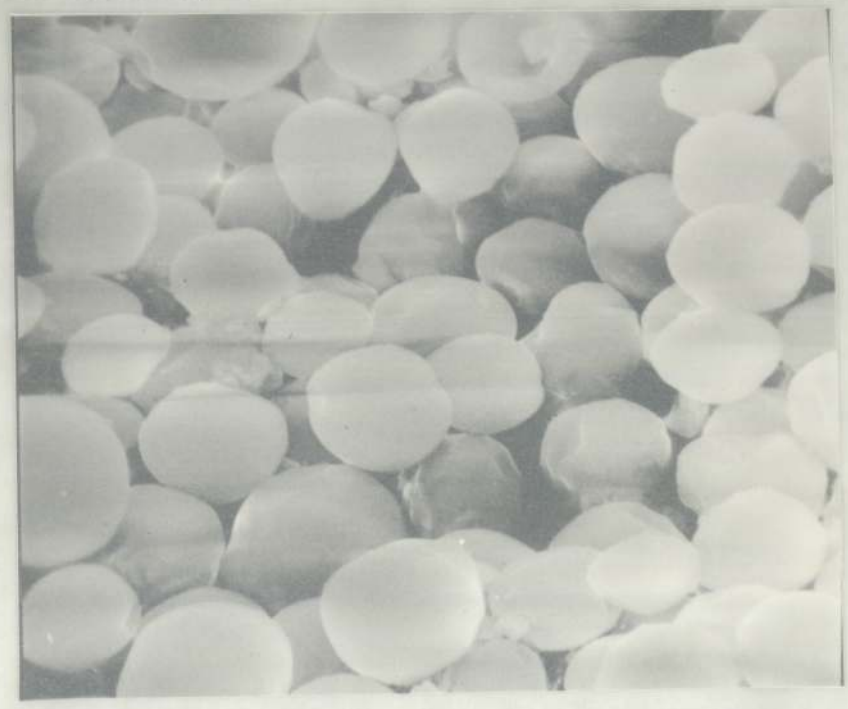
(a) glass/silica gels are prone to swell/contract depending on the chemical state of the system surrounding them and that they should not necessarily be considered as being a rigid matrix.

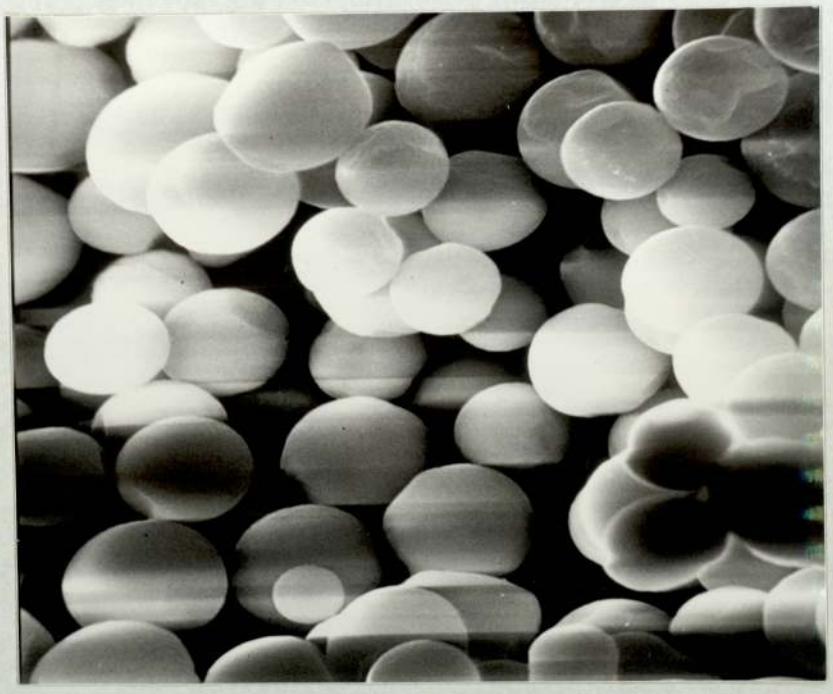
Fig 4.3 Scanning photomicrographs of Dupont SE packing materials.

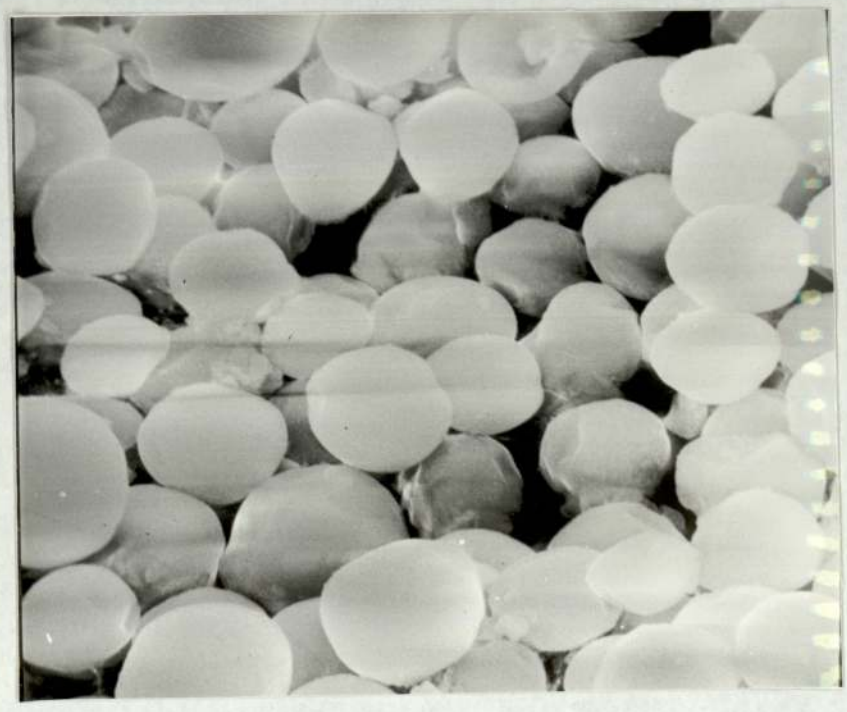
(a) SE 500x 2K



(b) SE 100x 2K





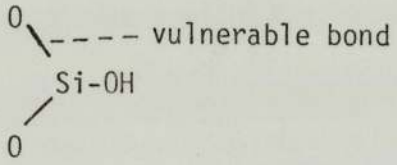


(b) the durability of the matrix can be affected by both the chemical state of the medium in which it is confined and also the mechanical stresses acting upon the system.

(c) inorganic impurities in the silica matrix may also reduce the durability.

(d) a combination of the large surface to volume ratio, high mechanical stresses (high pressures) and the chemical conditions present in the analysis of dextrans on microspheres of silica the risks of breaking up the silica matrix on both a chemical and physical scale were possible.

(e) with regards to silica dissolution it was the siloxane bond:-



that was the most vulnerable bond and if any surface treatment of the matrix were to be performed its objective should be to strengthen this bond.

(f) dissolution of silica from the matrix was occurring but not at the rate at which Iler (256) suggests.

The reduction in the column efficiency was considered to be too low to enable the conversion procedure into a molecular weight distribution form to be used.

A second pair of Du Pont S.E.C. columns reported to be packed with an 'ultra-pure' silica packing were obtained directly from E.I. Du Pont de Numours, Inst., Wilmington (257). The column efficiency quickly fell from about 17,000 to 5,000 at which value they remained

for approximately 300 hours.

Spheron P1000

Spheron was the only organic crosslinked polymer packing used in the research programme. It was described by Hradril (267) to be a poly(2-hydroxyethyl-methacrylate-co-ethylenedimethacrylate). The Spheron P1000 batch used was a 17 μm diameter research batch obtained from Prof. J. Kalal, Institute of Macromolecular Sciences, Prague.

The Spheron was packed as an aqueous slurry by upward delivery into a Shandon Southern H.P.L.C. column. Although good efficiencies were originally obtained (>2,500 plates) this could not be maintained for very long. The packed bed was found to compress and the efficiency reduce. The rate at which the bed compressed appeared to be proportional to the number of dextran samples applied and not necessarily to the quantity of water that passed through the column. After the packing had compressed, the column could be unpacked, redispersed and repacked into the column with the same initial efficiency as that previously achieved.

The repacking of the column did not necessitate a complete recalibration of the packing as the calibration curve was expressed in terms of the Wheaton-Bauman distribution coefficient, K_d. It was only necessary to establish the interstitial and total liquid volumes for the repacked column although well characterised dextran 'T' fractions were used occasionally to determine whether or not the calibration curve had changed.

4.1.3 Sample Detection

The eluate from the column passed into a differential refractometer model 1107LJ supplied by Laboratory Data Control, and

the resulting change in the eluate concentration registered on flat-bed recorders (Smiths Ltd., Venture Servoscribe, types 2 and 1b). The resulting chromatograms produced by these systems could then be treated to give a molecular weight distribution for the injected sample.

4.2 ANALYTICAL TECHNIQUES

The general analytical procedure for the analysis of the dextran samples is outlined below.

4.2.1 Sample Preparation

The preparation of a sample to be injected on to the analytical column fell into one of four categories. These were:-

(a) The preparation of a standard solution. A standard solution was prepared by dissolving a known amount of dextran powder in a precise volume of distilled or deionised water. The dextran powder was the same as the dextran used to make the feed solution so that the moisture content and the detector response would be identical. A glucose marker was added to those standards used on the high pressure systems to indicate the total inclusion volume.

(b) Solutions tooconcentrated for analysis. These were the feed solutions, that were diluted by ten-fold using standard volume flasks.

(c) Solutions too dilute for analysis. These were concentrated using a Büchi rotary evaporator.

(d) Solution of an acceptable concentration for analysis. These solutions were filtered before analysis, and if possible marked with glucose.

4.2.2 Sample Loading

The filtered sample was injected into a six port sample injection valve fitted with a constant volume sample loop. The chart recorder was adjusted to give an appropriate height for the baseline (usually set at 10% on the recorder response) and the position of the indicator was marked. When the sample was injected on to the column the movement of the chart paper was simultaneously started. The chart paper speed had been set to allow a convenient number of points to be taken for the data conversion procedure. After the glucose peak, if present, the detector was allowed to establish a baseline and the chart recorder stopped. Between each sample the sample loop was flushed out with distilled or deionised water.

4.2.3 Verification of Column Parameters

The measurement of the interstitial and total liquid volume for the fractionating system depended upon an accurate and precise measurement of the eluent flowrate. This was performed by weighing the eluate collected in a known time period. The void volume was taken to be at point of inflection on the leading shoulder of a dextran T 2000 chromatograph as shown in Fig. 4.4 and the total liquid volume of the fractionating solution was marked by the peak maximum of the glucose peak. The column was calibrated by the procedure described in Section 4.3.1 below, but a regular check on the column calibration was made by passing through a series of dextran T fractions having known molecular weight distributions.

The Spheron P 1000 packing had a particle diameter (17 μm) slightly too large for the column to exhibit the infinite diameter effect for which it had been designed. A front developed on the glucose peak as shown in Figure 4.5. When higher molecular weight sugars such

Fig 4.4 Void volume of column estimated from chromatograph of Dextran T 2000

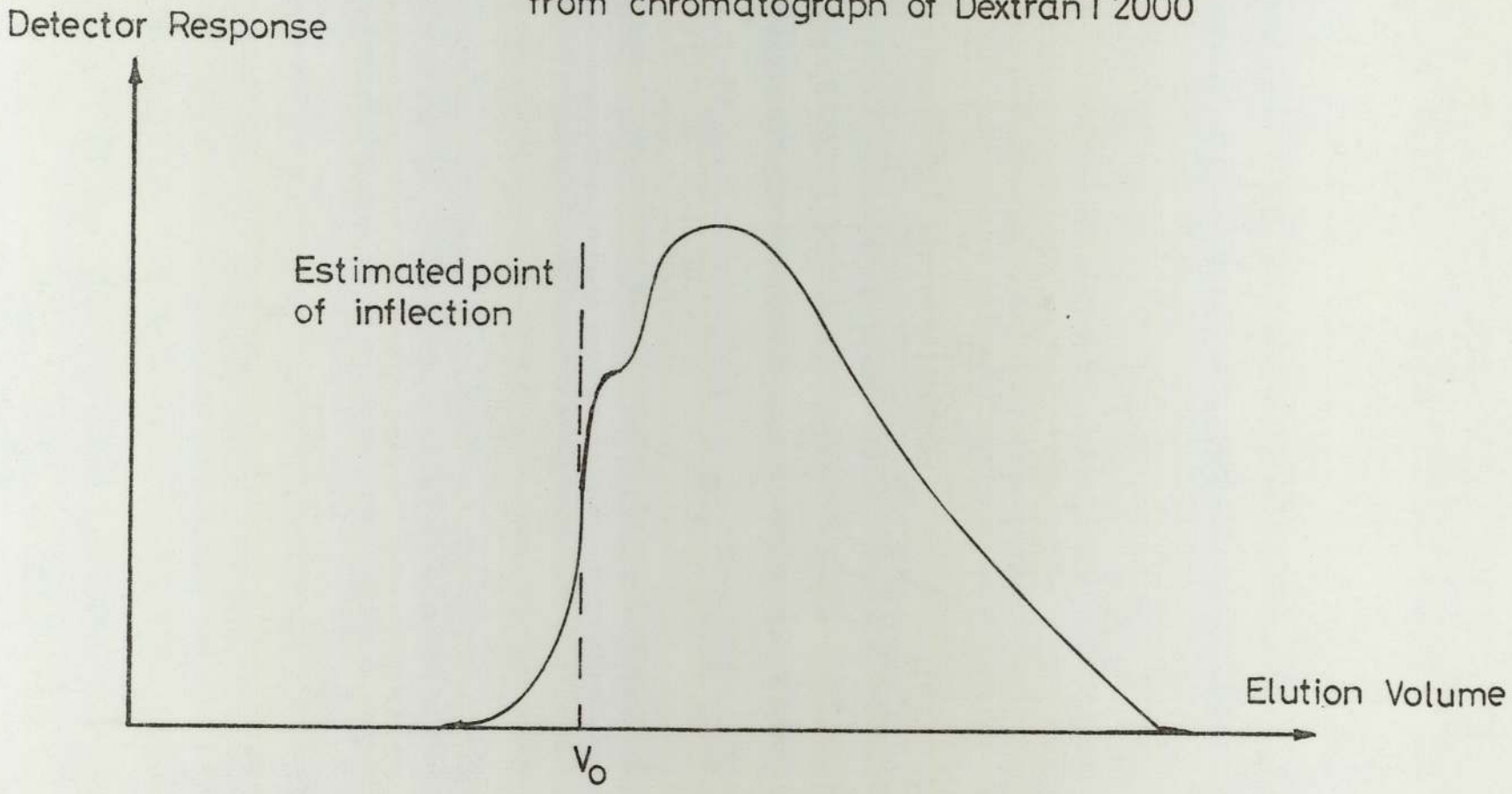


Fig 4.5 Elution profile for glucose on Spheron P1000 packed in a 8m.m. i.d. Shandon column.

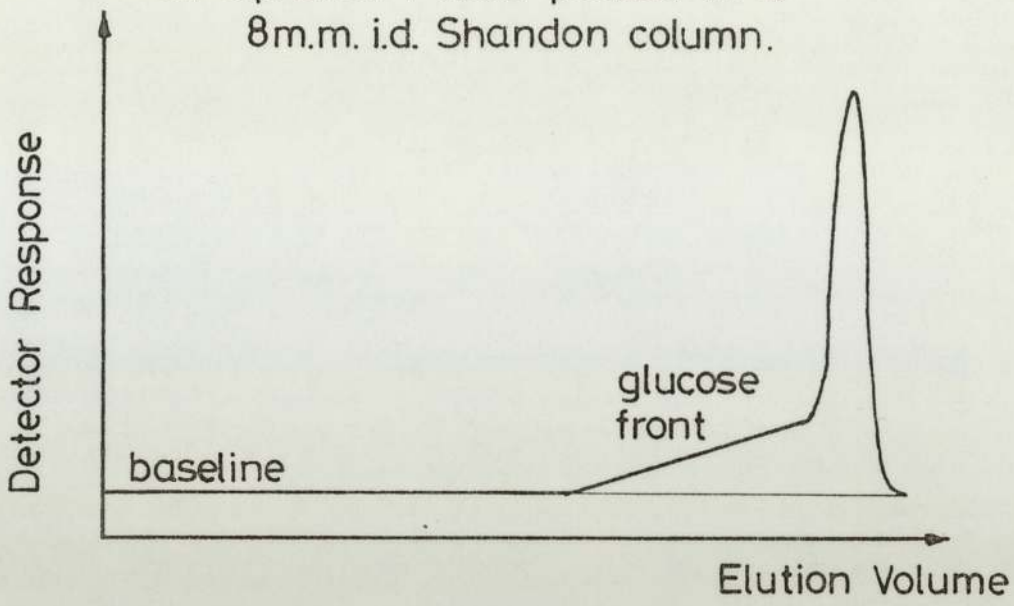
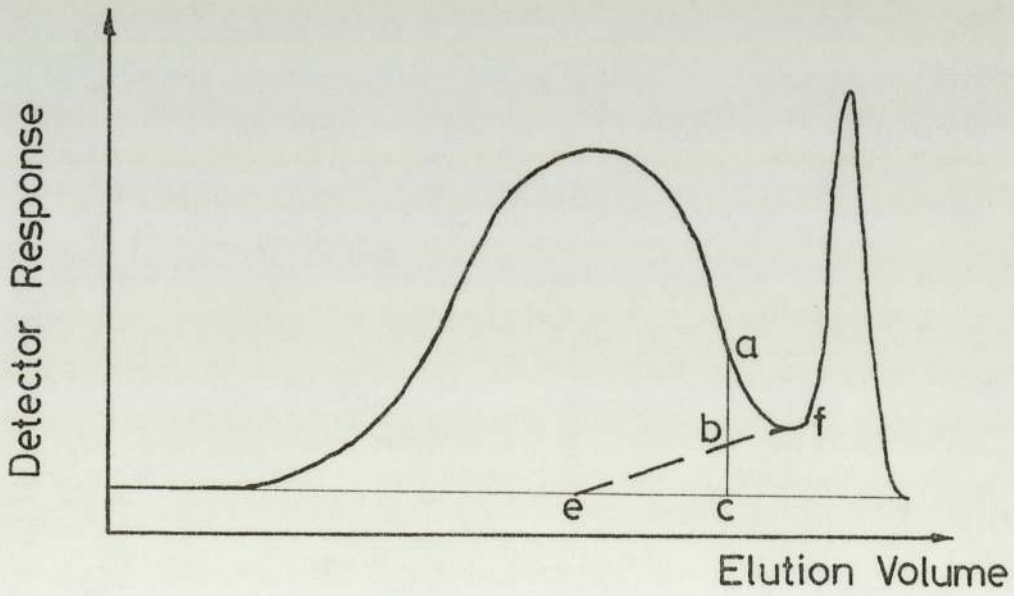


Fig 4.6 Elution profile for Dextran plus a glucose marker.



as sucrose and maltose were injected on to the column, it was noticeable that the front was very much reduced. Therefore even for the low molecular weight dextran molecules the fronting would be negligible and an infinite diameter effect is achieved. Since glucose was used as a marker it was necessary to remove the front of the glucose peak from the dextran elution curve. This was performed by passing several samples of glucose through the column at the same flowrate at which the dextran samples were to be analysed. The point at which the front began was measured and its height compared to the peak value. These results were averaged and the front of the glucose peak removed as shown in Fig. 4.6. The glucose front was approximately linear and therefore it was easy to construct the 'artificial' sloping baseline E F. Within this region the detector response showed a concentration proportional to the height AC, however, the response due to the dextran peak alone was AB. Therefore only these heights were measured for the molecular weight determinations and for the concentration analysis.

4.3 DATA CONVERSION

Before any qualitative information could be abstracted from a chromatogram it was necessary to calibrate the column packing. The calibration procedure used is described in 4.3.1. The other two sub-sections report how a chromatogram of a product is analysed in both a qualitative and quantitative manner.

4.3.1 Calibration Procedure

All three column packings used were calibrated using Dextran T fractions having known molecular weight distribution. For the two materials used in the high pressure systems the column efficiencies

were sufficiently high enough, greater than 2,500 plates per fractionating system to attempt conversion from a chromatogram to a molecular weight distribution. Chromatograms obtained by g.p.c. are adequate for comparing relative distributions of samples provided that they are determined under identical operating conditions. As the feed and products for an experiment are analysed on the same equipment at the same time and under the same conditions, the chromatograms produced can be compared directly as a measure of the relative distributions of the samples. However, this sort of analysis does not provide any quantitative assessment of the sample and cannot be compared with other samples analysed at different times. Therefore there is a need to convert the chromatograms into a molecular weight distribution by means of the calibration curve.

A polynomial of the type described by equation 2.39 was used. The coefficients were obtained by the following procedure:-

(1) A series of chromatograms were obtained for the following dextran T fractions 2000, 500, 150, 70, 40 and 20 along with a dextran 110 with a known molecular weight distribution. The eluate flowrate for each chromatograph was measured and for the higher T fractions glucose could be added as a marker.

(2) The chromatograms were measured and their areas calculated. The elution volumes at various fractions of the total area were taken, and the corresponding molecular weight noted from the calibration curve provided by Pharmacia Ltd..

(3) The elution volumes at the known molecular weights were averaged and converted into the Wheaton-Bauman distribution coefficient, K_d.

(4) The calibration curve was drawn.

(5) The coefficients a_i in equation 2.39 were estimated by using a Forsythe's Method of lease square fitting (258) which is contained in program E20ABF in the NAG' Library of the Aston University Computer. The information was fed into this program by program ENGLAND a copy of which is contained in Appendix A.1.

(6) The resulting calibration curve was checked to see if it described the input data.

(7) If the calibration curve fitted the input data the coefficients for a_i in equation 2.39 were incorporated into the computer program MWDC, and chromatograms for the dextran T fractions were converted into molecular weight distributions and compared against the distributions supplied by Pharmacia. If the molecular weight distributions obtained did not agree with those provided by Pharmacia, adjustments were made to the K_d coefficients in point (3) and the calibration procedure reiterated until satisfactory agreement was made between the calculated and original distributions. A listing of program MWDC is also contained in Appendix A.1.

4.3.2 Determination of Sample Concentrations

Sample concentrations were obtained by comparing the area of the chromatogram above the baseline of an injected sample against the area of a standard solution. The preparation of the solutions was described above in Section 4.2.1. The response of the refractometer was directly proportional to the concentration of the polymer in the eluate. Integrating the area of the chromatogram above the baseline implied that a value directly proportional to the weight of polymer injected was obtained. As a constant volume sample injector was used, this was also proportional to the concentration of

the injected sample.

The area of the chromatogram was evaluated by Simpson's rule. Originally this was performed using a hand calculator but was incorporated into the computer program MWDC. The procedure for the determination of the sample concentration was:

(1) Establish the pore and total liquid values for the column.

(2) Measure the height of the chromatogram above the baseline for regular intervals along the abscissa of the chart paper.

(3) Calculate the elution volume between the measured heights and convert this into an increment in the Wheaton-Bauman distribution coefficient.

(4) Calculate the area of the chromatogram by Simpson's rule.

(5) If the chromatogram is a standard solution then the area of the chromatogram was divided by the known concentration to provide a value of the area of response per unit mass of dextran. Or, if the chromatogram was an unknown concentration then the area was divided by the response area per unit mass to evaluate the quantity of dextran in the injection.

4.3.3 Determination of Molecular Weight Distributions

The high efficiencies and good resolving capacities of the two high pressure chromatographic systems allowed qualitative analyses to be made on the dextran samples. The data conversion procedure was a numerical interpretation of the Yau and Flemming technique (78) and was written as a computer program, called 'MWDC'. The algorithm for this program is sketched in Fig. 2.12. The computation procedure was as follows:-

(a) Using the heights measured at regular intervals along the chromatogram, a normalised chromatogram was calculated.

(b) For each of the measured values, the Wheaton-Bauman distribution coefficient was calculated and the value of the gradient of the calibration curve at this point, $d(\log M)/dK_d$.

(c) The product of the normalised height and the reciprocal of $d(\log M)/dK_d$ provided the required value of the ordinate for the differential molecular weight distribution curve.

(d) Molecular weight averages were calculated according to the expressions given in Section 2.7.1.

4.4 COMMENTS ON THE ANALYTICAL SYSTEM

The introduction of the molecular weight interpretation technique allowed products from different experiments to be compared directly by means of their molecular weight distributions or averages. The other major advantage of using the computer program MWDC for the analysis of a quantitative measure of a sample as well as a qualitative was the rapid, reproductible manner of the analysis. However the manual method of digitising the chromatogram using a pencil and ruler was thought to be poor and could cause errors in the analysis. If a computerised data handling method, for example an analogue electrical signal recorded on to a magnetic cassette were to be introduced, this would eliminate many of the errors in the present system.

5.0

REPETITIVE BATCH CHROMATOGRAPHY

5.1 PRINCIPLE OF OPERATION

Gel permeation chromatography was used to fractionate dextran. A large scale preparative column had repeated samples of dextran feed solution injected onto the column. Figure 5.1 illustrates the discontinuous sample inputs to the chromatograph and the resulting outlet concentration profile resulting from the fractionation of the dextran molecules. The column effluent alternates between a required acceptable product fraction and an unrequired waste fraction. Figure 5.2 shows the concentration profile for the column effluent in greater detail. From a short pulse injection at the inlet, the solution is fractionated between points a_n, where the highest molecular weight molecules are eluted, and d_n where the lowest molecular weight molecules present in the injected sample are eluted. The objective in fractionating the dextran molecules is to reduce the molecular weight range of the final product. Therefore the molecular weight range of interest is between the points b_n and c_n. By careful adjustment of the parameters, molecules that are too large for the desired product and therefore are eluted between point a and b could be eluted from the column at the same time as the unrequired low molecular weight molecules from a previous injection as illustrated by Fig. 5.1.

The objective of the repetitive batch fractionating column was to remove about ten percent of the dextran from each end of the molecular weight range when fractionated on Spherosil XOB075. As the nth injection in Fig. 5.2 interacts with only the two injections on either side, then a typical injection in a series could be represented by injecting only three samples. The first would represent the n-1th injection, in the experimental programme this injection was termed the

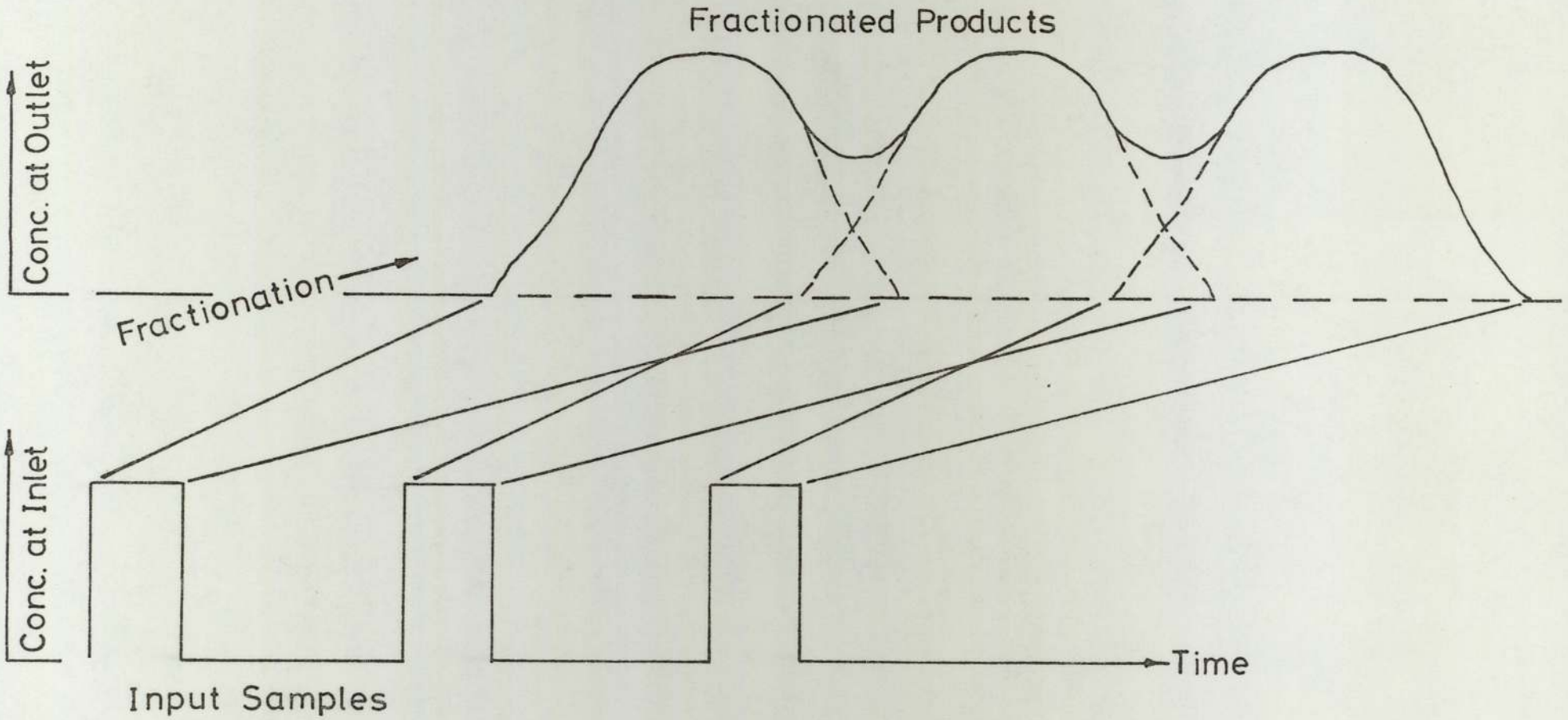
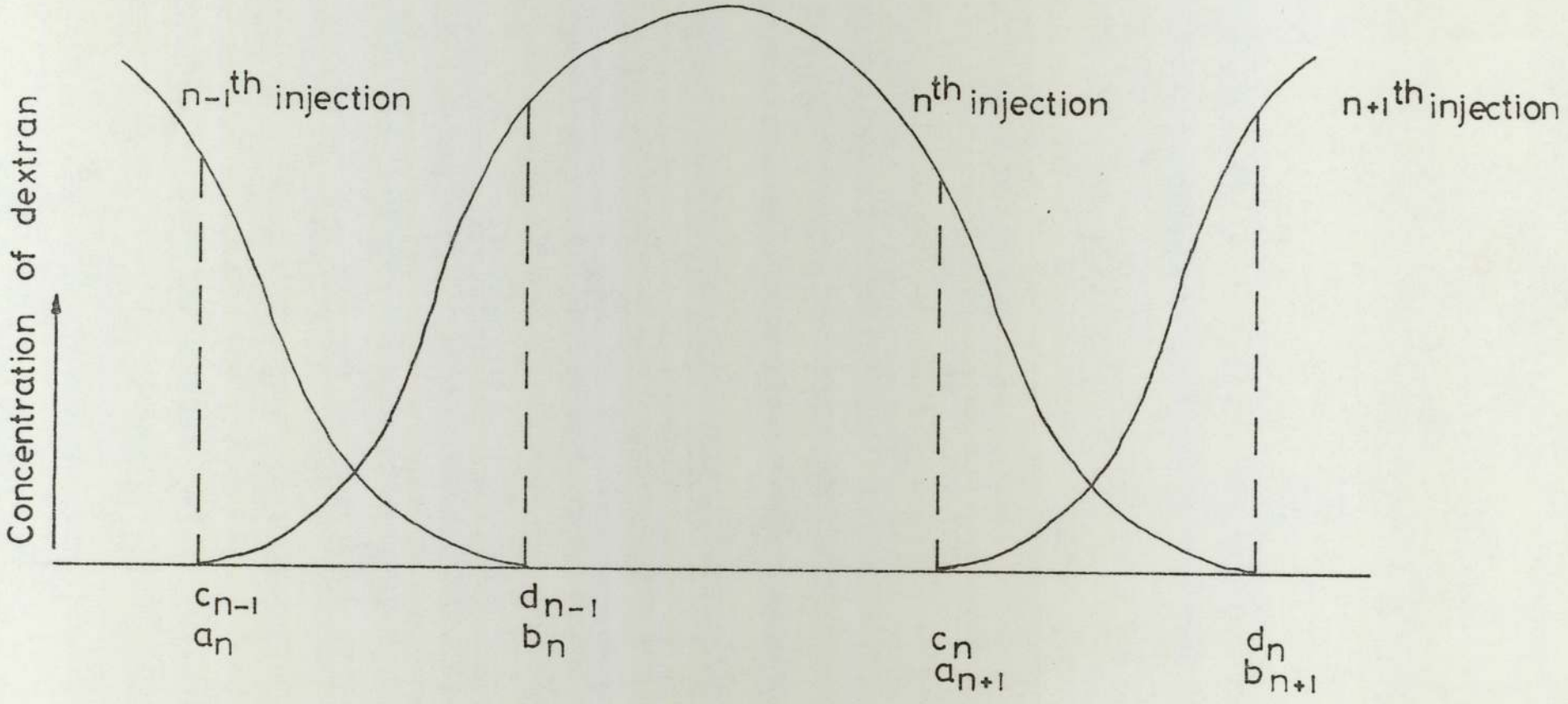


Fig 5.1 Sample Input and Product Collection Sequences

Fig 5.2 Concentration profile of column effluent.



initial peak; the second represented by the nth injection was bounded by two samples and was termed the product peak; and, the n+1th injection was termed the final peak. The waste fractions between the initial and product peaks and the product and final peaks were termed waste fractions 1 and 2 respectively.

The overlapping of successive injections allows both the unrequired high and the low molecular weight molecules to be removed from an injected sample in a single pass through the chromatograph. This is an obvious advantage for this type of chromatograph for the production of clinical dextran when compared to the semi-continuous equipment where two passes are required.

5.2 REPETITIVE BATCH CHROMATOGRAPHY

5.2.1 Eluent and Feed Systems

The repetitive batch chromatograph was a completely self-contained unit mounted on a movable metal-framed structure. Both the feed and eluent reservoirs were mounted on the structure. This equipment is illustrated by Fig. 5.3, and by a schematic diagram in Fig. 5.4.

Eluent passed from the reservoir via an in-line No.2 glass sinter to a positive displacement metering pump. Both the eluent and the feed solution were pumped by a 'M.P.L. KV TWIN METRIPUMP' supplied by Metering Pumps Limited, London, and fitted with a 2800 r.p.m. motor operating at 96 strokes per minute. The two plastic metering heads were type PG13G, having a polypropylene casing and a glass plunger. The eluent left the pump via 4mm i.d./6mm o.d. polyproplyene tubing, through a short section of loosely packed Spherosil XOB075 to two a.c. solenoid pinch valves where it was either directed onto the fractionating column or returned to the reservoir through a brass adjustable pressure

Fig 5.3 Photograph of repetite batch chromatograph

Key

C Control valves

Er Eluent reservoir

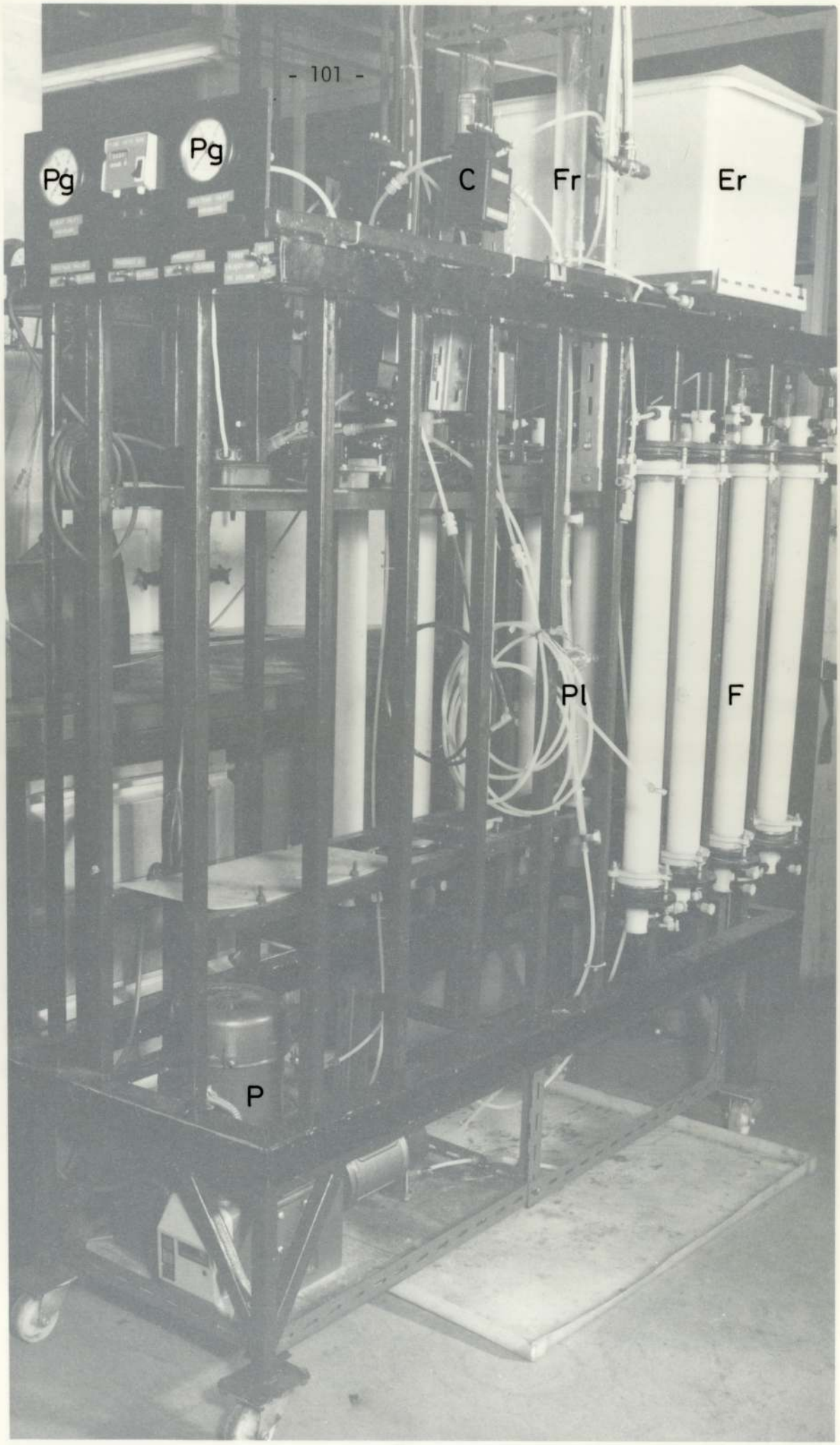
F Fractionating column

Fr Feed reservoir

P Pump

Pg Pressure gauge

Pl Product lines


Pg
Pg
C
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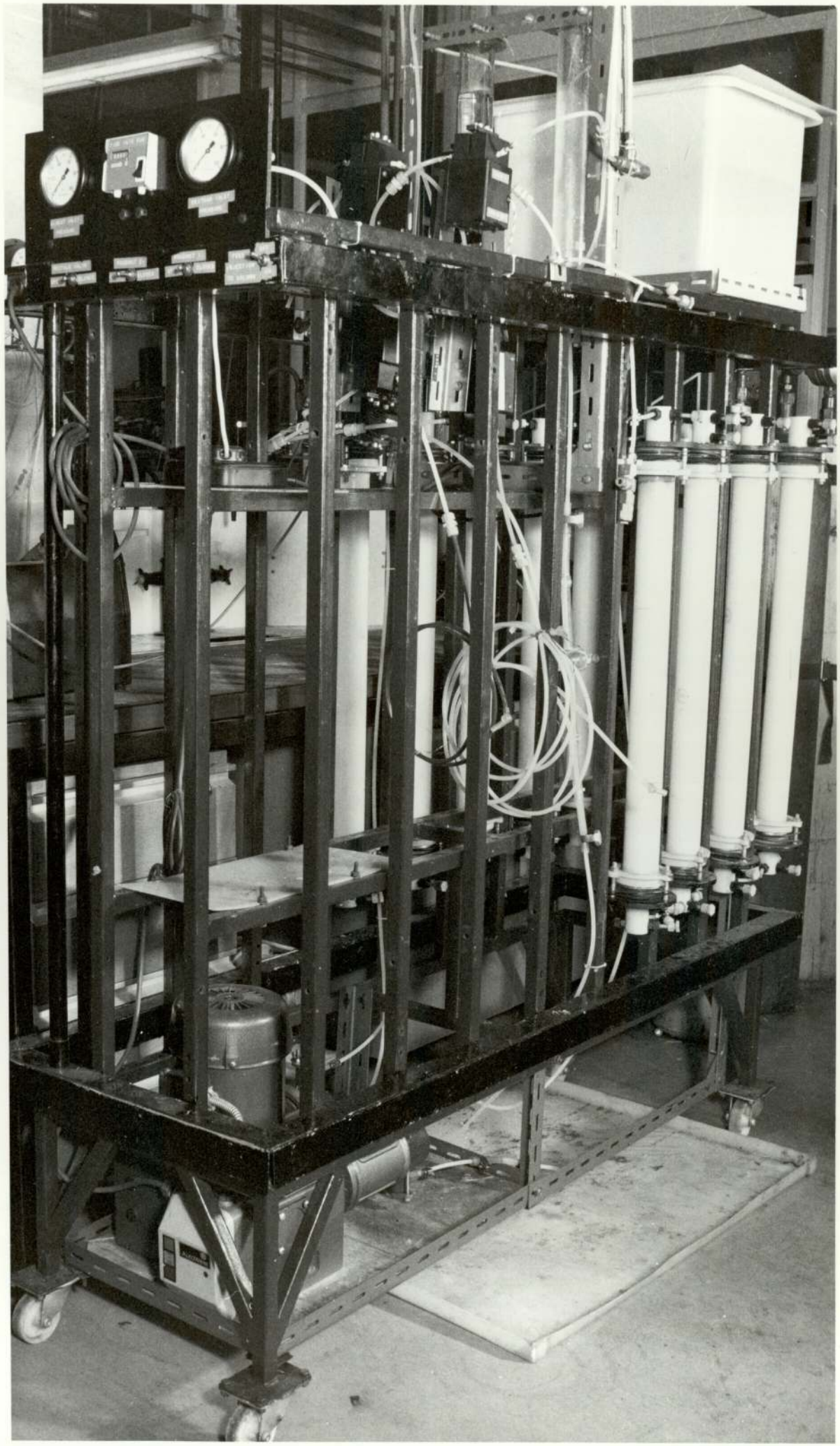


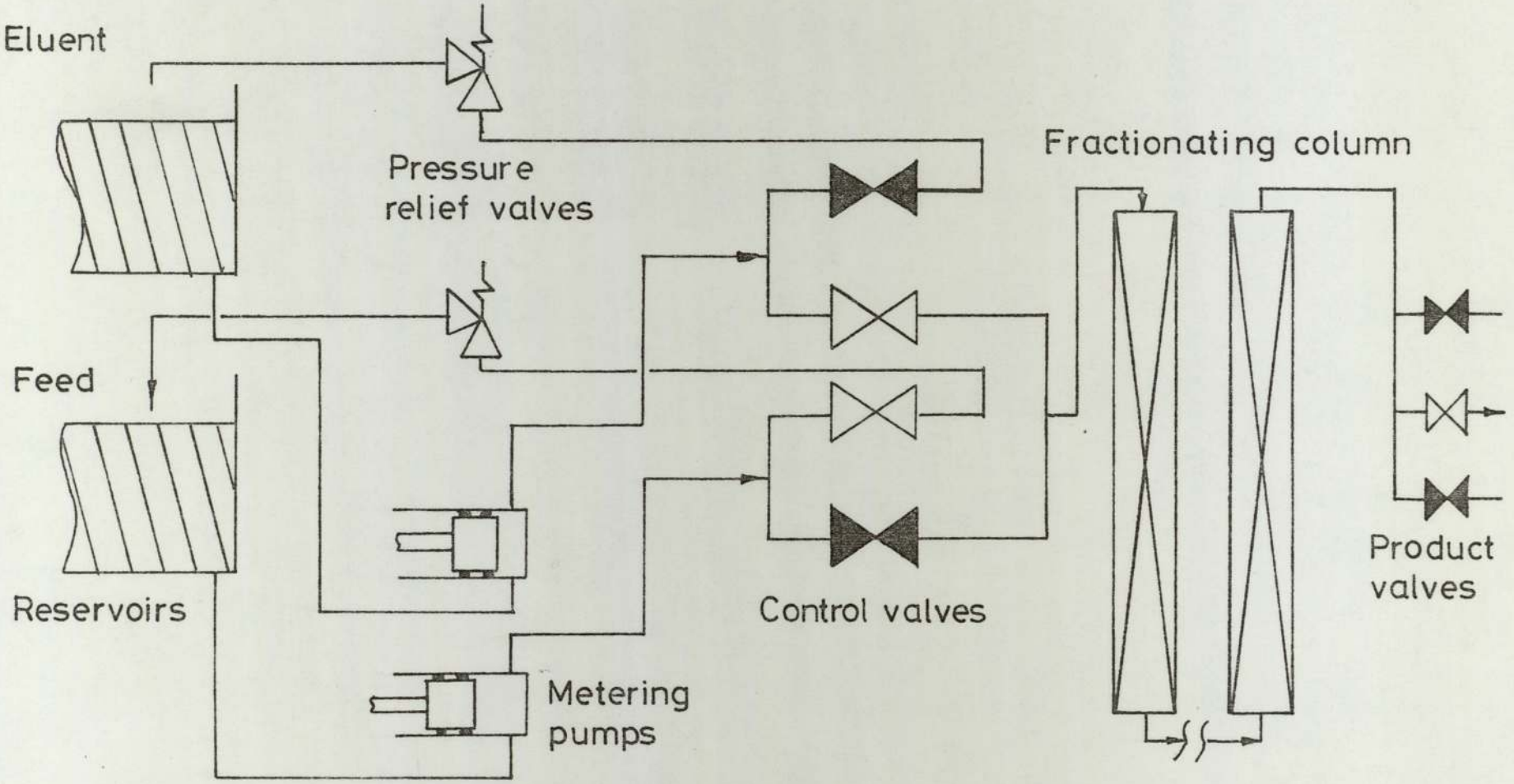


Fig 5.4 Schematic diagram of repetitive batch chromatograph in elution mode

relief valve supplied by Hoke International Ltd., Barnett. The feed solution was also loaded/recycled by a similar system.

The principle of operation of the a.c. solenoid pinch valves was to create a reliable seal in a flexible P.V.C. tube by pinching it between two metal rods, that were pulled together by the solenoid. These valves were developed by F.J. Ellison, and a comprehensive account of their development has already been reported (34). Control of the four a.c. solenoid pinch valves that constituted the eluent/feed loading system was achieved by a manually operated double pole/single throw control switch.

5.2.2 The Fractionating Column

The column was made by connecting ten borosilicate glass Q.V.F. columns 5.1 cm i.d. by 70 cm long packed with Spherosil XOB075 particles (200-400 μm). The columns were mounted vertically on a metal frame and linked in series connected alternately at the top and the bottom. The connections were made as short as possible in order to minimise the dead volume. These columns were originally used by F.J. Ellison (34) in a semi-continuous chromatograph. Figure 5.5 summarises the efficiencies, void and pore volumes for the columns.

After only four experimental runs, it was found that bacterial debris present in the dextran feed was beginning to be deposited at the inlet to the column. Therefore it was necessary to remove and replace the first 15-16 cm of the column packing to prevent a re-occurrence of this event. A 5.1 cm i.d. by 30 cm long loosely packed with Spherosil XOB075 was incorporated into the feed recycle loop as a filter. A similar column was also fitted to the eluent recycle loop. Figure 5.6 shows the molecular weight distributions of dextran samples simultaneously

Fig 5.5 Characteristics of columns used in the repetitive batch chromatograph.

Original column number	Void volume $cm.^3$	Pore volume $cm.^3$	Column volume $cm.^3$	H.E.T.P. cm.	Number of plates
1	544	622	1166	0·67	105
6	531	658	1189	0·60	117
7	556	596	1152	0·70	100
8	551	642	1193	0·66	106
12	540	636	1176	0·63	111
13	534	610	1144	0·64	109
14	527	643	1170	0·74	95
15	534	621	1155	0·58	120
17	529	635	1164	0·64	109
19	533	619	1152	0·64	108
Average	541	628	1169		

taken from the top and bottom of the filter. These results confirm that the composition of the feed solution was not changing with time.

Fig. 5.6 Comparison of molecular weight average before and after short filter

Sample No.	Top of Short Filter M_w	Bottom of Short Filter M_w
1	48800	47700
2	46000	47800
3	48500	46900
	M_n	M_n
1	20200	20800
2	20400	21800
3	21200	22100

The inclusion of this short section of packing prevented any further bacterial debris being deposited on the batch fractionating column. It was noticeable that as the dextran feed solution was recycled prior to an experiment, the colour of the bed turned from white to a dark grey and the solution became much clearer. At the end of each experiment the short section of packing was cleaned by flushing through with distilled water, this removed much of the grey colouration. On one occasion the Spherosil XOB075 was removed from the column and given an acid-wash.

5.2.3 Eluent Monitoring System

The eluent from the repetitive batch chromatograph was monitored by taking off a small side stream by means of a Watson-Marlow peristaltic pump and passing this stream through a Jobling refractive index detector. This system is illustrated by Fig. 5.7. The tubing between the refractometer and the sample point at the column outlet was kept as short as possible to minimise the lag in the detection system. Although the refractometer can only detect changes in concentration and cannot provide a measure of the average molecular weight of the effuent stream, it did provide useful information in deciding whether or not to make a change in the fraction collection as traces indicated by Fig. 5.1 were recorded. These traces were produced on either a Servo-scribe flat-bed or a Kent chart recorder. There was a lag of approximately between 0.8 and 1.2 minutes between recorder and the sample point.

5.2.4 Sample Collection

Samples were collected in light weight plastic containers of 10 l capacity. Three solenoid pinch valves, identical to those used on the eluent/feed loading system controlled the flow of the column eluate. Each valve was individually operated by a manual single pole switch, only one valve was open for any given time period. Sodium azide or chloroform was added to the collected fractions to prevent any microbial growth. Small quantities of concentrated product fractions along with a small quantity of diluted feed were kept in 5 or 10 ml vials and stored in a refrigerator.

Fig 5.7 Photograph of detector unit used with repetitive batch chromatograph.

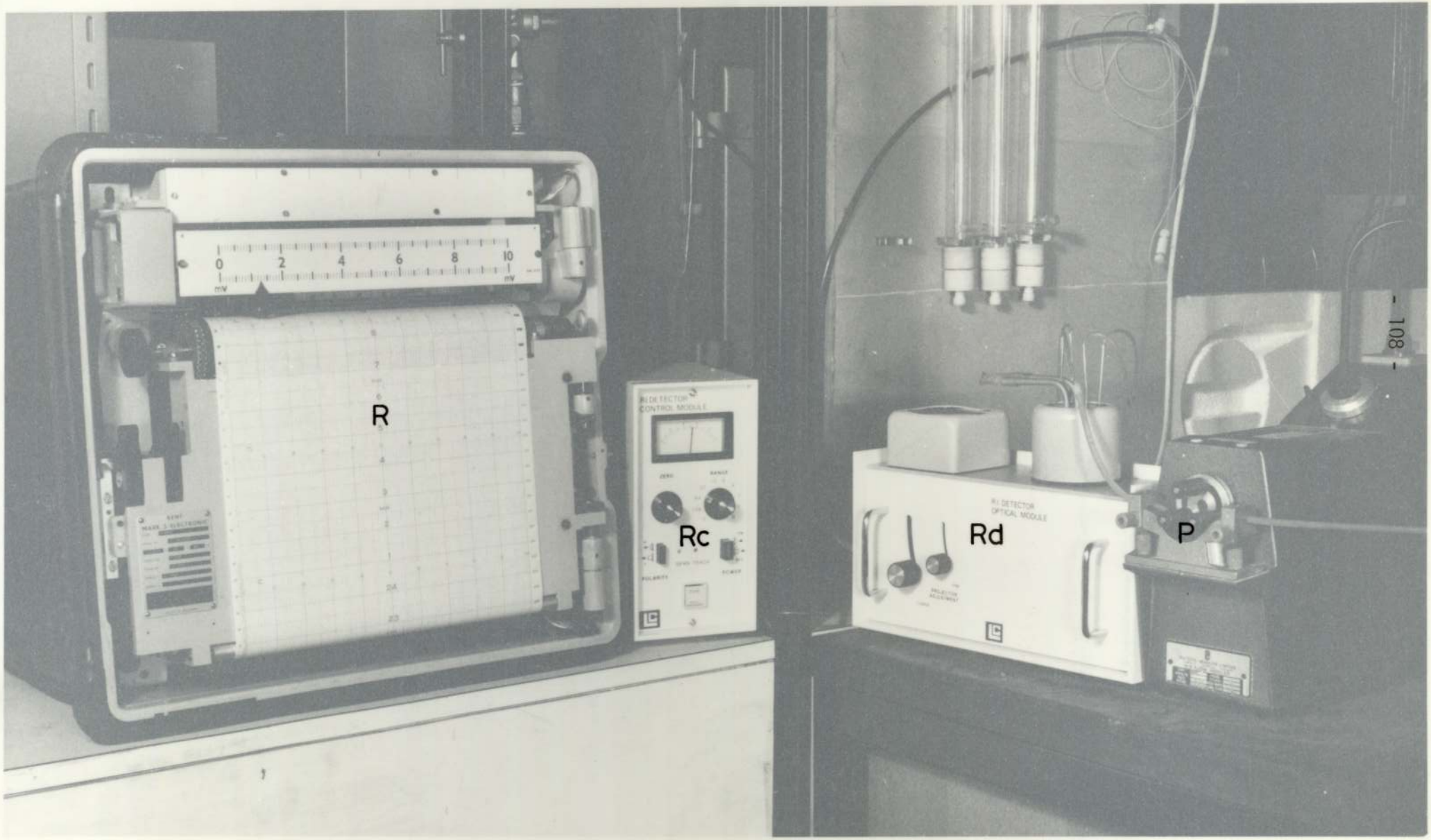
Key

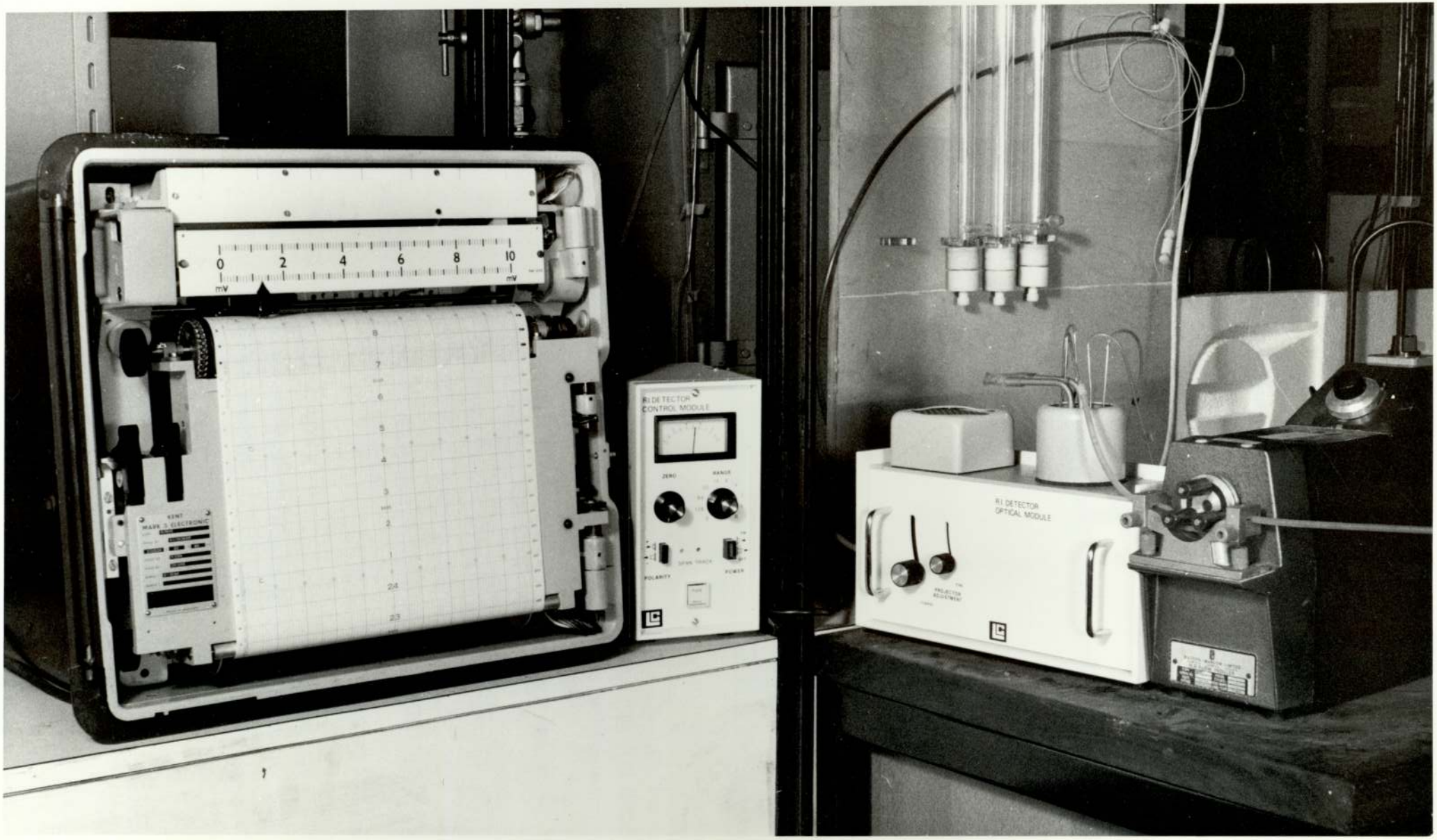
P Peristaltic pump

R Recorder

Rc Refractometer control unit

Rd Refractometer detector unit


R
Rc
Rd
P


0 2 4 6 8 10
mV
RI DETECTOR
CONTROL MODULE
ZERO
RANGE
POLARITY
POWER
RI DETECTOR
OPTICAL MODULE

5.3 EXPERIMENTAL TECHNIQUES

5.3.1 Preparations of Dextran Feed Solutions

The growth of micro-organism in carbohydrate solutions is a common problem and the recommended prevent-ive procedure for dextran solutions is to add sodium azide (NaN_3) at a concentration of 0.02% w/v (259). However this preventative procedure could not be used on the feed solution because the solution came into contact with a brass pressure relief valve and azides of copper and other heavy metals can be explosive (260). Therefore the feed solution had to be prepared on the day of the experiment and the sodium azide was added to the collected products. Usually 2 l of feed solution was sufficient for an experiment.

The ability of a dextran powder to dissolve depends upon the average molecular weight of the sample, the higher the molecular weight the more difficult it is to dissolve the dextran. Dissolution was aided by elevated temperatures.

As only small quantities of feed solution were required the solution was prepared in a beaker, a measured quantity of water was heated in a beaker on a heating block and a weighed amount of dextran powder slowly added whilst the solution was continuously stirred until all the dextran was dissolved. Before the dextran solution cooled it was filtered twice through a No.2 glass sinter in an effort to remove any particulate matter. Although the glass sinter did show signs of discolouration and the flowrate of the solution through the filter did decrease, indicating that the sinter was blocking, this procedure was clearly not sufficient to remove all of the biological debris present in the dextran feed and extra preventative measures outlined in section 5.2.2 were necessary.

5.3.2 Valve Maintenance

It was found necessary to replace the flexible P.V.C. tubing in the four solenoid valves in the eluent/feed loading section before every experiment. The three valves at the column outlet required little or no attention. This was because they were not subjected to the high pressures like pre-column valves. Occasionally during an experiment a tube would show signs of expansion, signifying it was about to burst. It was then necessary to stop the experiment and replace the faulty tube. With pre-prepared pieces of tubing and the experience gained in replacing the tubes regularly this was a relatively simple and speedy task. The new P.V.C. tube was lubricated with silicone grease and inserted between the cut-off bars. The adjusting bolts were then correctly set, to provide adequate force to pinch the tube together when the solenoid was activated.

5.3.3 Flowrate and Pressure Measurements

Frequent measurement of the eluent and the feed flowrates were necessary to ensure that the pumps were operating correctly. It has been the experience of previous workers in this laboratory that the discharge rate from this type of positive displacement pump changed when there was a change in the back pressure of the system. This is why pressure relief valves were fitted in the recycle loops to create an artificial back pressure so that the pump experienced little or no change in the back pressure and therefore provided a constant flowrate. The eluent and feed flowrates were measured using calibrated glass Q.V.F. 5.1 cm i.d. pipe sections. The pipe section used for the feed reservoir, whilst the eluent was measured by switching from its reservoir to the pipe section using a glass three way valve.

The discharge pressures from the feed and eluent pump-heads were continuously monitored by two Bourdon gauges, 100-690 kN M^{-2} (0-100 p.s.i.) supplied by Budenburg Gauge Ltd., Altrincham. The pulsations produced by the metering pump was minimised by the restriction of the fluid movement to the gauges, by using screw clips on the polypropylene tubing immediately before the pressure gauge. Particular attention was given to the eluent pressure just after a sample had been loaded as this was the period when the highest column pressure drop was recorded. A sudden reduction of pressure indicated by either gauge usually ment that the P.V.C. tubing in the solenoid valves had failed and required changing.

5.3.4 Sample Analysis

The feed, product and waste fractions were analysed by the g.p.c. technique, outlined in Chapter 4. Originally, only the chromatogram could be used as a means of quantifying the distribution of a sample. But the development of the molecular weight determination technique, developed during this experimental programme made the comparison of samples easier.

5.4 EXPERIMENTAL PROGRAMME

5.4.1 Scope

One of the principle aims of this experimental programme was to establish the rate of production and concentration of an acceptable dextran product fraction that could be obtained by repetitive batch chromatography for a given quantity of chromatographic packing. This information would then be used to compare repetitive batch chromatography with a semi-continuous method of operation. Although the same quantity

of chromatographic packing was used for both the repetitive batch and for the semi-continuous as well as the same column dimensions, these dimensions may not be the optimal configeration for either the batch or the semi-continuous methods of operation. The experimental programme concerned with the repetitive batch chromatography for the fractionation of dextran had the following objectives:-

(1) To product a suitable product having a narrower molecular weight distribution than the injected material. This was to be achieved by the removal of approximately ten percent of the injected dextran material from either end of the molecular weight range in a single pass of the feed solution through the apparatus.

(2) To determine the maximum rate at which dextran could be fractionated by a given quantity of chromatographic packing; bounded by the constraints of producing an acceptable product and within the pressure limitations of the apparatus.

Also to:-

(a) Investigate the effect of sample volume upon the fractionating ability of the apparatus and its effect upon the quality of the products.

(b) Investigate the effect of sample concentration upon the fractionating ability of the apparatus and its effect upon the quality of the products.

5.4.2 Experimental Conditions

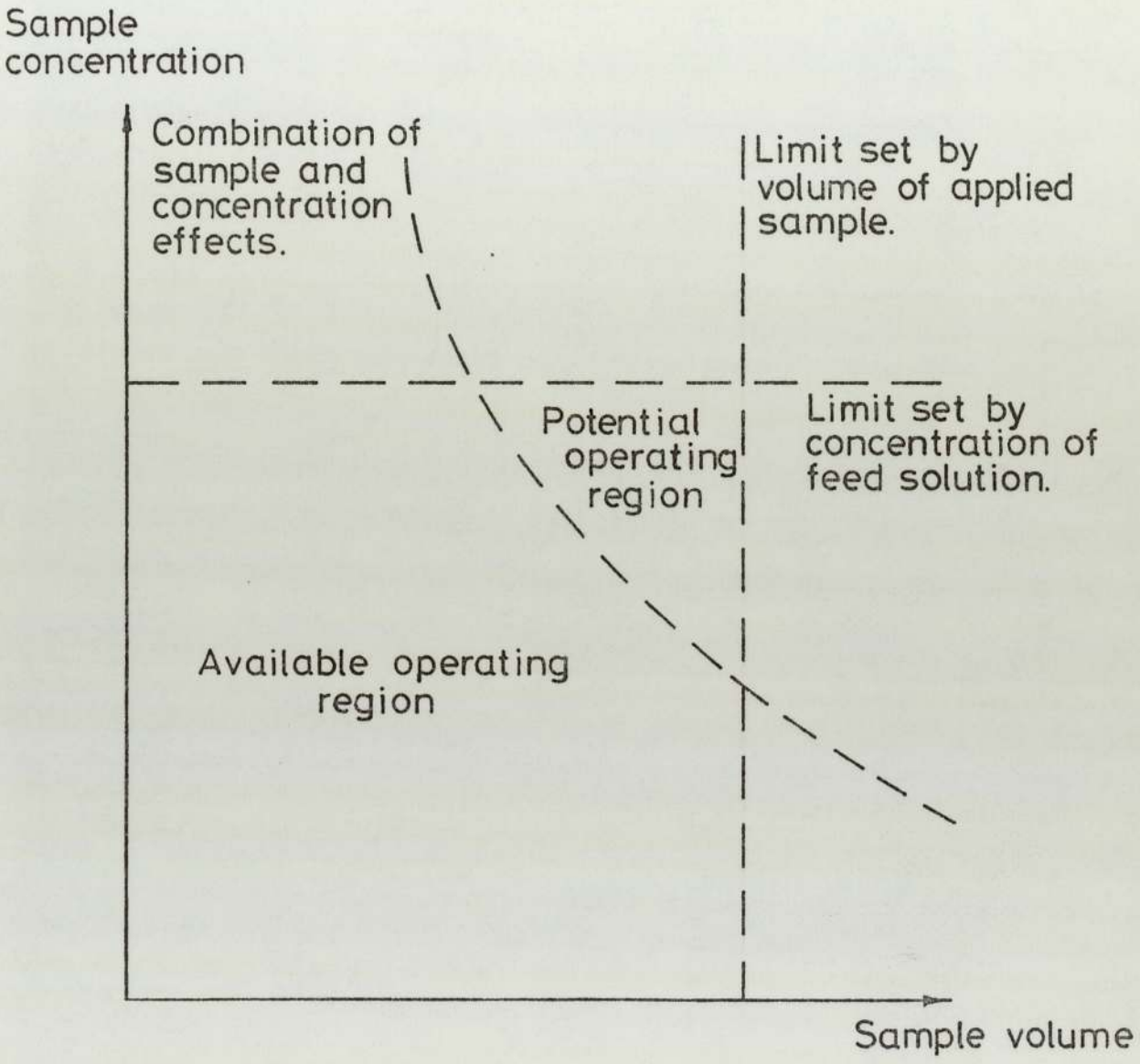
The experimental conditions for the repetitive batch study were dominated by the requirement to maximise the quantity of dextran that could be fractionated per unit of time. When these columns were used in a semi-continuous fashion, the average flowrate in the fractionating

section was approximately 50 cm^3 min^{-1}; but in a subsequent study using columns of similar dimensions but made from stainless steel and not borosilicate glass, dextran fractionation had been achieved with eluent flowrates of 100 cm^3 min^{-1} (261), as a result of which the initial volumetric flowrate in these runs was chosen to be 100 cm^3 min^{-1}. With the pressure drop limitation of the glass column set at 540 kN m^{-2} (75 p.s.i.) no increase in flowrate was possible when concentrated dextran solutions were injected onto the column. Figure 5.8 explains the type of operating region in which the repetitive batch experiments were performed. The maximum value on the ordinate is bounded by the maximum concentration that the metering pump was capable of pumping. The maximum value along the abscissa is bounded by the maximum volume of sample that could be injected onto the column that produced an acceptable product. A combination of the concentration sample volume interactions will further limit the practical operating region either by the injection causing an excessive pressure drop across the column or by the combination reducing the columns fractionating ability.

The volume of eluent pumped onto the column between the end of one sample injection and the start of the next was kept constant for eight of the ten reported experiments at 6.28 l. This volume was approximately equal to the total pore volume of the fractionating column. The two remaining experiments had either a slight increase or decrease to the volume of eluent pumped onto the column between successive injections.

The objective of collecting about 80% of the injected feed material in the product peak was obtained in a majority of cases. The decision when to change from collecting the waste fraction to collecting the product fraction was made by using information extracted

Fig 5.8 Practical operating region for the repetitive batch chromatograph



from the initial peak. From chromatograms of single injections fractionated on the batch chromatograph it was observed that they were approximately symmetrical, with the peak maximum at about fifty percent of the injected mass. Therefore during an experiment, once the initial peak maximum had been recorded the area proportional to the initial ten percent of the sample was estimated and the height of the detector response noted. Since the chromatographs were symmetrical, the height of the detector response when ninety percent of the injected sample had been eluted would be the about the same as at ten percent. Therefore when the chromatogram of the initial peak fell from the peak maximum to this value the waste fraction collection was begun, and finished when the chromatograph rose again to this value as shown in Fig. 5.1.

Reports in the literature vary as to what size the volume of the injected feed solution can be as a percentage of the total column volume. Therefore a wide variation in the volume of the injected samples, equivalent to 0.7 to 3.1% of the total column volume, or represented by 0.87 to 3.74% of the total liquid volume of the column, were used. Experimental runs No. 3, 2, 1, 8, 7 and 5 investigated the variation in sample volume and its effect on the fractionation of dextran, and also serve as an indication to the maximum quantity of dextran that can be fractionated whilst producing a clinically acceptable product.

The effect of increasing the concentration of the dextran feed solution was also investigated. The maximum limit of the dextran feed solution proved to be about 270 g l^{-1}. This was not because of the solubility limit, but because at higher concentrations pumping difficulties were encountered, eventhough the dextran reservoir was raised as high as

possible, at concentrations higher than this limit air was sucked in by the positive displacement pump past the shaft seal because the dextran was too viscous to flow into the pump. Two experiments, numbers 9 and 10 were performed with feed solutions about 270 g l^{-1} without any deterioration of the final product fractions. Higher sample loadings were not possible because of the pressure limitations of the column.

5.4.3 Feed Solution

The dextran feed used in this set of experiments was supplied by Fisons Ltd., (Pharmaceutical Division) the batch number was BT161D. All the feed for the batch fractionation came from one barrel. It was unfortunate that this barrel subsequently proved to have a much lower molecular weight average than the rest of the batch; the molecular weight average and the number average for the feed solution used in this set of experiments are:-

M_w	47500
M_n	20800
D	2.28

5.4.4 Results and Discussion

The loading procedure of the dextran solution onto the batch chromatograph for all the repetitive batch experiments together with a description of the fractionated samples are contained in Figs. 5.9 to 5.18, and a summary of the product fractions is contained in Fig. 5.19. The following discussion will centre on two main points:-

(a) What effect the volume of sample injected had upon the molecular weight distribution of the fractionated product.

(b) What effect the concentration of the feed solution had upon the fractionated product.

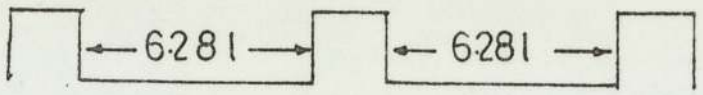
These topics will be discussed individually.

(a) Effect of Sample Volume

In experiments 1, 2 and 3 the quantity of dextran loaded onto the column during each injection ranged from 17.3 to 38.8 grams. The concentrations of the feed solutions for each of the experiments was approximately 170 g l^{-1}. The variation in the sample loading was by virtue of changing the volume of feed injected from about 100 to 230 cm^3. The quantity of the fractionated product collected was approximately the required 80% of the feed in each case. The molecular weight averages for the fractionated product increased with increasing the volume of the sample loaded as Fig. 5.20 shows. A possible explanation for this observation could be due to the interference introduced into the chromatography of the dextran by the use of 'large' injection volumes, that is, the technique for fractionating the dextran solutions in this programme was identical to the analytical system described in Chapter 4. But instead of a small pulse of sample being loaded, a larger volume of feed sample was used. If we can consider an idealised model where the larger samples in the repetitive batch chromatography programme are considered as a series of short non-interacting pulses as shown in Fig. 5.21 where each pulse is fractionated individually. As a constant volume was maintained between the end of one injection and the beginning of the next, which was equivalent to the total pore volume of the stationary phase, molecules that are totally

Figure 5.9
Experiment No. 1

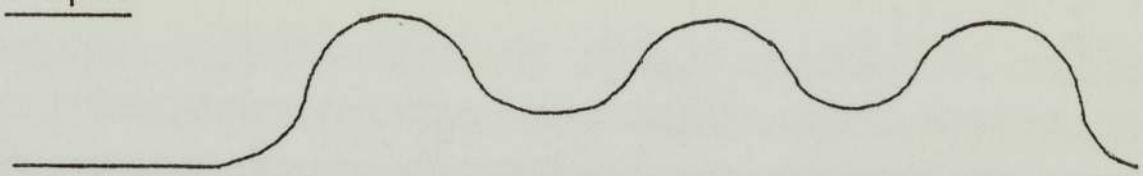
Input



39.2	38.8	40.5	Mass of Dextran, g
0.228	0.226	0.235	Volume Injected, l

Feed Concentration: 172 g/l
Feed Equivalent to : 1.6 % total volume
1.9 % liquid volume
Equivalent hourly feedrate: 35.7 g/h

Output

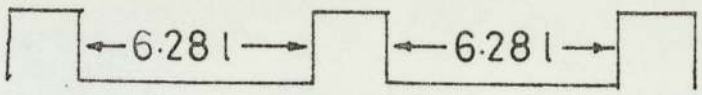


	Initial	Waste No.1	Product	Waste No.2	Final
Volume Eluted, l	11.53	1.51	5.02	1.51	6.28
Mass of Dextran, g		3.31	30.85	5.22	
Concentration, g/l		2.19	6.14	3.46	
Mol. Wt. Avs. $\times 10^{-3}$					
$\overline{M}_w$		67.8	36.3	94.5	
$\overline{M}_n$		12.8	20.6	9.7	
D		5.29	1.67	9.74	
Prod. % of feed			79.5		
Cumulative Mol. Wts. $\times 10^{-3}$ 5%		4	10	3	
10%		7	15	5	
90%		135	126	406	
95%		423	203	1013	

Equivalent hourly productrate: 24.7 g/h

Figure 5.10
Experiment No. 2

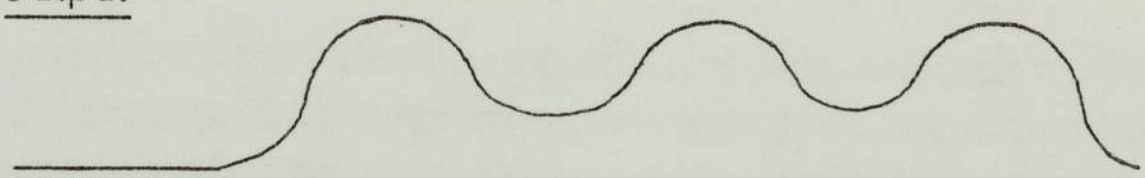
Input



23·3	23·8	22·6	Mass of Dextran, g
0·146	0·149	0·141	Volume Injected, l

Feed Concentration: 160 g/l
Feed Equivalent to : 1·0 % total volume
1·2 % liquid volume
Equivalent hourly feedrate: 22·2 g/h

Output

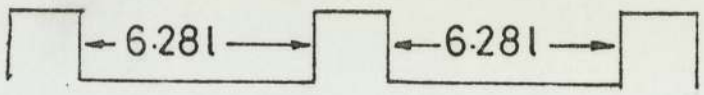


	Initial	Waste No.1	Product	Waste No.2	Final
Volume Eluted, l	11·39	1·41	5·02	1·40	6·28
Mass of Dextran, g		2·44	19·61	2·12	
Concentration, g/l		1·73	3·91	1·51	
Mol. Wt. Avs. x 10^{-3}					
$\overline{M}_w$		149·2	31·2	171·0	
$\overline{M}_n$		29·9	17·6	26·6	
D		4·99	1·77	6·43	
Prod. % of feed			82·4		
Cumulative Mol. Wts. x 10^{-3} 5 %		10	10	10	
10 %		17	14	15	
90 %		627	119	654	
95 %		1444	141	1454	

Equivalent hourly productrate: 18·3 g/h

Figure 5.11
Experiment No. 3

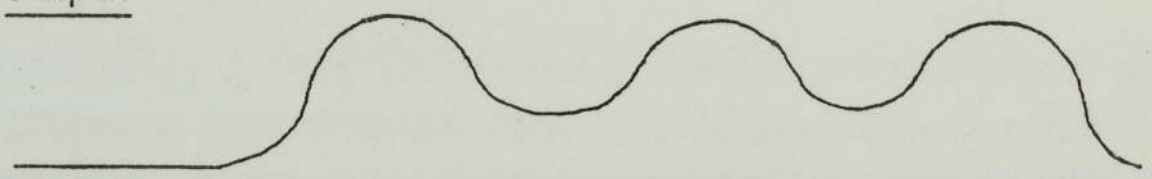
Input



18.4	17.3	16.9	Mass of Dextran, g
0.109	0.102	0.100	Volume Injected, l

Feed Concentration: 169 g/l
Feed Equivalent to : 0.71 % total volume
0.84 % liquid volume
Equivalent hourly feedrate: 16.4 g/h

Output

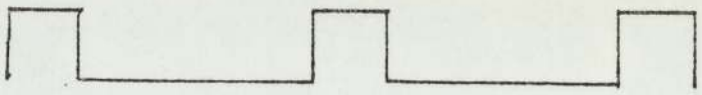


	Initial	Waste No.1	Product	Waste No.2	Final
Volume Eluted, l	11.21	1.36	5.02	1.36	6.28
Mass of Dextran, g		2.24	13.59	1.47	
Concentration, g/l		1.65	2.71	1.08	
Mol. Wt. Avs. $\times 10^{-3}$					
$\overline{M}_w$		154.3	28.1	164.4	
$\overline{M}_n$		41.8	16.5	42.6	
D		3.69	1.70	3.86	
Prod. % of feed			78.6		
Cumulative Mol. Wts. $\times 10^{-3}$ 5 %		14	9	13	
10 %		24	13	21	
90 %		669	68	660	
95 %		1298	94	1791	

Equivalent hourly productrate: 12.8 g/h

Figure 5.12
Experiment No. 4

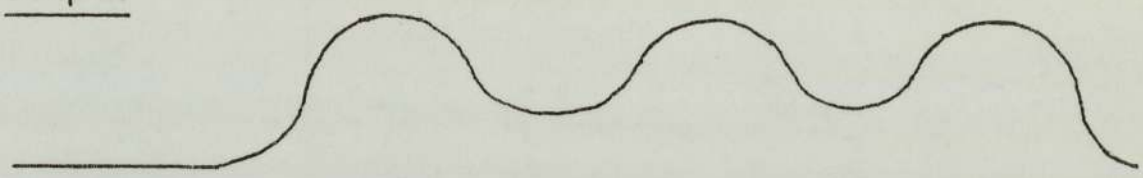
Input



48·1	49·2	53·6	Mass of Dextran, g
0·321	0·328	0·357	Volume Injected, l

Feed Concentration: 150 g/l
Feed Equivalent to: 2·3 % total volume
2·7 % liquid volume
Equivalent hourly feedrate: 45·2 g/h

Output

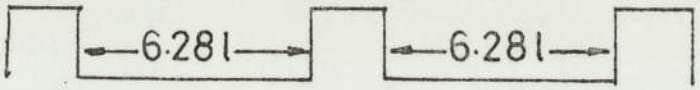


	Initial	Waste No.1	Product	Waste No.2	Final
Volume Eluted, l	11·53	1·51	5·02	1·51	6·32
Mass of Dextran, g		5·3	43·1	8·62	
Concentration, g/l		3·54	8·58	5·73	
Mol. Wt. Avs. $\times 10^{-3}$					
$\overline{M}_w$		50·4	40·2	43·8	
$\overline{M}_n$		9·0	20·7	10·1	
D		5·60	1·94	4·34	
Prod. % of feed			87·6		
Cumulative Mol. Wts. $\times 10^{-3}$ 5%		3	11	3	
10%		5	14	5	
90%		93	129	73	
95%		255	205	188	

Equivalent hourly productrate: 39·6 g/h

Figure 5.13
Experiment No. 5

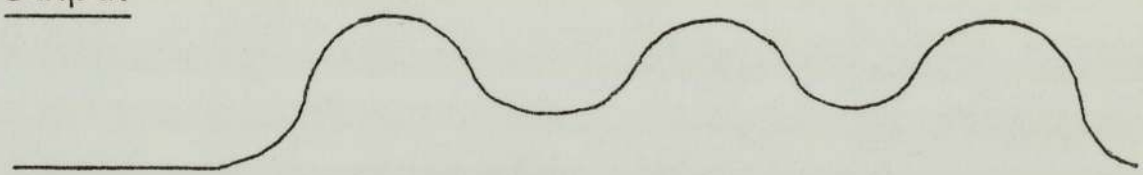
Input



82.9	81.9	78.1	Mass of Dextran, g
0.468	0.463	0.441	Volume Injected, l

Feed Concentration: 177 g/l
Feed Equivalent to : 3.1 % total volume
3.7 % liquid volume
Equivalent hourly feedrate: 73.4 g/h

Output

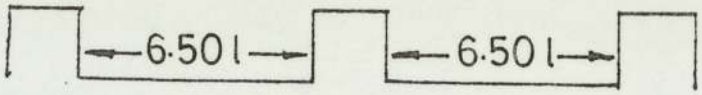


	Initial	Waste No.1	Product	Waste No.2	Final
Volume Eluted, l	11.49	1.33	5.02	1.33	6.37
Mass of Dextran, g		10.2	74.9	10.9	
Concentration, g/l		7.66	14.91	8.18	
Mol. Wt. Avs. $\times 10^{-3}$					
$\overline{M}_w$		36.2	61.2	38.9	
$\overline{M}_n$		13.4	24.6	14.2	
D		2.70	2.49	2.74	
Prod. % of feed			91.4		
Cumulative Mol. Wts. $\times 10^{-3}$ 5 %		4	11	5	
10 %		8	16	8	
90 %		60	237	70	
95 %		91	468	110	

Equivalent hourly productrate: 67.1 g/h

Figure 5.14
Experiment No. 6

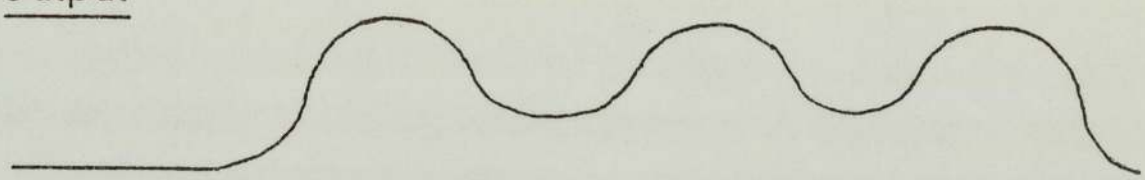
Input



39·2	39·6	48·3	Mass of Dextran, g
0·238	0·240	0·293	Volume Injected, l

Feed Concentration: 165 g/l
Feed Equivalent to: 1·6 % total volume
2·0 % liquid volume
Equivalent hourly feedrate: 35·2 g/h

Output

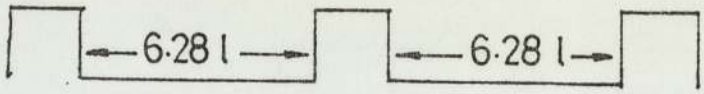


	Initial	Waste No.1	Product	Waste No.2	Final
Volume Eluted, l	11·53	1·51	5·02	1·51	6·53
Mass of Dextran, g		4·9	24·9	5·2	
Concentration, g/l		3·26	4·96	3·46	
Mol. Wt. Avs. $\times 10^{-3}$					
$\overline{M}_w$		43·3	54·9	35·3	
$\overline{M}_n$		12·9	30·6	14·0	
D		3·36	1·79	2·52	
Prod. % of feed			62·9		
Cumulative Mol. Wts. $\times 10^{-3}$ 5%		4	14	5	
10%		7	18	8	
90%		116	183	64	
95%		209	262	146	

Equivalent hourly productrate: 22·1 g/h

Figure 5.15
Experiment No. 7

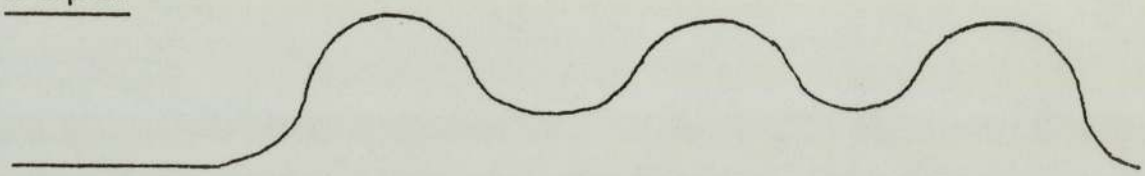
Input



62·1	70·3	72·8	Mass of Dextran, g
0·338	0·382	0·396	Volume Injected, l

Feed Concentration: 184 g/l
Feed Equivalent to: 2·7 % total volume
3·3 % liquid volume
Equivalent hourly feedrate: 63·3 g/h

Output

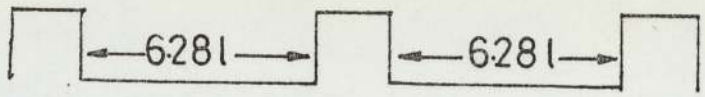


	Initial	Waste No.1	Product	Waste No.2	Final
Volume Eluted, l	11·67	1·51	5·02	1·51	6·28
Mass of Dextran, g		8·3	54·5	10·8	
Concentration, g/l		5·51	10·4	7·17	
Mol. Wt. Avs. $\times 10^{-3}$					
$\overline{M}_w$		116·7	39·2	53·2	
$\overline{M}_n$		23·1	22·8	23·9	
D		5·05	1·72	2·23	
Prod. % of feed			77·6		
Cumulative Mol. Wts. $\times 10^{-3}$ 5%		8	11	11	
10%		14	15	15	
90%		496	123	153	
95%		1123	208	230	

Equivalent hourly productrate: 49·1 g/h

Figure 5.16
Experiment No. 8

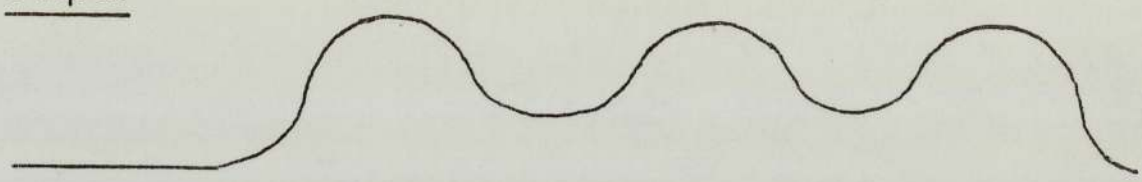
Input



51·1	52·4	50·9	Mass of Dextran, g
0·267	0·274	0·260	Volume Injected, l

Feed Concentration: 191 g/l
Feed Equivalent to : 1·9 % total volume
2·3 % liquid volume
Equivalent hourly feedrate: 48·0 g/h

Output

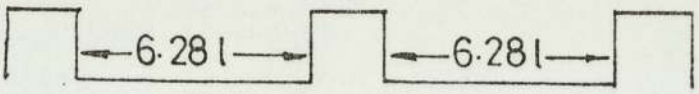


	Initial	Waste No.1	Product	Waste No.2	Final
Volume Eluted, l	11·53	1·51	5·02	1·51	6·17
Mass of Dextran, g		5·52	40·5	4·44	
Concentration, g/l		3·36	8·06	2·49	
Mol. Wt. Avs. $\times 10^{-3}$					
$\overline{M}_w$		115·7	34·7	98·5	
$\overline{M}_n$		23·8	19·9	22·7	
D		4·86	1·74	4·34	
Prod. % of feed			77·2		
Cumulative Mol. Wts. $\times 10^{-3}$ 5 %		8	10	7	
10 %		14	13	13	
90 %		446	124	197	
95 %		885	197	550	

Equivalent hourly productrate: 37·1 g/h

Figure 5.17
Experiment No. 9

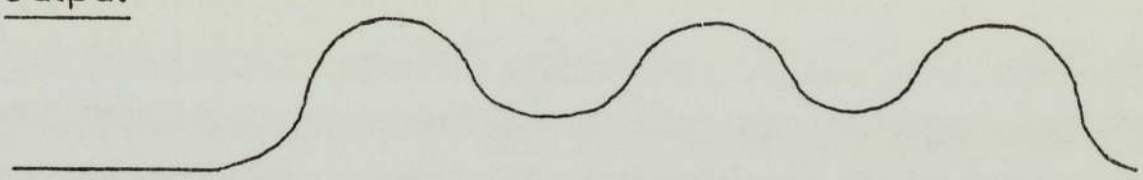
Input



62·9	62·9	60·3	Mass of Dextran, g
0·239	0·239	0·224	Volume Injected, l

Feed Concentration: 263 g/l
Feed Equivalent to: 1·6 % total volume
1·9 % liquid volume
Equivalent hourly feedrate: 57·9 g/h

Output

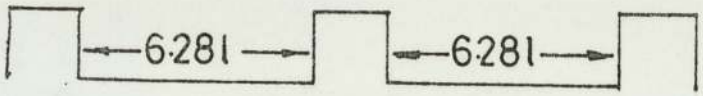


	Initial	Waste No.1	Product	Waste No.2	Final
Volume Eluted, l	11·67	1·33	5·17	1·50	6·32
Mass of Dextran, g		5·56	53·2	4·88	
Concentration, g/l		4·18	10·3	3·25	
Mol. Wt. Avs. $\times 10^{-3}$					
$\overline{M}_w$		136·8	37·8	143·5	
$\overline{M}_n$		22·7	21·7	24·7	
D		6·03	1·74	5·81	
Prod. % of feed			84·6		
Cumulative Mol. Wts. 5%		5	11	7	
10%		12	14	13	
90%		510	123	548	
95%		1263	208	1327	

Equivalent hourly productrate: 49·0 g/h

Figure 5.18
Experiment No. 10

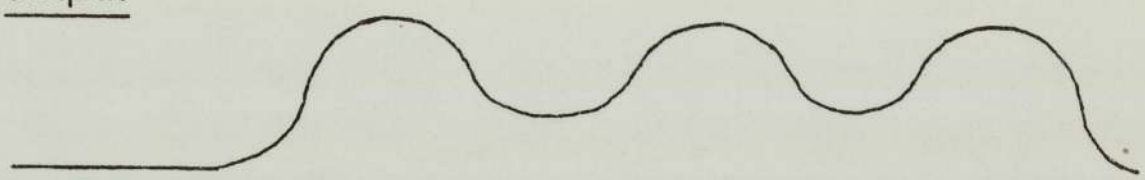
Input



37·3	38·8	36·4	Mass of Dextran, g
0·139	0·145	0·136	Volume Injected, l

Feed Concentration: 268 g/l
Feed Equivalent to: 1·0 % total volume
1·2 % liquid volume
Equivalent hourly feedrate: 36·3 g/h

Output



	Initial	Waste No.1	Product	Waste No.2	Final
Volume Eluted, l	11·26	1·41	5·02	1·40	6·29
Mass of Dextran, g		4·4	32·3	4·6	
Concentration, g/l		3·16	6·34	3·29	
Mol. Wt. Avs. $\times 10^{-3}$					
$\overline{M}_w$		100·5	36·2	69·5	
$\overline{M}_n$		16·4	23·0	25·3	
D		6·13	1·57	2·75	
Prod. % of feed			83·3		
Cumulative Mol. Wts. 5 %		5	11	9	
10 %		9	15	17	
90 %		436	144	204	
95 %		1200	200	490	

Equivalent hourly productrate: 30·2 g/h

Fig 5.19 Summary of the distributions for the product fractions from the repetitive batch chromatograph.

Product from exeriment	$\bar{M}_w$	$\bar{M}_n$	D	M_{10}	M_{90}
1	36300	20600	1·67	15000	126000
2	31200	17600	1·77	14000	119000
3	28100	16500	1·70	13000	68000
4	40200	20700	1·94	14000	129000
5	61200	24600	2·49	16000	237000
6	54900	30600	1·79	18000	183000
7	39200	22800	1·72	15000	123000
8	34700	19900	1·74	13000	124000
9	37800	21700	1·74	14000	123000
10	36200	23000	1·57	15000	144000

Fig 5.20 Effect of sample volume on molecular weight of product fraction

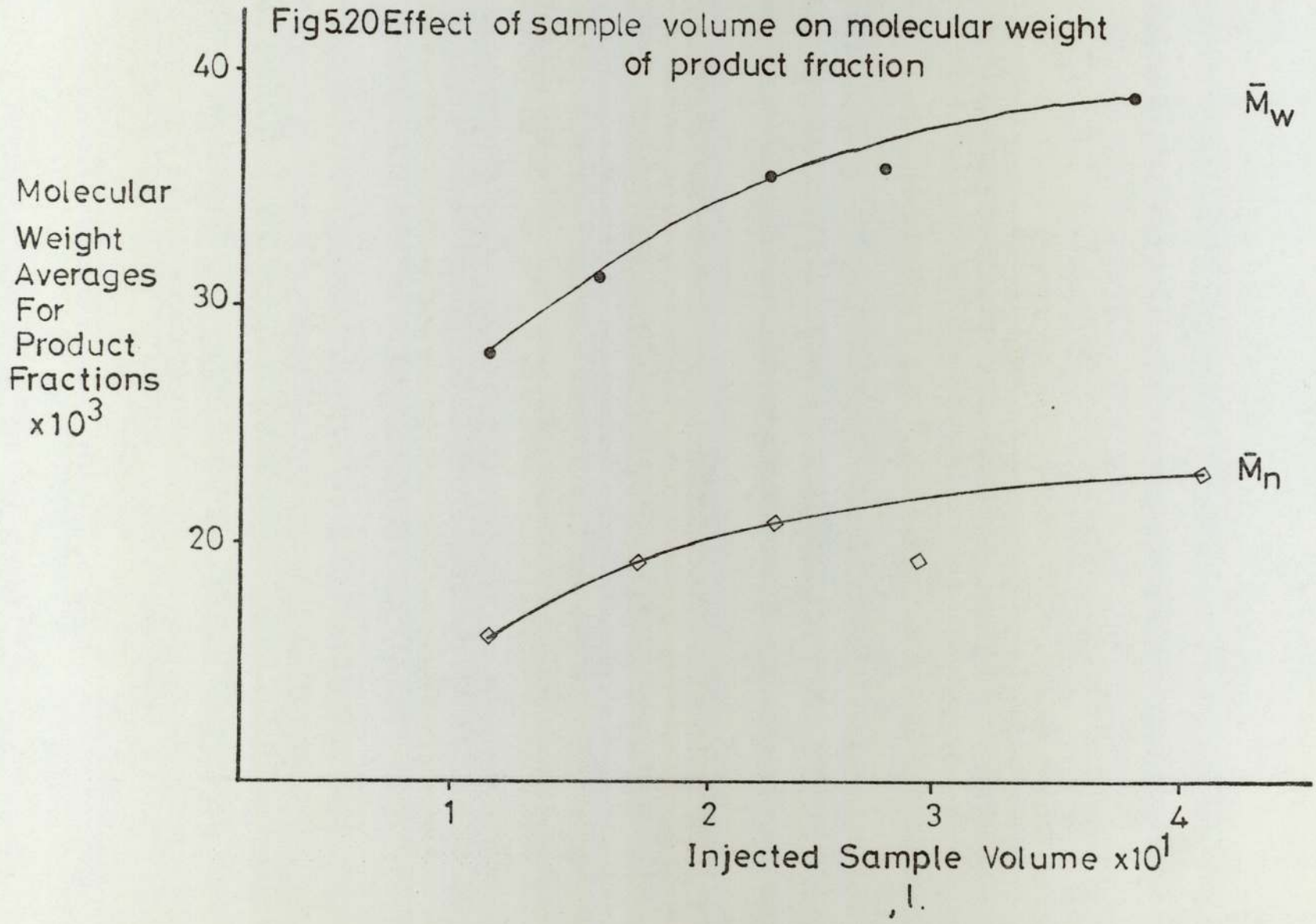
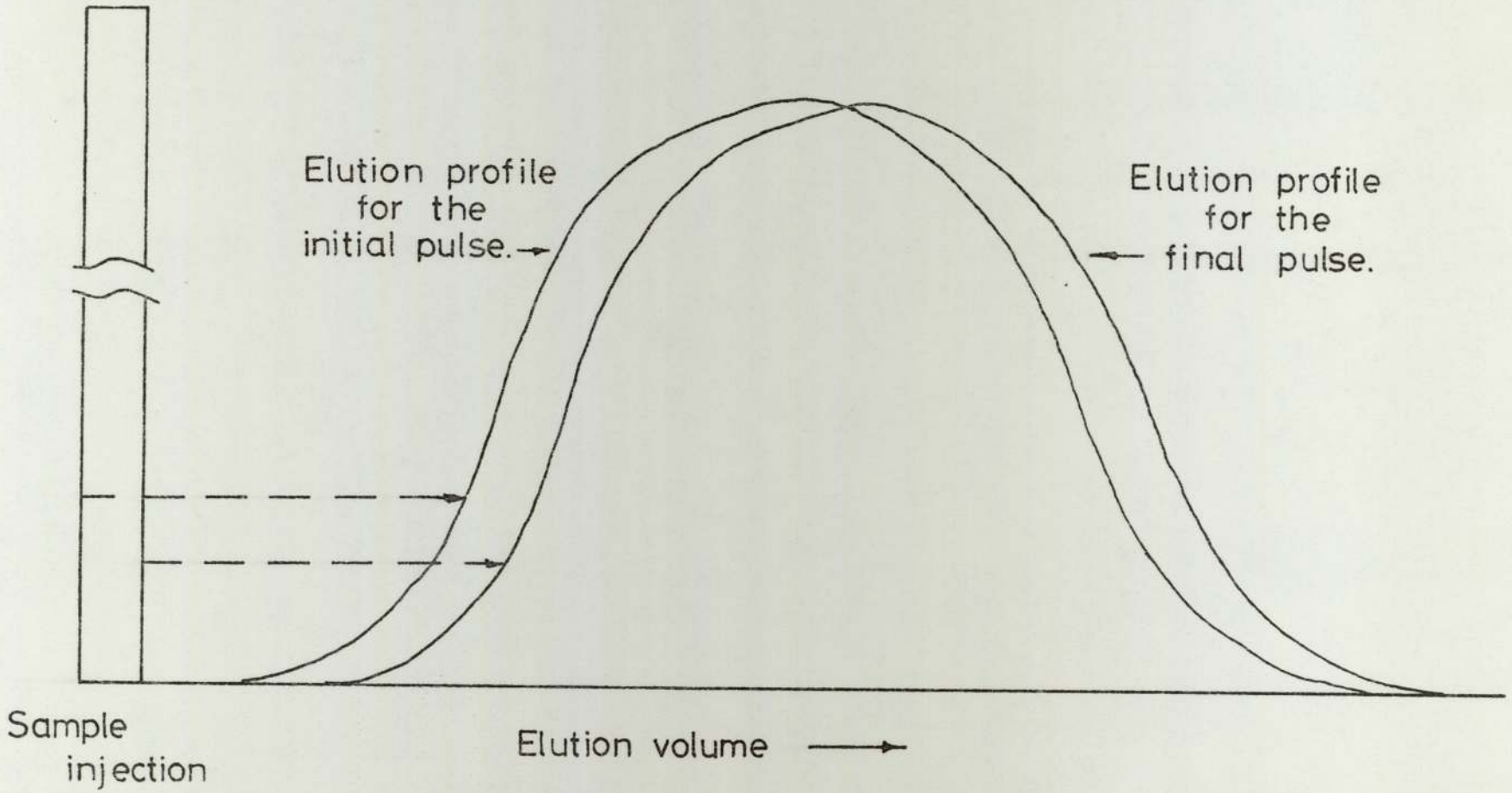


Fig5.21Fractionation of large volume sample



excluded from the stationary phase from the initial pulse of one injection should begin to elute the instant the totally included molecules have been eluted from the final pulse of the proceding injection. As the sample volumes increased the greater the difference in elution volumes between the first and final pulses for a sample became, as indicated by points 1 and 1' that represent the elution volumes for the totally included molecules in Fig. 5.22. Similarly for the high molecular weight material indicated by the points h and h'. Since the pulses are non-interacting the difference between the points 1 1' and h h' equals the volume of the injected sample. The effect on the product fraction would be to increase the molecular weight range present in the product fraction and because the observed elution profile for the dextran feed material was symmetrical the higher molecular weight molecules would have a greater effect on M_w and M_n averages of the product fraction thus increasing their values.

These effects were observed in experiments 1, 2 and 3 as Fig. 5.20 shows although corrective measures were taken by adjusting the volume eluted as measured from the start of the initial injection. As the volume of sample injected was increased the eluted volume prior to the collection of the product fraction also increased. The simple description of non-interacting pulse describing the large injection volumes suggests that the elution should increase by the same volume as the sample volume.

As Fig. 5.23 illustrates the increase in the volume eluted before the product fraction was collected was not linearly dependent upon the sample volume. For experiments 1, 2 and 3 the increase in the elution volume is between 3 and 5 times the increase in sample volume. If there were no interactions of the pulses then the effect of increasing

Fig 5.22 Overlapping sample injections

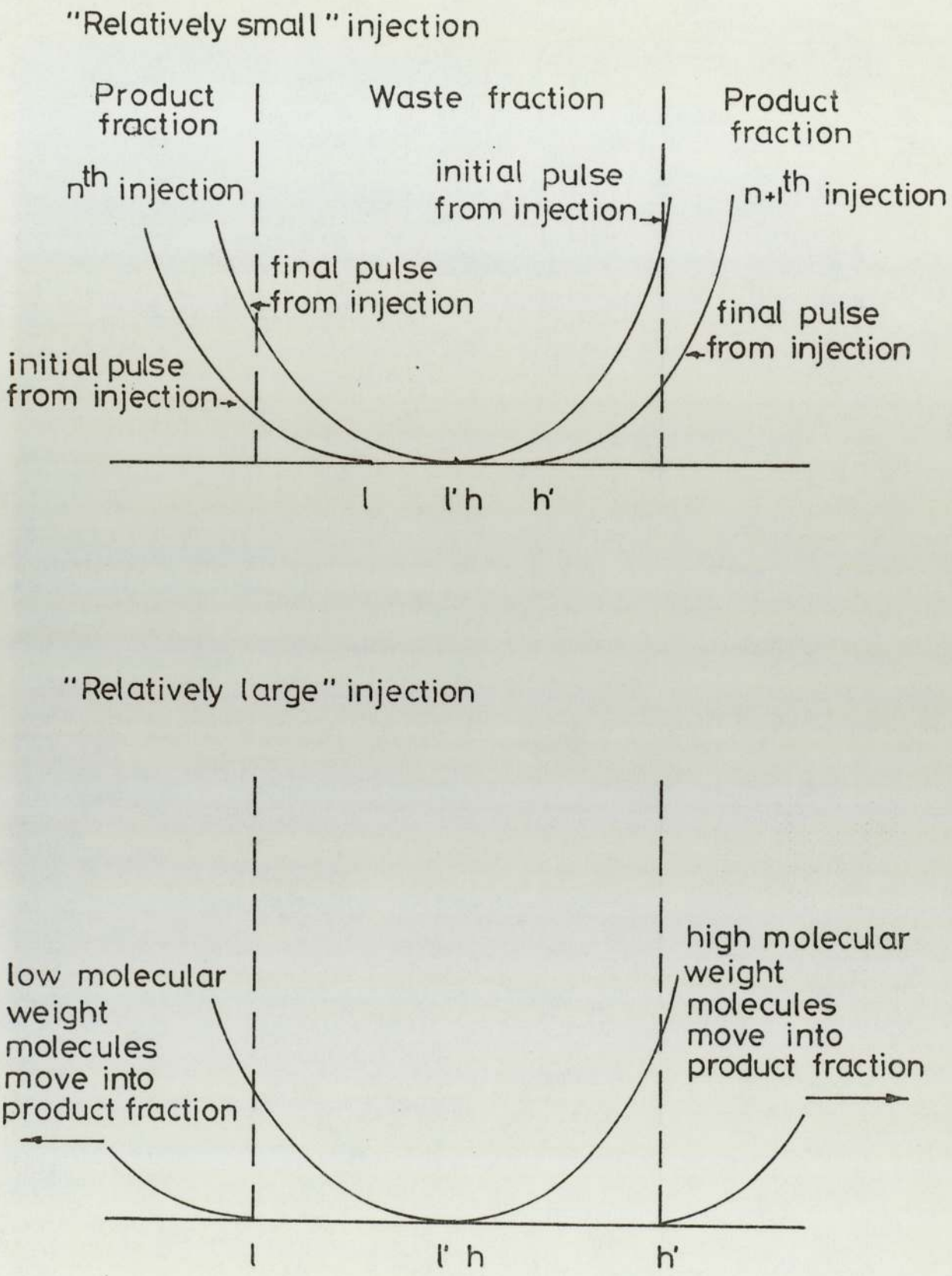
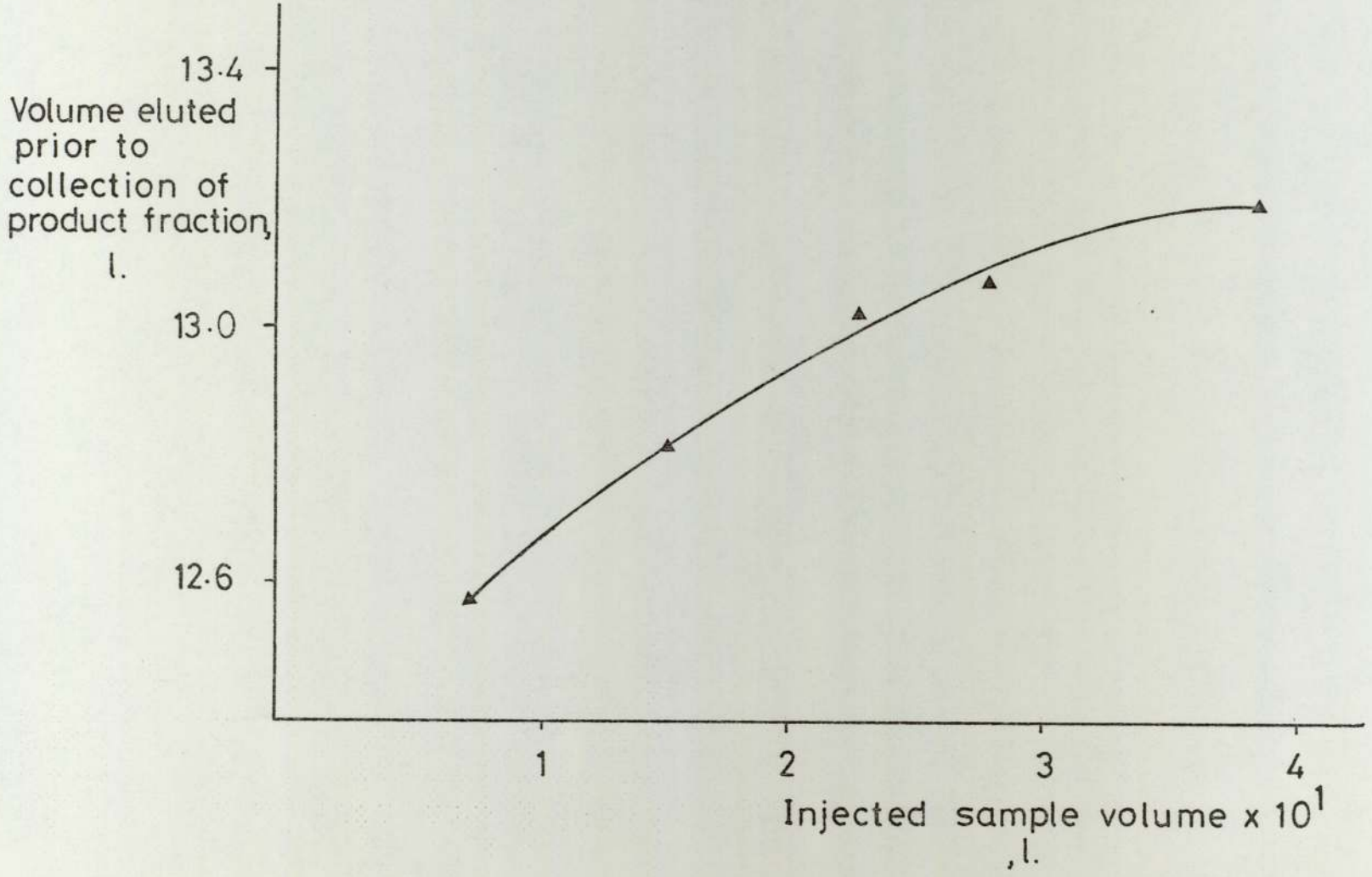


Fig 5.23 Effect of sample volume on elution volume of product fraction.



the elution volume by a greater amount than the increase in the sample weight of the collected product. But as Fig. 5.20 shows, the molecular weight averages of the product fractions increased, therefore other effects appear to be operating during the fractionation of the dextran molecules as well. The most obvious is the increase in the mass of the loaded sample. The increase in sample volume, increases the mass of dextran injected which in turn produces a localised higher on column concentration. Ellison (34) on a small scale batch chromatographic column demonstrated that mutual interations of dextran molecules on Porasil C have had the effect of altering the calibration curve for the column. There was an increase in the Wheaton-Bauman distribution coefficient with increase in polymer concentration in the mobile phase, this was accompanied by a broadening of the sample peaks, although it was not clear whether the broadening was due to the actual displacement of the eluted molecules or by concentration effects causing increased zone broadening. Observations on an analytical column packed with Porasil C (37-75 μm) used to determine the quality and concentration of the product samples for this experimental programme recorded that some dextran peaks were eluted beyond the total liquid volume of the column. Ellison in routine analysis on Spherosil XOB075 did not observe any such effects. The detection method used by Ellison was an automatic colorimetric method. This enables more dilute samples to be used than did the refractive index detection method used in conjunction with the Porasil C column. The increase in the concentration of the injected samples from approximately 0.08 w/v% to 4 w/v% and could account for the observed increase in the elution of the dextran peak. A similar effect may be occurring with the repetitive batch study. This may explain why the molecular weight of the product fraction increased

when the sample volume increased. Broadening of the fractionated peaks may be due to the actual displacement of the eluted molecules.

When the volume of the sample injected was increased in experiments 7 and 8 it was considered that the zone broadening for a particular molecular weight species also increased. This was concluded from the way the molecular weight distribution of the product fraction approached the molecular weight of the feed sample and by the waste fraction approaching the distribution of the product fraction. This can be summarised by the molecular weight averages for the experiments numbers 3, 1 and 7 as shown below:-

Experiment No.	Description	$\bar{M}_w$	$\bar{M}_n$	D
	Feed	47500	20800	2.28
3	Product Fraction	28100	16500	1.72
	Waste Fraction 2	164400	42600	3.86
1	Product Fraction	36300	20600	1.76
	Waste Fraction 2	84500	9700	9.64
7	Product Fraction	39200	22800	1.71
	Waste Fraction 2	53200	23900	2.23

where the respective volume of sample applied was increased.

A feature of the molecular weight distribution for the waste fractions for the three reported samples above was that the number average was much higher for experiment 3 than either of the other two samples. This may be because that experiment 3 had the smallest volume of feed solution applied and with reference to Fig. 5.22 the molecular weight distribution of the waste fraction had a broader distribution containing the very high and the very low molecular weight molecules but in the calculation of the molecular weight averages the very high molecular weight molecules would have a greater effect. As the sample volume increased and so the volume between the theoretical first and

final pulses in the eluted peaks also increased, as in Fig. 5.21, because of the log-linear relationship between molecular weight and elution volume for g.p.c. this had a greater affect on the high molecular weight molecules. When the sample volume was increased the number average molecular weight of the waste fraction was reduced. When the sample volume was increased still further in experiment 7 the number average for the waste fraction increased again to a value approximately the same as the product fraction.

The values for the points at the 5 and 10% molecular weights for the cummulative distribution for the waste fractions approached the values for the product fractions too. This suggested that there was poor fractionation of the low molecular weight molecules for experiment 7.

The largest volume of feed solution applied to the repetitive batch column was in experiment 5 where the volume injected was equivalent to 3.7% of the total liquid volume of the column. The trend exhibited by experiments 1, 2, 3, 7 and 8 showed that the elution volume for experiments increased with an increase in sample volume. Unfortunately, this was not the case in experiment 5 because the product fraction was collected after only 12.82 l, and if the curve illustrating the relationship between the volume of sample loaded and the volume eluted from the column prior to the collection of the product fraction in Fig. 5.23 is extended through the absissca valve of 0.44 l, it suggests that the collection volume should have been approximately 13.2 l. This error in the collection volume of the product fraction explains why the experiment succeeded in removing only 9% of the injected feed material, and as the molecular weight averages for obtained product suggests,

Experiment	$\bar{M}_w$	$\bar{M}_n$	D
5	61200	24600	2.49
feed	47500	20800	2.28

the material removed was only low molecular material because both the molecular weight averages have increased. Therefore this experiment failed to determine whether the fractionating column was still capable of reducing the molecular weight range of an injected sample where the sample volume had been increased from 0.38 to 0.44 litres.

The experiment that recorded a satisfactory reduction in the amount of dextran in the product fraction both at the high and low molecular weight range and had the maximum quantity of dextran injected was experiment 7. The quantity of dextran injected was 70 grams in a 184 g l^{-1} concentrated solution. Experiment 7 showed that the maximum sample volume injected was equivalent to 3.3% of the total liquid volume of the column, this result supports the 'rule of thumb' estimations suggested by Brewer (172) that the maximum volume of sample that can be applied when using gel filtration (g.p.c.) is approximately 3% of the total bed volume. Conder and Purnell (262) who have had greater experience in operating a large-scale preparative batch chromatograph using a gas-liquid system suggests that for a large volume sample three operating modes occur. Classification of these operating modes can be described by the value of the product of the number of plates occupied by the feed inlet band and the reciprocal of the root of the total number of plates in the column as shown below:-

Mode	Range
Elution	$\frac{1}{2} > \theta$
Overload Elution	$6 > \theta > \frac{1}{2}$
Eluto-frontal	$\theta > 6$

where $\theta = N_f \cdot N_t^{-\frac{1}{2}}$

N_f - No. of plates occupied by feed band

N_t - No. of plates in column.

Figure 5.24 illustrates these three operating modes for a binary system, the descriptions of the outlet profiles for the elution and overload elution modes are,

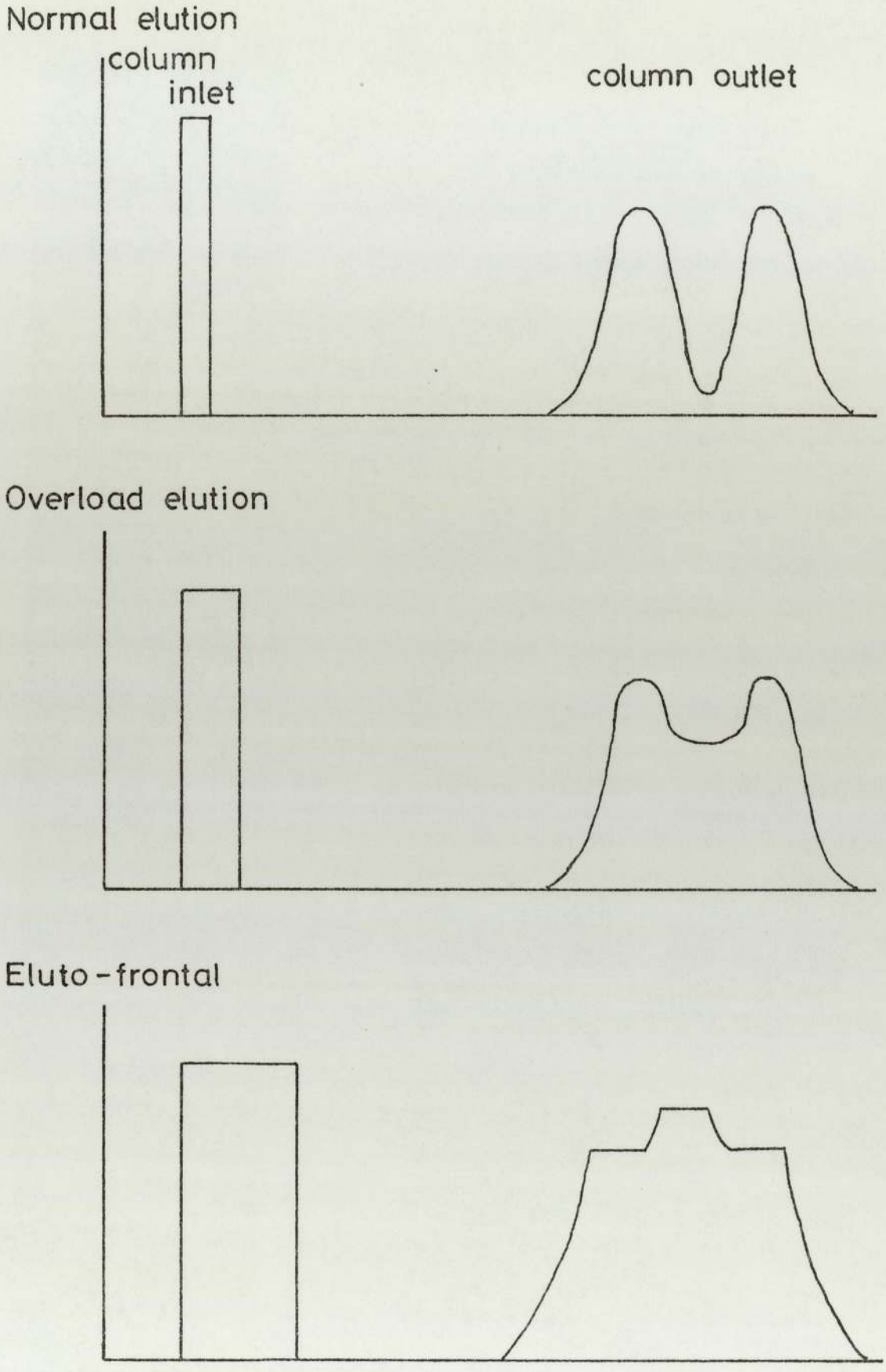
(a) Elution. This mode has the shortest sample injection and the outlet profiles are gaussian if there is a linear type of partition isotherm. These peaks get broader as the value of θ approaches $\frac{1}{2}$.

(b) Overload Elution. The increase in the sample volume increases the broadening of the eluted peaks so that in this mode the outlet profile is intermediate between the Gaussian peaks and the flat-topped profile.

For the experimental programme the size of the injected sample volume in terms of θ ranged from 0.27 to 1.15 thus the type of operating mode changed from elution to overload elution. The value of θ for experiment 7 was approximately 1 and therefore in the overload elution mode. It was expected that the fractionation of dextran could operate in the overload elution mode because it was the objective of the project to remove only relatively small quantities from the extremes of the outlet profile and the broad profile for a molecular weight species could be contained within the waste fraction.

Two experiments were performed that had a deviation in the volume of eluent applied to the column between successive injections. This was a result of mis-calculation of the injection times rather than a planned operation. The two experiments were, experiment 6 that had an increase of 3.5% in the standard pore volume used and experiment 4 that had a slight reduction of 1.4%.

Fig 5.24 Elution modes for a binary system.



Experiment 6 aimed to repeat the operating conditions of experiment 1 where the same product collection sequence and similar feed concentrations were used. The slight increase in the volume of eluent applied to the column between injections caused a change in the molecular weight distributions of the product and waste fractions as shown below:-

Collected Fraction	Experiment 6			Experiment 1		
	$\bar{M}_w$	$\bar{M}_n$	D	$\bar{M}_w$	$\bar{M}_n$	D
Waste 1	43300	12900	3.36	67800	12800	5.29
Product	54900	30600	1.79	36300	20600	1.67
Waste 2	35300	14000	2.52	94500	9700	9.74

Both experiments used the same collection sequence. A higher molecular weight product fraction was expected for experiment 6 because relative to the injected sample, the product fraction was collected earlier. This experiment emphasises the fact that a small variation of 3.5% in the eluent volume between successive injections can cause a large change in the molecular weight distribution of the product fraction.

For experiment 4 a slight reduction of 1.4% was made in the standard eluate volume of 6.28 l between successive injections. The volume of solution injected was approximately 0.32 l. The product fraction for experiment 4 had higher molecular weight averages than those experiments that used a smaller sample volume. Only 12.4% of the feed material was removed from the feed injection which accounts for the broad molecular weight distribution of the product fraction. A comparison of the product fraction for experiment 4 with experiments 7 and 8 that had similar injection volumes is presented below:-

Experiment	Sample Volume,l	$\bar{M}_w$	$\bar{M}_n$	D	M_{10}	M_{90}
4	0.330	40200	20700	1.94	14100	128900
7	0.382	39200	22800	1.72	14500	122800
8	0.274	34700	19900	1.74	13200	124400
Feed		47500	20800	2.29	13300	133000

When comparing the M_{10} and M_{90} values which are the points at 10 and 90% of the cumulative distribution it can be seen that although only 12.4% has been removed, this consists of both the high and the low molecular weight material. The ability to minimise the volume of eluent applied between successive injections will obviously aid the overall product throughput rate. But, any increase in the feed throughput rate by this method is thought to be small compared to maximising the sample injection volume of the feed solution concentration.

The collection of 80% of the injected feed material was achieved in a majoritory of experiments as shown by Figs. 5.9 to 18. Unfortunately the quality of the product fractions changed. This was inherent in the technique used for determining when to change the eluate collection because the refractometer system could only provide quantitative information. As further experimentation is recommended, a technique for monitoring an intensive property of the eluate be used in order to product a more constant quality product fraction. For example a low angle laser could monitor the number average of the eluate.

(b) Effect of Feed Concentration

An increase in the throughput of the batch chromatographic equipment was also attempted by increasing the concentration of the

feed solution from approximately 170 to 265 g l^{-1}. The maximum concentration of the feed solution that could be handled was approximately 265 g l^{-1}. Two experiments were performed at higher concentrations in order that the effect on fractionation might be investigated. Previous experiments had already used feed concentrations far higher than most preparatives studies and had demonstrated that fractionation could be achieved.

Experiments 10 and 2 loaded almost identical volumes of feed solution, although experiment 10. loaded almost 63% more dextran than experiment 2. The mass of dextran loaded in experiment 10 was similar to the quantity injected in experiment 1. A comparison of the molecular weight distribution for the product fractions for experiments 10 and 2 showed that the higher concentration feed solution gave slightly higher molecular weight averages but a lower polydispersity, indicated below:-

Experiment	Feed Concentration	$\bar{M}_w$	$\bar{M}_n$	D
10	268	36200	23000	1.57
2	160	31200	17600	1.77

Although the molecular weight averages for experiment 10 was higher it was still within the criteria for an acceptable product of having an $\bar{M}_w$ less than 40000, and a polydispersity of less than 1.8.

When a larger sample volume of feed was used, the result of using a higher feed concentration appeared to have a less sensitive effect on the product distribution. This was also supported by the results from experiments 8 and 9 where similar loadings give similar product fractions in spite of the difference in volume and concentration. Thus there appeared to be less effect of sample volume and of sample concentration on the product fractions when the overall loading of dextran

was high. Therefore, but for the pressure limitation of 480 kN m^{-2} it was felt that the volume of feed solution that could be applied at 265 g l^{-1} concentration could have been increased to the 3.3% of the total liquid volume as recorded in experiment 7.

5.4.5 Conclusions

The use of g.p.c. to fractionate broad dextran fractions on a preparative scale has been demonstrated. Both high and low molecular weight material was removed from the input sample in a single pass through the column and produced a product fraction having a narrower molecular weight distribution. The chromatographic column was operated in a repetitive batch manner. The process made use of large sample volumes of concentrated feed, up to 268 g l^{-1}, in order to achieve high throughputs. The maximum hourly product rate recorded was 49 g h^{-1}. The maximum sample volume applied to the column that produced a satisfactorily reduced product fraction was equivalent to 3.3% of the total liquid volume.

There appeared to be no significant adverse effect on the quality of the fractionated product when the concentration of the feed samples were increased from 170 to 265 g l^{-1}. But for the pressure limitations on the glass fractionating column it was anticipated that the quality of feed solution injected at 265 g l^{-1} could have been increased to the equivalent of 3.3% of the total liquid volume of the column.

6.0

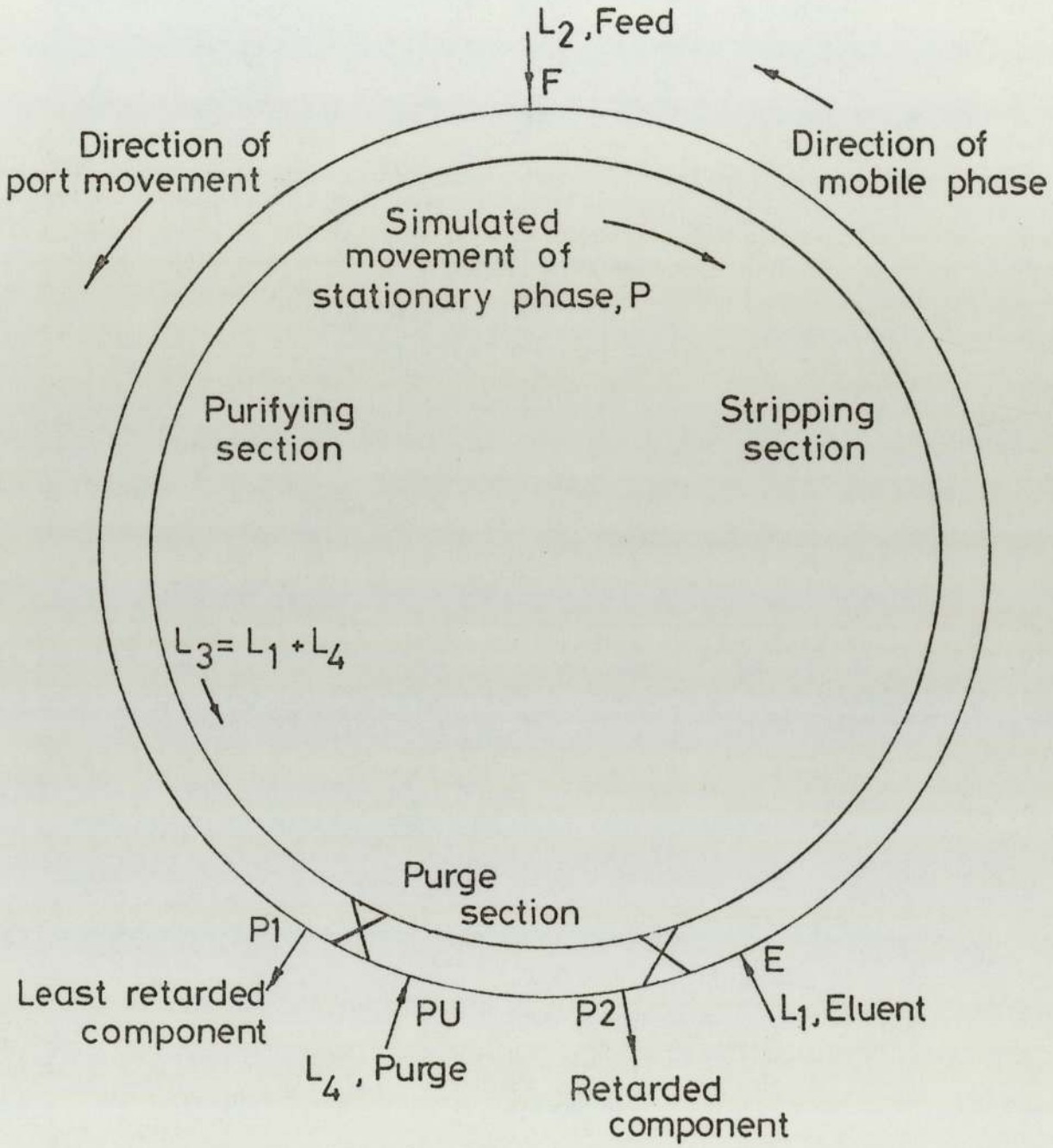
SEMI-CONTINUOUS CHROMATOGRAPHY

6.1 INTRODUCTION

The theories about g.p.c. fractionation and the principles of semi-continuous chromatographic equipment have already been discussed in Chapters 2 and 3, respectively. A schematic diagram of the semi-continuous chromatographic refiner No. 5 (SCCR5) is given in Fig. 6.1 that allows various features of the equipment to be illustrated. The equipment can be considered to be in two sections, the purge and fractionating sections. The purge section, between PU and P2 in Fig. 6.1 is where material that has a preferential affinity for the stationary phase is removed by flushing through with excessive quantities of the mobile phase. The fractionating section, between the points E and P1 can be considered to have two parts, rather like a multistage liquid-liquid extraction process. A stripping section between E and F and a purifying section between F and P1. Pure eluent enters at point E and moves towards point F stripping out any molecules that have a preference for the mobile phase. At F there is a step change increase in the mobile phase velocity and any material having a preference for the mobile phase is carried forward, and eventually eluted from point P1. The input and the product ports remain in a set position for a period of time, termed the switch time, before the functions of the ports are indexed by one column in the same direction as the mobile phase flow thus the process simulates the packing moving in the opposite direction. When all the columns in the chromatograph have operated as the purge section, the chromatograph is then said to have completed one cycle.

The volumetric throughput of the eluent, feed, and the purge are denoted by the symbols L_1, L_2 and L_4 respectively. L_3 is the sum of eluent and feed flowrates. An effective velocity can also be

Fig 6.1 Schematic diagram of the S.C.C.R.5.



assigned to the 'apparent' movement of the stationary phase, P, when measured from the feed point. From a knowledge of the column's characteristics and the relative velocities of the mobile and stationary phases theoretical cut positions can be applied to the stripping, purifying and purge sections which help in the selection of the operating conditions. Measurement of the column characteristics were only made at near infinitely dilute conditions, but when feed solution concentrations of up to 200 g l^{-1} were fed continuously onto the columns, deviations between the calculated theoretical cut points and the actual cut positions were expected.

6.1.1 Principle of Operation

The principle of operation of the SCCR5 machine is best explained using a quaternary system. Consider that, initially it is required to fractionate the feed into two products each containing two components. Fig. 6.2a shows diagrammatically how the components in the feed will begin to be distributed. The least-retained components will move preferentially with the mobile phase and progress further towards the outlet port P1. Components having a greater affinity for the packing will still move in the direction of the mobile phase but with a lower velocity. Fig. 6.2b shows the distribution of the components after half-a-cycle. Note how the purge section advances towards the greater retained components although they are still moving with the mobile phase. The least retained components appear to move towards the outlet port P1. Fig. 6.2c shows how the components are distributed at the pseudo-equilibrium condition.

Now consider that a change in the fractionating performance of the rig is required and that only one component is to be removed from

Fig 6.2 Principle of operation for the S.C.C.R.5.

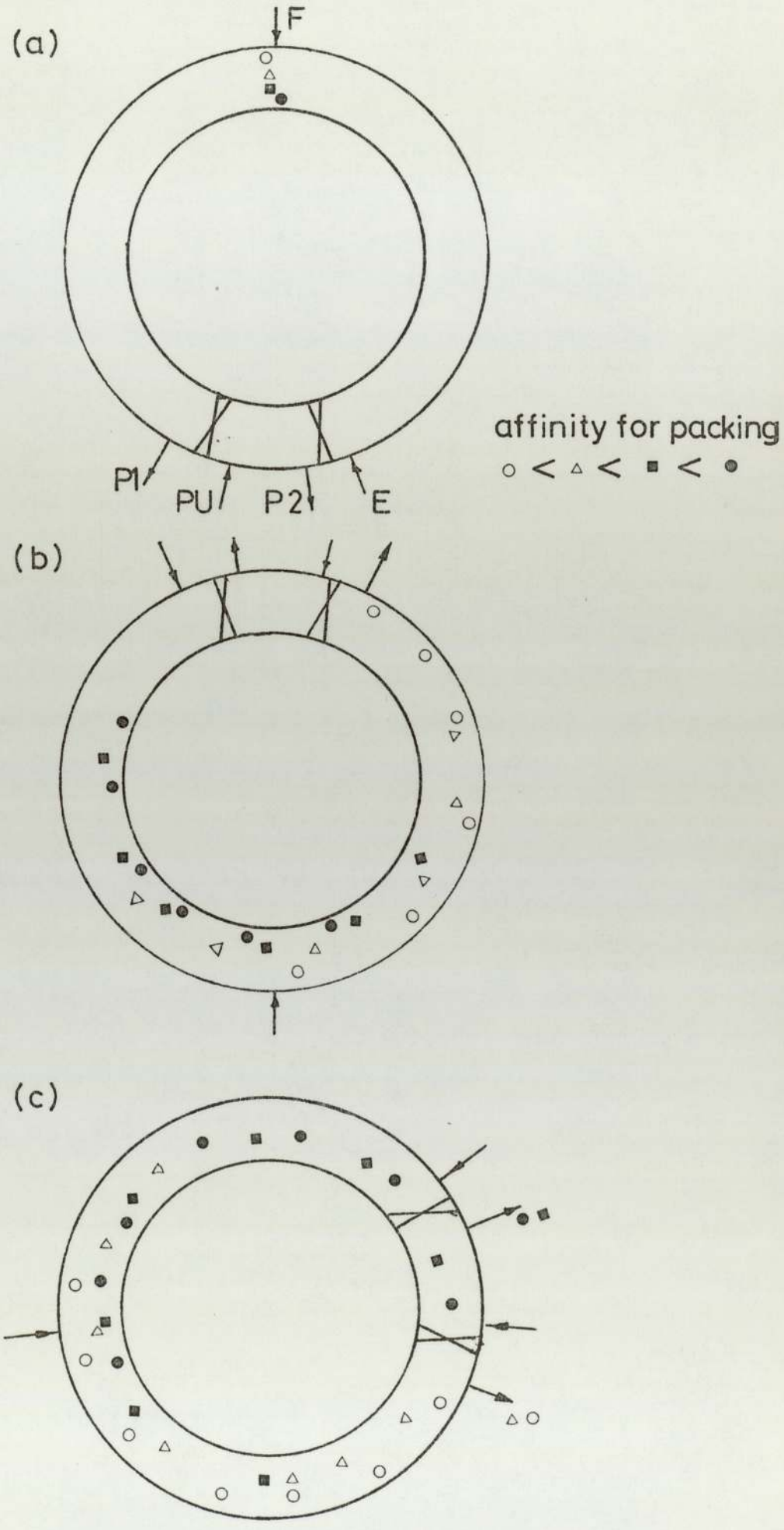


Fig 6.3 Pseudo-steady state distribution for high molecular weight removal.

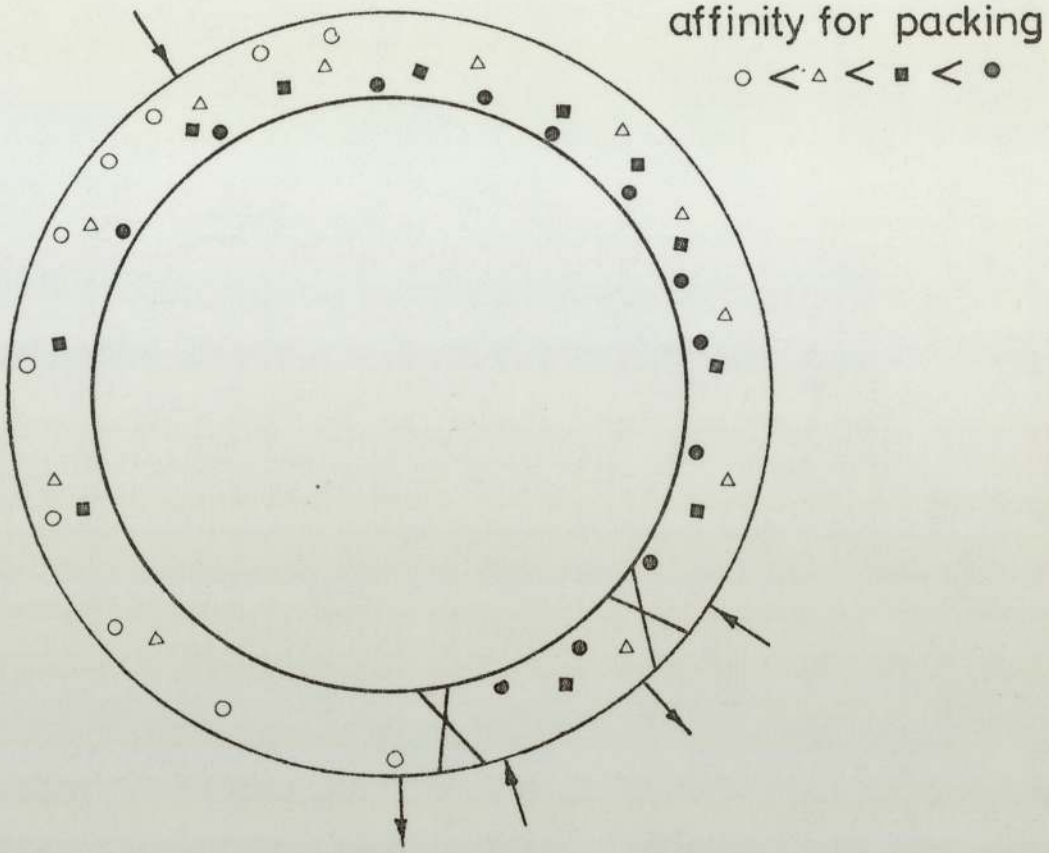
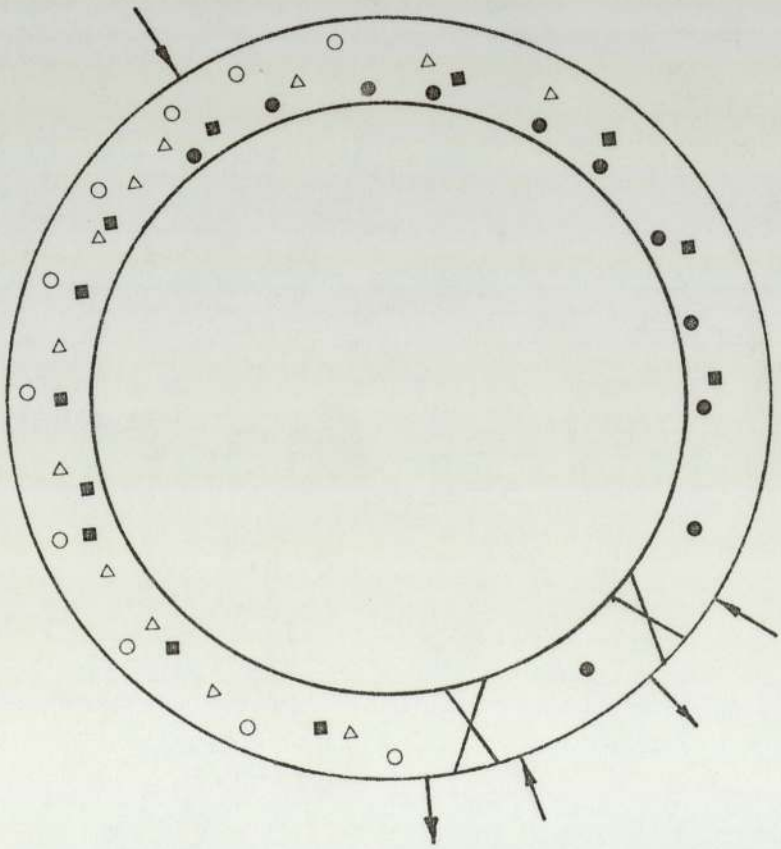


Fig 6.4 Pseudo-steady state distribution for low molecular weight removal.



the feed material. This can be achieved by adjusting the relative velocities of the mobile and stationary phases. Figures 6.3 and 6.4 indicate the pseudo-steady state distribution profiles that can be expected when high and low molecular weight fractions are removed from the feed. The component distribution profiles changes when the type of fractionation changes from a high to a low fractionation.

6.1.2 Idealised Calculation of Cut Positions

The rate of movement of a component in the mobile phase r'_{mi}, is proportional to the probability that the molecule can be found in the mobile phase. Similarly the rate of movement of a component in the stationary phase r'_{si} relative to a fixed feed position is proportional to the probability that the molecule can be found in the stationary phase. Thus,

$$r'_{mi} = \bar{v}_m \cdot R_{mi} \tag{6.1}$$

and

$$r'_{si} = \bar{v}_s \cdot R_{si} \tag{6.2}$$

where $\bar{v}_m$ and $\bar{v}_s$ are the average volumes of the mobile and stationary phases, respectively. See section 2.3.3 for the definition of the retention parameter R. So therefore,

$$r'_{mi} = \bar{v}_m \cdot \frac{v'_o}{v'_o + K_{di}v'_i} \tag{6.3}$$

and

$$r'_{si} = \bar{v}_s \cdot \frac{K_{di}v'_i}{v'_o + K_{di}v'_i} \tag{6.4}$$

where v'_o and v'_i are the average pore and interstitial volumes per unit length of column (i.e. $m^3.m^{-1}$). The average velocity of the stationary phase is

$$= \frac{P}{v'_o} \tag{6.5}$$

At the end of each switch interval the stationary phase appears to move by one column in a direction opposite to the mobile phase. But, the operation also transfers a quantity of mobile phase liquid, which reduces the effective mobile phase rate to;

$$L'_i = L_i - \frac{v'_o l}{s} \tag{6.6}$$

where l - length per column

s - switchtime,

and so the average velocity of the mobile phase is:-

$$\bar{v}_m = \frac{L'_i}{v'_o} \tag{6.7}$$

If a component were to be retained by the stationary phase;

$$r'_{si} > r'_{mi}$$

therefore

$$\frac{P}{v'_i} \cdot \frac{K_{di} v'_i}{v'_o + K_{di} v'_o} > \frac{L'_i}{v'_o} \cdot \frac{v'_o}{v'_o + K_{di} v'_o} \tag{6.8}$$

or

$$P K_{di} > L'_i$$

or

$$K_{di} > \frac{L'_i}{P} \tag{6.9}$$

Thus for compounds to be retained by the packing, they must have a distribution coefficient, K_d, greater than the ratio of the mobile phase to stationary phase flowrates. Conversely for molecules to be eluted from the column they must have a distribution coefficient less than the ratio of the mobile phase to the stationary phase flowrates, that is:-

$$K_{di} < \frac{L'_i}{P}$$

So for the separation of two components having distribution coefficients K_{d1} and K_{d2} the ratio of mobile phase to stationary phase must fall between these limits,

$$K_{d1} < \frac{L'_i}{P} < K_{d2} \qquad (6.10)$$

The calculation of the experimental cut-points were based on distribution coefficients calculated from data obtained under near-infinity dilute conditions. The SCCR5 was built and commissioned by Dr. Holding. The results of the column characteristics are contained in Fig. 6.5. The void and interstitial values for the columns were averaged so that they might be used to calculate the average cut-points, the values obtained by Dr. Holding (251) after verification were used throughout this work.

The theoretical cut point for the eluting section was calculated by expression 6.9 with the inequality sign replaced by an equality.

Fig 6.5 Characteristics of the columns used in the S.C.C.R. 5.

Column number	Original Liquid volume cm^3	Measured		Liquid volume $V_o + V_i$	$\frac{V_i}{V_o}$	Number of plates	H.E.T.P. cm
		Void volume V_o, cm^3	Pore volume V_i, cm^3				
1	1250	576	564	1140	0.978	123	0.52
2	1216	543	678	1219	1.224	107	0.64
3	1300	599	618	1217	1.031	157	0.45
4	1121	552	610	1162	1.106	86	0.80
5	1190	602	574	1176	0.953	132	0.52
6	1223	638	596	1234	0.933	94	0.73
7	1194	595	584	1179	0.982	77	0.89
8	1203	563	597	1160	1.060	80	0.85
9	1214	507	654	1161	1.290	72	0.95
10	1144	563	593	1159	1.053	89	0.78
Average	1203	574	607	1181	1.061	107	0.71

6.1.3 Cut Points for Polymer Fractionation

For a multi-component feed the differences in the distribution coefficients for the molecules in ascending order in the polymer chain are very small. It is impractical to completely resolve two molecules that have distribution coefficients either side of the theoretical cut point because other factors associated with the non-ideality of the separation become dominant. In practice a degree of overlap in the molecular weight distribution of the products occurs.

6.1.4 Non-idealities Occurring in a Practical System

A number of factors influence the ability of the SCCR equipment, to separate a feed mixture. Some of the factors that cause departures from the idealised case are:-

(a) Concentration interactions

(b) Zone broadening

(c) Discontinuous operation

(d) Finite feed flowrate

These factors are briefly discussed below. Barker, Hatt and Williams (15) have discussed in detail the theoretical aspects of continuous chromatography.

(a) Concentration Interactions

Distribution coefficients particularly in g.p.c. have been shown to be affected by concentration, see section 2.10 although the precise effect is as yet unknown. Ellison (34) operating a similar semi-continuous chromatograph showed how the product distributions can change when increasing the feed concentration from 10 to 200 g l^{-1}.

(b) Zone Broadening

Zone broadening depends upon several factors, such as particle size, eluent velocity, packing density and the physical properties of the system being fractionated, the same as when using batch columns. The greater the zone broadening the poorer the fractionation.

(c) Discontinuous Operation

Packing material is simulated to move in the opposite direction to the mobile phase in a discontinuous manner by the sequencing of the inlet and outlet ports. The degree to which this discontinuity affects the system would be reduced if the number of sequencing steps (columns) were increased.

(d) Feed Flowrate

The inequality shown in equation (6.10) has to be extended to include the interaction of a finite volume of feed. Hence the limits of the practical cut positions are;

$$K_{di} < \frac{L_1'}{P} < \frac{L_3'}{P} < K_{d2} \qquad (6.11)$$

Thus the closer the distribution coefficients, the closer the values of L_1' and L_3' have to be, thereby limiting the feed flowrate.

6.2 SEMI-CONTINUOUS CHROMATOGRAPH

6.2.1 Introduction

The SCCR5 included three special features compared to previous equipment for dextran fractionation:-

(1) The material of construction for the fractionating column was stainless steel that allowed greater eluent pressures.

(2) Pneumatic valves increased the reliability of the equipment when working at higher pressures.

(3) The incorporation of an electrical/pneumatic timing unit allowed greater flexibility and easier reproductibility of the equipment.

The SCCR5 is illustrated by Fig. 6.6 and shown schematically in Fig. 6.7. The fractionating column is mounted on a tubular frame construction made from 'Handy' square tube and joints, and consists of ten 5.1 cm i.d. x 70 cm long stainless steel columns connected to each column where six pneumatically operated poppet valves. The flow of liquid into each column was controlled by the opening of the eluent, transfer, feed or purge valve. The control of fluid out of the column was to be either of the product valves opening or by the opening of the transfer valve associated with the next column. Only the feed column had more than one inlet valve open at any instant, these were the feed and transfer valves. Isolation of the purge column was achieved by the closing of both the transfer valves either side. The actuation and sequencing of the valve functions were controlled by a electrical/pneumatic timing unit.

6.2.2 Pneumatic Valves

The double pneumatically actuated poppet valves used in the SCCR5 were developed by C.B. Ching (169). The valves were manufactured by Aston Technical Services Ltd., a company associated with Aston University. An illustration of a typical valve is shown in Fig. 6.8 and a drawing along with a list of the materials of construction in Fig. 6.9. Ching (169) describes in detail the fabrication of this type of valve.

With the exception of those valves controlling the transfer of liquid between columns, the poppet valves had a pneumatic pressure

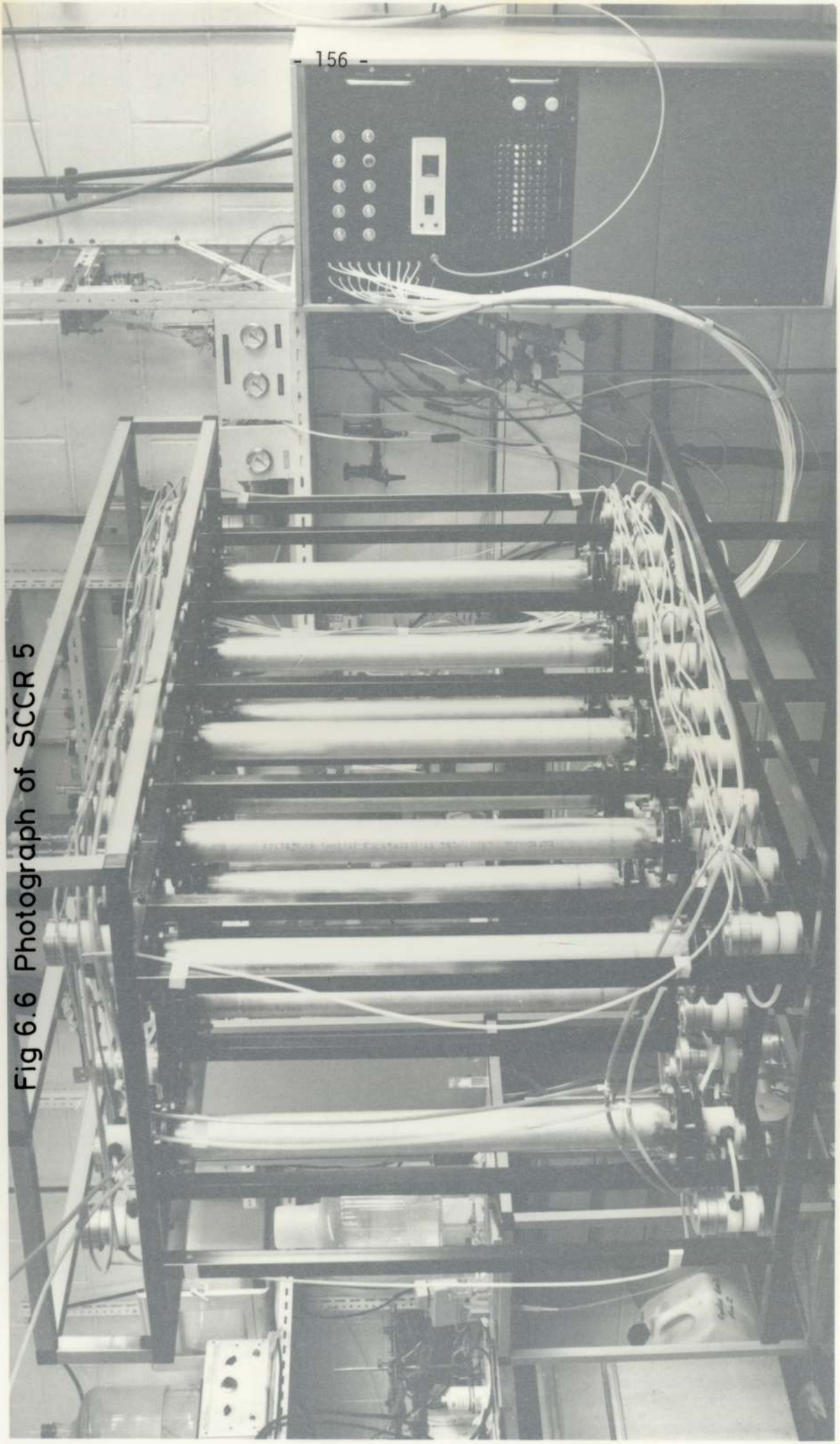


Fig 6.6 Photograph of SCCR 5

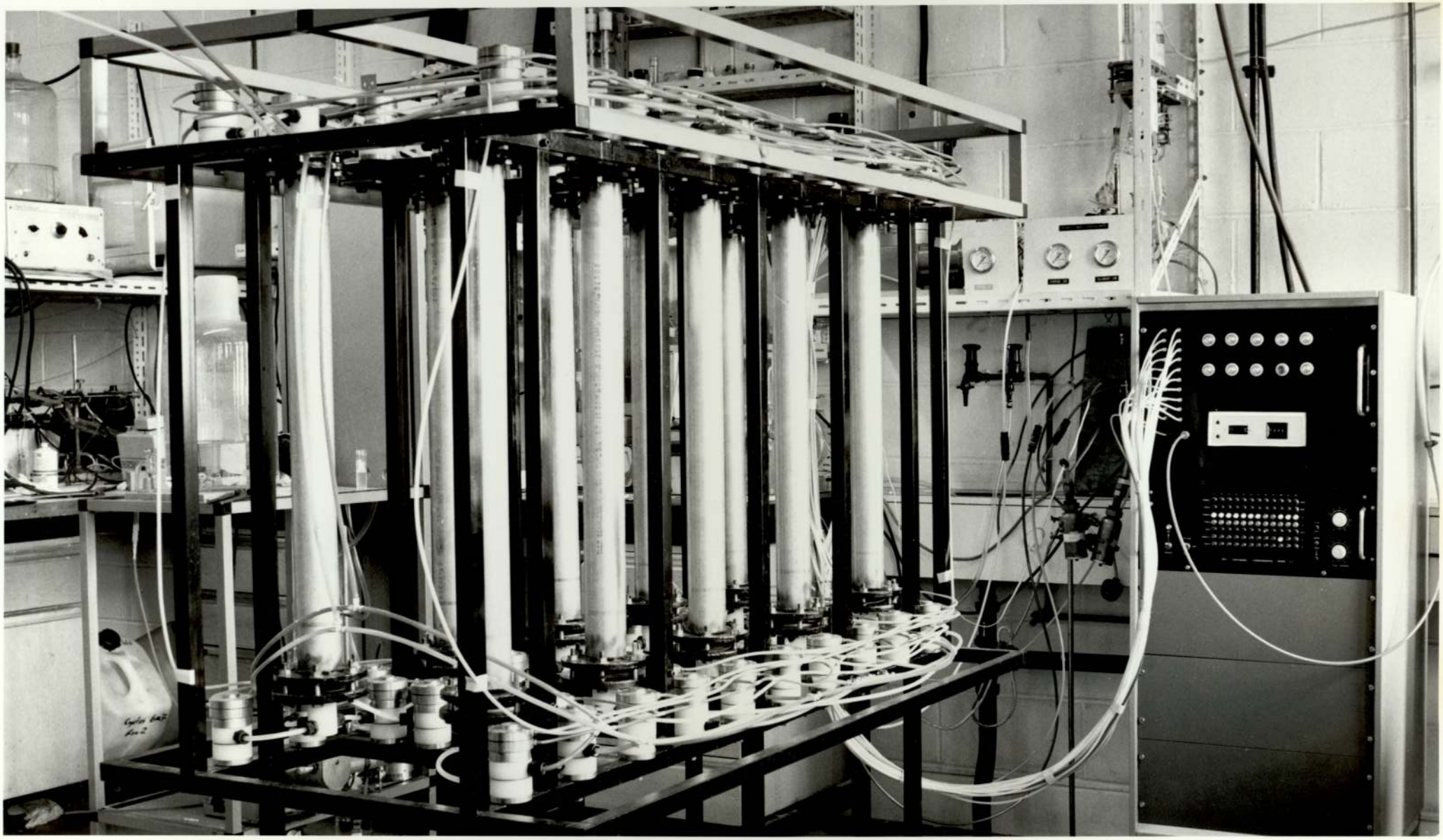


Fig 6.7 Schematic diagram of the S.C.C.R.5.

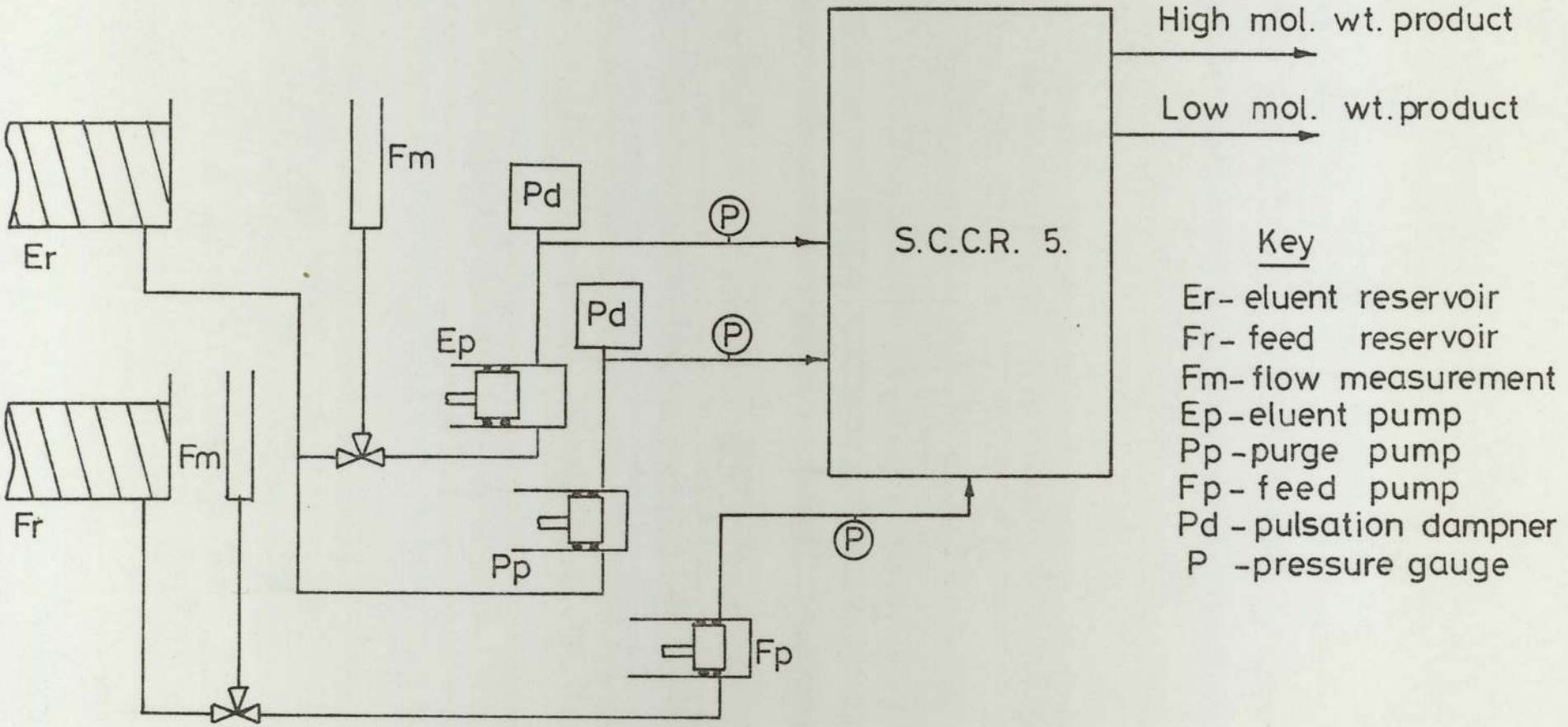


Fig6.8 Photograph of pneumatic poppet valve

Item No.	Description	Material
1	Diaphram chamber -upper	Brass
2	Diaphram	Viton
3	Body gasket	Viton
4	Diaphram chamber -lower	Brass
5	Diaphram backing plate	Brass
6	Sealing ring washer	Brass
7	Body	Polypropylene
8	Inlet chamber	Polypropylene
9	Mounting plate	Brass
10	Poppet	P.T.F.E.glass filled
11	Adjustment nut	Brass
12	Valve stem	Brass
13	Thrust washer	Brass
14	0 Ring BS 007	Neoprene
15	0 Ring BS 008	Neoprene
16	O Ring BS 110	Viton
17	4 B A Screw	Brass
18	2 B A Cheesehead screw	Brass
19	4 B A Hex. nut	Brass

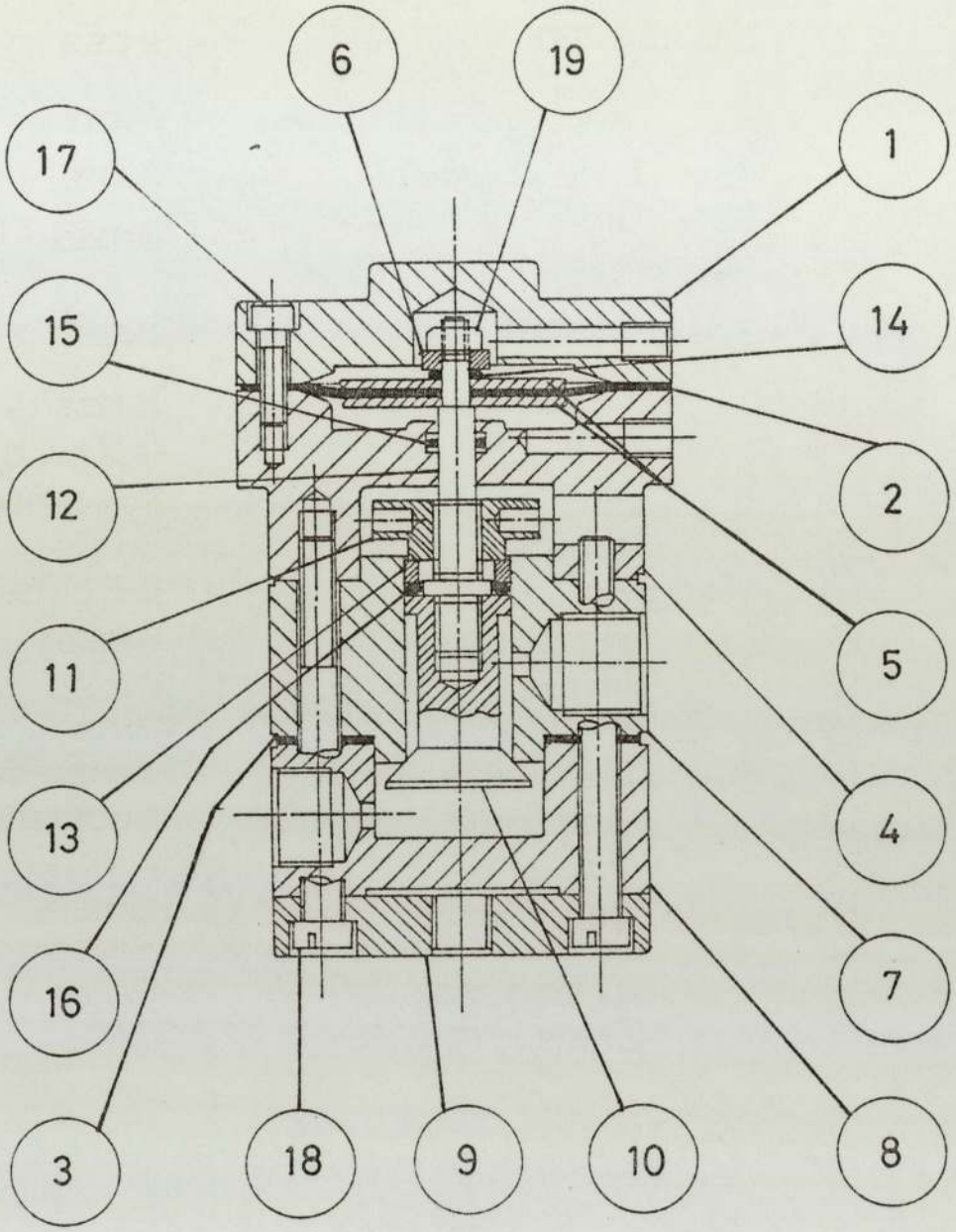


Fig 6.9 Cross-sectional view of pneumatic poppet valve.

(termed bias pressure) in the chamber below the pneumatic diaphram, number 2 in Fig. 6.9 thus ensuring that the valve would remain closed. When the valve was required to open, a higher (actuating) pressure was applied to the upper chamber. For the transfer valves the bias and actuating pressure chambers were reversed because unlike the rest of the valves it was usual for the transfer valves to remain open unless a column was to be isolated, in which case the transfer valve was activated to close. It was usual for the bias pressure to be about half the actuating pressure.

6.2.3 Columns

The overall fractionating column was made up from ten stainless steel columns each of 5.1 cm i.d. by 70 cm long packed with Spherosil XOB075 (200-400 μm). These were mounted vertically on the 'Handy' tube framework and connected in series via the transfer valves. The packing was held in place by a polypropylene mesh having a 150 micron aperture backed by a coarser 1680 micron aperture mesh. The meshes were sandwiched between two 'O' shaped pieces of polyproplyene and either araldited or welded together. A rubber 'O' ring gasket was placed on either side of the polypropylene mesh to provide an effective seal between the column end and the end fitting. The column end fittings were manufactured from 6.4 cm diameter polyproplyene rod, into which fitted 0.635 cm (1/4") B.S.P. parallel plastic compression fittings. These fittings were connected to 6 mm o.d. x 4 mm i.d. nylon tubing which was colour coded. The colours used for the various streams were:-

Green	eluent
Yellow	feed
Brown	purge
Red	high molecular weight product

Blue	low molecular weight product
White	transfer lines

6.2.4 Sequencing Unit

An electrical signal from a digital timer was sent to an incremental sequencing unit. The sequencing unit was designed and constructed by Mr. M. Lea the departmental electronics technician. A detailed wiring diagram and explanation of the principle of operation was reported by Deeble (263). The output from this sequencing unit then went to a bank of solenoid valves where each valve was actuated in turn. The output from the solenoid valve then actuated the various inlet and outlet streams in the chromatograph. A side-stream was taken off from each of the actuating lines and via a shuttle-valve to actuated the necessary transfer valves to close. A shuttle-valve received an input signal from two sources but had only one output signal. These valves were essential to the operation of the sequential unit because the transfer valve between, say, columns 4 and 5 had to be closed when both column 4 and column 5 were being purged. Therefore the pneumatic signals that actuated the purge valves in columns 4 and 5 were connected to the two inlets of the shuttle valve, so that when either of these lines were actuated the output from the shuttle valve was such that the transfer valve closed. A second pneumatic line was also taken off to indicate the position of the purge column. The output from the digital timer could have been over-riden by a manual re-set button.

6.2.5 Pumps and Liquid Storage

The distilled or de-ionised water that was used as the eluent for the chromatograph was stored in a set of plastic vessels having

capacities of 46 and 75 l each. The total system had a storage capacity of about 500 litres. The storage reservoirs were housed approximately 4.5 m above the SCCR5. This storage system was the source of the mobile phase and the purge. Both the streams were pumped using a positive displacement pump type Twin K metripump supplied by Metering Pumps Ltd., London. For the eluent stream a plunger type head was used, which had a glass plunger and polypropylene casing. For the purge stream a diaphram type head, which had a polypropylene easing with a P.T.F.E. diaphram, was used.

The dextran feed solution was stored in a 40 l glass reservoir and was pumped into the SCCR5 by a M.P.L. series 2 pump, fitted with two number 4 polypropylene heads. At higher flowrates both heads were used with their stroke mechanism out of phase, so that a more even flow of solution was delivered to the chromatograph. The flow of the streams from the Twin K metripump were smoothed using nitrogen charged stainless steel pulsation dampeners fitted with a nitrile rubber diaphram; these dampeners were supplied by Fawcett Engineering Ltd., Brombrough.

6.2.6 Sample Collection

Product streams from the chromatograph were collected each cycle or pair of cycles in light plastic containers of 30 and 110 l. The switching of products from one pair of containers to the next was done simultaneously using a fraction collector.

6.3 EXPERIMENTAL TECHNIQUES

6.3.1 Preparation of Feed and Eluent

Eluent for the experiments was either distilled water produced by a 8 l h^{-1} automatic water still (Fisons Scientific Apparatus,

Loughborough) or, de-ionised water produced by a 240 l h^{-1} Elgastat B224 water purification unit (Elgar, High Wycombe). The eluent was then stored in the plastic reservoirs.

The same water was also used to make-up the feed solution. A measured quantity of water was put into a 50 l isomantle and heated, and a known amount of dextran powder added. The solution was continuously agitated by compressed air until all the dextran had been dissolved. The hot solution was then vacuum-filtered by a No. 2 glass sintered filter, stored in a 110 l plastic container and allowed to cool. Sodium azide was added to the solution at 0.02% w/v concentration to prevent the growth of micro-organisms.

6.3.2 Valve Maintenance

Before the start-up of every experimental run, the sequencing operation of the SCCR5 was checked by passing eluent through the rig. This also removed any of the dextran that may have been trapped in the dead volumes of the valves or columns from the previous run. It was occasionally necessary to alter the tension on the poppet valve (10) or the 'O' ring seal (16) by means of the adjustment nut (11) shown in Fig. 6.9, so that the valve could open and close easily and effectively. It was noticeable that as the number of runs increased the necessity to make adjustments to the valves decreased.

6.3.3 Start-up and Shut-down Procedure

The experimental runs performed at ambient temperature lasted up to 40 hours. Each run was divided up into daily operations ranging from 8-10 hours. It had been established by previous workers (34, 169) that no detectable change in the dextran distributions around the chromatography occurred during the period the machine was shut down.

Details of the start-up procedure were as follows:-

(a) The pneumatic supply to the timer unit was turned on and the pressures of the bias and actuating pressures to the rig were set at the correct value.

(b) The product lines were connected to the fraction collector which were positioned to deliver to the correct containers.

(c) The delivery lines to the eluent and feed pumps were opened.

(d) The digital timer was set to the required time interval, and approximately the correct percentages for the stroke mechanisms of the mobile phase, purge and feed pumps set. Final adjustments to the stroke mechanisms were made when the machine was in operation.

(e) The digital timer, the pneumatic supply to the chromatograph and the pumps were then started in quick succession.

Shut-down procedure included turning off the pumps and immediately closing the delivery lines from the storage reservoirs, to prevent any liquid siphoning through the rig. The liquid pressure in the columns were allowed to decay before the pneumatic supply to the chromatograph was switched off.

6.3.4 Flowrate and Pressure Measurements

For a constant quality product it was desirable that the flowrates of the various input streams be kept as constant as possible. Flowrate measurements were taken at the inlet and the outlet of the rig at regular intervals, usually every two or three switches when the machine had reached pseudo-steady state, although more frequent measurements were taken during the first few cycles. Measurements at the inlet to the apparatus consisted of measuring the time taken for liquid to pass

between two points in a calibrated tube. The mobile phase and the feed flowrates were measured in this manner. The purge flowrate to the rig was not measured.

The high and the low molecular weight products were measured at the outlet by collecting the products in measuring cylinders for a given time period. The volumetric flowrate of the high molecular weight product should have been equal to the sum of the feed and the mobile phase flowrates. Any significant difference usually indicated that a valve(s) was not functioning correctly. The purge flowrate was controlled by measuring the low molecular weight product. The input and product streams were measured simultaneously to ensure a correct volumetric balance was recorded.

The pressure at the inlet to the fractionating and purge sections were taken, and also at the feed point. These pressures varied during the course of the switch. So that the pressures could be compared between different cycles they were taken at a set number of seconds after the valves had switched.

6.3.5 Sampling Techniques

The plastic vessels containing the products were weighed after each cycle or pair of cycles. A 1 l sample was taken after the solution had been weighed and mixed. The rest of the product was disposed. The retained samples were then analysed for molecular weight distribution and concentration of dextran.

At the end of each experimental run, the mobile phase and feed pumps were isolated, and the contents of each of the columns were purged out individually into 10 l plastic containers. This process was performed manually. The samples were weighed and after the sample had

been thoroughly mixed a small sample was taken for analysis. This enabled profiles of the average column concentrations and molecular weight distributions to be constructed.

6.3.6 Dextran Mass Balance

The criterion that the dextran input for a complete cycle to the SCCR5 should equal the output had to be met if the process was considered to be in a pseudo-steady state. Dextran mass balances were carried out for each cycle or pairs of cycles after the break through of the dextran to the products. The mass of dextran fed to the SCCR5 was calculated from the product of the average feed flowrate for the time period and the average concentration of the feed solution. Similarly, for the dextran output from the unit.

6.3.7 End of Run Profiles

Samples taken by purging the individual columns at the end of each run were analysed to give the mass and the molecular weight distribution of the dextran present. Unfortunately the precise distribution of the molecules between the stationary and mobile phases was not known. For this reason the average column concentrations are represented by the total mass of dextran in the column in the total liquid volume of the column.

6.4 EXPERIMENTAL PROGRAMME

6.4.1 Scope

The experimental programme was concerned with the following objectives:-

(i) To remove high molecular weight material from a dextran

feed using the SCCR5.

(ii) To remove low molecular weight material from a concentrated, approximately 220 g l^{-1}, dextran feed.

(iii) To investigate the effect of increasing the post feed cut positions, that is increase feed rate whilst maintaining the preferred theoretical cut position constant.

6.4.2 Experimental Operating Conditions

Four experiments were performed at ambient temperatures for the fractionation of dextran. Two experiments attempted the removal of high molecular weight material from the feed and two attempted the removal of low molecular weight material. A summary of the operating conditions is presented in Fig. 6.10. An illustrated example of the calculation of the theoretical cut position is contained in Appendix A.3.

Prior to this research programme, Dr. S.R. Holding had used the SCCR5 to investigate the effect of the eluent-to-feed ratio on the removal of high molecular weight material from a dextran feed. The first experiment in my research programme, experiment A, used similar operating conditions that had been used over a year earlier to determine if the fractionating capabilities of the SCCR5 had deteriorated. Experiment B also attempted to remove high molecular weight material from the feed solution. This experiment had the highest rate of feed throughput in all the experiments performed at ambient temperatures.

The removal of low molecular weight material from a dextran feed solution had only been performed using a dilute feed solution of 10 g l^{-1} prior to this study. Ellison (34) observed an increase from 19 to 78% of the feed material present in the low molecular weight product when increasing the feed concentration from 10 to 220 g l^{-1} for the same

Fig 6.10 Summary of operating conditions for the S.C.C.R. 5.

Experiment	Switch-time s.	Conc. of feed $g.l^{-1}$	Feedrate per		Eluent to feed ratio	Flowrates $cm^3.min^{-1}$			Theoretical cut position		
			cycle	hour		eluent	feed	purge	pre-feed	post-feed	purge
A	450	192	282·7	226·1	4·96	99·6	20·1	455	0·286	0516	4376
B	450	231	689·0	551·2	2·54	100·4	39·6	413	0·290	0·747	3·892
C	300	236	201·8	242·1	12·26	209·6	17·1	508	0·746	0·877	3·035
D	1200	215	463·3	139·0	4·94	52·2	10·6	130	0·737	1·063	3·127

operating conditions. Choosing a pre-feed theoretical cut position of 0.75 and an eluent-to-feed ratio of 12.35 experiment C attempted to remove low molecular weight material from a concentrated feed solution of 236 g l^{-1}. Experiment D had the same objective where the eluent-to-feed ratio was reduced to 5.

Figure 6.10 presents the average operating conditions for the final six cycles of the experiment where the SCCR5 was approaching or had achieved pseudo-equilibrium. Variations in the operating conditions and measured parameters for each cycle or pair of cycles are contained in Figs. 6.11 to 6.22.

6.5 RESULTS AND DISCUSSION

6.5.1 Mass and Composition Profiles at the End of Experiments

The pseudo-steady state distribution profiles for experiments A, B, C and D are shown in Figs. 6.24 and 6.27, respectively. The calculations for the 'concentrations' of the reported samples have been described in section 6.3.7. These diagrams show the distribution of the dextran at the end of any switch time after the chromatograph has reached pseudo-equilibrium. A description of how the concentration profiles are drawn is aided by Fig. 6.23, eluent enters the fractionating section via column 1 and moves from left to right as shown in the diagram passing through columns 2, 3, and so on. The feed solution enters the column number 5 on the diagram and therefore increases the eluate flowrate in columns between 5 and 9. The product containing the high molecular weight material is eluted from column number 9. The purged column, column number 10 has had all the low molecular weight material removed from it during the switch and is not shown in the subsequent concentration profiles.

Fig 6.11 Operating conditions for experiment A.

cycle	Pressures			Temperatures				Input flowrates			Product flows		Cut-positions		
	eluent	feed	purge	purge	eluent	feed	in-line	L_1	L_2	L_3	H.M.W.	L.M.W.	pre-feed	post-feed	purge
	kN.m^{-2}	kN.m^{-2}	kN.m^{-2}	°c	°c	°c	°c	ml. min^{-1}	ml. min^{-1}	ml. min^{-1}	ml. min^{-1}	ml. min^{-1}			
9	580	275	200	20	20	20	20	101	19·9	120·9	119·3	449·5	0·297	0·526	4·312
10	585	265	170	20	20	20	20	99·4	19·9	119·3	114·4	444·	0·279	0·508	4·429
11	555	250	170	20	20	20	20	98·1	20·7	118·8	116·7	453·	0·264	0·478	4·353
12	552	240	166	20	20	20	20	97·6	19·8	117·4	116·0	449·	0·258	0·470	4·306
13	570	260	167	20	20	20	20	102·1	19·8	121·9	121·3	446·	0·310	0·531	4·272
14	563	265	170	20	20	20	20	97·9	20·1	118·0	119·3	444·	0·262	0·526	4·429

Fig 6.12 Products from experiment A.

Cycles	Dextran Input		High Mol. Wt. Product			Low Mol. Wt. Product			H.M.W.P	L.M.W.P	Mass
	Per Cy.	Per hr.	Conc.g.l.	Vol. l.	Mass. g.	Conc.g.l.	Vol. l.	Mass g.	Output	Output	Balance
9 + 10	286·6	229·2	4·548	17·70	80·5	7·336	66·22	485·8	0·142	0·858	0·988
11 + 12	274·3	219·4	4·393	17·46	76·7	7·120	66·00	469·9	0·140	0·860	0·935
13 + 14	287·3	229·8	3·983	17·70	71·5	7·157	65·32	467·5	0·133	0·867	0·938

	High Mol. Wt. Product			Low Mol. Wt. Product		
	M_w	M_n	D	M_w	M_n	D
9 + 10	179 000	45 400	3·943	51 300	25 000	2·053
11 + 12	179 200	45 300	3·951	53 800	25 900	2·073
13 + 14	179600	45000	3·991	52 700	24 600	2·114

Fig 6.13 Purged products from experiment A.

Column	Purged Volume, l	Purge Conc., $g\,l^{-1}$	Dextran Mass, g	Column Conc., $g\,l^{-1}$	Col. conc. / Feed conc.	M_w	M_n	D
1	3·300	7·658	25·27	20·80	0·108	50700	25800	1·97
2	2·982	36·81	91·60	75·39	0·393	56200	27500	2·04
3	2·683	34·14	109·8	90·33	0·471	60700	27300	2·23
4	3·330	39·01	129·9	106·91	0·557	61000	27700	2·20
5	3·116	32·22	100·4	82·63	0·430	67900	29400	2·31
6	3·263	9·920	32·37	26·64	0·139	—	—	-
7	3·237	5·860	18·97	15·61	0·081	102700	29600	2·91
8	3·286	4·239	13·93	11·46	0·060	103700	35300	3·50
9	3·250	3·289	10·69	8·80	0·046	120900	42000	2·88

Fig 6.14 Operating conditions for experiment B.

cycle	Pressures			Temperatures				Input flowrates			Product flows		Cut-positions		
	eluent	feed	purge	purge	eluent	feed	in-line	L_1	L_2	L_3	H.M.W.	L.M.W.	pre-feed	post-feed	purge
	kN.m-2	kN.m-2	kN.m-2	°c	°c	°c	°c	ml.m-1	ml.m-1	ml.m-1	ml.m-1	ml.m-1			
5 + 6	1380	625	138	20	20	20	20	99.5	39.7	139.2	138.7	397	0.28	0.737	3.707
7 + 8	1450	685	138	20	20	20	20	100.3	40.1	140.4	138.5	406	0.289	0.729	3.811
9 + 10	1530	725	148	20	20	20	20	100.6	39.9	140.5	138.4	409	0.293	0.728	3.846
11 + 12	1510	730	138	20	20	20	20	100.0	39.4	139.4	141.8	404	0.286	0.767	3.788
13 + 14	1505	730	138	20	20	20	20	101.1	40.0	141.1	138.8	416	0.298	0.733	3.926

Fig 6.15 Products from experiment B.

Cycles	Dextran Input		High Mol. Wt. Product			Low Mol. Wt. Product			H.M.W.P. Output	L.M.W.P. Output	Mass Balance
	Per Cy.	Per hr.	Conc. g.l.$^{-1}$	Vol. l.	Mass. g.	Conc. g.l.$^{-1}$	Vol. l.	Mass g.			
9+10	691·3	553·0	8·603	20·76	178·6	17·81	61·05	1087·5	0·141	0·859	0·916
11+12	682·6	546·1	10·67	21·27	226·9	17·40	60·63	1054·9	0·177	0·823	0·939
13+14	693·0	554·4	9·366	20·82	195·0	18·22	6239	1136·7	0·146	0·854	0·961

	High Mol. Wt. Product			Low Mol. Wt. Product		
	M_w	M_n	D	M_w	M_n	D
9+10	120 500	38300	3·13	46900	23100	2·03
11+12	116 300	37100	3·13	44800	22700	1·97
13+14	123600	39000	3·17	49300	23700	2·03

Fig6.16 Purge products from experiment B.

Column	Purged Volume, l	Purge Conc., $g\,l^{-1}$	Dextran Mass, g	Column Conc., $g\,l^{-1}$	Col. conc. / Feed conc.	M_w	M_n	D
1	3·025	18·63	56·36	46·39	0·201	46000	22600	2·03
2	3·005	43·53	130·8	107·7	0·466	42300	21100	1·99
3	2·930	62·73	183·8	151·3	0·655	40400	20400	1·98
4	3·080	59·81	184·2	151·6	0·656	41300	20600	2·00
5	3·195	48·01	153·4	126·3	0·547	45300	20100	2·26
6	2·815	29·89	84·13	69·24	0·300	53350	24200	2·21
7	3·130	17·68	55·35	45·56	0·197	73000	29500	2·47
8	2·845	13·92	39·61	32·60	0·141	79700	34100	2·34
9	3·205	9·82	31·48	25·91	0·112	94500	34800	2·72

Fig 6.17 Operating conditions for experiment C.

cycle	Pressures			Temperatures				Input flowrates			Product flows		Cut-positions		
	eluent	feed	purge	purge	eluent	feed	in-line	L_1	L_2	L_3	H.M.W.	L.M.W.	pre-feed	post-feed	purge
	kN.m2	kN.m-2	kN.m-2	°c	°c	°c	°c	ml.m-1	ml.m-1	ml.m-1	ml.m-1	ml.m-1			
7 + 8	705	550	150	20	20	20	20	212·2	16·9	229·1	236·6	504	0·763	0·951	3·000
9 + 10	910	660	150	20	20	20	20	207·5	17·0	224·5	229·4	515	0·727	0·896	3·089
11 + 12	1170	920	150	20	20	20	20	209·7	16·9	226·6	232·8	501	0·741	0·922	2·982
13 + 14	1370	1080	150	20	20	20	20	210·4	16·8	227·2	229·8	504	0·750	0·899	3·000
15 + 16	1490	1170	150	20	20	20	20	208·8	17·5	226·3	225·1	501	0·733	0·863	2·982

Fig 6.18 Products for experiment C.

Cycles	Dextran Input		High Mol. Wt. Product			Low Mol. Wt. Product			H.M.W.P. Output	L.M.W.P. Output	Mass Balance
	Per Cy.	Per hr.	Conc. g.l.$^{-1}$	Vol. l.	Mass. g.	Conc. g.l.$^{-1}$	Vol. l.	Mass g.			
11+12	199.4	239.3	12.02	23.28	279.9	0.717	50.12	35.95	0.886	0.114	0.792
13+14	198.2	237.9	14.21	22.98	326.5	0.664	50.35	33.43	0.907	0.093	0.908
15+16	206.5	247.8	14.48	22.51	326.0	0.858	49.44	42.40	0.885	0.115	0.892

	High Mol. Wt. Product			Low Mol. Wt. Product		
	M_w	M_n	D	M_w	M_n	D
11+12	79400	29700	2.67	39800	24100	1.65
13+14	77000	31500	2.45	38300	21700	1.76
15+16	78700	29700	1.98	33100	19000	1.74

Fig 6.19 Purge products for experiment C.

Column	Purged Volume, l	Purge Conc., $g\,l^{-1}$	Dextran Mass, g	Column Conc., $g\,l^{-1}$	Col. conc. / Feed conc.	M_w	M_n	D
1	2·642	1·260	3·33	2·741	0·012	28100	16 100	1·75
2	2·494	10·42	26·01	21·41	0·091	27500	16000	1·73
3	2·486	28·89	71·81	59·10	0·250	29400	18100	1·62
4	2·447	51·94	127·1	104·6	0·443	34300	20100	1·70
5	2·165	79·68	172·5	142·0	0·602	36200	20500	1·76
6	2·616	57·53	150·5	123·9	0·525	41400	21000	1·97
7	2·564	44·07	113·0	93·00	0·394	36500	21800	1·68
8	2·566	34·99	89·78	73·89	0·313	40400	23000	1·76
9	2·401	22·87	54·92	45·20	0·192	44400	24600	1·81

Fig 6.20 Operating conditions for experiment D.

cycle	Pressures			Temperatures				Input flowrates			Product flows		Cut-positions		
	eluent	feed	purge	purge	eluent	feed	in-line	L_1	L_2	L_3	H.M.W.	L.M.W.	pre-feed	post-feed	purge
	kN.m-2	kN.m-2	kN.m-2	°C	°C	°C	°C	ml.m-1.	ml.m-1.	ml.m-1.	ml.m-1.	ml.m-1.			
1 + 2	460	330	50	20	20	20	20	51·4	11·0	62·4	64·3	130	0·711	1·051	3·127
3 + 4	1270	900	50	20	20	20	20	51·2	10·6	61·8	60·6	123	0·707	1·032	2·912
5 + 6	1580	1020	50	20	20	20	20	51·7	10·4	62·1	61·5	126	0·722	1·041	3·005
7 + 8	1410	1120	30	20	20	20	20	53·0	10·5	63·5	61·6	134	0·762	1·084	3·250
9 + 10	1430	1145	30	20	20	20	20	51·4	10·5	61·9	61·2	123	0·713	1·082	2·912
11 + 12	1530	1290	20	20	20	20	20	52·1	10·7	62·8	63·0	132	0·734	1·063	3·189

Fig 6.21 Products for experiment D.

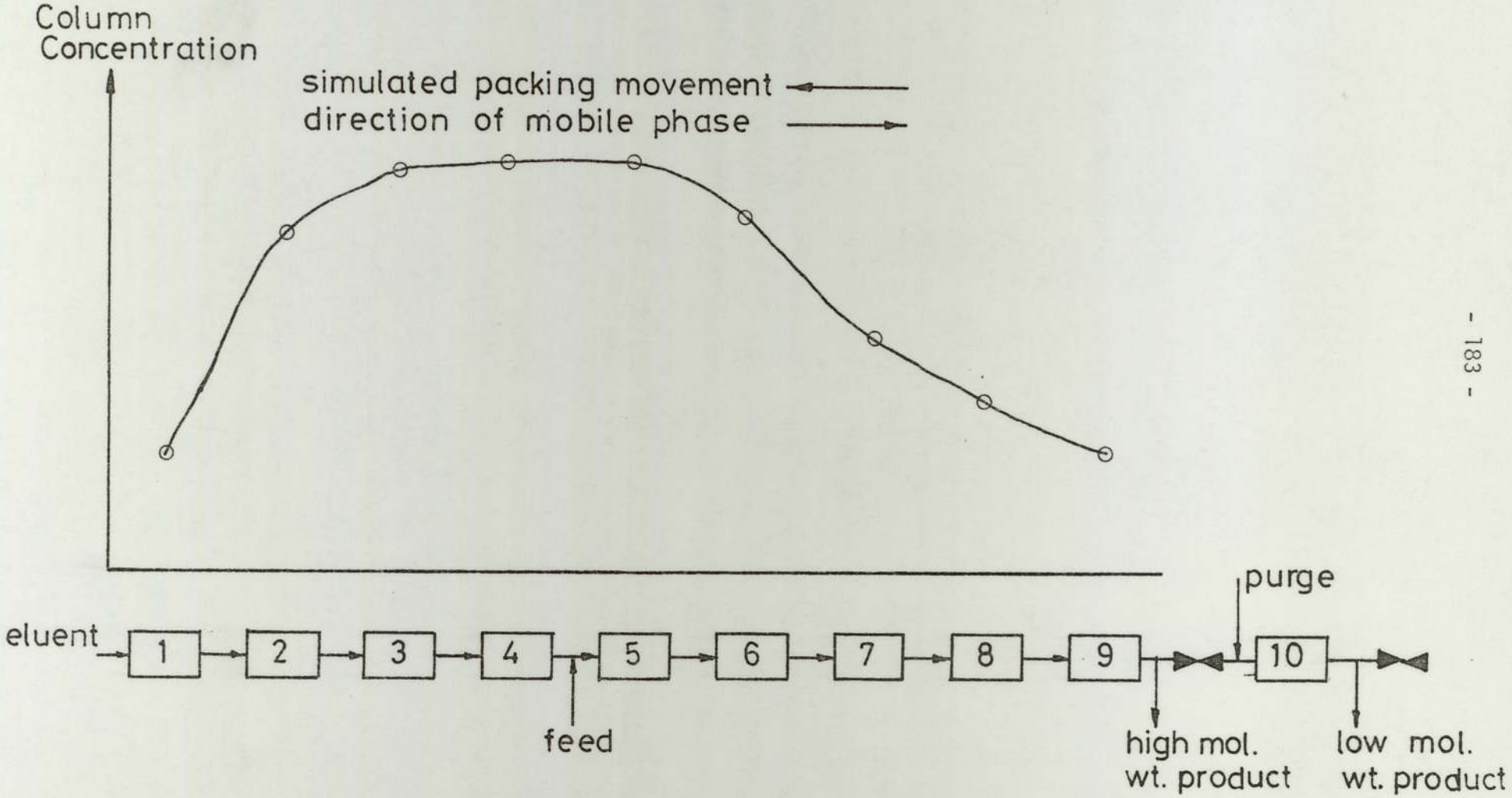
Cycles	Dextran Input		High Mol. Wt. Product			Low Mol. Wt. Product			H.M.W.P.	L.M.W.P.	Mass
	Per Cy.	Per hr.	Conc. g/l.	Vol. l.	Mass. g.	Conc. g/l.	Vol. l.	Mass g.	Output	Output	Balance
7+8	465	155	34·45	24·38	840	1·380	52·16	72	0·921	0·079	0·980
9+10	451	150		23·47	–		51·71	–			
11+12	474	158	31·21	25·47	795	1·639	53·07	87	0·902	0·098	0·957

	High Mol. Wt. Product			Low Mol. Wt. Product		
	M_w	M_n	D	M_w	M_n	D
7+8	72000	30700	2·35	37900	20 300	1·87
9+10						
11+12	73400	30900	2·38	39300	20700	1·90

Fig 6.22 Purge products for experiment D.

Column	Purged Volume, l	Purge Conc., $g\,l^{-1}$	Dextran Mass, g	Column Conc., $g\,l^{-1}$	Col. conc. / Feed conc.	M_w	M_n	D
1	2·133	1·078	2·30	1·893	0·009			
2	2·415	15·20	36·71	30·21	0·140	26800	16600	1·62
3	1·750	41·80	73·15	60·21	0·280	35900	19600	1·83
4	2·000	61·80	123·6	101·7	0·472	36200	21300	1·70
5	1·659	67·87	112·6	92·67	0·430	39600	22300	1·78
6	1·834	55·73	102·2	84·12	0·391	42300	23300	1·82
7	2·202	43·20	95·13	78·30	0·364	42100	22500	1·87
8	1·888	46·40	87·60	72·10	0·335	44800	23500	1·91
9	1·861	43·40	80·77	66·47	0·309	44800	23800	1·87

Fig 6.23 Description of concentration profiles.



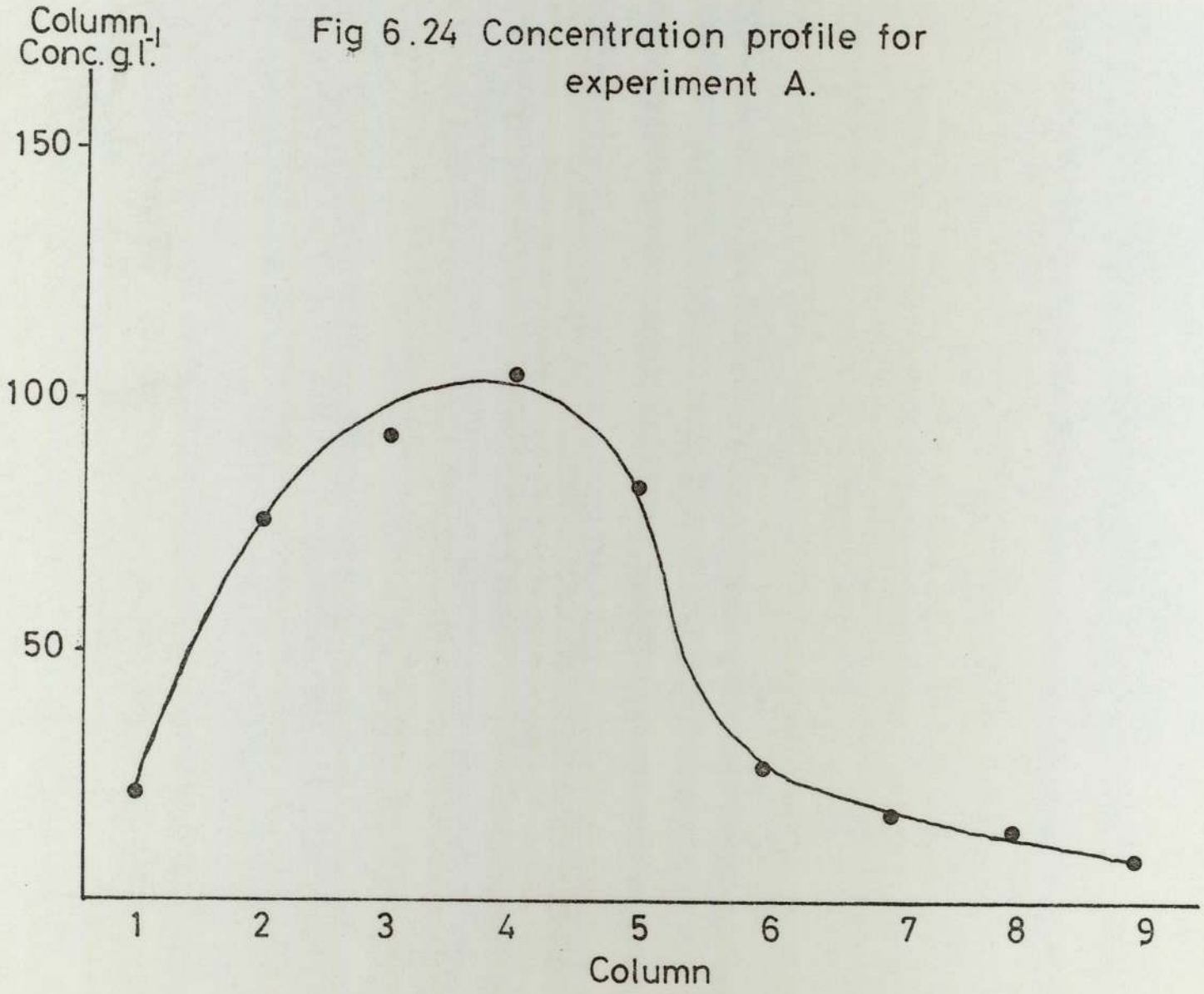


Fig 6.24 Concentration profile for experiment A.

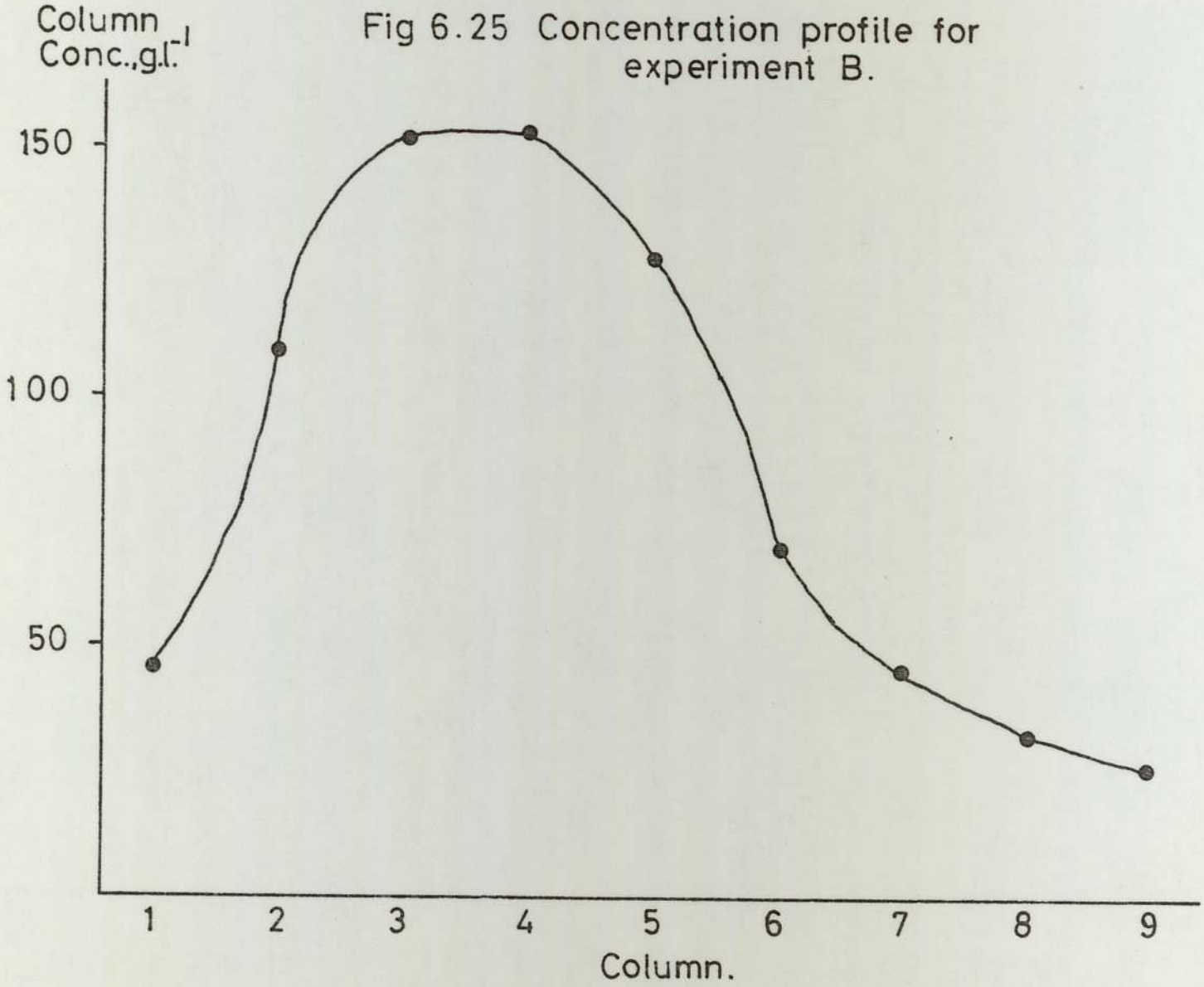


Fig 6.25 Concentration profile for experiment B.

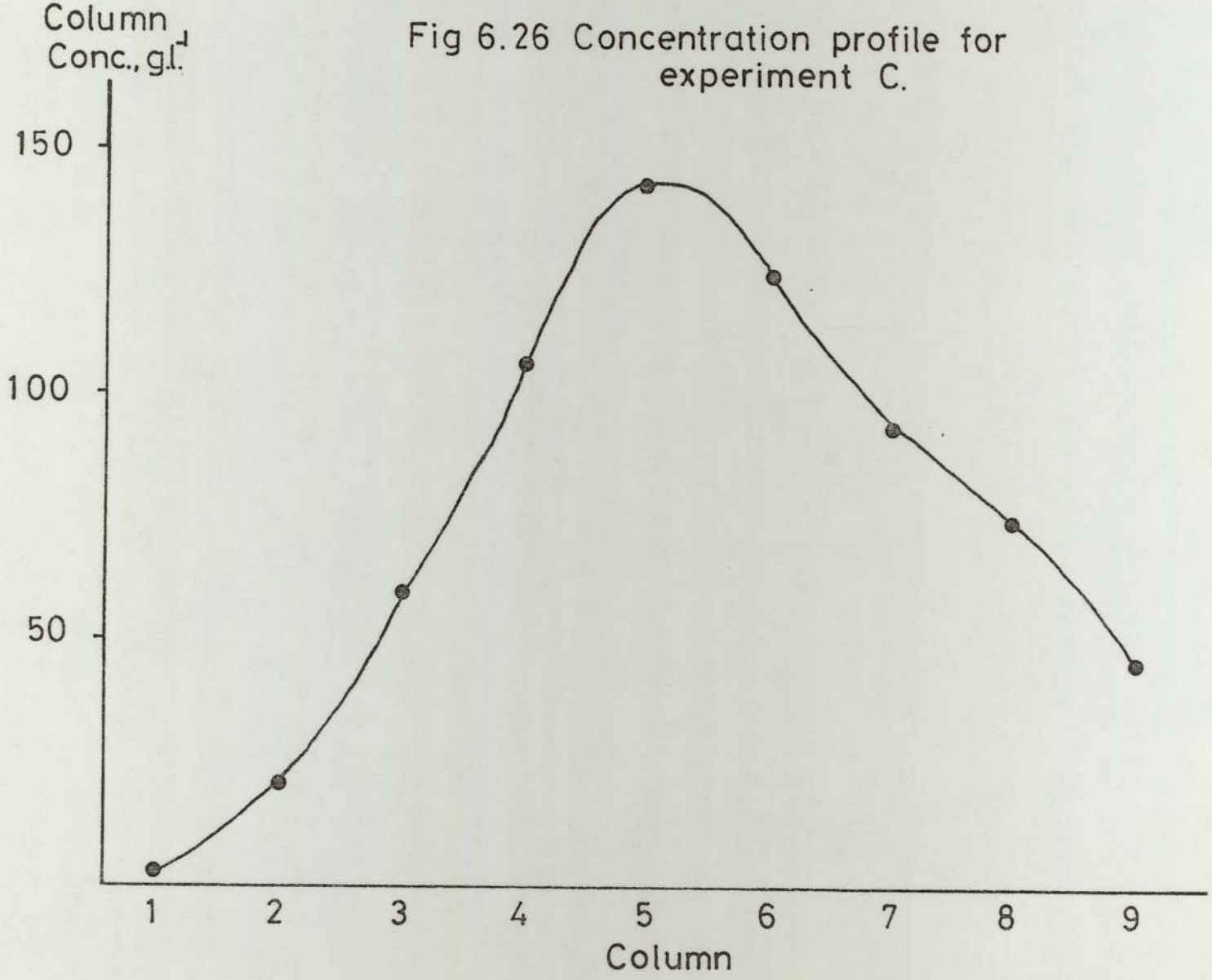


Fig 6.26 Concentration profile for experiment C.

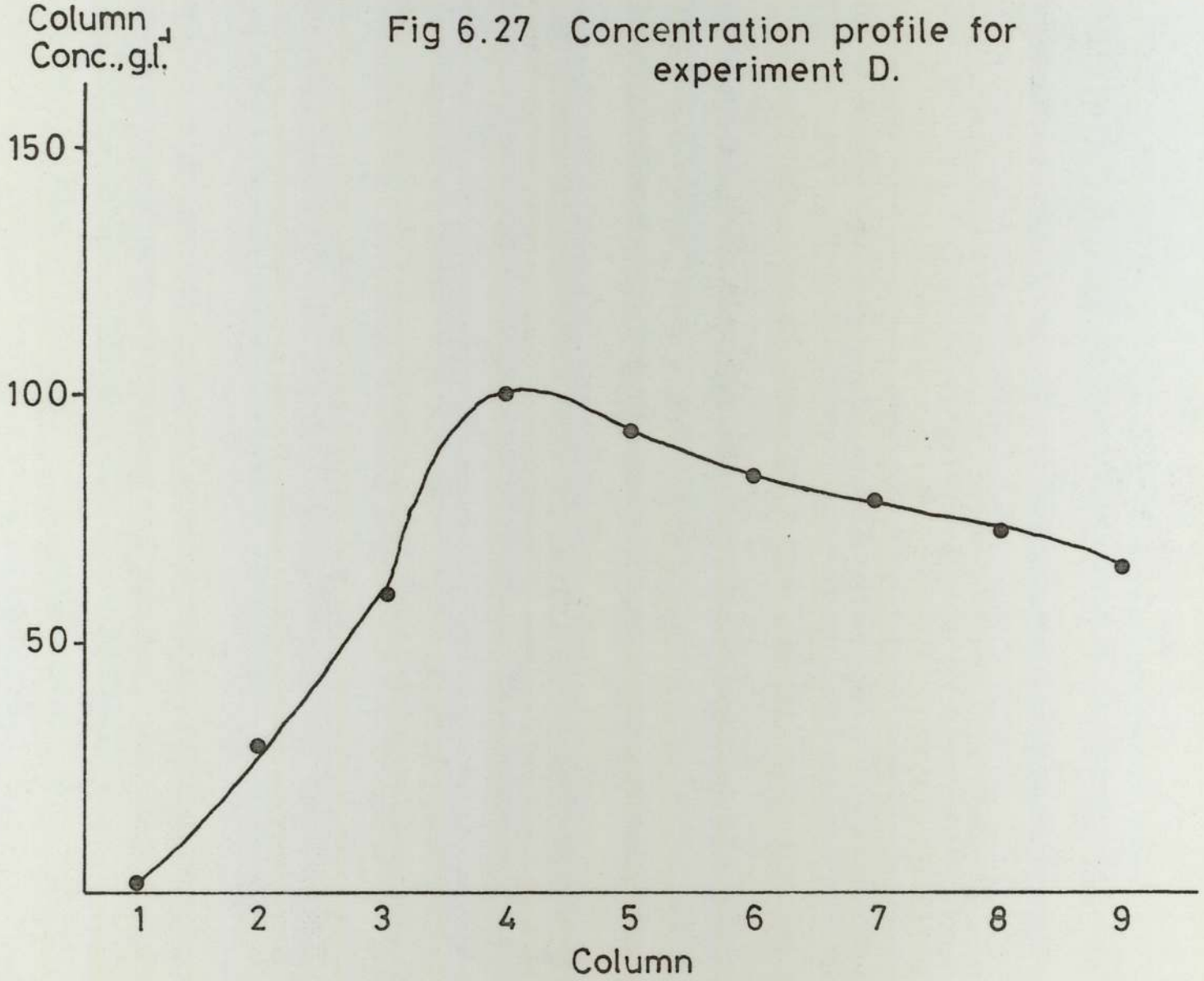


Fig 6.27 Concentration profile for experiment D.

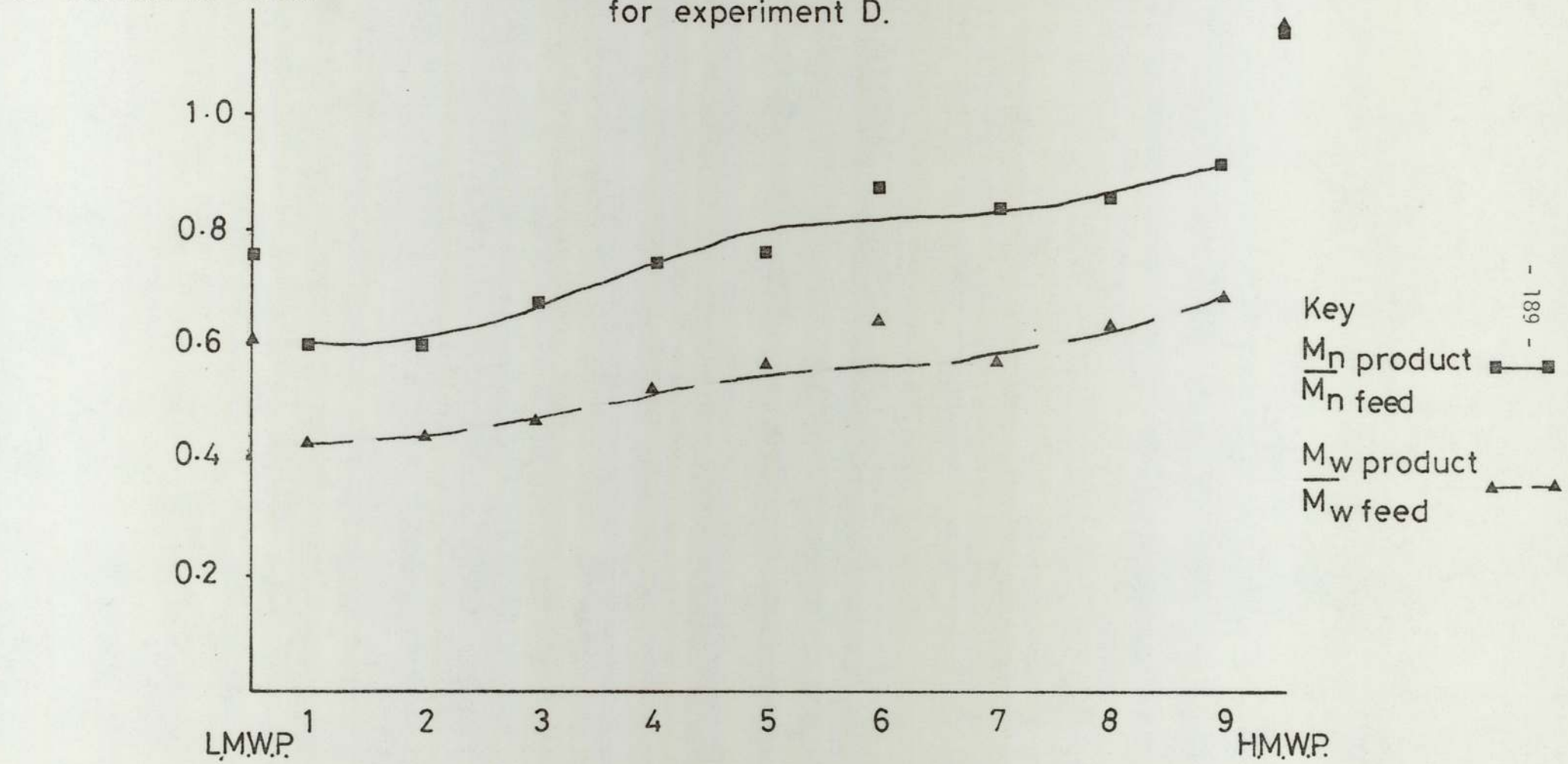
The functions of the various inlet and outlet port functions then index by one column moving in the direction of the eluent flow which simulates a movement of the packing from right to left (the opposite direction to the mobile phase flow) as seen on the diagram. As the switch time progresses, there is a net movement of the dextran from left to right.

As expected the concentration profiles for the removal of high molecular weight fractions are different to those runs where low molecular weight material was removed. Figs. 6.24 and 6.25 show that most of the dextran feed moves with the stationary phase leaving a minor portion of high molecular weight material to be eluted with the mobile phase, whilst in Figs. 6.26 and 6.27 the major part of the dextran feed moves with the mobile phase.

An interesting feature about all the profiles presented is that they exceed a relative average on-column concentration of 100 g l^{-1}. This value is much greater than on conventional chromatographic columns.

When experiment B and D are compared with experiment A and C, respectively, the regions of high concentration flatten for the lower eluent-to-feed ratio experiment. This suggested that the column was beginning to be overloaded. However, it was considered that there was still a difference in the composition in the mobile and stationary phases, and that fractionation was occurring. For example, Fig. 6.28 plots the weight and number molecular weight averages for the purged products of experiment D divided by the corresponding average of the feed solution. The values for the samples in the purge section are approximately constant but their values are significantly lower compared to the product eluted from this section. This suggests that there is a difference in the molecular weight distributions of the dextran in the

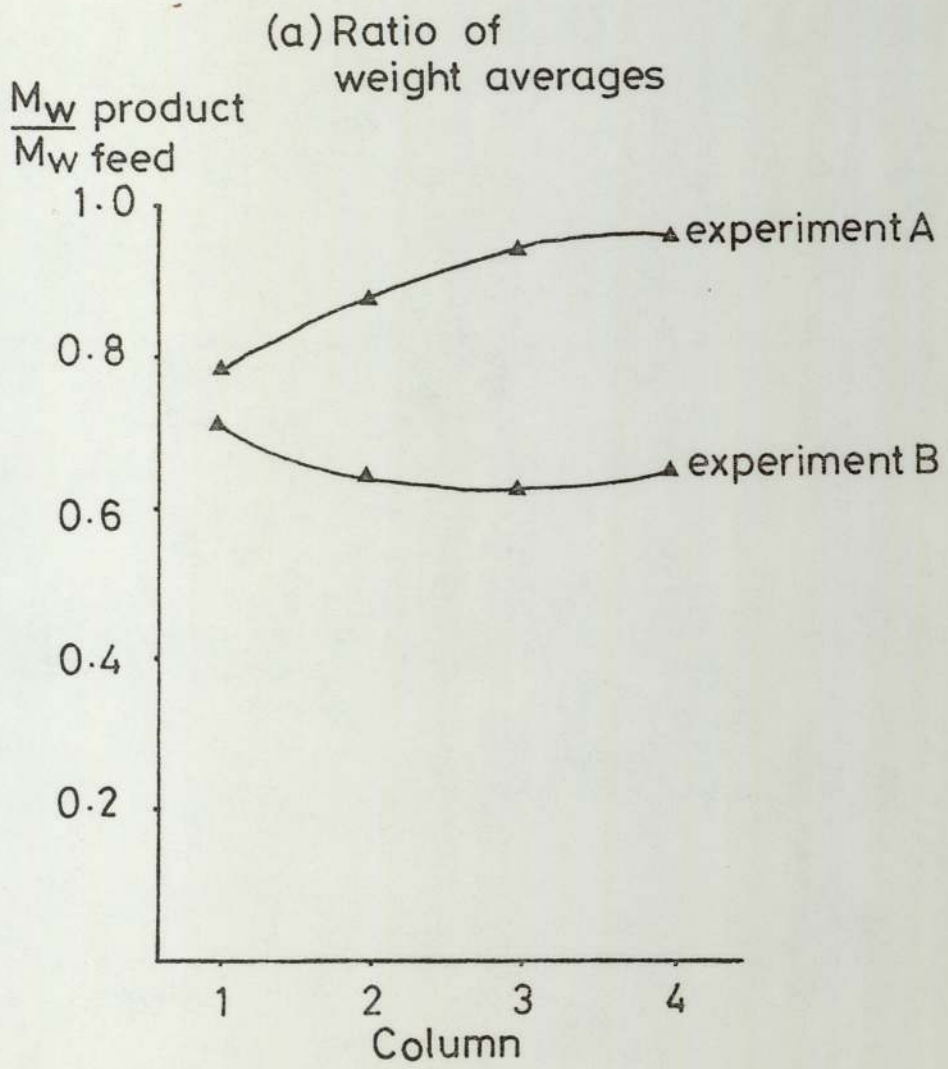
Fig6.28 Ratio of molecular weight averages of purge products to feed for experiment D.

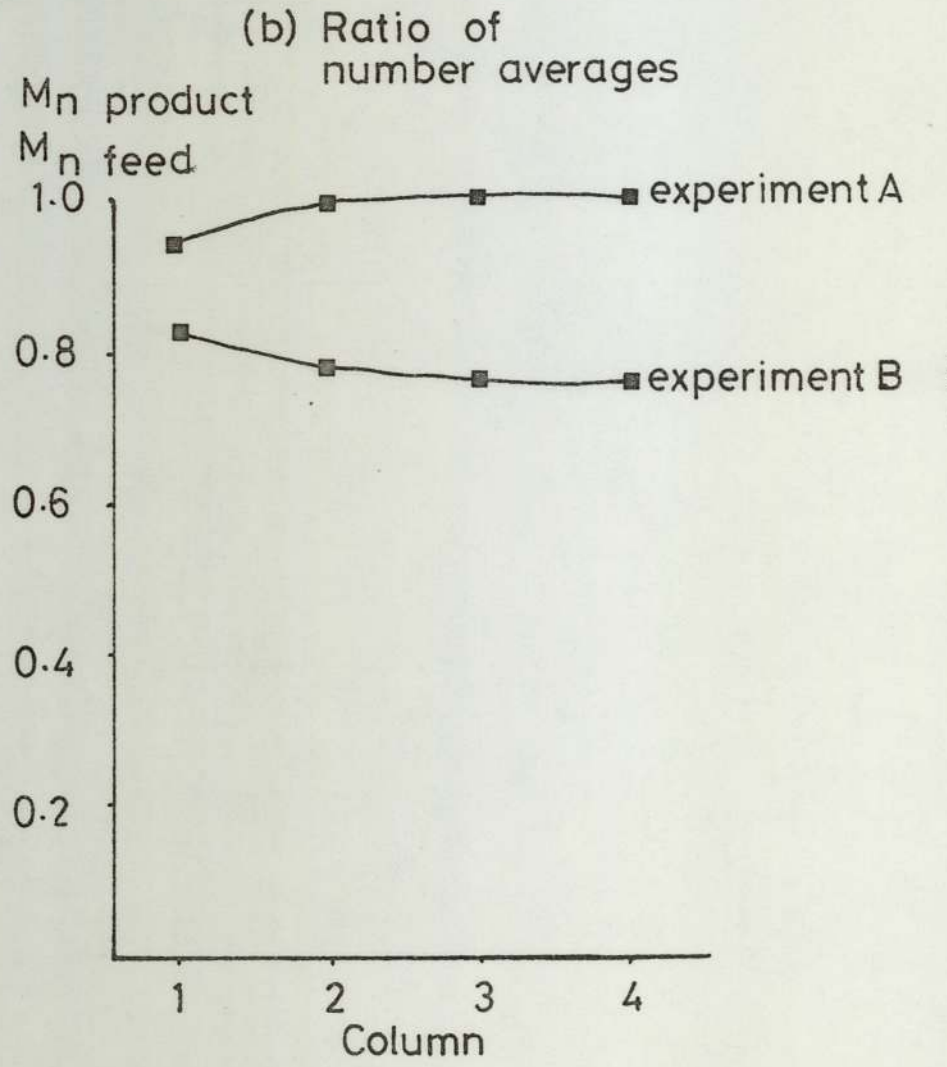


mobile and stationary phases and that the stationary phase contains a predominance of low molecular weight material. As the switch time progresses all the dextran molecules in the column move in the direction of mobile phase but in keeping with the principles of conventional batch g.p.c. the high molecular weight molecules migrate at a faster rate. The observed high molecular weight product distribution is the average for the material eluted during the switch time. When the dextran is first eluted from the column the material has a higher molecular weight distribution which falls as the switch time progresses, this phenomena has been observed by Williams (216).

The molecular weight distributions of the material held up in the stripping section of the fractionating column for experiments A and B had different molecular weight averages. The molecular weight averages for these columns relative to the feed are illustrated by Fig. 6.29 where it can be seen that the molecular weight averages for experiment B are lower than experiment A. An explanation for this could be due to the lower eluent-to-feed ratio increasing the concentration in the fractionating column during experiment B. Consider temporarily that the mechanism of gel permeation to be ideal where there are no molecular interactions and that steric exclusion alone is controlling the fractionation. In experiment B the higher concentration of eluate provided a greater potential driving force for those molecules that can enter the pores and this allowed a greater number of the small molecules to do so. If we now consider molecules interactions, as the concentration of the eluate is increased, this causes a reduction in the hydrodynamic volume of the dextran molecules and therefore allow them to behave as an apparently lower weight molecule. This would mean that the molecular weight averages should increase for those columns in experiment B where

Fig 6.29 Ratio of molecular weight averages of purge products to feed for experiments A & B.





the higher column concentrations were observed. Therefore either the reduction of the hydrodynamic volume is not occurring with increased concentration, or, there is another phenomena present. There is evidence to support the theory that the hydrodynamic volume of the molecules are decreased as the eluate concentrations are increased from dilute conditions. For example Ellison (34) found that the quantity of low molecular weight material increased from 19 to 78% when the feed concentration increased from 10 to 220 g l^{-1} whilst keeping the same operating conditions. Although experiments A and B where both feeds were introduced in concentrated solutions a different phenomena is considered to be affecting the mechanism of g.p.c.. The description of steric exclusion alone does not describe the mechanism of g.p.c. under the concentrated column conditions present in the SCCR5, and the diffusion of the molecules within the stationary phase should be considered. This description of steric exclusion being coupled with the diffusion of molecules in the stationary phase has been supported by several people notably by Yau (108). This model of the g.p.c. mechanism could explain why the molecular weight averages in the stripping section are lower for experiment B than for experiment A. The high concentration of dextran in the mobile phase means that there is a high potential driving force for those molecules that can enter the pores to do so. Molecular interactions causes them to become smaller. As the diffusion coefficients for small dextran molecules are relatively high they will be able to penetrate the whole of the available stationary phase volume. Higher molecular weight molecules that can be included in the stationary phase pore volume will experience similar molecular interactions that will cause them to become smaller and therefore able to penetrate deeper into the stationary phase. However because of the higher concentration of the lower molecular weights the diffusion of these molecules is

reduced. Laurent, Sundelöf, Wik, and Wärmegard (264) reported the diffusion of dextran in concentrated solutions for two molecular weight samples ($\overline{M}_w$ 150,000 and 20,000) and showed that for an increase in the average concentrations of the dextran solutions the diffusion rate was reduced far more in the higher molecular weight sample. Therefore because of the reduced mobility of the large dextran molecules in the stationary phase, they cannot penetrate as readily as under dilute conditions and so there is a self exclusion process operating.

For those molecules that do penetrate into the stationary phase and are moved closer to the eluent port by the indexing of the port functions, as the column concentration decreases the elution volume of some of the higher molecules will increase. This effect was observed in experiment B, as shown in Figure 6.30. Although the actual mass of the higher molecular weight dextrans (mol. wt. > 50,000) falls as it approaches the elution port, their relative percentage with respect to the lower molecular weights increase.

6.5.2 Product Concentrations and Compositions

For the four experiments performed at ambient temperatures experiments A and B aimed to remove high molecular weight material from the feed and so the low molecular weight product was the main product of interest. Similarly for the experiments C and D where low molecular weight material was removed, the main product of interest was the high molecular weight product. Details of the products are summarised in Fig. 6.31.

Fig 6.30 Actual and relative variations in dextran masses with column position for experiment B.

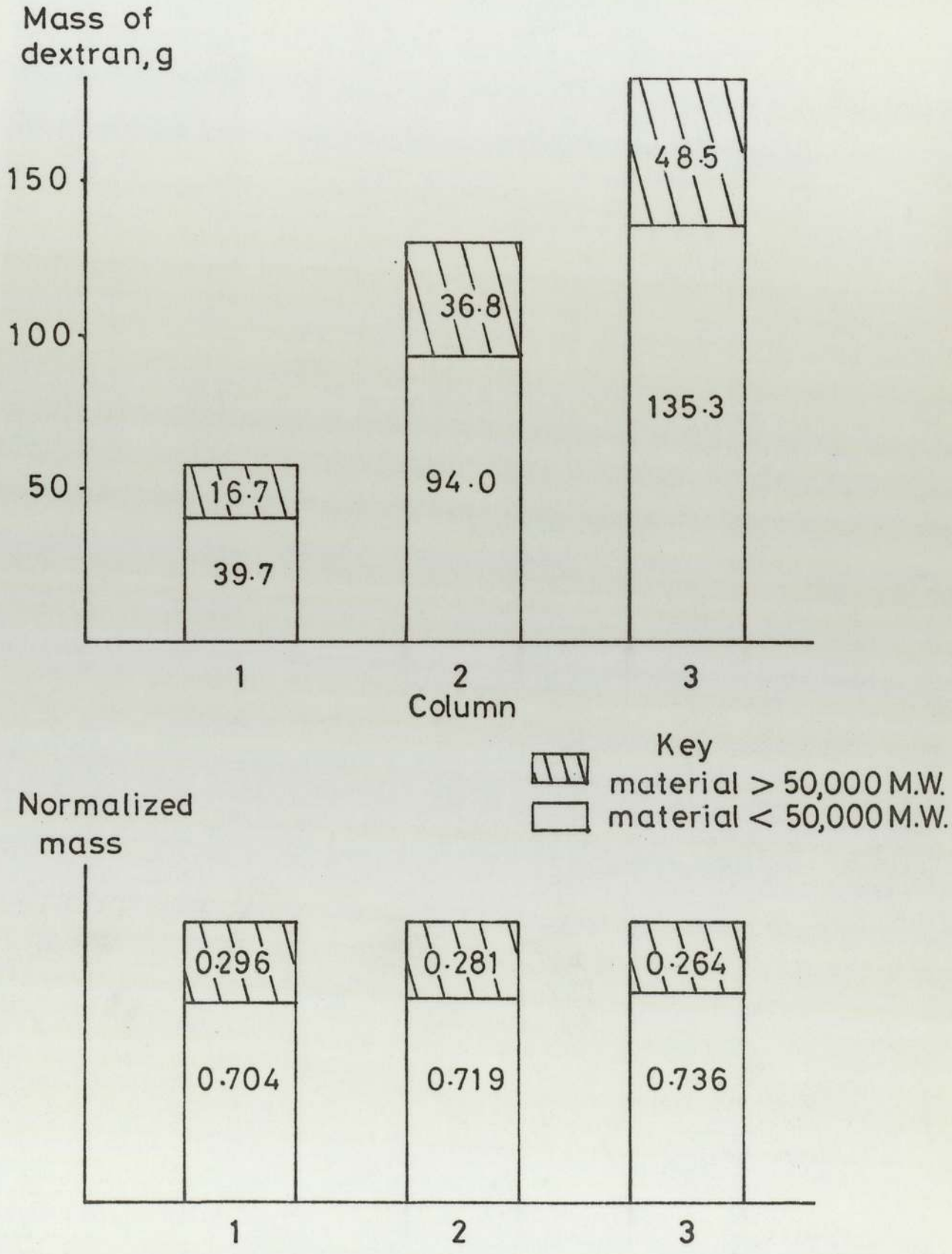


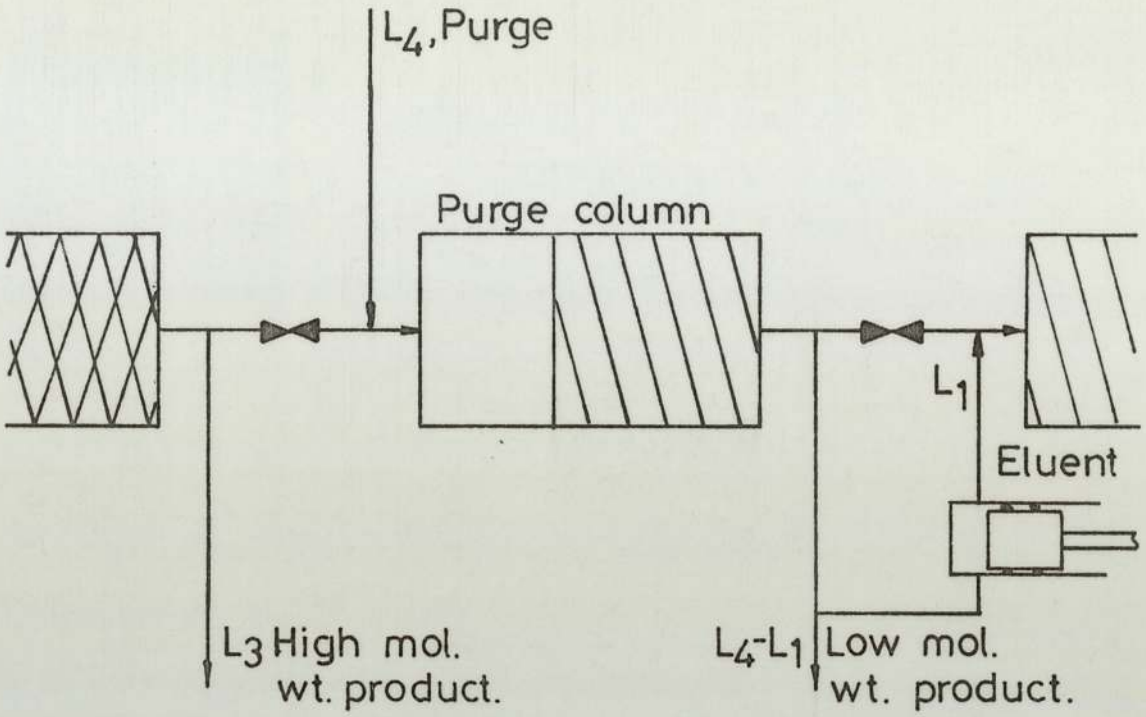
Fig 6.31 Summary of products for the S.C.C.R. 5.

Experiment	Input mass, g.	High mol. wt. product					Low mol. wt. product				
		Mass g.	Conc. $g.l^{-1}$	Mol. Wt. Distribution			Mass g.	Conc. $g.l^{-1}$	Mol. Wt. Distribution		
				M_w	M_n	D			M_w	M_n	D
A	282·7	35·8	3·98	179,600	45,000	3·99	233·8	7·16	52,700	24,600	2·11
B	693·0	97·5	9·37	123,600	39,000	3·17	568·4	18·22	49,300	23,700	2·03
C	206·5	163·0	14·48	78,700	29,700	1·98	21·2	0·86	33,100	19,000	1·74
D	474·0	397·5	31·21	73,400	30,900	2·38	43·5	1·64	39,300	20,700	1·90

The main products for experiments A and B both show a reduction in the molecular weight averages. The molecular weight averages for experiment B being lower than those for experiment A. This is probably due to the 'self exclusion effect' that was observed in the stripping section of the column, which was aided by the increase in the concentration of the feed solution by 20% from 192 to 231 gl^{-1}. The percentage of low molecular weight product for experiments A and B were very similar, even though the nominal feedrate was increased in experiment B to 40 cm^3 min^{-1} from 20 $cm^{-3}min^{-1}$ which raised the post-feed theoretical cut position to 0.746 compared with 0.516 for experiment A. This suggests that the post-feed theoretical cut position does not have a significant effect on the product split within these limits. The concentration of the lower molecular weight product for experiment B was 17.5 gl^{-1}. This value could be increased by a reduction in the quantity of the purge liquid used because 3.8 times the required theoretical value was used. In theory, therefore, the low molecular weight product for this experiment could be increased to 66.5 gl^{-1}. Alternative schemes to increase the product concentrations have been proposed. The simplest of which would be to reduce the quantity of purge liquid. However in these experiments the concentration of the products was secondary to the quality so to ensure complete removal of the low molecular weight molecules. Another of the proposed schemes is illustrated in Fig. 6.32. The objective of this arrangement was to increase the concentration of both products. Minimising the eluent and purge streams would increase the product concentrations. Refluxing part of a product stream could be possible. The eluent fed into the fractionating section, could be replaced by refluxing part of the low molecular weight product, as illustrated by Fig. 6.32(a). This would mean that the concentration in

Fig 6.32 Proposed purge scheme.

(a) Initial purge mode



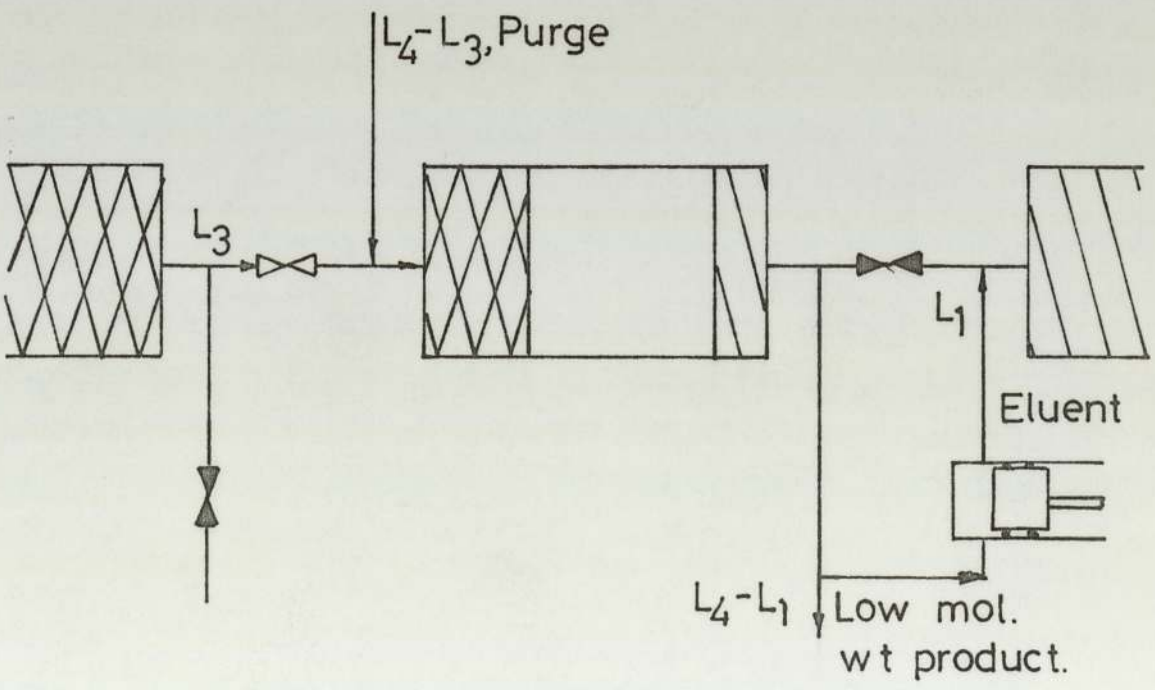
Key

eluent

low mol. wt.

high mol. wt.

(b) Secondary purge mode



the stripping section would not fall as much as at present and possibly aid fractionation by allowing the self exclusion phenomena to occur in the column into which the eluent (reflux) would be flowing. The high molecular weight product could initially be taken off as in the present system. But in the next switch the high molecular weight product will begin to elute from the column that has been totally purged. If high molecular weight material could be introduced into this column during the purging operation it would have the following effects:-

(a) the additional column length should aid the fractionating capabilities of the chromatograph

(b) less eluent would be required therefore an overall increase in the product concentrations would be expected.

A delay in refluxing the high molecular weight product into the purging column would be essential because the high molecular weight material would migrate at a faster rate than low. Therefore as in the repetitive batch chromatography a volume equivalent to the total pore volume would have to be eluted (in practice a volume in excess of the total pore volume would be used) to ensure that there was no mixing of the high and the low molecular weight molecules. After an appropriate time delay a secondary timer unit could simultaneously close the high molecular product and open the transfer valve between the fractionating and purge sections. This timer could also be used to reduce the flow of eluent to the column as shown in Figure 6.32 (b). A disadvantage of this system is that a secondary timer unit would be required and a more complex system for controlling the isolation of the purge column.

An advantage of the low molecular weight removal experiments was that the main product of interest was eluted from the fractionating section and higher eluent-to-feed ratios could be used whilst still maintaining similar concentrations for the main product as experiment

A and B. Experiment C produced a main product having 10.7% of the lower molecular weight dextrans removed. This experiment demonstrated that the SCCR5 machine could remove low molecular weight dextrans from a concentrated feed solution. A decrease in the eluent-to-feed ratio was attempted in experiment D. It was anticipated that if the eluent-to-feed ratio were to be decreased the pressure drop across the fractionating column would be increased. As the pressure drop had reached 1550 KNm^{-2} (225 p.s.i.) in the previous experiment, for experiment D the mobile phase flowrates were reduced and the eluent-to-feed ratio reduced from 12.3 to 5. Thus, the post-feed theoretical cut position was increased from 0.88 to 1.07. Experiment D removed 8.9% of the feed solution in the low molecular weight product. This product split was similar to experiment C. This suggests that the product split was insensitive to the post-feed theoretical cut position.

6.6 CONCLUSIONS

Experiments A and B for the removal of high molecular weight dextrans from a feed solution using the SCCR5 were in agreement with similar experiments performed by Dr. S. R. Holding. The pressure drops across the various sections of the chromatograph were consistent with these earlier experiments, therefore there was no detectable change in the condition of the porous glass packing. A solution containing 0.55 kg h^{-1} of dextran could be fed to the chromatograph and a satisfactory product which had a necessary quantity of high molecular weight material removed obtained.

Experiments establishing that the removal of low molecular weight dextrans from a concentrated (approximately 220 gl^{-1}) feed solution were performed. The necessary pre-feed theoretical cut position for the removal of the low molecular weight dextrans restricted

the flowrate of the dextran feed solution to less than half that for the high molecular weight fractionations. Thus a feedrate of only 0.24 kgh^{-1} of dextran was achieved with the Spherosil XOB075. It was considered that this value could be increased if the packing material were to be changed. Changing the packing material would also have other advantages as discussed in section 8.4.3.

The product split was relatively insensitive to a change in the post-feed cut positions for experiments A and B and for experiments C and D. The concentration of the major product for experiment D was between 4 and 5 times the concentration of the main product for experiment A despite the fact that the same eluent-to-feed ratio was used. This is because the main product from experiment A was eluted from the purge section. In a commercial application of this unit operation the economic success or failure of this process might depend on whether the concentrations of the product streams are high enough. As the comparison between experiments A and D show, when only the least retained product is important a higher eluent-to-feed ratio may successfully be used. If the required product is the retained component then an emphasis on obtaining a more concentrated product should be made either by using one of the proposed schemes or an alternative arrangement.

7.0

COMPARISON OF REPETITIVE BATCH AND SEMI-CONTINUOUS CHROMATOGRAPHS

7.1 INTRODUCTION

The comparison of the operation of the repetitive batch and semi-continuous chromatographs may be made in several ways, such as, the quality of product, rate of feed throughput, concentration of final products, profitability of process, versatility of equipment, reliability, ease of automation, and the degree of labour required. The objective of both the batch and the semi-continuous studies was the maximisation of the rate of feed throughput whilst maintaining the standards set by the hypothetical ideal product fractions given in section 1.0. The molecular weight distributions of the feed materials were different for the repetitive batch and the SCCR5 equipment. This is reflected in the specifications of the ideal product standards. The same amount of packing material was used in both chromatographs, which had identical column dimensions, although the actual column dimensions used may not be the optimal configuration of either of the operating methods. Therefore this comparison is only a preliminary study and an investigation into a variety of column geometrics is required before a complete study of the comparison of the repetitive batch and SCCR equipment can be concluded. A difference in the material of construction for the columns of the chromatographs; glass for the repetitive batch chromatograph, and stainless steel for the SCCR5, allowed a higher pressuredrop limitation to be set for the SCCR5. This consequently allowed greater eluent and feed velocities to be used which inturn led to an increase in feed throughput. An allowance in the rate of feed throughput has been made for the difference in pressure drop limitation of the chromatographs. The rate of feed throughput for the semi-continuous chromatograph has been reduced by the ratio of the observed operating pressure divided by the maximum safe operating

pressure of the repetitive batch chromatograph. That is, by the relationship;

$$F_{R_c} = F_{R_{ob}} \times \frac{P.d._{ob}}{P.d._{safe}} \qquad (7.1)$$

where F_{R_c} rate of feed throughput for the SCCR5 machine at a pressure drop comparable to the repetitive batch rig,

$F_{R_{ob}}$ observed rate of feed throughput for the SCCR5 machine,

$P.d._{ob}$ observed pressure drop for the SCCR5 machine

$P.d._{safe}$ maximum safe operating pressure for the repetitive batch rig.

It is envisaged that if a g.p.c. method of fractionating dextran were used for an industrial scale process, the equipment should have the capability of fractionating up to a hundred tonnes per annum. Therefore the comparison of the operating methods should include speculation on the potential scale-up of the two modes of operation. Alternative applications for these process techniques will be considered and discussed.

7.2 DISCUSSION

The main points for the comparison of the repetitive batch and semi-continuous methods of operating a chromatograph are as follows:-

(a) Quality of product

The inherent nature of the operations of the chromatographs for the fractionation of dextran does not allow a direct comparison of

the fractionated products to be made. Various parameters attempting to characterise the product fractions have been used, such as, weight average molecular weight, $\bar{M}_w$, number average molecular weight, $\bar{M}_n$, and polydispersity the ratio of $\bar{M}_w/\bar{M}_n$ but none are completely suitable for the present purpose. There have also been several attempts to express the efficiency of a polymer fractionation as shown in section 2.5. But, there remains a need for more definitive methods of characterisation to be proposed in the absence of a parameter that allows a direct comparison for the product fractions, the quality of the eluted product was compared against a hypothetically ideal product specification. Tolerance values were given for the weight and number average molecular weights. These values were compared with the product fraction and it was this comparison that decided if the product was on specification. A majority of the reported experiments produced on specification products. The experiments that produced the maximum feed throughput rate whilst maintaining the specifications set by the hypothetically ideal products were as follows;

Repetitive batch	Experiment 7
SCCR5 performing:-	
Low molecular weight	
removal	Experiment C
High molecular weight	
removal	Experiment B

Although the product fraction for the repetitive batch experiment 7 satisfied the product specification there was an indication that poor fractionation of the low molecular weight material was occurring. This was inferred from the similar values at 5 and 10 percent on the cumulative distribution curve for both the waste fractions

and the product fraction. This was assumed to be a feature of the Spherosil XOB075 packing. Subsequent experiments on the SCCR5 equipment supported this observation. Therefore it could be calculated that the fractionating range of the Spherosil XOB075 packing was only just broad enough for the operating conditions of the repetitive batch chromatograph and as a utility packing for the SCCR5.

A broad fractionating range for the column packing of an industrial-scale repetitive batch chromatograph completely covering the molecular weight range of the feed under the expected operating conditions is a fundamental requirement. This is analogous to most analytical requirements. Some problems that may be encountered by having a fractionating material of too narrow a molecular weight range have been discussed in section 2.7.2. However, if a SCCR chromatograph were used the fractionating range of the packing material could be much narrower. It has been established that if a SCCR chromatograph were to be used for the production of clinical dextran two passes through the machine would be required. If economically feasible, two fractionating materials could be used. If the original feed material were to be considered as three fractions of low, middle, and high molecular weight molecules, the first packing could totally include the low and middle molecular weight material and the unrequired high molecular weight molecules could be removed in the high molecular weight product. Whilst the second packing material could totally exclude the remaining high and middle molecular weight materials and remove the unrequired low molecular weight molecules in the purge stream. Therefore, the selection of packing materials having suitable chosen exclusion limits and narrower fractionating ranges may aid the SCCR equipment to produce a product bounded by a more exact molecular weight range. However, in this

experimental programme the Spherosil XOB075 packing used in both chromatographs has produced products that have complied with the specifications set by the hypothetically ideal standards as defined in Fig. 1.2.

(b) Rate of feed throughput

The SCCR5 chromatograph was capable of fractionating dextran with a higher rate of feed throughput than the repetitive batch chromatograph. In experiment B a feed throughput of 550 g h^{-1} was achieved with a back pressure of 1.55 x 10^5 N m^{-2}. Making the necessary allowance for the difference in pressure drop limitations of the chromatographs by equation (7.1) the expected throughput for the same theoretical cut positions as experiment B, but at the maximum safe operating pressure for the glass columns would be 175 g h^{-1}. Similarly for experiment C, where the observed feed throughput was 240 g h^{-1} with a pressure drop of 1.03 x 10^5 N m^{-2} an equivalent experiment on glass columns would have had an expected throughput of 112 g h^{-1}. Experiments B and C were the highest feed throughput experiments performed on the SCCR5 chromatograph at ambient temperatures for the removal of high and low molecular weight material, respectively.

To produce a clinical fraction the SCCR5 would be required to operate under both sets of operating conditions. If the chromatograph removed high molecular weight material at a feed rate of 175 g h^{-1} for three time periods and then fractionate the intermediate product under the same conditions as experiment C for four time periods then the removal of both high and low molecular weight molecules would be accomplished. The net average feed throughput would be 75 g h^{-1} and the pressure limit of the glass columns would not be exceeded. This value can be directly compared to the 63.3 g h^{-1} feed throughput observed in

experiment 7 performed on the repetitive batch chromatograph. Therefore for the fractionation of dextran into a hypothetical clinical product, the operation of a chromatograph in a semi-continuous mode has allowed an increase of 18.5% in the feed throughput rate compared to repetitive batch mode. For the production of a clinical dextran the repetitive batch chromatograph has the inherent advantage of only requiring a single pass and so minimising the increase in the rate of feed throughput of the SCCR equipment. It is clear however that if only a single product-cut were required then as the above results indicate, the rate of feed throughput for the SCCR chromatograph has a value of at least two or three times greater than that of the repetitive batch chromatograph.

Apart from determining the product quality, the porosity of the column packing would also influence the rate of feed throughput. The type of packing used for the chromatographs, Spherosil XOB075, had a wide porosity range. It was just broad enough to fractionate the complete molecular weight range of the feed. But it was considered when used in a preparative column, the packing fractionated the high molecular weight molecules better. This allowed a lower eluent-to-feed ratio to be used for the high molecular weight removal experiments and as a result influenced the rate of feed throughput. On an equivalent basis the rate of feed throughput for experiments B and C were 175 and 112 $g\ h^{-1}$, respectively, an increase of 56.3%. Thus the choice of packing materials having suitable chosen exclusion limits would also aid the rate of feed throughput for the SCCR chromatograph.

(c) Concentrations of final products

The dilution of the feed solution by the eluent is important particularly when the final product of the process is to be recovered as a highly concentrated solution or as a solid. The high costs of the

energy requirements of the subsequent concentrating and/or drying operations underlines the importance in obtaining as high a concentrated product from the chromatographic process as possible.

The feed solutions applied to both chromatographs were far higher than is usually found in conventional analytical chromatography and most preparative applications. As a result the products from both chromatographs were relatively concentrated. The concentrations of the products from the repetitive batch chromatograph were directly related to the weight of solute injected. Experiments 7 and 9 produced the maximum average concentration for products produced by repetitive batch fractionation. The observed maximum concentrations were approximately 10 g l^{-1}. For the repetitive batch chromatograph these values cannot be improved upon for a particular throughput.

The concentrations of the main products from the SCCR5 chromatograph were higher than those of the repetitive batch chromatograph for the highest feed throughput experiments. The main product for experiment B had a concentration of 17.5 g l^{-1}. This theoretically could have been increased to 66.5 g l^{-1} by reducing the volume of eluent in the purge section from 3.9 column volumes to one, and with the inclusion of recycle streams increasing the on-column concentrations at the extremities of the fractionating section even higher values could be possible.

The concentration of the main products for the low molecular weight removal experiments C and D on the SCCR5 were 14.5 and 31.1 g l^{-1}, respectively. In both these experiments relatively high eluent-to-feed ratios were necessary because of the characteristics of the Spherosil XOB075. Apart from the advantages described in the above sections, if a packing having more suitable characteristics for low molecular weight

removal were used an increase in the concentration of the main product would also be possible.

A measure of the operating cost could be directly related to how much water it would be necessary to remove to produce a gram of final product. In order to increase the concentration of the products to 200 g l^{-1} the following quantities of water will be required to be removed;

(i) for repetitive batch experiment 7, 19.2 grams per gram of dextran

(ii) for experiment B, 11.2 grams per gram of dextran

(iii) for experiment C, 14.7 grams per gram of dextran

Therefore the semi-continuous method of operation will require the removal of 25.9 grams of water per gram of dextran compared to 19.2 grams for the repetitive batch method, equivalent to an increased cost of 35%. However the concentration of the products from the repetitive batch chromatographic method cannot be improved upon for a particular throughput. But changes in the quantity of purge for experiment B could reduce the quantity of water that would have to be removed to 2.87 grams. Changes in the packing material could enable low molecular weight removal experiments to be performed with an eluent-to-feed ratio of 2.5. This would reduce the quantity of water that would have to be removed in the final product from 14.7 grams to 3.00 grams. Thus the semi-continuous method of operation would require the removal of 6 grams of water per gram of dextran, a reduction of 77%. This would be equivalent to only 31% of the quantity of water that would have to be removed by the repetitive batch method. A full economic evaluation of these processes remains to be carried out. As a sequel to this Ph.D. thesis, an economic feasibility study is to be carried out by Fisons Ltd., Pharmaceutical Division.

(d) Other considerations

In the above sections the discussion has centred upon the composition, quality and rate of the products eluted from the chromatographs with respect to dextran fractionation. However the comparison of batch and continuous chromatographs may be made on many bases and should include such topics as, scale of operation, capital cost, reliability, ease of automation and the versatility of the chromatograph.

The scale of operation and the capital cost of the chromatographs are closely linked. For both methods of operating a chromatograph the single most expensive item of capital equipment to be purchased, would probably be the packing material. Other costs that would be incurred by both processes would be the cost of columns, tubing and fittings. In addition to this the SCCR equipment would have the cost of the sequencing equipment and the valves. A comparison of the chromatographic processes could be made using a non-discounting technique for their profitability. A useful technique is the "return on original investment" which is a ratio of the profit of the process to the fixed capital costs. This could be simplified to the ratio of rate of feed throughput to the cost of the packing material for the batch process or the cost of the packing material plus the cost of the valves for the SCCR equipment. That is,

$$P_c = \frac{F_c}{\left(1+\frac{C_v}{C_p}\right)C_p} = \frac{F_c}{(1+x)C_p}$$

$$P_b = \frac{F_b}{C_p}$$

where P is the profitability of the process

F is the feed throughput

C is the capital cost

x is the capital cost of the valves divided by the capital cost of the packing

and the subscripts

c is the continuous chromatograph

b is the batch chromatograph

p is the packing

v is the valves

therefore for

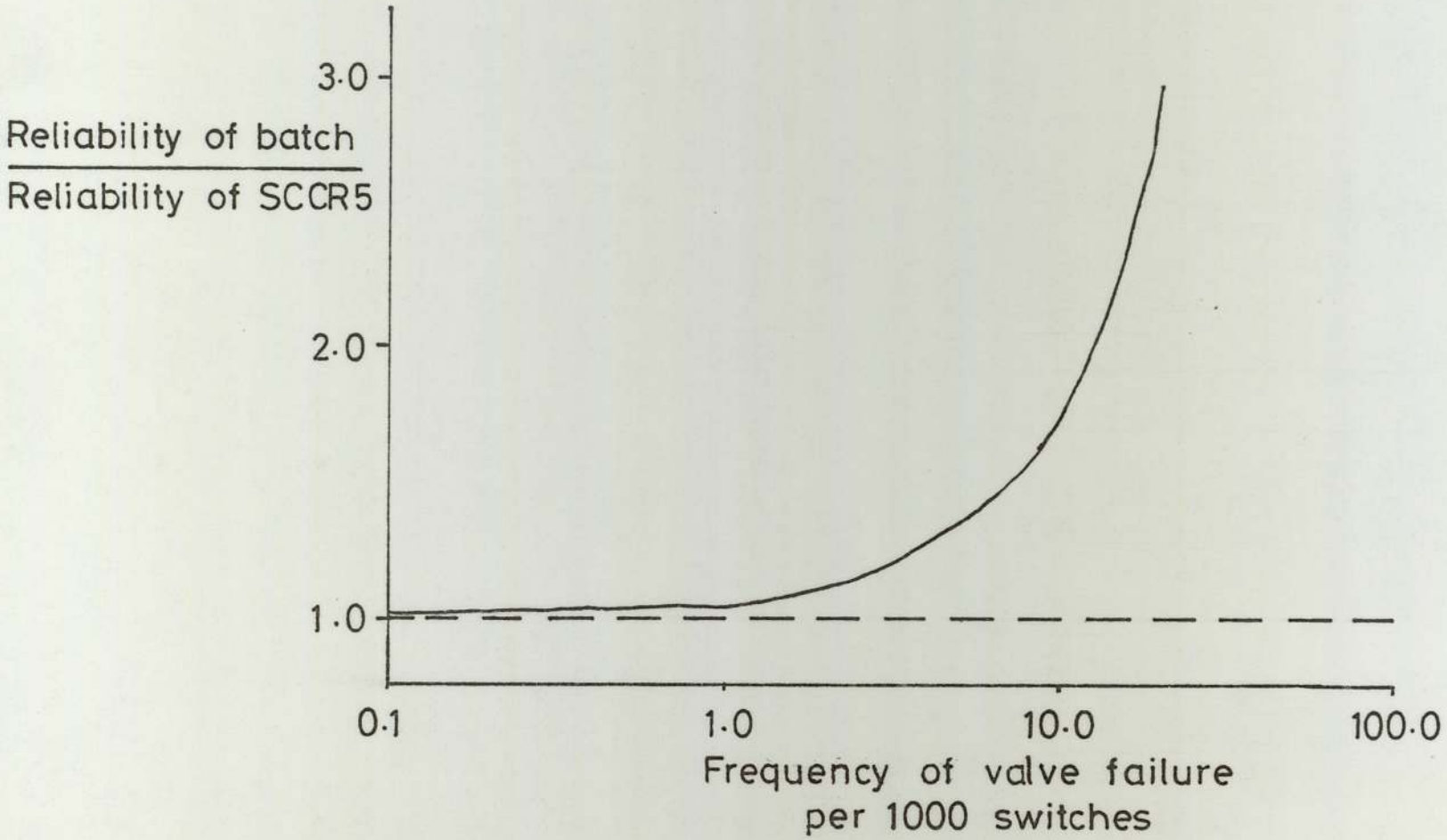
$$P_c > P_b$$

$$F_c > F_b (1+x)$$

The capital cost of the packing and the rate of feed throughput are directly related. The cost of the valves is relatively insensitive to the size of operation. Similar valves, therefore similar costs, could be used on a chromatograph having an increase in column diameter of five or ten times. Therefore semi-continuous chromatography would be more applicable to large scale production chromatography whilst batch chromatography would be more useful for small production or preparative applications.

The successful operation of the SCCR equipment depends upon the ability of the valves to open or close in a predetermined sequence. As an exercise to show how the reliability of the repetitive batch chromatograph compared to the SCCR5 chromatograph Fig. 7.1 shows the relationship for valves having a probability of failing to work correctly ranging from 0.02 to 0.0001. The repetitive batch chromatograph requires fewer actuating valves and is therefore always more reliable.

Fig 7.1 Reliability of batch chromatograph compared to SCCR 5.



The inclusion of suitable high or low pressure sensing alarms to the SCCR equipment would indicate whether a valve or valves has failed to operate correctly would prompt the necessary corrective action.

Automation of both chromatographic processes is possible. The present SCCR equipment is already semi-automated with the indexing of the column post function being fully controlled by an electrical timer. Only the flowrates of the process streams were manually controlled. The addition of flow controllers for a production scale chromatograph to this equipment is a simple task. The repetitive batch system used was manually controlled but fully automated controllers for this type of equipment have been reported (176, 177).

If the chromatographic system were to be used to separate or fractionate more than one system the packing material may have to be changed. Packing chromatographic columns is an "art", and so the emptying and the repacking of columns for a chromatographic system would be costly in both time and labour and would precent uncertain column characteristics. The SCCR equipment in its present form allows the complete column to be changed in a simple operation. Thus, increasing the versatility of the equipment. This feature could also be incorporated into a repetitive batch system.

7.3 CONCLUSIONS

Both the repetitive batch and the semi-continuous chromatographic methods produced dextran products that complied with the set standards. The semi-continuous chromatographic method was capable of fractionating dextran at slightly higher feed flowrates than the repetitive batch chromatographic method. The SCCR5 equipment also produced slightly higher product concentrations. Although if the experimental conditions were

to be used in a commercial process to produce clinical dextran, because the semi-continuous method requires the concentration of an intermediate product, this process requires 35% more water to be removed. But the SCCR equipment has the capabilities of producing highly concentrated products that will require the removal of less than one-third the quantity of water that will have to be removed from the product of the repetitive batch chromatograph.

The lower capital cost and the less complex method of operation of the repetitive batch chromatography suggests that this type of equipment would be more useful as a laboratory preparative tool. The SCCR equipment is more applicable to large-scale production equipment.

8.0

SEMI-CONTINUOUS DEXTRAN FRACTIONATIONS

AT ELEVATED TEMPERATURES

8.1 MODIFICATIONS TO THE SCCR5 EQUIPMENT

The fractionation of dextran by the SCCR5 at ambient temperatures has been reported in section 6. The pressure limitation of the apparatus restricted the quantity of dextran that could be fractionated. The observed high pressures were due to the flow of the viscous mobile phase solution. Elevating the operating temperature of the fractionating columns would reduce the viscosity of the mobile phase and therefore allow an increase in the product throughput, provided all other conditions were the same as previous. The literature that discussed the effect of temperature on g.p.c. has been presented in section 2.10. The fractionating performance of the SCCR5 was studied at three temperatures 20, 45 and 60°C. Operating conditions were chosen with the aim of removing high and low molecular weight fractions from the feed material. The building and testing of the modifications to the SCCR5 along with the additional experimental techniques and safety precautions that were necessary are reported below.

8.1.1 Control and Heating of the Mobile Phase

The first step to increasing the operating temperature of the apparatus was to provide a heat source for the mobile phase. Electrical immersion heating was chosen. Because a 10 cm diameter chromatograph was planned to be built in the laboratory that would require a large supply of heated water the mobile phase heater was over sized for use with the SCCR5 in order to accommodate the potential demand. It was also planned to use an ion exchange resin as a stationary phase for the larger diameter equipment and therefore this restricted the choice of constructional materials for the eluent heater to those that would not liberate ions that might affect the ion exchange

resin. Hence stainless steel was chosen as the construction material.

Heating of the mobile phase was performed in two stages, as Fig. 8.1 shows. Primary heating of the water took place in a 30 cm i.d. by 31 cm long stainless steel vessel fabricated by the Department's workshop. Incorporated into the vessel were three all stainless steel immersion heaters each of 5 kWatt capacity. The temperature in the tank was controlled by a thermostat linked to a four pole contactor. For safety a relief line was taken from this vessel and looped back over the mobile phase reservoir.

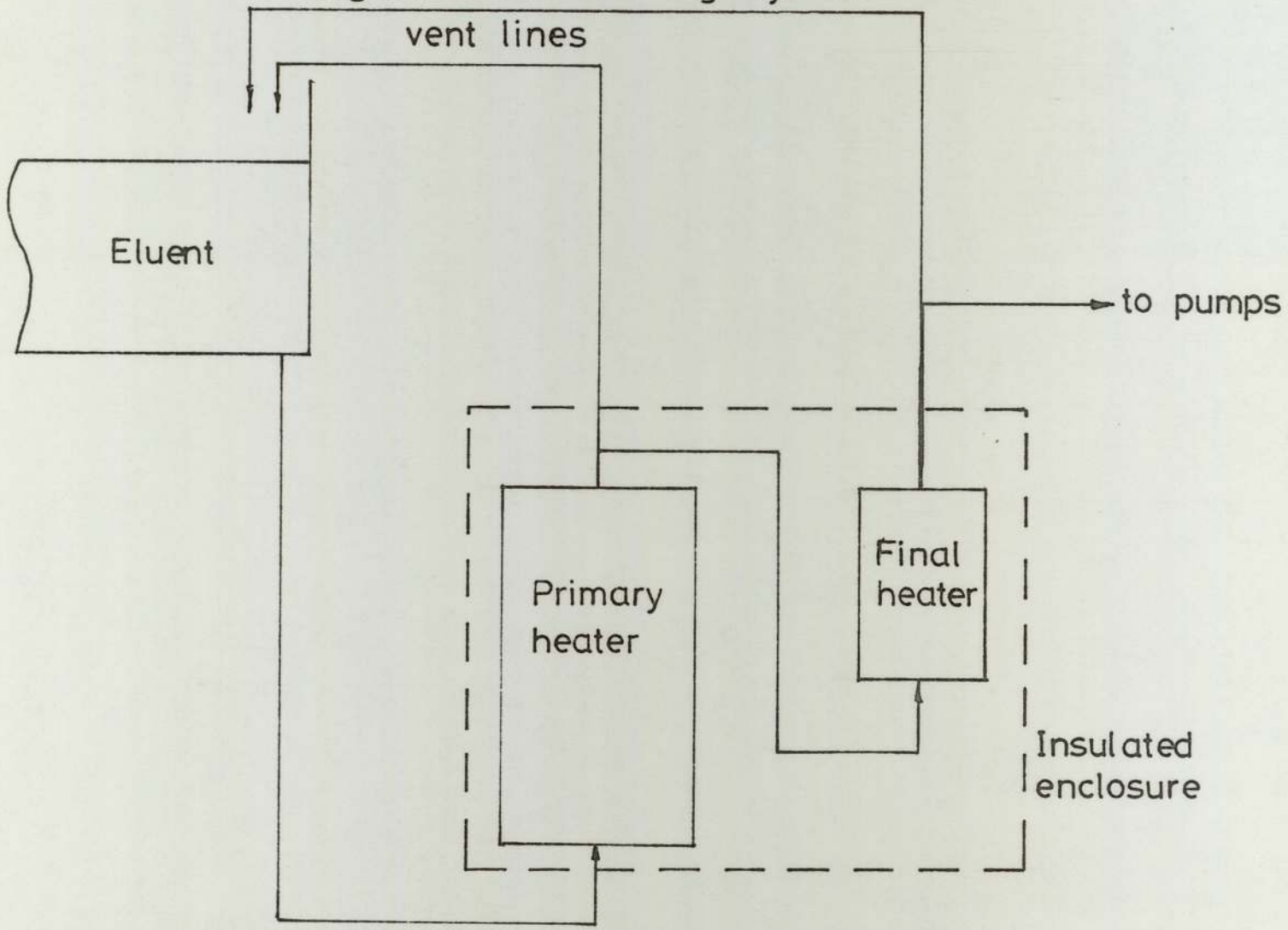
The second stage in the heating of the mobile phase was performed in a smaller vessel, 13 cm i.d. by 38 cm long. Accurate and precise control of the mobile phase temperature was achieved by controlling the 3 kWatt stainless steel heater in the vessel by a three term digital set point temperature controller, supplied by Pye Ether Ltd., Stevenage. The input to the controller was taken from the purge line by a hypodermic thermocouple see section 8.1.4. A thermostat in the immersion heater was also connected to the power supply to prevent the water in the vessel from overheating. As an additional safety feature a vent line was taken from the top of the heating vessel.

Both heating vessels were mounted in a dexian frame surrounded by blockboard and filled with vermiculite. The vessels were then sited directly below the metering pumps to allow short delivery lines to be used.

8.1.2 Control and Heating of the Dextran Feed Solution

The dextran feed heater consisted of a 5 cm i.d. by 25.4 cm long glass Q.V.F. pipe section mounted horizontally with a side-arm. At one end a hose connection was attached and at the other a reducing

Fig 8.1 Eluent heating system



piece was connected to a screwthread joint through which a 1 kWatt sheathed glass immersion heater fitted. The immersion heater was connected in series with a variable resistor that allowed the power output from the heater to be reduced. Liquid flowed in by a hose connection and out from the side arm. The temperature of the dextran solution to the rig was controlled using a two term controller supplied by Diamond H. Controls Ltd., Norwich. The input to the controller was measured as close as possible to the outlet arm of the feed heater by means of a hypodermic thermocouple. All the glassware was covered with copper gauze as a safety precaution and safety spectacles were worn at all times when the feed heater was switched on.

8.1.3 Enclosure for the SCCR5

An enclosure for the SCCR5 was built around the chromatographic columns so that the air temperature surrounding them could be maintained at approximately the same temperature as the mobile phase and feed streams. After some slight modifications to the original framework the enclosure was mounted upon it. The final selection for the materials of construction for the enclosure were resin bonded fibreglass slabs backed by laminated fibreglass sheet. The corners of the sheets were protected by 'L' shaped aluminium angle. The laminated fibreglass sheet was purpose-built from chopped glass matt.

The interior surface of the enclosure was coated with a special heat and chemical resistant resin to prevent any of the liquid penetrating the insulation should a liquid line break. A finned air heater used for heating and maintaining the temperature inside the enclosure. The air was circulated using two airotors. The control of the air temperature was by a digital set point two term controller

Fig 8.2 Photograph of SCCR 5

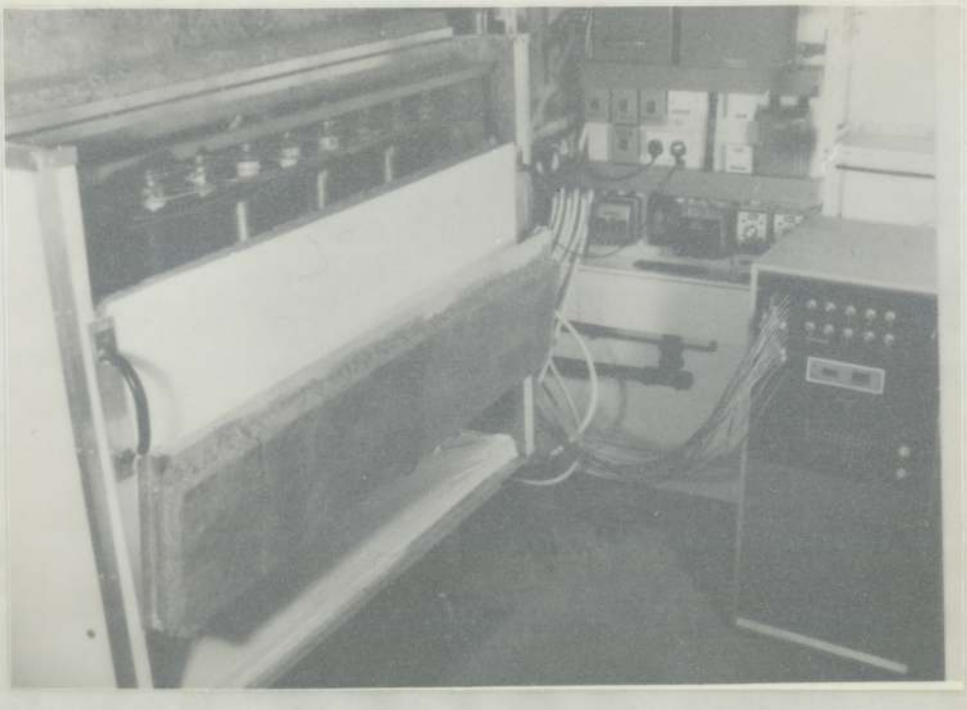
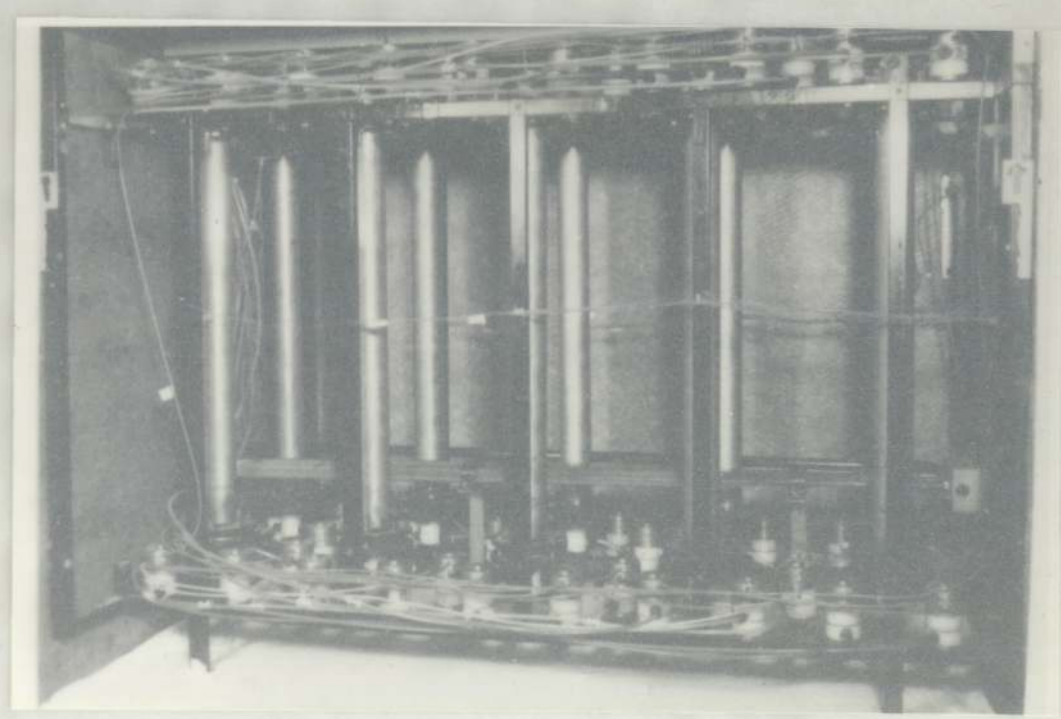
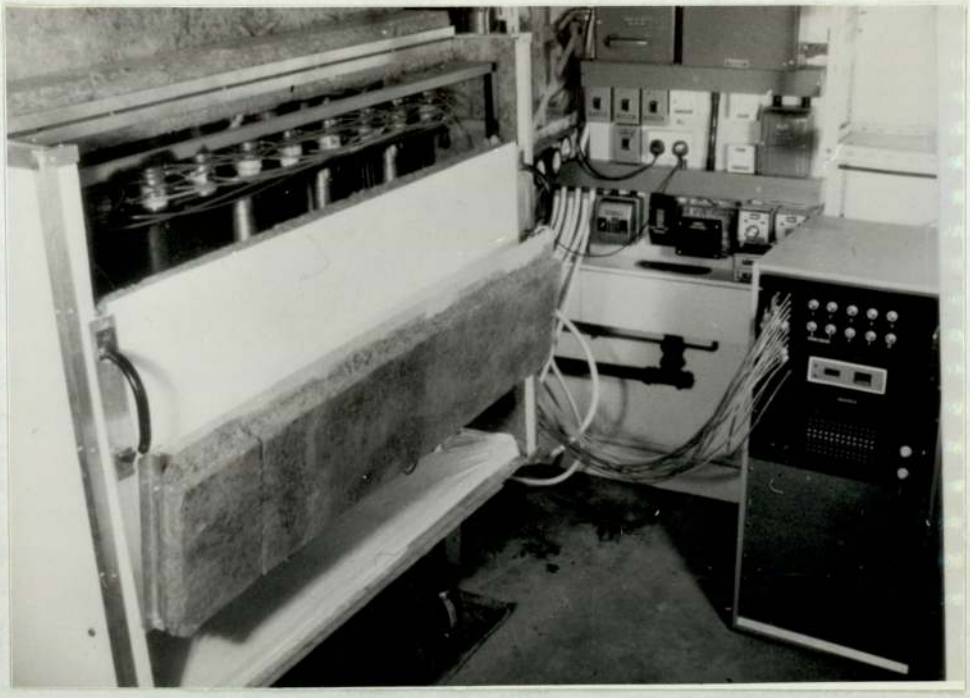
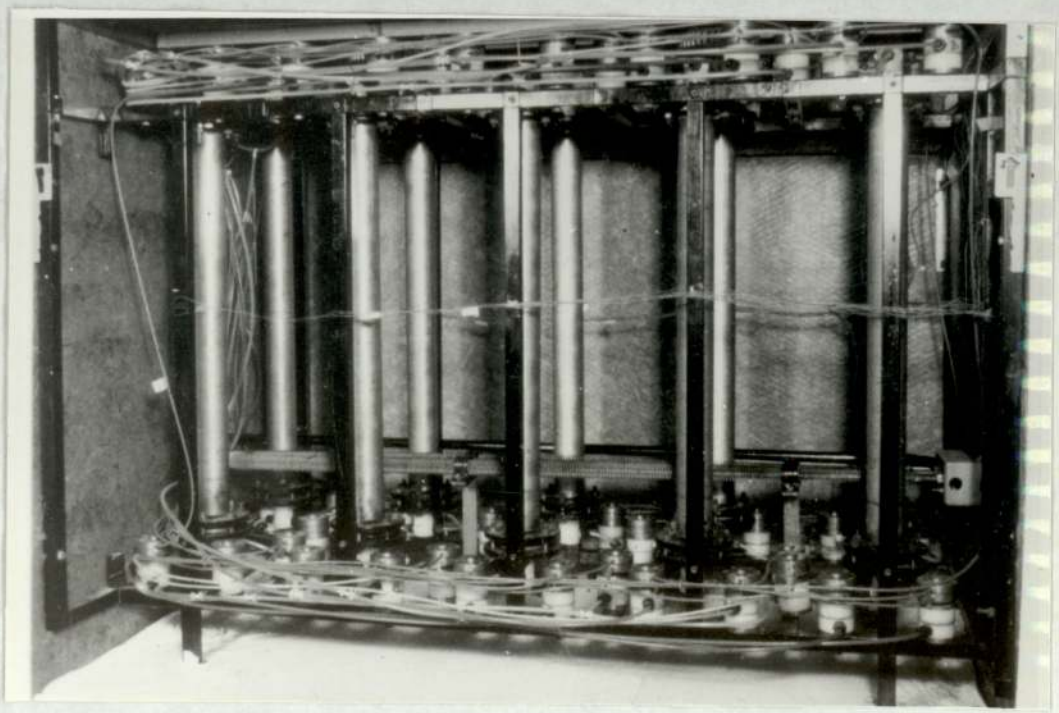


Fig 8.3 Photograph inside heating enclosure







supplied by Diamond H. Controls Ltd., Norwich. The input to the controller was from a mineral insulated thermocouple inserted through the enclosure wall.

Four hinged sections allowed easy access to the valves at the top and the bottom of the two rows of columns, as shown in Fig. 8.2. If necessary it was a simple matter to remove the complete side of the enclosure as Fig. 8.3 indicates.

8.1.4 Temperature Indication and Control

All the temperature indications and controller inputs were from nickel-chromium/nickel-aluminium thermocouples supplied by Comark Electronics Ltd., Rustington. Three different types of thermocouple were used; hypodermic probes, similar to hypodermic syringe needles that were inserted through the rubber septum sample points in the various inlet and outlet lines; a mineral insulated thermocouple, a probe approximately 25 cm long that was inserted through the enclosure wall to measure the inside air temperature; and self-adhesive patch thermocouples used to monitor the temperature of each of the individual columns. The electrical potentials from the thermocouples were sent either to the respective temperature controller or to an electronic thermometer where the temperature could be recorded.

8.2 ADDITIONAL EXPERIMENTAL TECHNIQUES

The operation of the fractionating columns at an elevated temperature added a further degree of complexity to the equipment. The various isolation switches and controls for the pumps and heaters are contained on the board to the right of the SCCR5 equipment shown in Fig. 8.2. The additional experimental techniques are reported in section 8.2.3.

8.2.1 Commissioning of Heating Equipment

A preliminary inspection ensured that the mobile phase heaters were correctly sealed; each heater was tested in turn to make sure that it was functioning and that the thermostat cut-out operated. Confirmation of to operability of the airotors and the air heater was also made. Simultaneous measurements were made of the air temperature at the top and the bottom of the enclosure when the controller set point was at 35°C and found to be 41°C and 39°C respectively. Water was then pumped to the column with the digital set point on the controller set at 37°C. The average volumetric flowrates to the columns were, from the purge head 353 cm^3 min^{-1} and from the eluent head 92.7 cm^3 min^{-1}. Fig. 8.4 shows the average temperature variation for a complete cycle of the SCCR5 equipment. It was also verified that the feed heat could maintain a constant temperature.

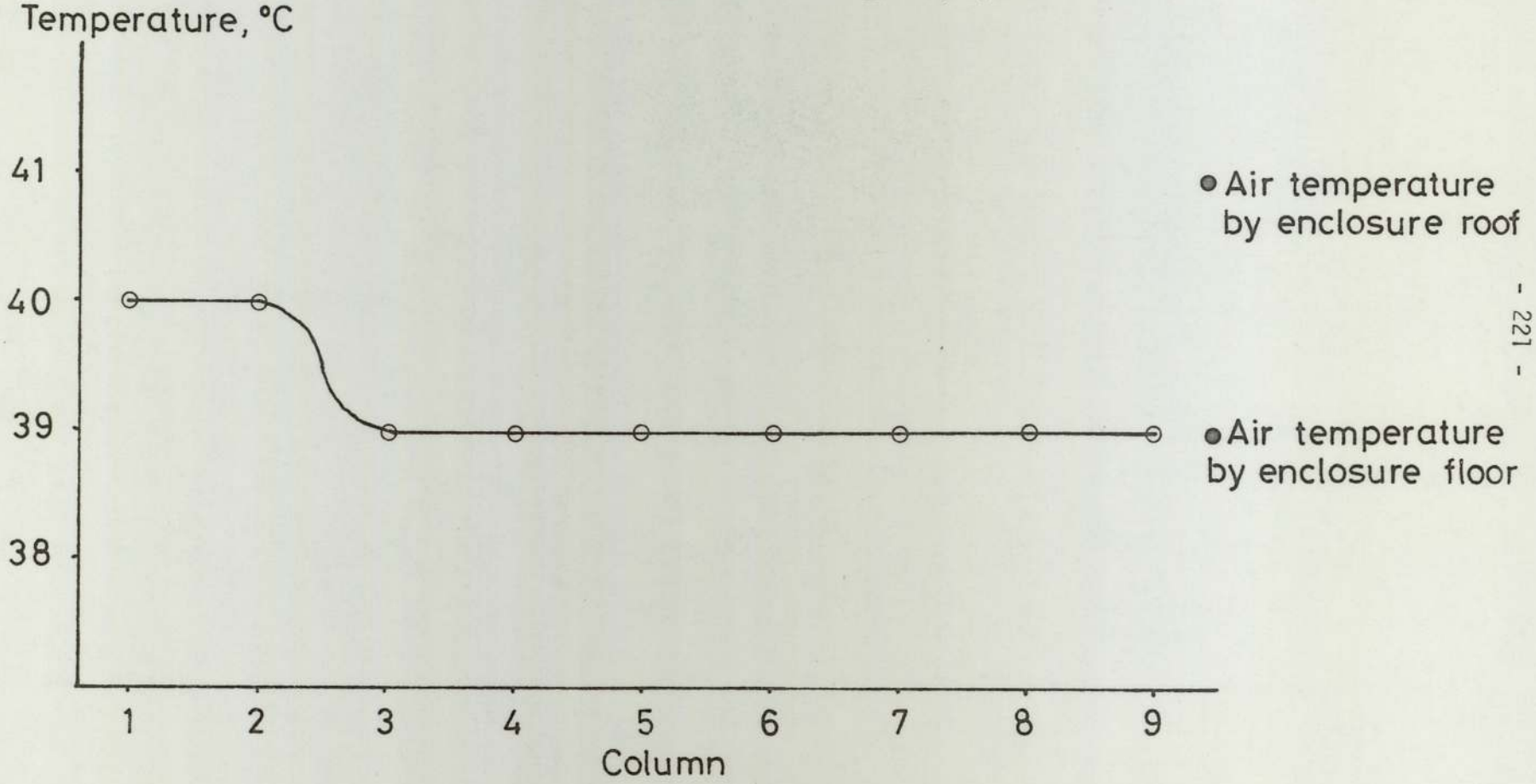
8.2.2 Temperature Measurements

Regular monitoring of the temperature of the inlet lines, the columns and a transfer line was performed. As indicated by Fig. 8.4 the temperatures of the columns altered slightly with their relative position from the inlet ports, because of this transient state temperatures were recorded at the same time through a switch. It was usual for the temperatures to be recorded at least three times per cycle.

8.2.3 Start-up and Shut-down Procedures

Amendments to the procedures outlined in section 6.3.3 for the start up of the SCCR5 are as follows:

Fig 8.4 Average column temperatures for commissioning of heating equipment.



(a) The experimental runs performed at elevated temperatures lasted for up to 56 hours. These experiments were performed continuously.

(b) The airotors and air heater inside the enclosure were turned on, and the temperature controller set.

(c) The pneumatic supply to the chromatograph was turned on and the mobile phase and purge pumps started. The water heaters were turned on and the temperature controller set. Two cycles of the chromatograph were made during which time the flowrates were adjusted.

(d) The start of the experiment was marked by switching feed pump on. The variable rheostat on the feed heater was adjusted.

At the end of the experiment the liquid heaters were turned off approximately one minute before the end of the final switch. The pumps were turned off at the end of the switch and the delivery lines closed. The liquid pressures were allowed to decay. The stroke mechanism for the mobile phase pump was zeroed and purging of the equipment began immediately. The delivery line to the purge head was opened, the water heater turned on and the individual columns were purged.

In the event of a failure of a component inside the enclosure both the liquid heaters were switched off along with the pumps and a note of the switch time was made. The actuating pressure to the machine was turned off isolating the columns. When the machine was operating at 45°C valves could be repaired in situ but at 60°C or above they were removed and replaced.

8.3 EXPERIMENTAL PROGRAMME

8.3.1 Scope

The industrial interest associated with this project required that an evaluation of the SCCR5's capabilities to fractionate dextran at both extremes of the molecular weight range at an elevated temperature be made. Two batches of dextran feed material were used. The aims of the experimental programme were:-

(1) to use experiment B, reported in Chapter 6, as a datum to evaluate the effect of elevating the temperature of the fractionating column upon the pseudo-steady state condition of the equipment or the final quality of the fractionated material.

(2) to determine if the feed to eluent ratio could be increased at an elevated temperature for high molecular weight removal and what effect this would have upon the final product quality.

(3) to study the effect of the theoretical cut position and temperature upon low molecular weight fraction removal.

(4) to reduce the volume of liquid passed through the purge section.

8.3.2 Experimental Operating Conditions

Fig. 8.5 summarises the average operating conditions for experiments with an aim of removing a high molecular weight fraction from the feed material. Fig. 8.6 contains the average experimental conditions for the experiments where the removal of low molecular weight dextran was attempted.

Experiments E and J repeated the same operating conditions as experiment B, reported in section 6, at 45 and 62°C respectively. A reduction in the eluent-to-feed ratio from a nominal 2.5 to 1.8 was made

Fig 8.5 Summary of operating conditions for high molecular weight removal at elevated temperatures.

Experiment	Switch-time s.	Conc. of feed $g.l.^{-1}$	Feedrate per		Eluent to feed ratio.	Flowrates $cm.^3 min.^{-1}$			Theoretical cut-position		
			cycle	hour		eluent	feed	purge	pre-feed	post-feed	purge
E	450	229	677·1	541·7	2·57	101·5	39·5	303	0·303	0·758	2·624
G	600	232	956·7	574·0	1·82	74·9	41·2	269	0·284	0·917	3·265
J	450	224	674·6	539·7	2·49	100·0	40·1	307	0·286	0·748	2·671

Fig 8.6 Summary of operating conditions for low molecular weight removal at elevated temperatures.

Experiment	Switch-time. s.	Conc. of feed. g. l^{-1}	Feedrate per		Eluent to feed ratio.	Flowrates $cm.^3 min.^{-1}$			Theoretical cut-position		
			cycle	hour		eluent	feed	purge	pre-feed	post-feed	purge
F	1200	232	492·2	147·7	4·94	52·4	10·6	177	0·743	1·069	4·571
H	1100	256	492·8	161·3	4·96	52·1	10·5	177	0·601	0·897	4·118
I	450	256	234·3	187·4	11·45	139·7	12·2	345	0·743	0·883	3·108
K	450	234	238·9	191·1	9·94	135·2	13·6	319	0·691	0·848	2·809
L	450	285	292·7	234·1	9·89	135·5	13·7	301	0·695	0·853	2·601

for experiment G whilst keeping the same pre-feed theoretical out-position as experiment B. Experiment G was performed at 62°C.

Five experiments were performed at elevated temperatures with the aim of the removal of a low molecular weight fraction from the feed. Three of these experiments, H, K and L only suceeded in fractionating the feed material into two products of approximately equal masses although the molecular weight distributions of the products were different. The same operating conditions as experiment D were repeated at 45°C in experiment F to study the effect of the elevated temperature on the fractionating capabilities of the SCCR5. In an attempt to remove slightly more of feed material in the low molecular weight product a change in both the theoretical cut positions were made in experiment H. Experiment I used a new batch of feed material BT216G and repeated the theoretical cut positions of experiment C. Experiment K use a pre-feed theoretical cut position that was expected to remove more of the low molecular weight material in the purge stream than experiment I. Experiment L repeated the flow conditions of experiment K at 62°C.

Figs. 8.7 and 8.8 summarises the changes that were made in the experimental operating conditions and the objectives, for the high and the low molecular weight removal programmes. Details of the experimental operating conditions, products and purged products are contained in Figs. 8.9 to 8.32.

8.4 RESULTS AND DISCUSSION

8.4.1 High Molecular Weight Fractionations

The pseudo-steady state concentration profiles for experiments B, E and J are illustrated in Figs. 8.33 to 8.35. The outstanding feature of the three concentration profiles was the variation in the

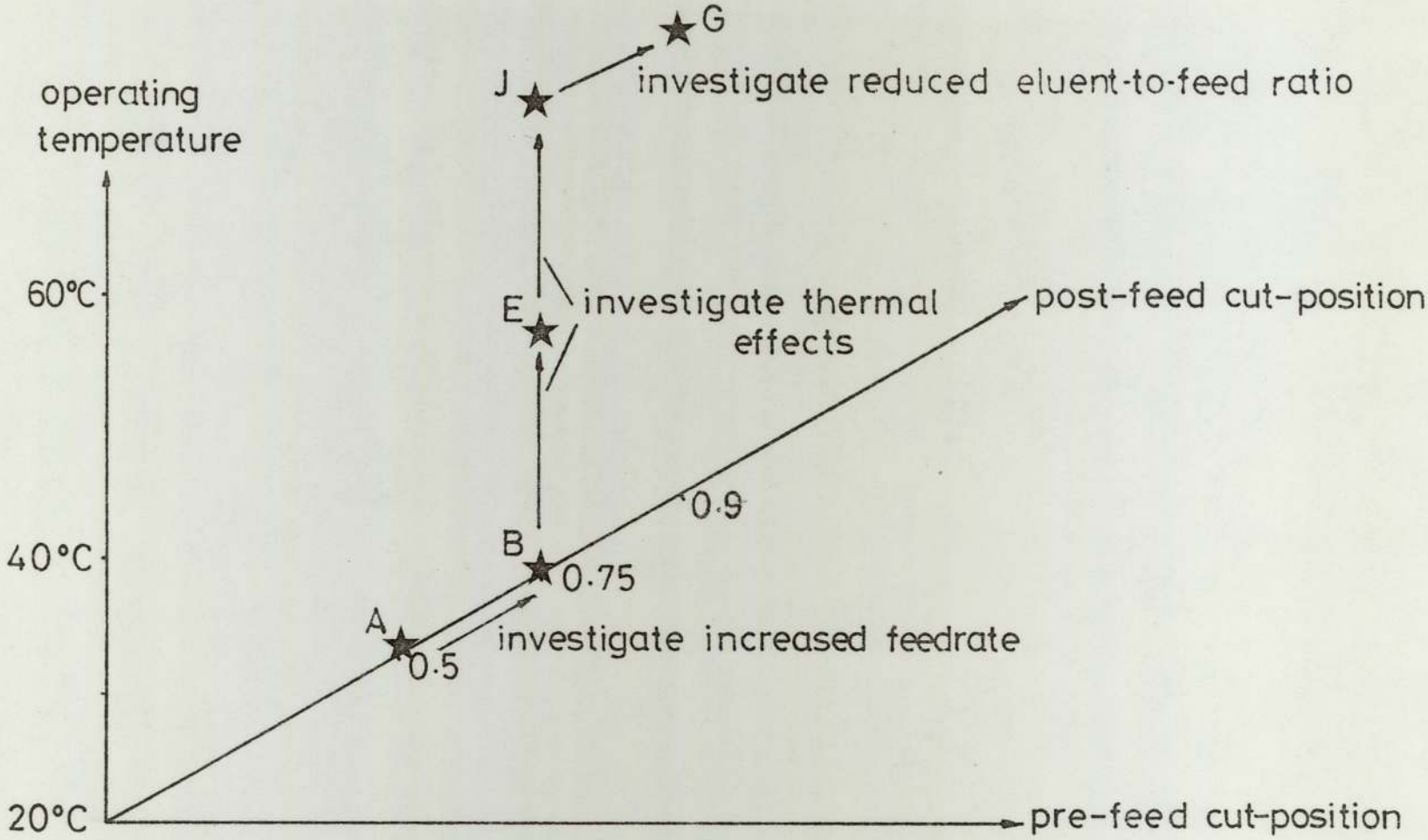


Fig 8.7 High molecule weight removal experiments.

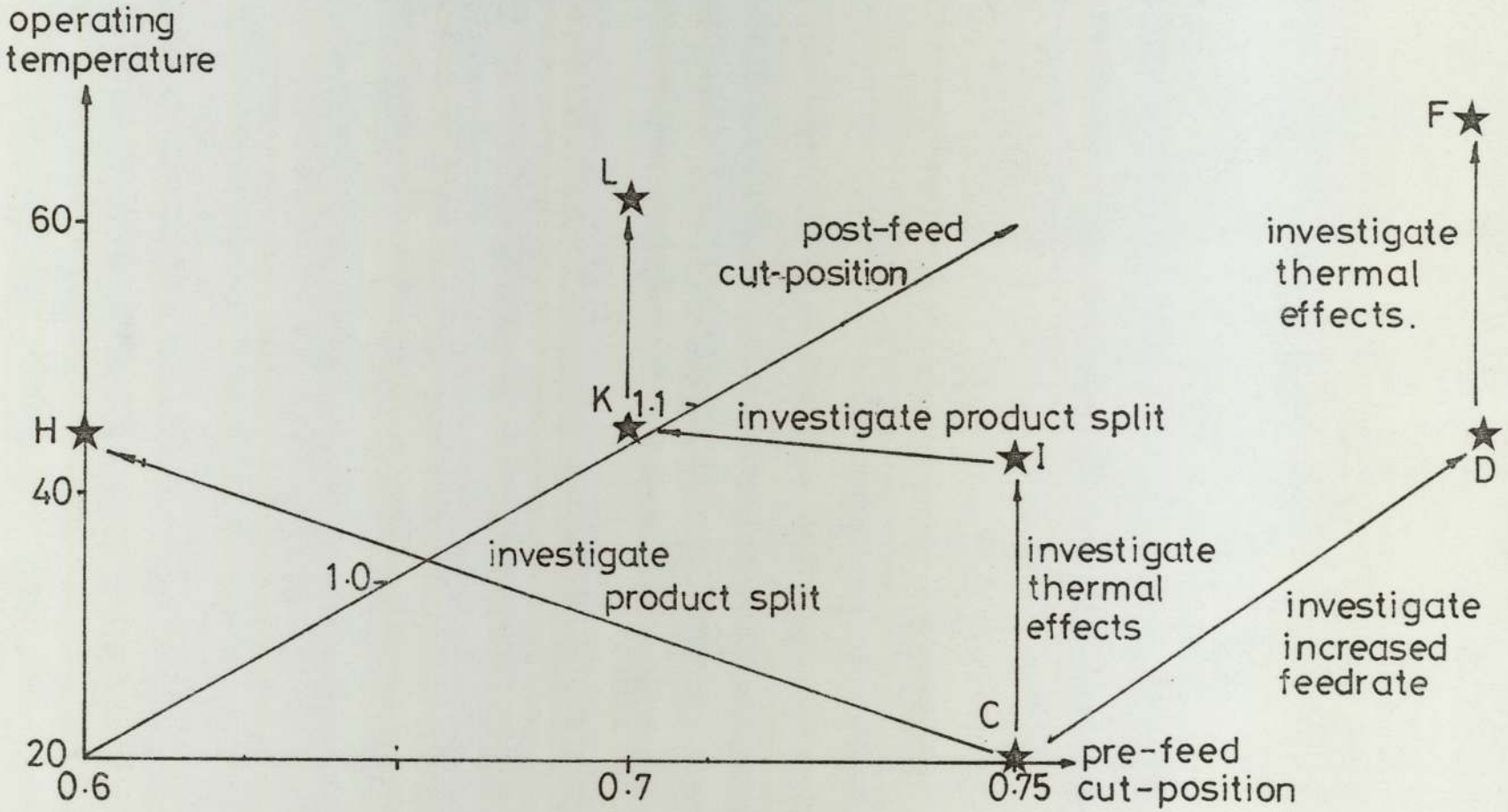


Fig 8.8 Low molecular removal experiments.

Fig 8.9 Operating conditions for experiment E.

cycle	Pressures			Temperatures				Input flowrates			Product flows		Cut-positions		
	eluent	feed	purge	purge	eluent	feed	in-line	L_1	L_2	L_3	HMW	LMW	pre-feed	post-feed	purge
	kN.m2	kN.m2	kN.m2	°c	°c	°c	°c	ml.m^{-1}	ml.m^{-1}	ml.m^{-1}	ml.m^{-1}	ml.m^{-1}			
11 + 12	1080	520	55	43	46	37	46	102·3	38·7	141·0	141·7	299	0·312	0·758	2·578
13 + 14	960	440	55	43	46	37	45	101·8	36·8	138·6	132·5	306	0·306	0·730	2·659
15 + 16	960	440	55	43	46	45	46	102·0	38·6	140·6	137·8	302	0·309	0·753	2·613
17 + 18	1020	480	55	42	46	44	45	101·5	39·9	141·4	138·9	303	0·303	0·763	2·624
19 + 20	1010	490	55	43	46	43	45	101·0	40·0	140·0	139·3	304	0·297	0·738	2·636

Fig 8.10 Products from experiment E.

Cycles	Dextran Input		High Mol. Wt. Product			Low Mol. Wt. Product			H.M.W.P. Output	L.M.W.P. Output	Mass Balance
	Per Cy.	Per hr.	Conc. g.l.$^{-1}$	Vol. l.	Mass. g.	Conc. g.l.$^{-1}$	Vol. l.	Mass g.			
13 + 14	631·0	504·8	7·460	19·84	148·0	26·79	44·23	1185·0	0·111	0·899	1·056
15 + 16	661·5	529·2	10·28	19·84	204·0	26·70	43·55	1163·0	0·149	0·851	1·033
17 + 18	684·5	547·6	9·778	19·84	194·0	28·53	44·23	1262·0	0·133	0·867	1·064
19 + 20	685·5	548·4	10·72	20·53	220·0	27·88	40·60	1132·0	0·163	0·837	0·987

	High Mol. Wt. Product			Low Mol. Wt. Product		
	M_w	M_n	D	M_w	M_n	D
13 + 14	136 800	46 700	2·929	47 300	25 500	1·855
15 + 16	129 100	45 500	2·836	50 700	26 500	1·910
17 + 18	135 000	43 300	3·120	44 300	23 800	1·858
19 + 20	145 700	44 900	3·247	51 100	27 300	1·873

Fig 8.11 Purge products from experiment E.

Column	Purged Volume, l	Purge Conc., $g\,l^{-1}$	Dextran Mass, g	Column Conc., $g\,l^{-1}$	Col. conc. / Feed conc.	M_w	M_n	D
1	2·025	22·22	49·0	40·33	0·176	44 500	24000	1·86
2	2·222	76·06	169·0	139·1	0·609	50400	24400	2·06
3	2·166	110·5	239·0	196·7	0·861	51900	25 200	2·06
4	2·172	114·7	249·0	204·9	0·897	57700	25700	2·25
5	2·160	102·9	222·0	182·7	0·799	58400	26500	2·21
6	2·219	63·86	142·0	116·9	0·512	69600	29500	2·36
7	2·245	35·33	79·0	65·02	0·284	78800	32800	2·40
8	2·308	22·09	51·0	41·98	0·184	82800	35500	2·33
9	2·281	14·46	33·0	27·16	0·119	88600	36400	2·44

Fig 8.12 Operating conditions for experiment F

cycle	Pressures			Temperatures				Input flowrates			Product flows		Cut-positions		
	eluent	feed	purge	purge	eluent	feed	in-line	L_1	L_2	L_3	HMW	LMW	pre-feed	post-feed	purge
	kN.m-2	kN.m-2	kN.m-2	°C	°C	°C	°C	ml.m-1	ml.m-1	ml.m-1	ml.m-1	ml.m-1			
7+8	930	830	30	45	43	45	45	51·8	10·6	62·4	61·3	177	0·725	1·051	4·571
9+10	1020	910	30	44	43	45	45	53·1	10·5	63·6	63·6	176	0·765	1·078	4·531
11+12	990	910	30	44	43	45	45	53·0	10·7	63·7	66·7	183	0·753	1·091	4·747
13+14	810	720	30	45	43	45	45	52·4	10·7	63·1	63·0	175	0·734	1·063	4·500
15+16	860	740	30	46	43	46	46	52·1	10·4	62·5	61·3	179	0·725	1·045	4·623

Fig 8.13 Products from experiment F.

Cycles	Dextran Input		High Mol. Wt. Product			Low Mol. Wt. Product			H.M.W.P. Output	L.M.W.P. Output	Mass Balance
	Per Cy.	Per hr.	Conc. g.l^{-1}	Vol. l.	Mass. g.	Conc. g.l^{-1}	Vol. l.	Mass g.			
9 + 10	487.5	146.3	35.28	25.74	908.0	0.697	70.31	49.0	0.949	0.051	0.981
11 + 12	521.5	156.5	44.24	26.99	1194.0	0.868	72.58	63.0	0.950	0.050	1.205
13 + 14	497.0	149.1	46.02	25.51	1174.0	0.659	69.85	46.0	0.963	0.037	1.227
15 + 16	483.0	144.9	35.20	24.83	874.0	0.911	69.17	63.0	0.933	0.063	0.970

	High Mol. Wt. Product			Low Mol. Wt. Product		
	M_w	M_n	D	M_w	M_n	D
9 + 10	61 800	24000	2.58	41 200	18 200	2.26
11 + 12	68 400	24000	2.85	38 700	16 800	2.30
13 + 14	69 000	25000	2.76	43 200	18 800	2.30
15 + 16	69 300	25 600	2.71	36 200	16 800	2.15

Fig 8.14 Purge products from experiment F.

Column	Purged Volume, l	Purge Conc., $g\,l^{-1}$	Dextran Mass, g	Column Conc., $g\,l^{-1}$	Col. conc. / Feed conc.	M_w	M_n	D
1	3.045	2.036	6.20	5.103	0.022			
2	3.136	24.21	75.90	62.47	0.269	24800	13800	1.80
3	2.905	54.33	157.8	129.9	0.559	31800	16300	1.95
4	3.091	75.67	233.9	192.5	0.829	37200	19300	1.94
5	3.273	74.52	243.9	200.7	0.865	41200	19200	2.14
6	3.010	85.58	257.6	212.0	0.913	42100	19700	2.14
7	3.098	83.96	260.1	214.1	0.922	43100	20100	2.14
8	2.895	89.64	259.5	213.6	0.920	42700	20000	2.14
9	3.431	79.63	273.2	224.9	0.969	42700	20400	2.09

Fig 8.15 Operating conditions for experiment G.

cycle	Pressures			Temperatures				Input flowrates			Product flows		Cut-positions		
	eluent	feed	purge	purge	eluent	feed	in-line	L	L	L	HMW	LMW	pre-feed	post-feed	purge
	kN.m-2	kN.m-2	kN.m2.	°c	°c	°c	°c	ml.m-1.	ml.m-1.	ml.m-1.	ml.m-1	ml.m-1.			
5+6	730	430	35	61	63	63	61	74·1	42·0	116·1	109	256	0·272	0·917	3·066
7+8	790	490	40	60	64	64	63	75·6	41·3	116·9	112	278	0·295	0·929	3·404
9+10	780	490	35	61	64	64	63	75·7	40·9	116·6	107	261	0·296	0·925	3·143
11+12	740	480	40	60	63	62	61	74·8	41·7	116·5	104	278	0·283	0·923	3·404
13	720	460	40	61	62	63	62	75·7	39·1	114·8	101	284	0·296	0·897	3·496

Fig 8.16 Products from experiment G.

Cycles	Dextran Input		High Mol. Wt. Product			Low Mol. Wt. Product			H.M.W.P. Output	L.M.W.P. Output	Mass Balance
	Per Cy.	Per hr.	Conc. g/l.	Vol. l.	Mass. g.	Conc. g/l.	Vol. l.	Mass g.			
7 + 8	959.0	575.4	13.88	20.75	288.0	25.07	55.57	1393.0	0.171	0.829	0.876
9 + 10	949.5	569.7	13.31	20.75	276.0	25.64	51.71	1326.0	0.172	0.828	0.844
11 + 12	968.5	581.1	12.03	20.75	250.0	26.75	55.34	1480.0	0.145	0.855	0.893
13	908.0	544.8	11.61	9.98	116.0	25.49	28.12	717.0	0.139	0.861	0.917

	High Mol. Wt. Product			Low Mol. Wt. Product		
	M_w	M_n	D	M_w	M_n	D
7 + 8	158700	45700	3.47	48200	25000	1.96
9 + 10	162400	48700	3.34	45400	24200	1.87
11 + 12	165200	48400	3.41	48000	24900	1.93
13	173500	49600	3.50	48000	25500	1.88

Fig 8.17 Purge products from experiment G.

Column	Purged Volume, l	Purge Conc., $g\,l^{-1}$	Dextran Mass, g	Column Conc., $g\,l^{-1}$	Col. conc. / Feed conc.	M_w	M_n	D
1	2·875	27·04	77·74	63·98	0·276	49900	25700	1·94
2	2·970	56·09	166·5	137·0	0·590	53400	25700	2·08
3	2·945	62·08	182·8	150·5	0·648	53900	26000	2·08
4	2·565	73·32	188·1	154·8	0·667	53 400	25 200	2·12
5	2·820	65·28	184·1	151·5	0·653	65600	26700	2·46
6	3·008	38·97	117·2	96·46	0·416	71600	29600	2·42
7	2·740	30·85	84·53	69·57	0·300	77900	34400	2·27
8	2·645	24·14	63·85	52·55	0·227	80 800	35600	2·27
9	2·923	13·84	40·45	33·29	0·143	88100	36500	2·42

Fig 8.18 Operating conditions for experiment H.

cycle	Pressures			Temperatures				Input flowrates			Product flows		Cut-positions		
	eluent	feed	purge	purge	eluent	feed	in-line	L_1	L_2	L_3	HMW	LMW	pre-feed	post-feed	purge
	kN.m-2	kN.m-2	kN.m-2	°C	°C	°C	°C	ml.m-1	ml.m-1	ml.m-1	ml.m-1	ml.m-1			
5 + 6	540	400	20	44	44	46	46	51·5	10·6	62·1	63	177	0·584	0·887	4·118
7 + 8	620	460	20	44	44	46	46	51·6	10·6	62·2	63	175	0·586	0·885	4·062
9 + 10	690	490	20	44	44	46	46	51·3	10·4	61·7	62	180	0·578	0·871	4·203
11 + 12	720	520	20	46	46	46	46	52·9	10·5	63·4	63	177	0·623	0·919	4·118
13 + 14	720	550	20	45	45	46	45	52·0	10·6	62·6	63	174	0·598	0·897	4·034

Fig 8.19 Products from experiment H.

Cycles	Dextran Input		High Mol. Wt. Product			Low Mol. Wt. Product			H.M.W.P.	L.M.W.P.	Mass
	Per Cy.	Per hr.	Conc.g.l.$^{-1}$	Vol. l.	Mass. g.	Conc.g.l.$^{-1}$	Vol. l.	Mass g.	Output	Output	Balance
7 + 8	497.0	162.7	19.57	23.02	450.4	5.50	63.28	348.0	0.564	0.436	0.806
9 + 10	488.0	159.7	19.81	23.02	456.0	7.43	63.73	473.5	0.491	0.509	0.952
11 + 12	493.0	161.3	19.77	23.25	459.6	7.16	63.50	454.7	0.503	0.497	0.927
13 + 14	497.0	162.7	20.65	23.02	475.4	6.28	65.77	413.0	0.535	0.465	0.894

	High Mol. Wt. Product			Low Mol. Wt. Product		
	M_w	M_n	D	M_w	M_n	D
7 + 8	42 900	25 600	1.677	27 200	19 500	1.393
9 + 10	46 000	25 900	1.778	30 300	20 500	1.477
11 + 12	45 700	26 400	1.732	29 200	20 200	1.448
13 + 14	45 800	25 900	1.770	28 300	19 600	1.442

Fig 8.20 Purge products from experiment H.

Column	Purged Volume, l	Purge Conc., $g\,l^{-1}$	Dextran Mass, g	Column Conc., $g\,l^{-1}$	Col. conc. / Feed conc.	M_w	M_n	D
1	3.280	4.622	15.16	12.48	0.049	28600	20200	1.41
2	2.995	44.00	131.8	108.5	0.434	27400	19900	1.38
3	3.215	76.81	246.9	203.2	0.794	28100	20200	1.39
4	3.160	78.40	247.7	203.9	0.797	33800	20700	1.65
5	3.140	81.87	257.1	211.6	0.827	32900	20500	1.61
6	3.075	83.08	255.5	210.3	0.822	31700	19900	1.60
7	3.100	76.65	237.6	195.6	0.764	28600	18400	1.58
8	3.135	71.70	224.8	185.0	0.723	34000	21900	1.55
9	3.105	57.14	177.4	146.0	0.570	33000	20900	1.58

Fig 8.21 Operating conditions for experiment I.

cycle	Pressures			Temperatures				Input flowrates			Product flows		Cut-positions		
	eluent	feed	purge	purge	eluent	feed	in-line	L_1	L_2	L_3	H.M.W.	L.M.W	pre-feed	post-feed	purge
	kN.m-2	kN.m-2	kN.m-2	°c	°c	°c	°c	ml.m-1	ml.m-1	ml.m-1	ml.m-1	ml.m-1			
7 + 8	480	410	70	43	45	46	46	138·7	12·3	151·0	154	345	0·732	0·873	3·108
9 + 10	580	510	70	43	45	46	46	140·5	12·2	152·7	156	347	0·752	0·893	3·131
11 + 12	630	560	70	43	45	46	46	139·2	12·2	151·4	156	347	0·737	0·878	3·131
13 + 14	660	580	70	44	45	46	45	139·7	12·2	151·9	155	348	0·743	0·883	3·143
15 + 16	700	610	70	43	45	46	46	140·3	12·2	152·5	153	340	0·750	0·891	3·051

Fig 8.22 Products from experiment I.

Cycles	Dextran Input		High Mol. Wt. Product			Low Mol. Wt. Product			H.M.W.P. Output	L.M.W.P. Output	Mass Balance
	Per Cy.	Per hr.	Conc. g.l.$^{-1}$	Vol. l.	Mass. g.	Conc. g.l.$^{-1}$	Vol. l.	Mass g.			
9 + 10	234.3	187.4	15.01	23.70	355.7	0.451	50.80	22.9	0.940	0.060	0.808
11 + 12	234.3	187.4	14.16	23.25	329.2	0.429	50.80	21.8	0.938	0.062	0.737
13 + 14	234.3	187.4	16.82	23.25	391.1	0.390	50.80	19.8	0.952	0.048	0.877
15 + 16	234.3	187.4	17.88	23.70	423.8	0.390	51.26	20.0	0.955	0.045	0.947

	High Mol. Wt. Product			Low Mol. Wt. Product		
	M_w	M_n	D	M_w	M_n	D
9 + 10	36300	23800	1.53	26600	18800	1.41
11 + 12	35500	23100	1.54	25900	19000	1.36
13 + 14	36800	23600	1.56	25800	19500	1.32
15 + 16	36600	23900	1.53	26000	19000	1.36

Fig 8.23 Purge products from experiment I.

Column	Purged Volume, l	Purge Conc., $g\,l^{-1}$	Dextran Mass, g	Column Conc., $g\,l^{-1}$	Col. conc. / Feed conc.	M_w	M_n	D
1	2·700	0·70	1·89	1·56	0·006	26600	19100	1·39
2	2·726	7·17	19·55	16·09	0·063	26600	16100	1·65
3	2·835							
4	2·623	31·78	83·36	68·16	0·268	24000	18700	1·28
5	2·750	67·27	185·0	152·2	0·595	25400	19500	1·30
6	2·175	68·42	185·8	152·9	0·597	27400	20600	1·33
7	2·720	66·54	181·0	149·0	0·582	28700	21200	1·35
8	2·858	59·06	168·8	138·9	0·543	29200	21600	1·35
9	2·975	40·39	120·2	98·93	0·386	28000	21400	1·31

Fig 8.24 Operating conditions for experiment J.

cycle	Pressures			Temperatures				Input flowrates			Product flows		Cut-positions		
	eluent	feed	purge	purge	eluent	feed	in-line	L_1	L_2	L_3	HMW	LMW	pre-feed	post-feed	purge
	kN.m-2	kN.m-2	kN.m2	°c	°c	°c	°c	ml.m-1	ml.m-1	ml.m-1	ml.m-1	ml.m-1			
7 + 8	580	330	40	62	62	62	62	101·7	40·7	142·4	137	298	0·305	0·774	2·578
9 + 10	620	330	40	63	64	63	62	99·3	40·7	140·0	135	295	0·278	0·747	2·532
11 + 12	670	370	50	63	63	63	62	99·5	41·7	141·2	136	309	0·280	0·760	2·694
13 + 14	690	360	50	62	62	64	62	100·0	40·0	140·0	134	305	0·286	0·747	2·647
15 + 16	670	370	50	63	63	63	62	100·6	38·6	139·2	133	306	0·293	0·737	2·659

Fig 8.25 Products from experiment J.

Cycles	Dextran Input		High Mol. Wt. Product			Low Mol. Wt. Product			H.M.W.P. Output	L.M.W.P. Output	Mass Balance
	Per Cy.	Per hr.	Conc.g/l.	Vol. l.	Mass. g.	Conc.g/l.	Vol. l.	Mass g.			
9 + 10	684.7	547.8	7.44	20.07	149.3	25.32	44.00	1114.1	0.118	0.882	0.923
11 + 12	701.5	561.2	8.25	20.07	165.6	24.51	46.72	1145.1	0.126	0.874	0.934
13 + 14	672.9	538.3	7.93	19.85	157.3	24.76	45.59	1128.7	0.122	0.878	0.956
15 + 16	649.4	519.5	8.30	19.85	164.7	25.48	46.04	1173.1	0.125	0.875	1.032

	High Mol. Wt. Product			Low Mol. Wt. Product		
	M_w	M_n	D	M_w	M_n	D
9 + 10	160600	45900	3.501	44500	23500	1.893
11 + 12	153600	48300	3.178	44900	23900	1.882
13 + 14	133400	44900	2.970	47300	24900	1.898
15 + 16	151700	47500	3.197	49500	24600	2.010

Fig 8.26 Purge products from experiment J.

Column	Purged Volume, l	Purge Conc., $g\,l^{-1}$	Dextran Mass, g	Column Conc., $g\,l^{-1}$	Col. conc. / Feed conc.	M_w	M_n	D
1	2·775	18·27	50·70	41·73	0·181	54200	22600	2·40
2	2·725	32·81	89·42	73·60	0·319	53300	21000	2·54
3	3·075	42·24	129·9	106·9	0·477	56700	21100	2·69
4	3·270	38·78	126·8	104·4	0·465	57800	21600	2·68
5	3·215	36·86	118·5	97·53	0·435	64800	23000	2·82
6	3·035	25·64	77·82	64·05	0·286	70300	24800	2·84
7	3·025	15·60	47·19	38·84	0·173	94000	29500	3·19
8	2·800	12·43	34·80	28·64	0·128	97500	31100	3·14
9	3·360	8·78	29·50	24·28	0·108	106500	31000	3·44

Fig 8.27 Operating conditions for experiment K.

cycle	Pressures			Temperatures				Input flowrates			Product flows		Cut-positions		
	eluent	feed	purge	purge	eluent	feed	in-line	L_1	L_2	L_3	HMW	LMW	pre-feed	post-feed	purge
	kN.m-2	kN.m-2	kN.m-2	°c	°c	°c	°c	ml.m-1	ml.m-1	ml.m-1	ml.m-1	ml.m-1			
7 + 8	770	610	60	46	46	45	45	135·2	13·7	148·9	147	323	0·691	0·849	2·855
9 + 10	930	580	60	46	46	44	45	135·0	13·8	148·8	147	316	0·689	0·848	2·774
11 + 12	1010	640	60	46	46	44	45	135·6	13·8	149·4	148	319	0·696	0·855	2·809
13 + 14	1050	650	60	46	45	44	45	135·0	13·6	148·6	147	320	0·691	0·846	2·820
15 + 16	1020	620	60	45	46	45	46	134·9	13·5	148·4	147	318	0·688	0·843	2·797

Fig 8.28 Products from experiment K.

Cycles	Dextran Input		High Mol. Wt. Product			Low Mol. Wt. Product			H.M.W.P. Output	L.M.W.P. Output	Mass Balance
	Per Cy.	Per hr.	Conc. g.l.$^{-1}$	Vol. l.	Mass. g.	Conc. g.l.$^{-1}$	Vol. l.	Mass g.			
9 + 10	241·8	193·4	10·81	21·89	236·6	3·16	46·72	147·6	0·616	0·384	0·795
11 + 12	241·8	193·4	12·40	22·34	277·0	3·83	47·85	183·3	0·602	0·398	0·952
13 + 14	238·3	190·6	11·78	22·11	260·5	5·12	46·72	239·2	0·522	0·479	1·049
15 + 16	236·5	189·2	11·75	22·11	259·8	4·75	47·85	227·3	0·533	0·467	1·030

	High Mol. Wt. Product			Low Mol. Wt. Product		
	M_w	M_n	D	M_w	M_n	D
9 + 10	41900	25500	1·64	21700	17300	1·25
11 + 12	42600	25400	1·68	23300	18300	1·27
13 + 14	39400	24700	1·59	24900	19000	1·31
15 + 16	40100	24900	1·61	23300	18800	1·24

Fig 8.29 Purged products from experiment K.

Column	Purged Volume, l	Purge Conc., $g\,l^{-1}$	Dextran Mass, g	Column Conc., $g\,l^{-1}$	Col. conc. / Feed conc.	M_w	M_n	D
1	3·140	2·80	8·80	7·24	0·031	23700	18100	1·31
2	3·315	27·22	90·2	74·24	0·317	27000	19200	1·41
3	3·095	62·16	192·4	158·4	0·676	27200	19300	1·41
4	3·070	76·92	236·1	194·3	0·831	27800	19300	1·44
5	3·278	73·29	240·2	197·7	0·848	28500	19500	1·46
6	3·125	67·02	209·4	172·3	0·736	32400	22800	1·42
7	3·250	49·23	160·4	132·0	0·565	32900	21700	1·51
8	3·180	39·01	124·1	102·1	0·437	33500	18400	1·83
9	3·450	23·42	80·8	66·50	0·287	32500	23800	1·36

Fig 8.30 Operating conditions for experiment L.

cycle	Pressures			Temperatures				Input flowrates			Product flows		Cut-positions		
	eluent	feed	purge	purge	eluent	feed	in-line	L_1	L_2	L_3	H.M.W.	L.M.W.	pre-feed	post-feed	purge
	kN.m-2	kN.m-2	kN.m2	°c	°c	°c	°c	ml.m-1.	ml.m-1.	ml.m-1.	ml.m-1.	ml.m-1.			
7 + 8	700	510	40	61	62	65	62	135·0	13·5	148·5	147	300	0·689	0·847	2·590
9 + 10	820	520	40	61	61	64	62	135·4	13·8	149·2	148	314	0·694	0·854	2·751
11 + 12	840	540	40	62	62	65	62	135·5	13·7	149·2	148	301	0·695	0·854	2·601
13 + 14	840	520	40	61	61	65	61	135·3	13·7	149·0	145	302	0·692	0·850	2·163
15 + 16	830	520	40	61	61	65	61	135·6	13·7	149·3	147	300	0·696	0·854	2·590

Fig 8.31 Products from experiment L.

Cycles	Dextran Input		High Mol. Wt. Product			Low Mol. Wt. Product			H.M.W.P. Output	L.M.W.P. Output	Mass Balance
	Per Cy.	Per hr.	Conc. g.l.$^{-1}$	Vol. l.	Mass. g.	Conc. g.l.$^{-1}$	Vol. l.	Mass g.			
9 + 10	294·8	235·8	11·40	21·67	246·9	6·16	45·59	280·8	0·469	0·532	0·898
11 + 12	292·7	234·1	12·48	21·89	273·1	5·20	44·00	220·9	0·553	0·447	0·844
13 + 14	292·7	234·1	11·33	21·21	240·3	7·61	43·77	333·1	0·419	0·581	0·980
15 + 16	292·7	234·1	12·65	21·89	276·9	5·76	48·76	280·6	0·497	0·503	0·953

	High Mol. Wt. Product			Low Mol. Wt. Product		
	M_w	M_n	D	M_w	M_n	D
9 + 10	37700	24700	1·53	24800	18500	1·34
11 + 12	38400	24500	1·57	22900	18500	1·24
13 + 14	39900	25100	1·59	23700	18900	1·25
15 + 16	39500	25100	1·57	23500	19000	1·24

Fig 8.32 Purged products from experiment L.

Column	Purged Volume, l	Purge Conc., $g\,l^{-1}$	Dextran Mass, g	Column Conc., $g\,l^{-1}$	Col. conc. / Feed conc.	M_w	M_n	D
1	3·248	2·85	9·26	7·62	0·027	23900	18700	1·28
2	3·147	27·12	85·35	70·25	0·247	25000	19200	1·30
3	3·345	64·19	214·7	176·7	0·621	25900	19200	1·35
4	3·065	83·11	254·7	209·6	0·736	26300	19000	1·39
5	3·305	76·82	253·9	209·0	0·734	29200	20200	1·45
6	3·450	63·78	220·0	181·1	0·636	31400	21000	1·50
7	3·570	48·79	174·2	143·4	0·503	31400	21400	1·47
8	3·155	45·91	144·9	119·2	0·419	30400	21400	1·42
9	3·180	32·11	102·1	84·04	0·295	32800	22200	1·48

Fig 8.33 Concentration profile for experiment B.

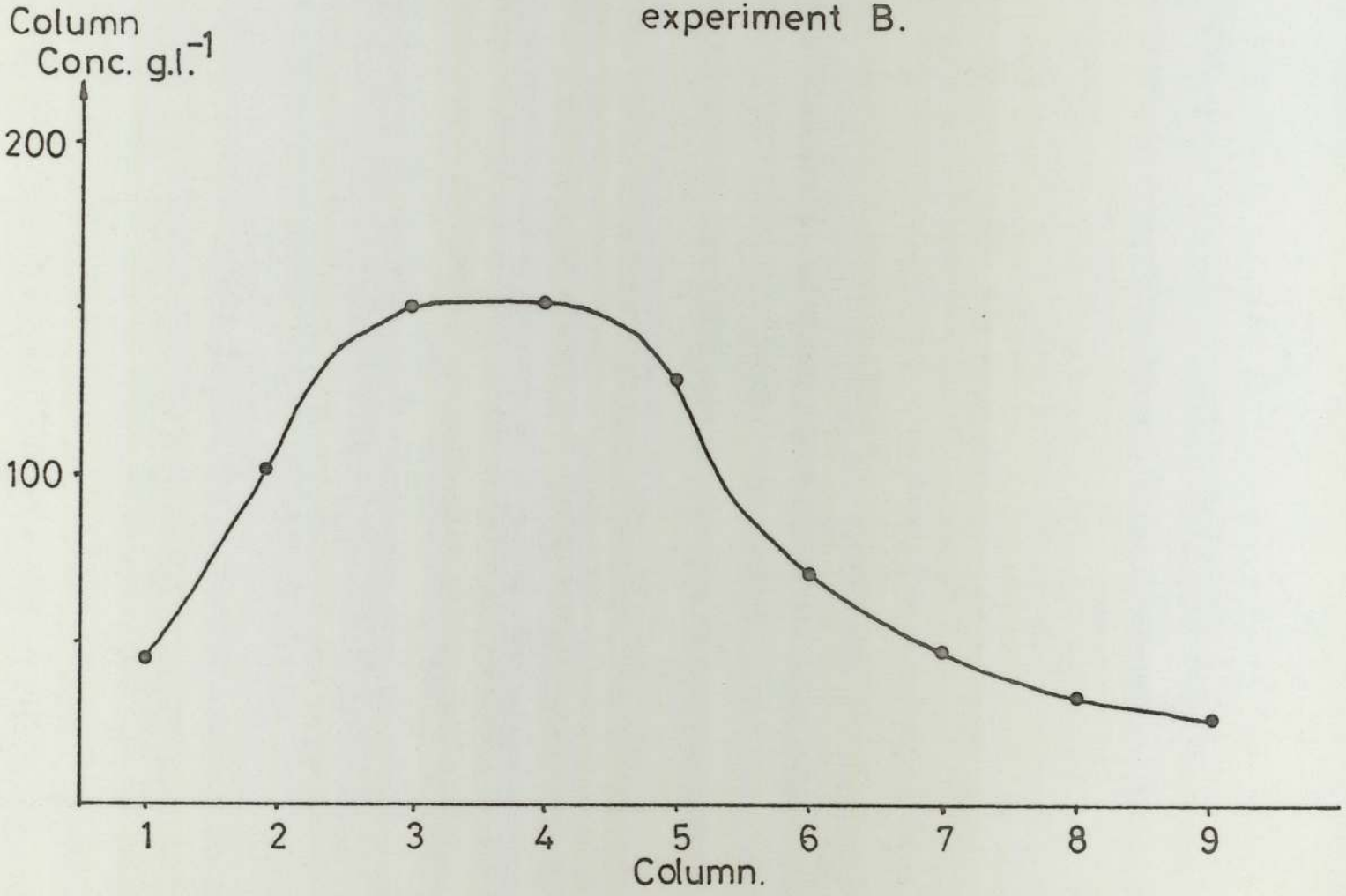


Fig 8.34 Concentration profile for experiment E.

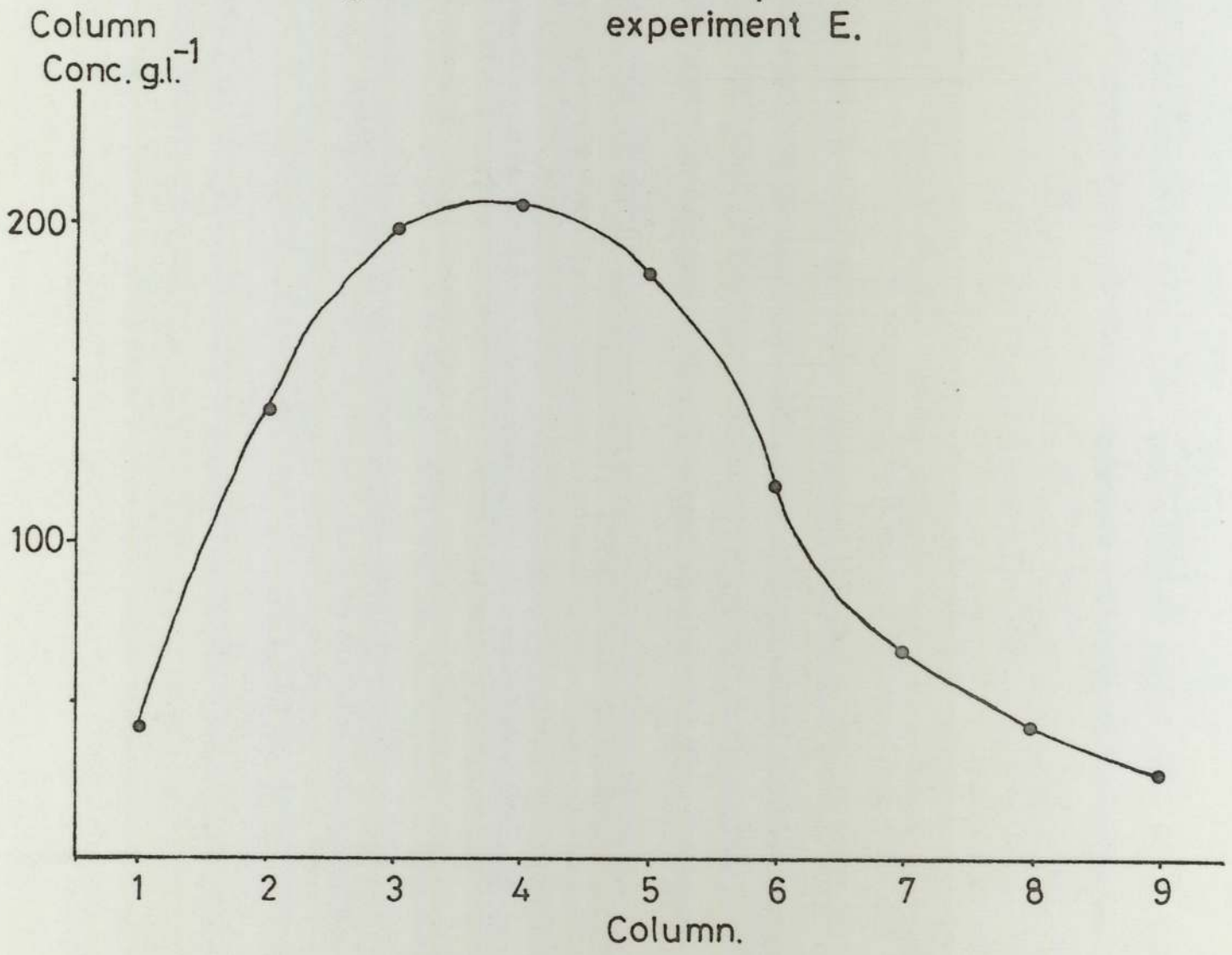
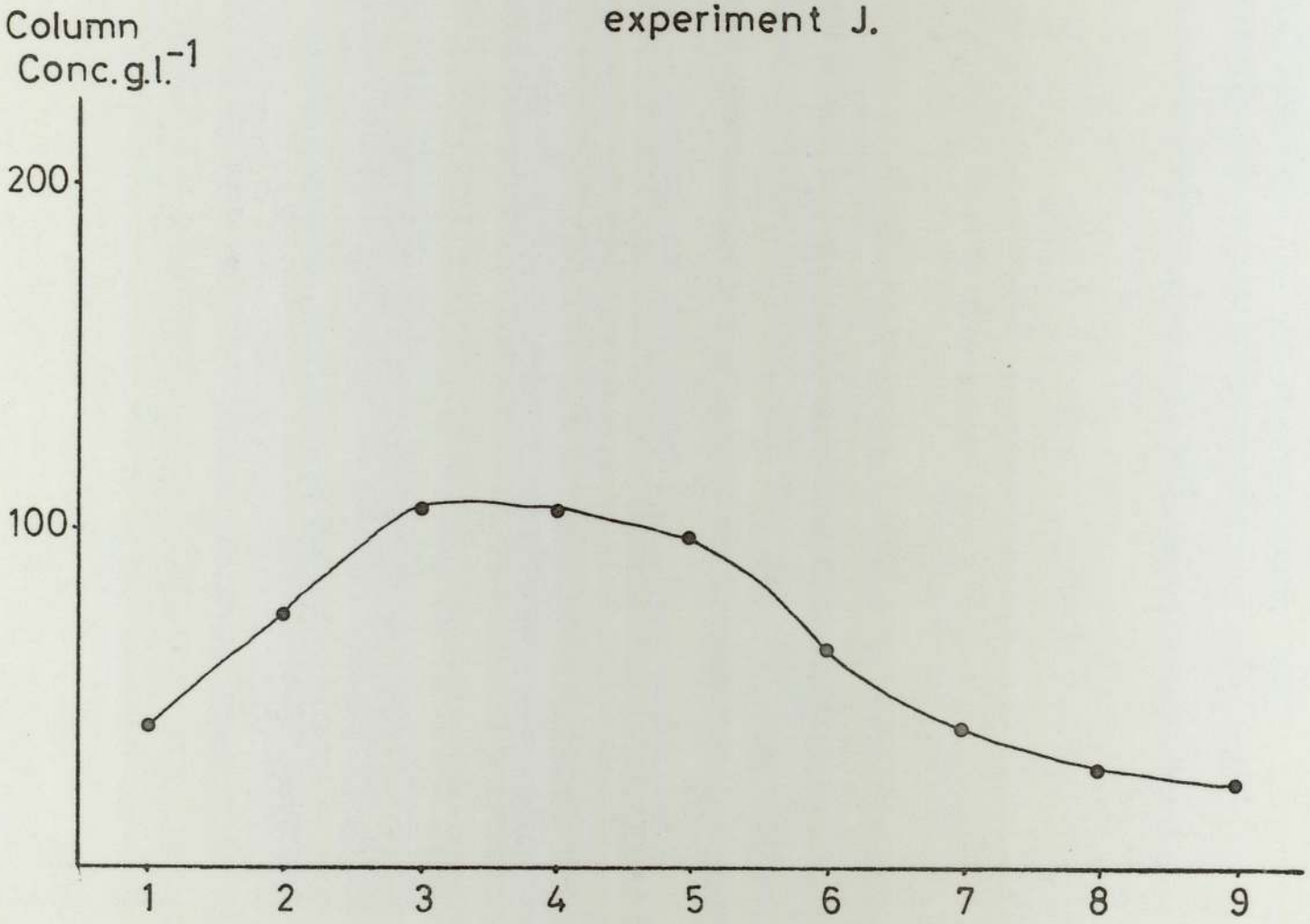


Fig 8.35 Concentration profile for experiment J.

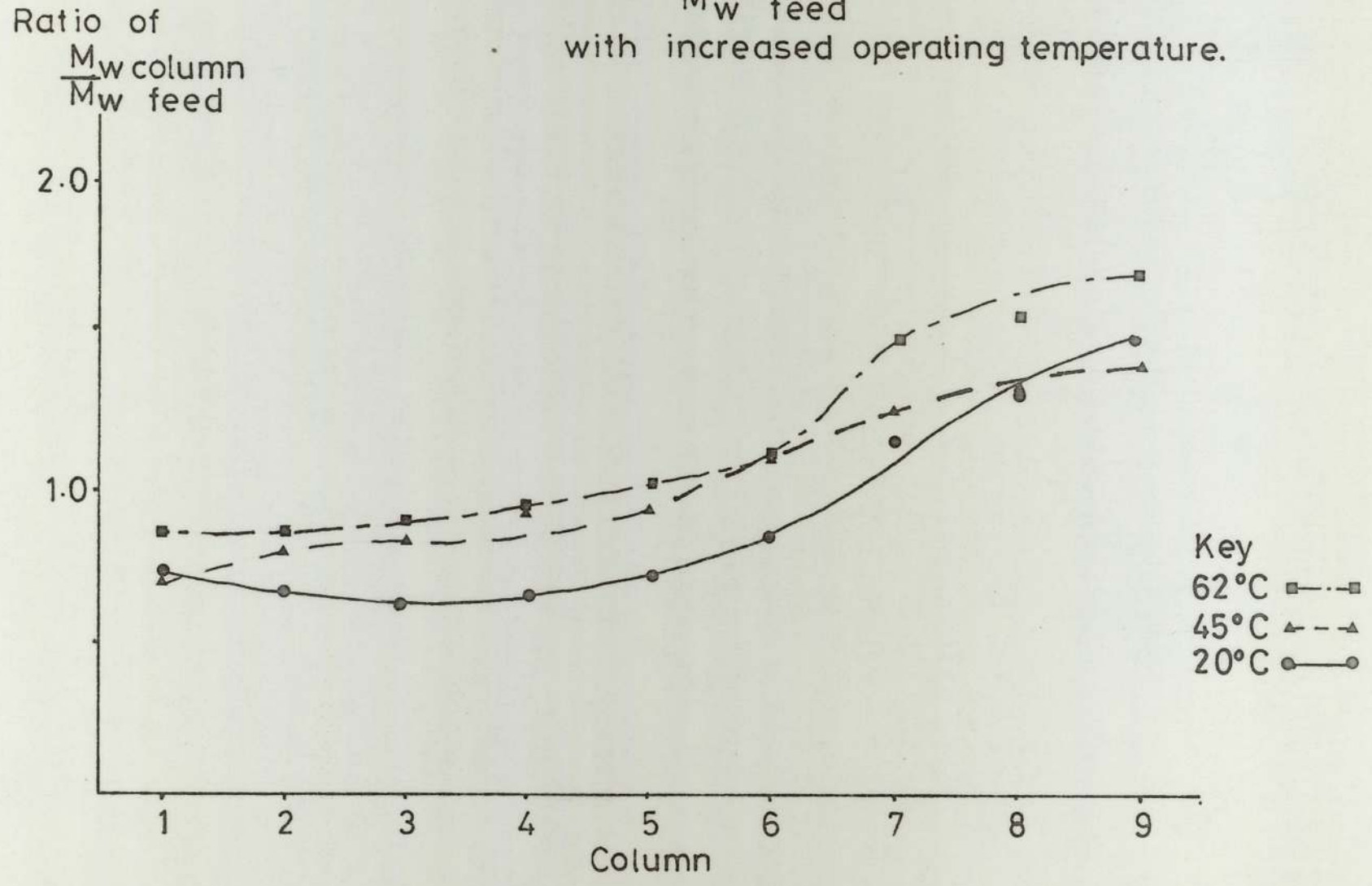


concentration of columns 3, 4 and 5. This variation is too large to be assigned to an experimental error and it is considered to be due to the temperature/concentration interactions of the dextran molecules. The concentrations at the extremes of the fractionating column, where relatively low concentrations occur, were approximately constant with the increase in temperature.

The structure of the Spherosil XOB075 packing was assumed to be rigid and any temperature/concentration interactions were assigned to changes in the physical properties of the dextran in solution. It is considered that the separation/fractionation in g.p.c. is dependent upon two mechanisms, primarily the steric exclusion of the molecules by virtue of their molecular size in solution and secondly by the mobility of the molecules in both the mobile and stationary phases.

For this description to be true deviations in the concentration profiles for experiments B, E and J should be observed, and accompanied by a change in the composition of the material held up. Fig. 8.36 shows the variation of the molecular weight averages normalized by the feed solution for the purged columns from experiments B, E and J. The ordinate can be considered to be an indicator of the relative quantity of "higher" molecular weight molecules present in the column. The greater this value is than unity, there are more "higher" molecular weight molecules present, and vice versa. It was suggested in section 6.5.1 that a "self exclusion effect" of the higher molecular weight molecules occurred in experiment B. This effect was indicated by Fig. 8.36 in the concaving of the curve between columns 1 and 5, that is columns 2, 3 and 4 contained relatively less high molecular weight molecules than column 1. When the operating temperature of the SCCR5 was increased to 45°C in experiment E the weight average for the dextran

Fig 8.36 Variation of $\frac{M_w \text{ column}}{M_w \text{ feed}}$ in fractionating column with increased operating temperature.

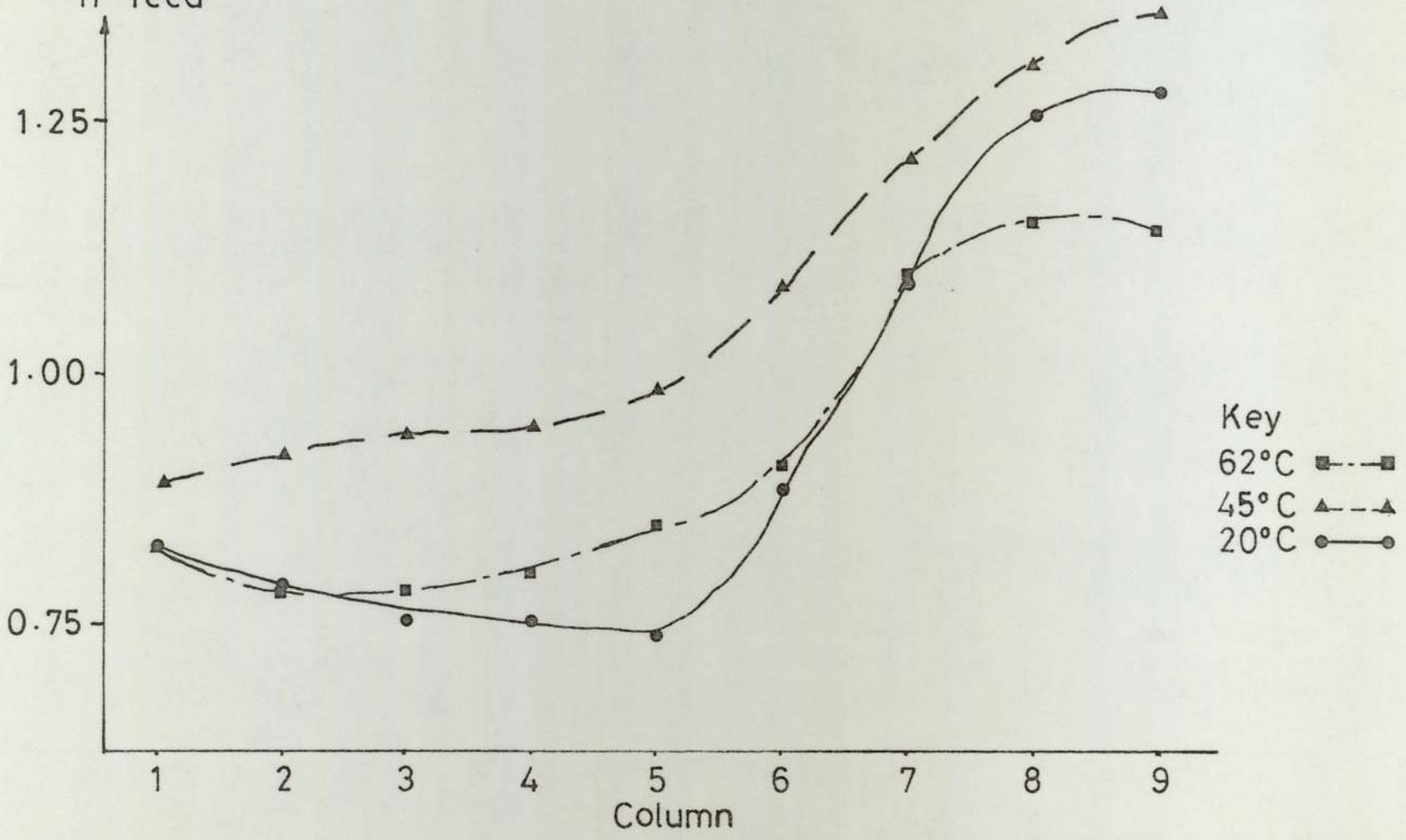


held up in the stripping section increased. Suggesting that accompanying the reduction in viscosity of the mobile phase the increase in temperature also allowed the dextran molecules greater mobility to penetrate the Spherosil XOB075 matrix. When the operating temperature was increased to 62°C in experiment the weight average of the purge samples also increased but, there was a reduction in the column concentrations. This may have been due to the increased hydrodynamic volume of the molecules. The molecules that could have entered the gel occupied a greater volume and so allowed fewer molecules to enter the gel.

If the increase in temperature increased the hydrodynamic volume of the dextran molecule then because of the log-linear relationship between molecular weight and elution volume, a small molecule would have experienced a greater change in elution volume. Fig. 8.37 illustrates the number average normalized by the dextran feed for the purged columns at the end of experiments B, E and J in the same manner as Fig. 8.36 showed the weight averages. The ordinate in Fig. 8.37 can be considered to be an indicator of the relative quantity of "smaller" molecules present in the column, and like Fig. 8.36 the larger the value, the more "smaller" molecules are present. For experiment J, relatively more "smaller" molecules were present in the purifying section of the fractionating column than either experiment B or E. This suggested that the smaller molecules had lower retention volumes at the higher temperature, and was consistent with the concept of the uncoiling of the dextran molecules at higher temperatures.

The "self exclusion effect" exhibited by experiment B was not observed in the experiments E and J,that used the same flow conditions at elevated temperatures. The composition and concentration profile

Fig 8.37 Variation of $\frac{M_n \text{ column}}{M_n \text{ feed}}$ in fractionating column with increased operating temperature.



for experiment J was similar to experiment A that had the same pre-feed theoretical cut-position but a higher eluent-to-feed ratio, therefore at the higher temperature of 60°C it was anticipated that a lower eluent-to-feed ratio could be used. Increases in the concentration about the feed point with the lower eluent-to-feed ratio experiments was expected to have two effects;

(i) the increase in mobile phase concentration would reduce the mobility of the higher molecular weight molecules and so help the dextran solution to exhibit the "self exclusion effect" and,

(ii) the increase in mobile phase concentration would cause a reduction in the hydrodynamic volume of the dextran molecules opposing the effect of the elevated temperature. This would effect the low molecular weight molecules in particular by increasing their partition coefficient.

Experiment G used a pre-feed theoretical cut position of 0.28, the same as experiments B, E and J but reduced the eluent-to-feed ratio from a nominal 2.5 to 1.8. Fig. 8.38 shows the end of run pseudo-steady state concentration profile. Compared to experiment J the maximum concentration in the stripping section for experiment G increased. Fig. 8.39 plots normalized molecular weight averages for experiment G and shows that the distributions resembles those obtained for experiment E.

The increase in the operating temperature to 62°C enabled an increase in the quantity of dextran that could be fractionated per cycle. Theoretical cut-positions similar to those used in experiment E had been used in an experiment performed at ambient temperatures by Dr. S.R. Holding who observed very little fractionation of the products (261). Fig. 8.40 shows the observed concentration profile for the ambient temperature experiment. There is very little similarity between Fig. 8.38 and 8.40.

Fig 8.38 Concentration profile for experement G.

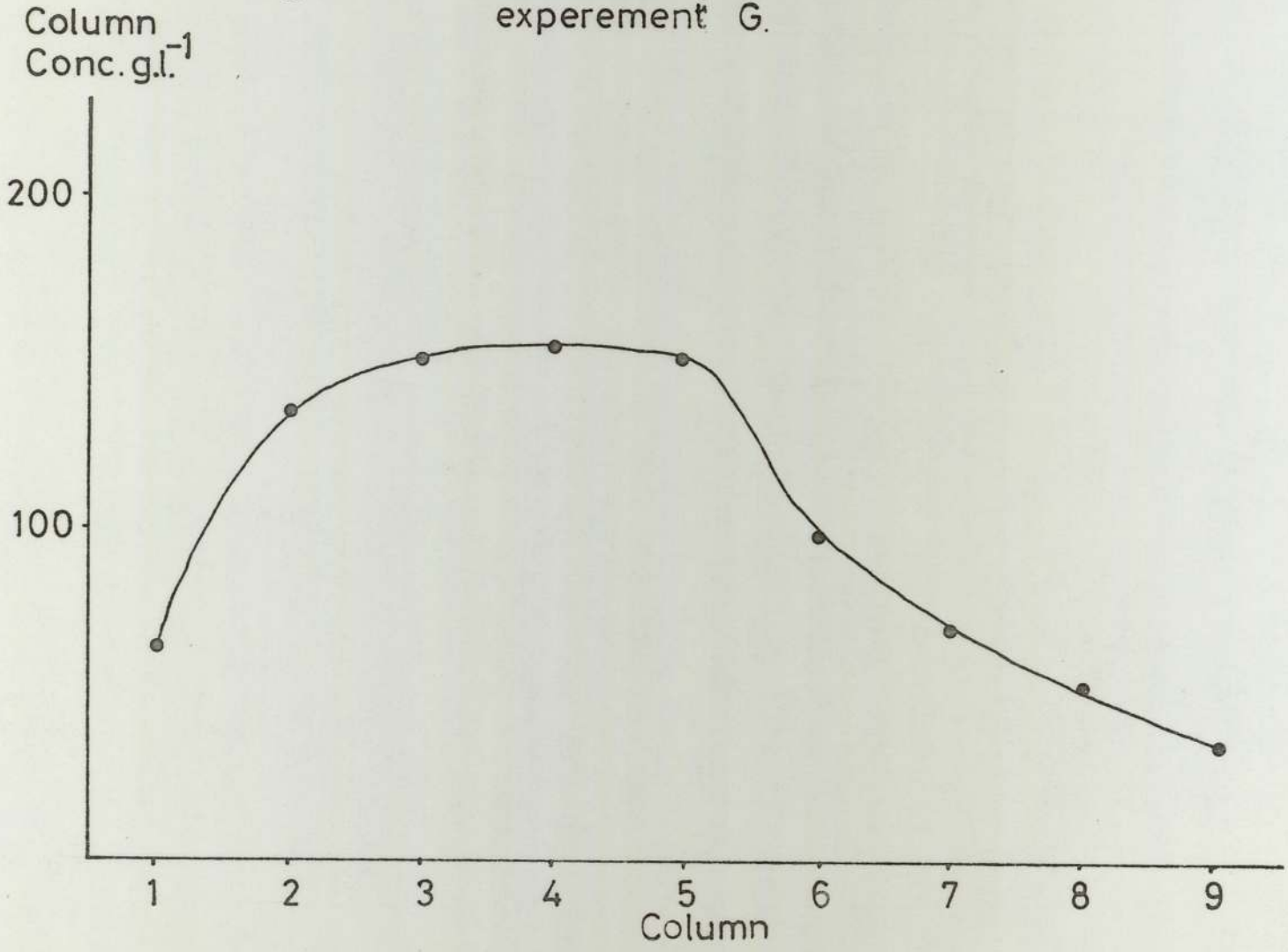


Fig 8.39 Variation of average molecular weights for experiment G.

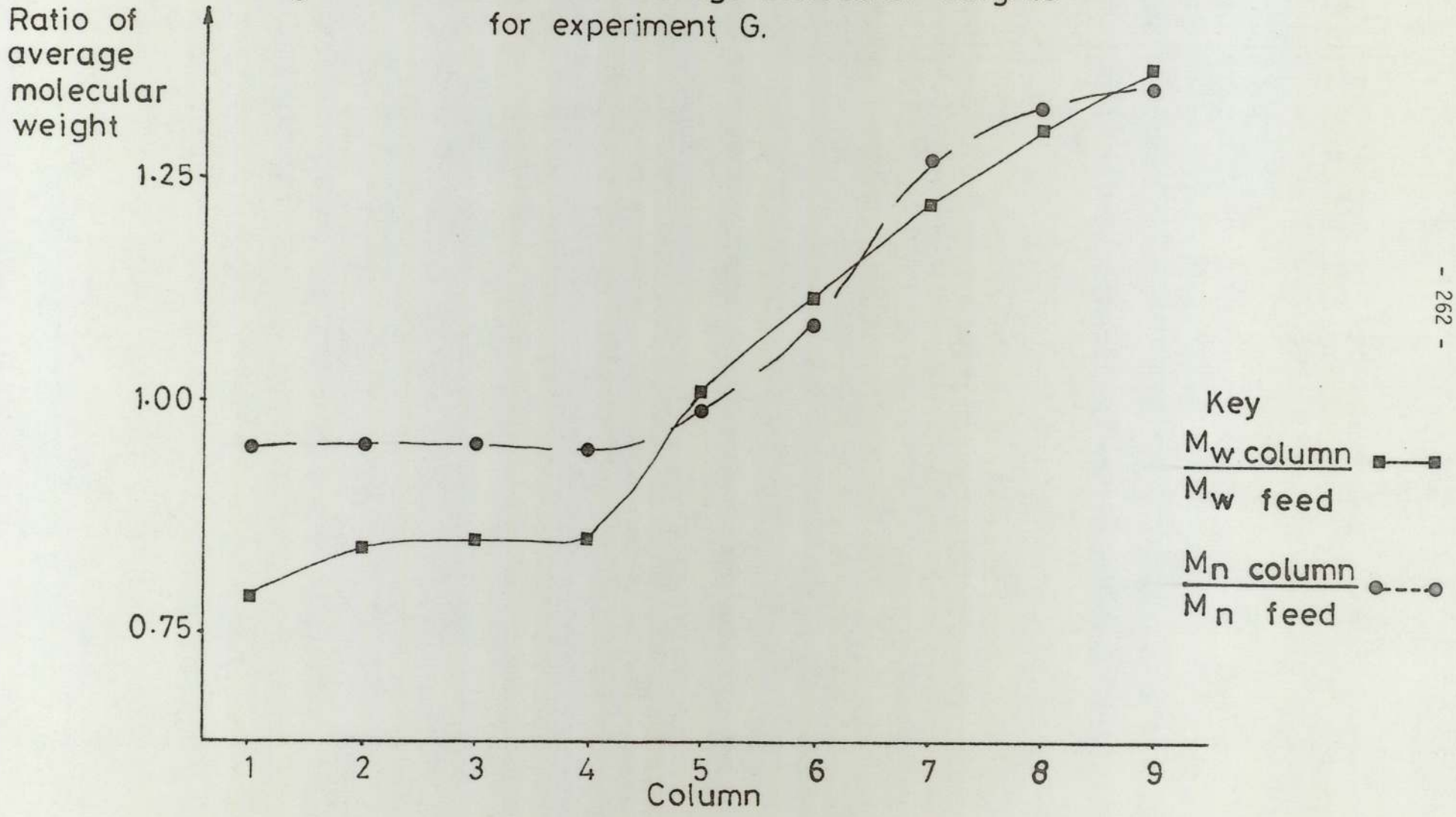
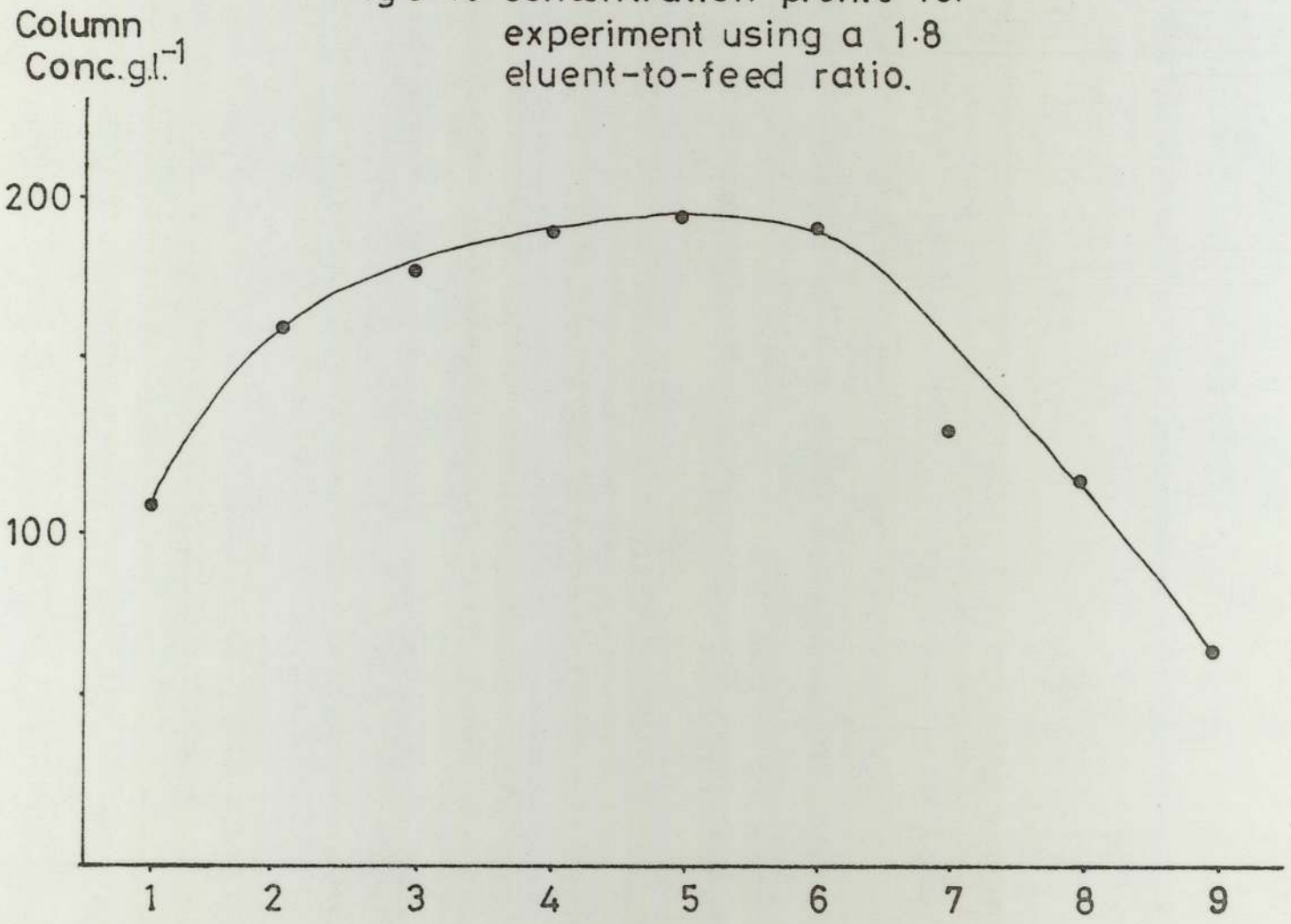


Fig 8.40 Concentration profile for experiment using a 1·8 eluent-to-feed ratio.

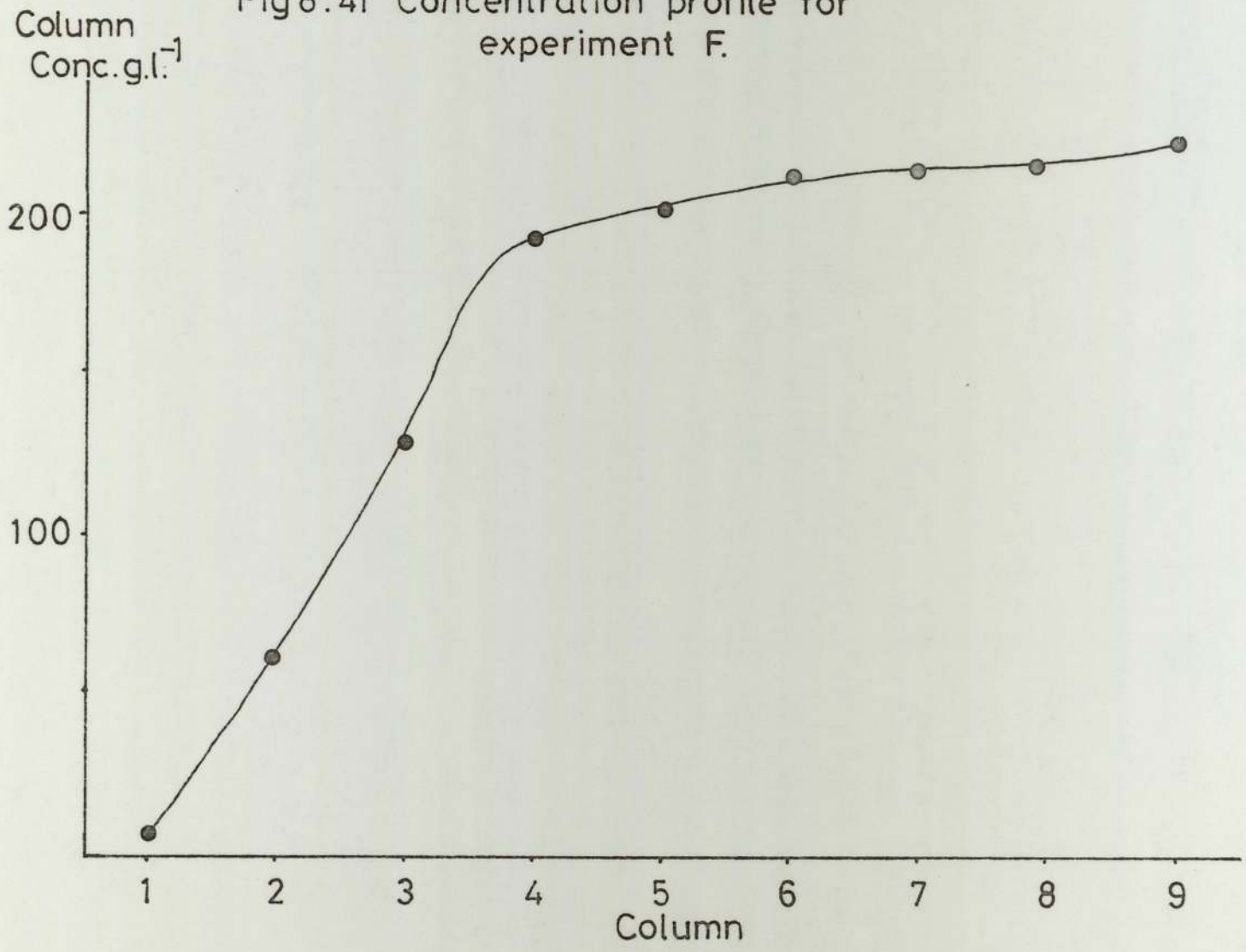


Operating the chromatograph at the eluated temperature allowed lower eluent-to-feed ratios to be used whilst maintaining the fractionation of the products.

8.4.2 Low Molecular Weight Fractionations

Concentration profiles of experiments where low molecular weight removal was attempted or achieved are illustrated in Fig. 8.41 to 8.45. Experiments D and F used similar operating conditions and show the effect of increasing the operating temperature. The affect was that the quantity of dextran hold-up increased. The average column concentration rose to 97% of the feed concentration for experiment F. Fig. 8.46 shows the relative concentration profiles for experiments D and F. A slight reduction in the quantity of low molecular weight fraction removed accompanied the increase in operating temperature, from 8.9 to 6.7% of the feed input. Experiment H attempted to increase the quantity of low molecular weight removal by reducing the pre-feed theoretical out position from 0.75 to 0.6. The feed material for experiment H was BT216G. Experiment H removed 46.5% of the feed material in the low molecular weight product. This was greater than the desired value of 10 to 15%. Therefore a pre-feed theoretical cut position between 0.6 and 0.75 was required to remove the required amount of low molecular weight dextran. The change to a lower molecular weight feed material may have contributed to the increase in the quantity of low molecular weight product observed in experiment H. In order to compare the two feed materials, experiment I repeated the theoretical cut-positions used in experiment C at 45°C. Experiment I removed approximately 5% of the feed material in the low molecular weight product. Experiment C removed approximately 10%.

Fig 8.41 Concentration profile for experiment F.



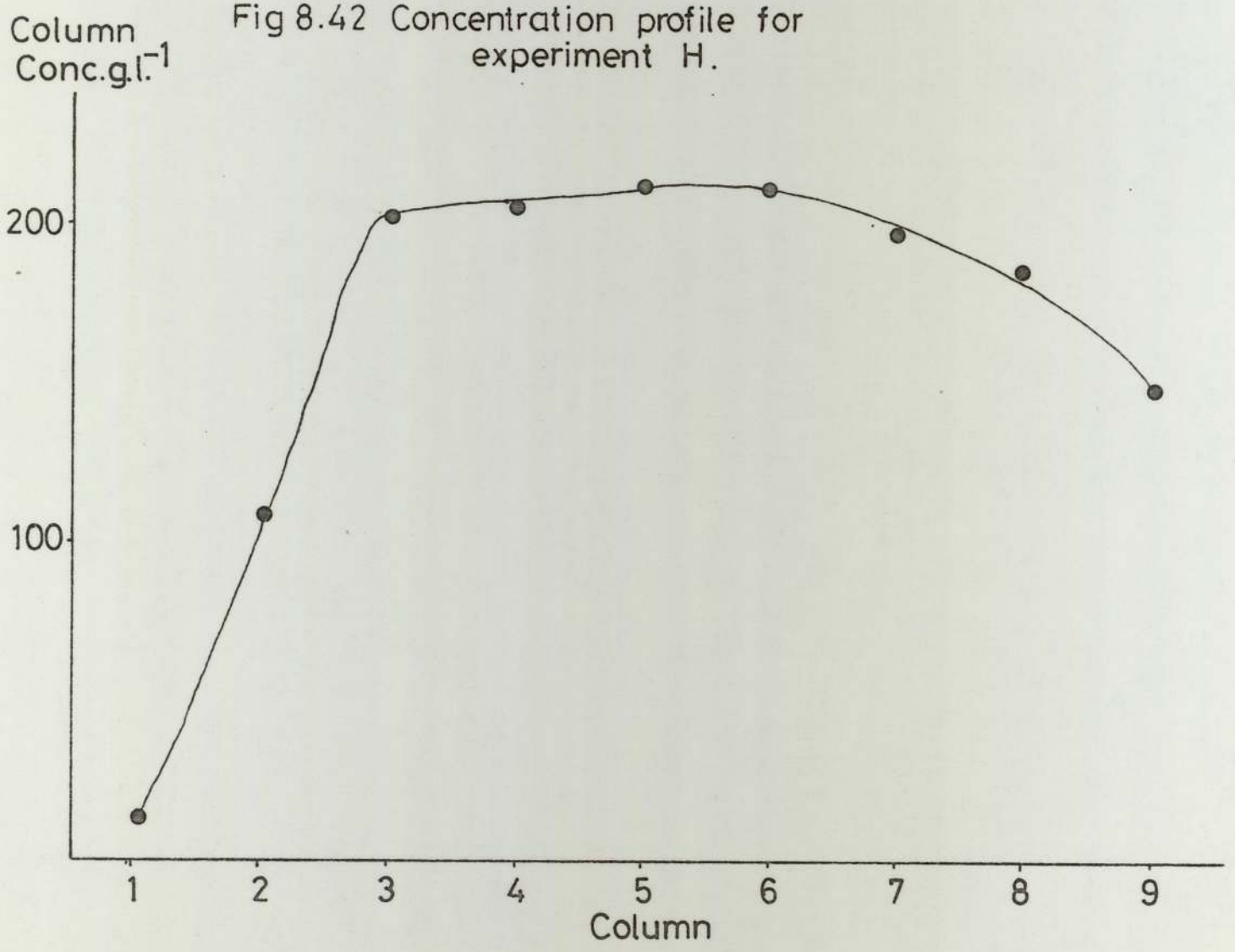
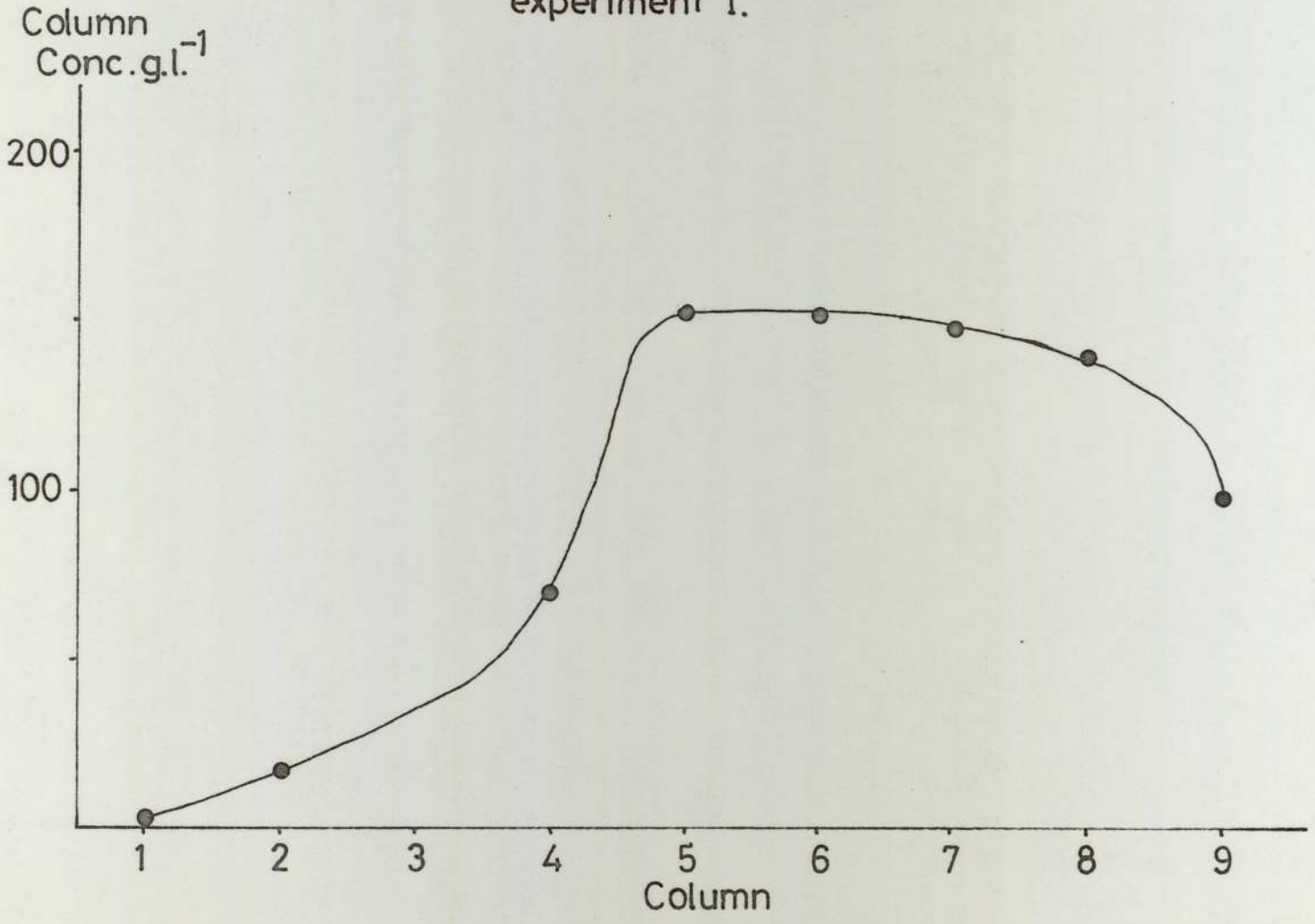


Fig 8.42 Concentration profile for experiment H.

Fig 8.43 Concentration profile for experiment I.



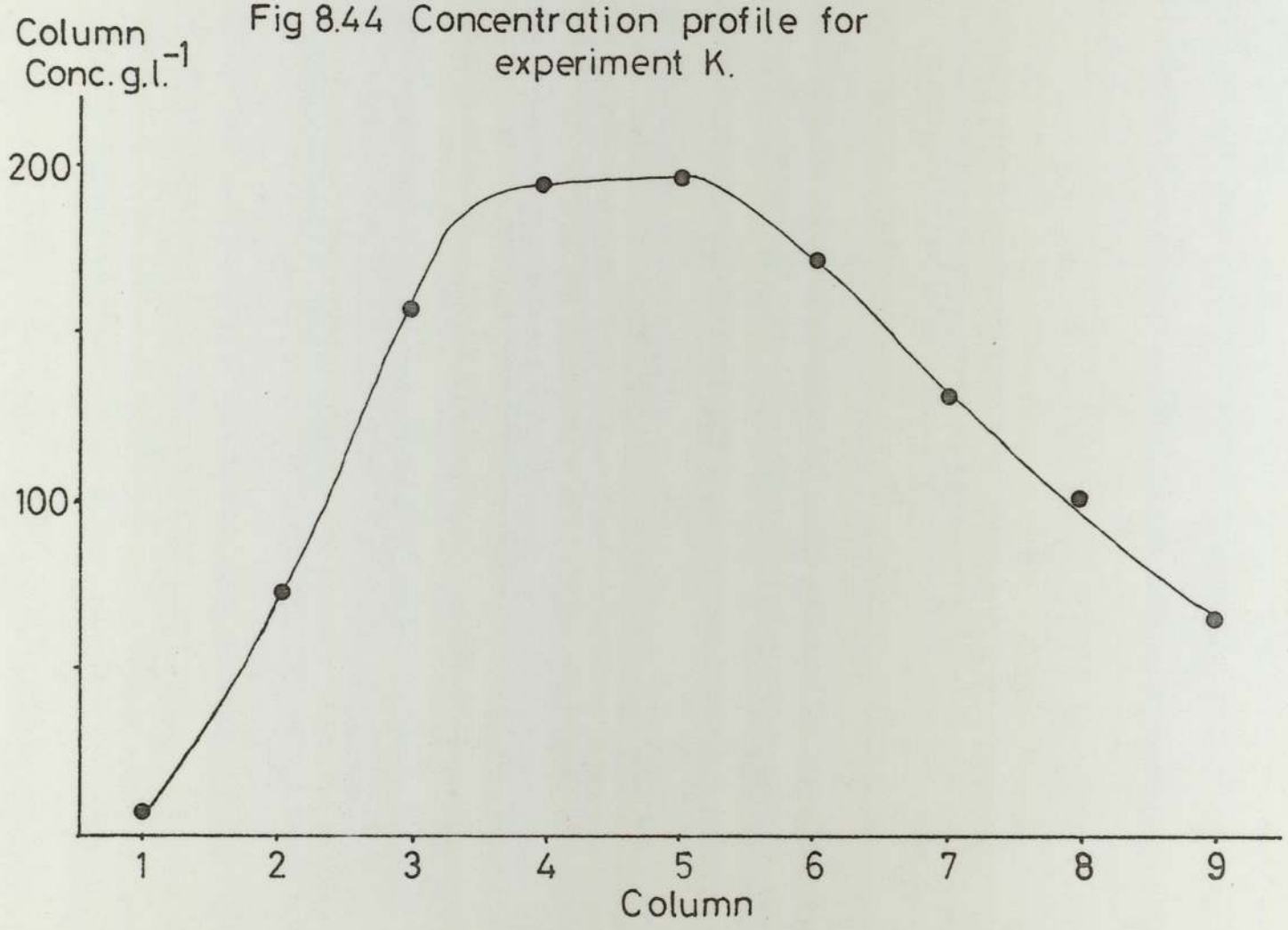


Fig 8.44 Concentration profile for experiment K.

Fig 8.45 Concentration profile for experiment L.

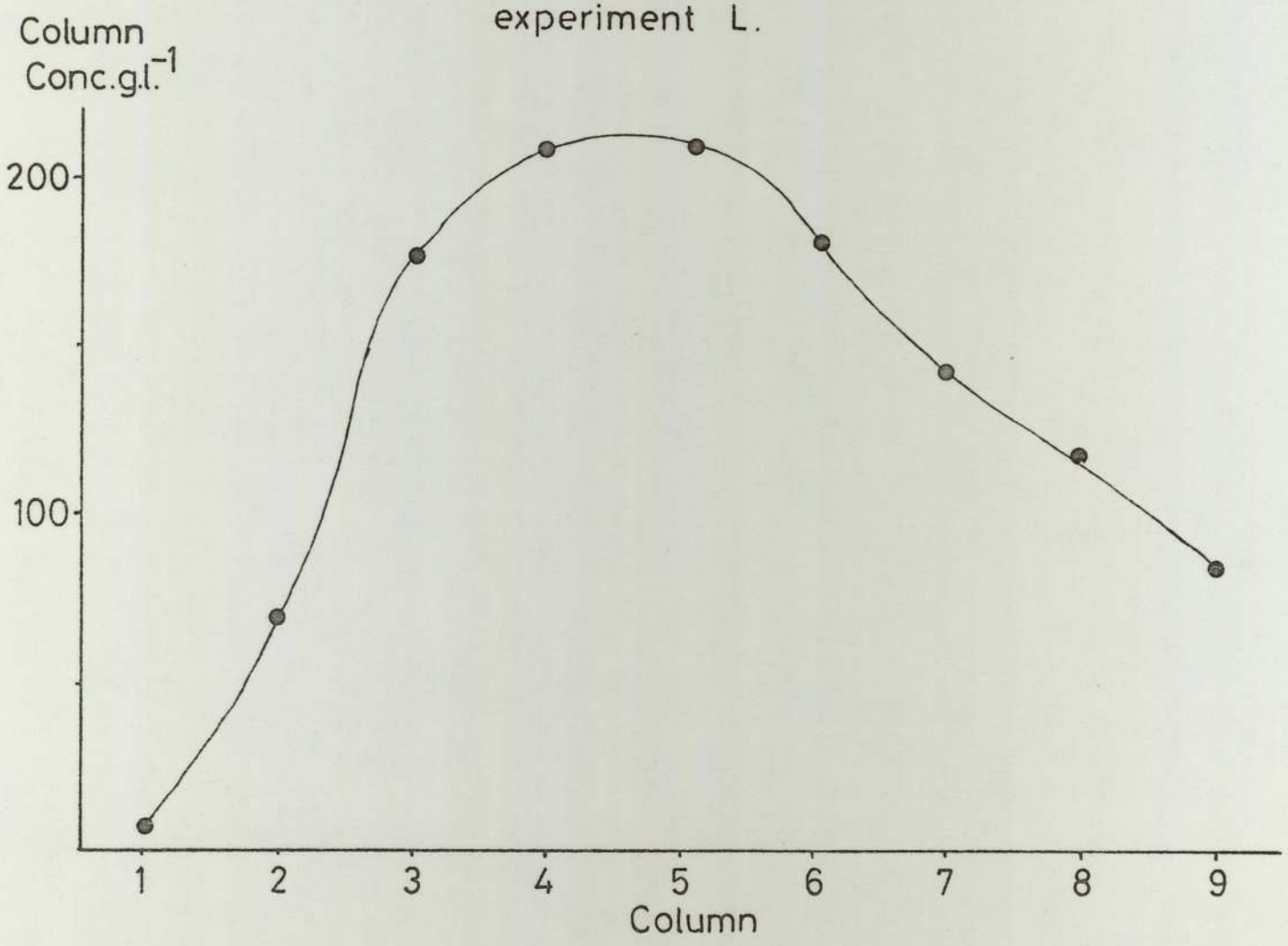
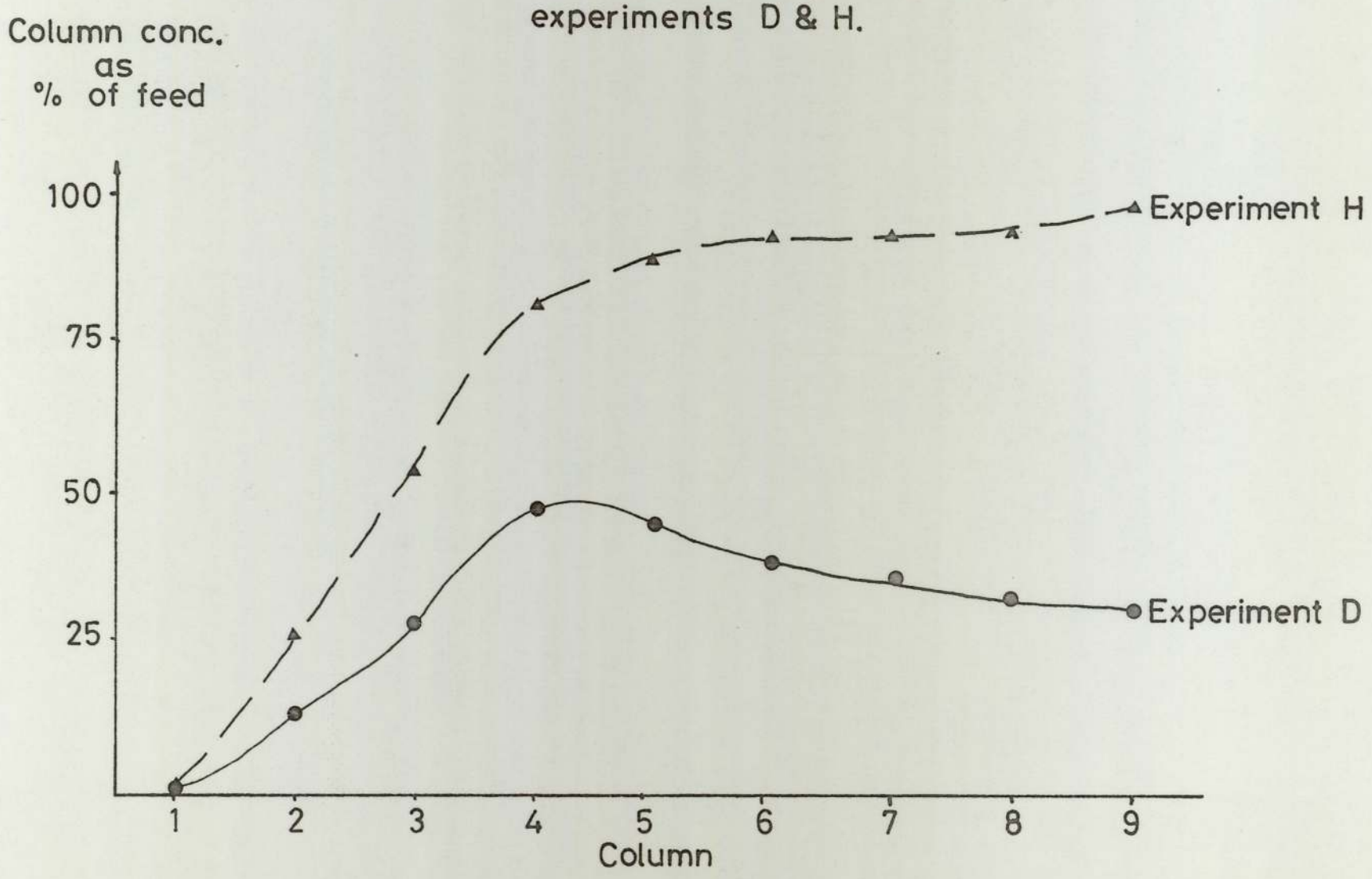


Fig 8.46 Relative concentration profiles for experiments D & H.



The reduction in the quantity of feed material removed in the low molecular weight product was due to increase in operating temperature, also observed by experiments D and F. Therefore it was considered that for experiment H, the lower molecular weight distribution of the BT216G feed did not contribute significantly to the increase in low molecular weight product and that the dominant factor controlling the product split was the pre-feed theoretical cut-position. A comparison between the relative pseudo-steady state concentration profiles for experiments C and I illustrated in Fig. 8.47 shows an increase in the dextran hold-up with increased operating temperature. The quantity of dextran hold-up increased significantly in the section of the fractionating column in which the majority of the feed was migrating. As with other experiments comparing similar operating condition at ambient and 45°C the increase in temperature resulted in a slight increase in the quantity of the major product removed.

Experiment K used a pre-feed theoretical cut-position of 0.7 where it was expected that 10 to 20% of the feed material in the low molecular weight product would be removed. This did not happen. The low molecular weight product contained approximately 47% of the feed material, similar to experiment H. A comparison of the normalized concentration profiles for experiments H and K is illustrated in Fig. 8.48. The on column concentrations for experiment H are higher than those for experiment K. This was probably because the eluent-to-feed ratio for experiment K was twice that in experiment H. The composition of the dextran hold-up in the stripping section for experiment K had a lower molecular weight distribution than for experiment H. The molecular weight distribution for the column closest to the eluent inlet port is significantly lower for experiment K as shown below:-

Fig 8.47 Relative concentration profiles for experiments C & I.

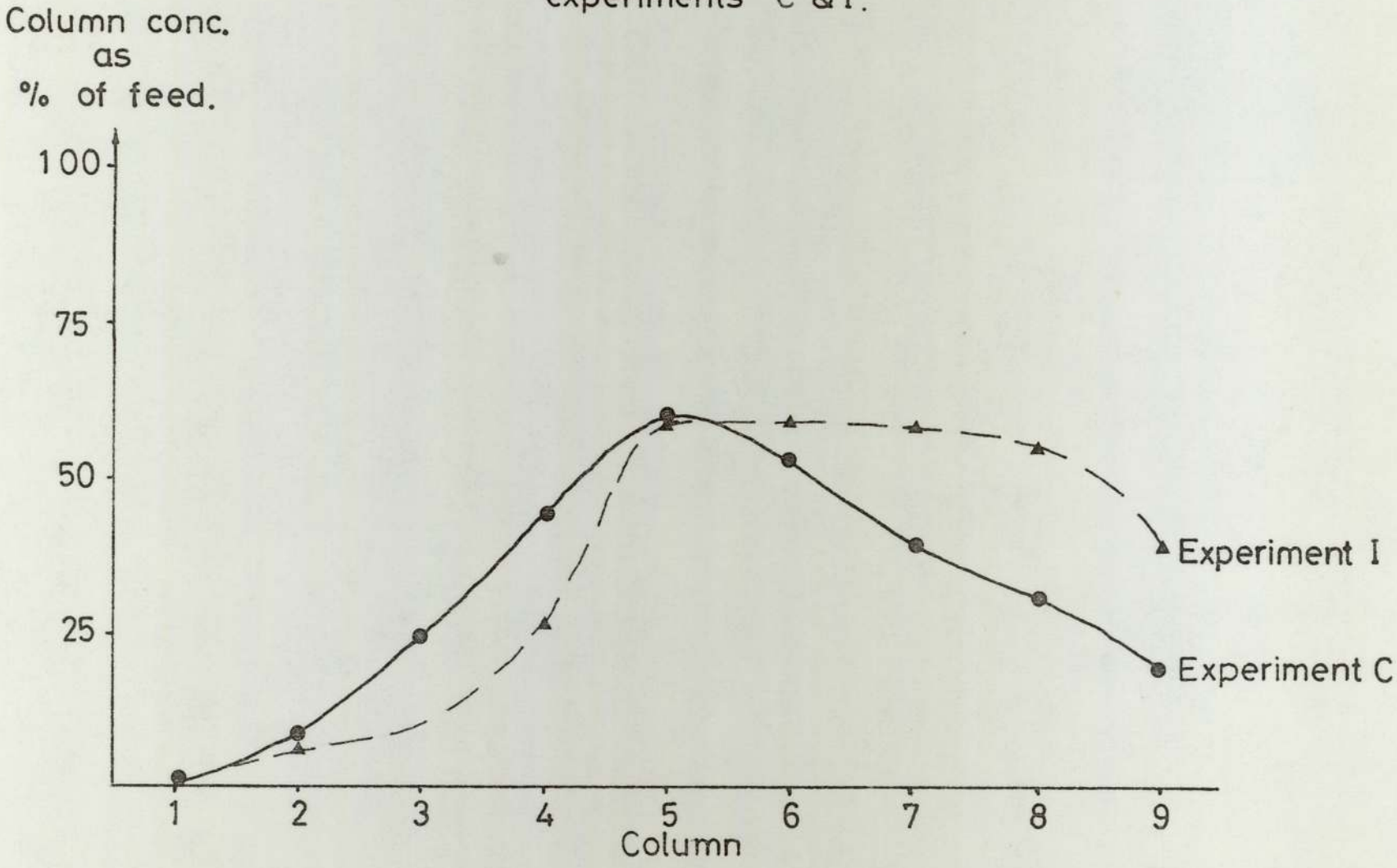
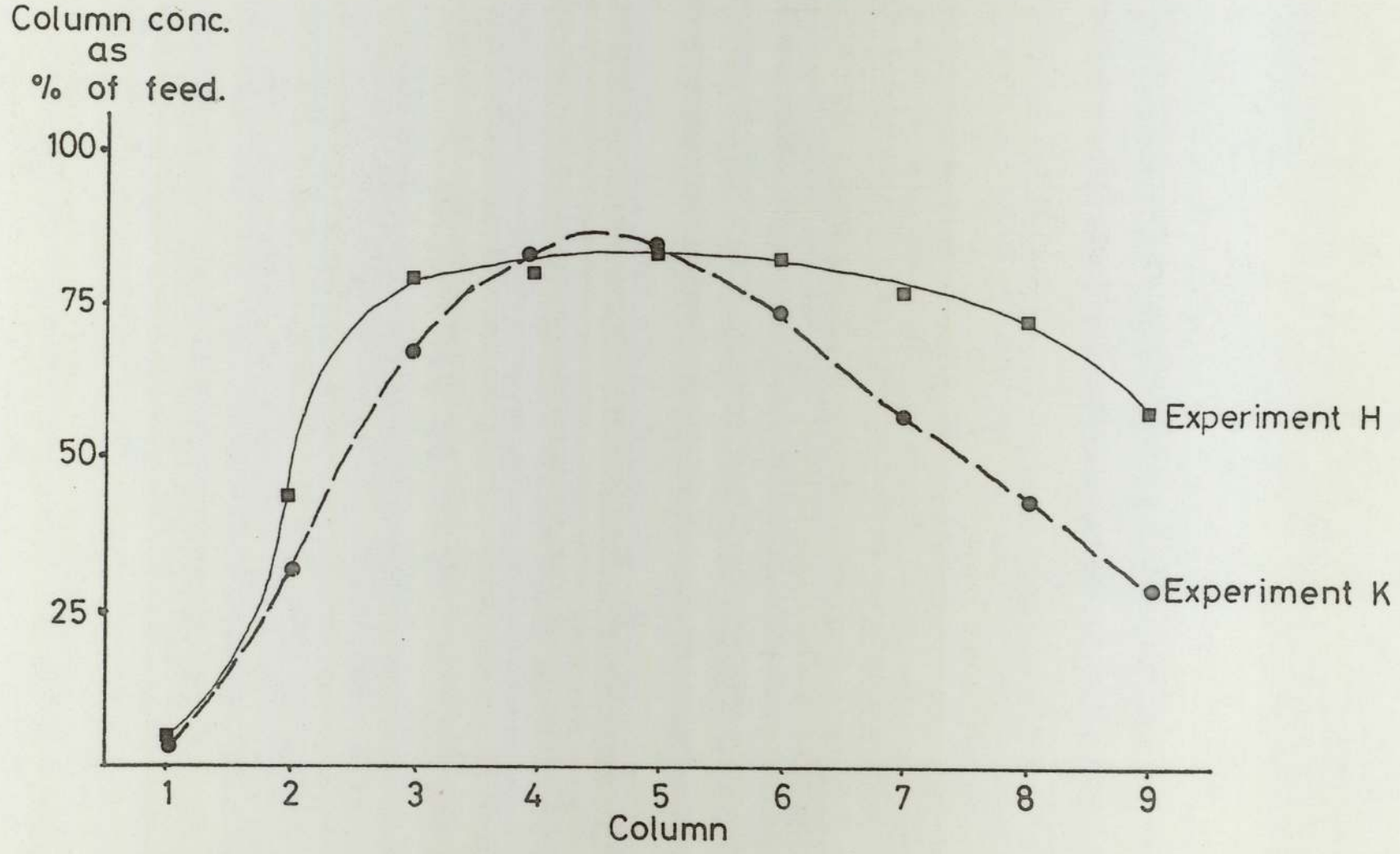


Fig 8.48 Relative concentration profiles for experiments H & K.



	Experiment I	Experiment K
$\bar{M}_w$	28600	23700
$\bar{M}_n$	20200	18100

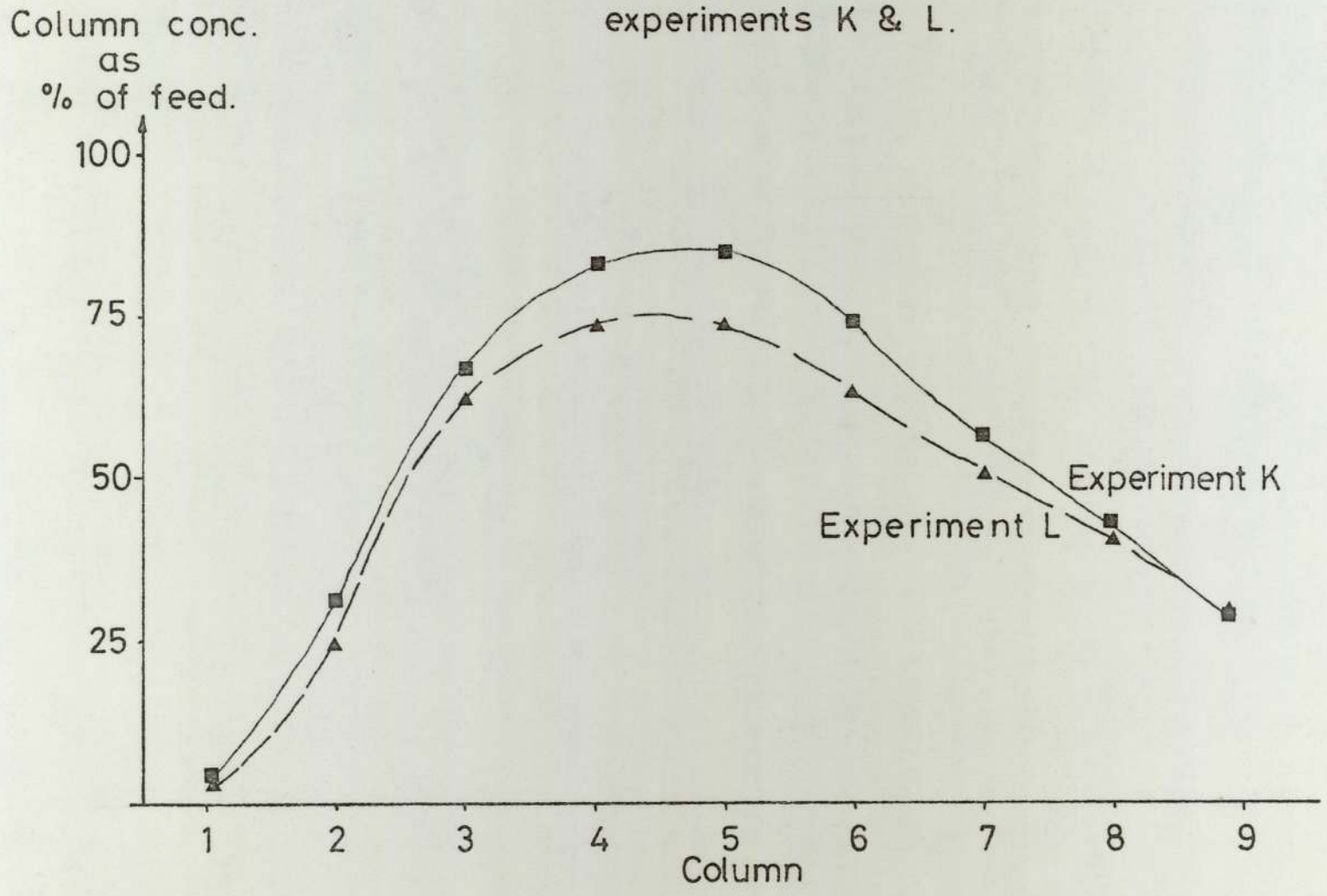
This was also apparent in the molecular weight distributions of the low molecular weight products for these experiments although their percentage product splits are similar.

An overall observation of the effect of heating the chromatograph upon the fractionation of the dextran solutions was that the product containing the major proportion of the feed material increased slightly with an increase in temperature. As experiment K fractionated the feed solution into approximately two equal masses the effect of elevating the temperature to 60°C upon the product split in experiment L was observed. The concentration of the feed solution was 285 g l^{-1}, the highest concentration used in the semi-continuous programme. The maximum on column concentrations were similar for both experiments byt the normalized column concentrations for experiment L showed a 13% reduction as illustrated in Fig. 8.49. This observation was also made for experiments E and F. The increased operating temperature in experiment L increased the fraction of feed eluted from the low molecular weight product port from approximately 47 to 50%.

8.4.3 Product Concentrations and Compositions

The concentrations of the main product for the experiments E and J compared with experiment B increased because of a reduction in the purge rate from 3.9 times to 2.6 times the required theoretical value. This was one of the proposed schemes for increasing the concentration of the products of the SCCR5 as suggested in section 6.5.2. Further reductions in the purge rate are thought possible but experimental

Fig 8.49 Relative concentration profiles for experiments K & L.



verification of this has yet to be proved. Alternative schemes for partially refluxing the product streams to increase the concentration of the final product have been proposed in section 6.5.2. The quality of the major product was not significantly altered by the elevation in temperature of the chromatograph for the experiments B, E and J. As the operating temperature of the SCCR5 was increased, the quantity of the major product increased slightly but the quality of the product remained constant suggesting that the resolution of the high and the low molecular weight products was increased with increasing temperature. The effect of increasing the post-feed theoretical cut position in experiment G compared to experiment J caused a slight increase the quantity of the high molecular weight materials removed. Despite removing more high molecular weight material the molecular weight averages for the main product of experiment G were greater than those experiments B, E and J. A small adjustment could be made to the operating conditions to allow slightly more dextran to be removed in the high molecular weight product than in experiment G, thereby producing a major product with a lower molecular weight distribution.

In experiments D and F the quantity of low molecular weight material removed fell by nearly 25% when the temperature of the chromatograph was increased from 20 to 45°C. This reduced the molecular weight averages for the main products as shown below:-

	Experiment D	Experiment F
M_w	72000	69300
M_n	30700	25600
Low molecular weight product removed	8.9%	6.7%

The quality of the low molecular weight products also showed a reduction in the number average:-

	Experiment D	Experiment F
M_n	20300	15800

which suggested that only the very small molecules that were present in the feed were penetrating the pores and were eluted in the low molecular weight product.

Experiment H was the first experiment to use the feed material BT216G and it failed in its original objective to remove a small quantity of the feed material from the low molecular weight end. Although this experiment failed in its original aim, experiment H demonstrated that the SCCR5 could fractionate a narrow molecular weight feed material into two products having different molecular weight ranges. Increasing the pre-feed theoretical cut position from 0.6 to 0.7 had no effect on the quantity of low molecular weight product collected, but did have a significant change on the quality of the product. The molecular weight average for the low molecular weight products of experiments H and K were:-

	Experiment H	Experiment K
M_w	28300	23600
M_n	19600	19000

The reduction of the low molecular weight product distribution was expected because the pre-feed theoretical cut position was increased to 0.7 but it was also expected that this would also reduce the quantity of dextran. The increase in temperature to 60°C in experiment L whilst maintaining the same operating conditions as experiment K caused no change in the quality of the fractionated products although there was a slight increase in the quantity of the low molecular weight product from 47 to 50% of the feed.

Repeating the same operating conditions as experiment C at 45°C and with the BT216G feed solution, experiment I succeeded in

removing only 5% of the low molecular weight material from the feed. As a result the molecular weight averages of the major product showed only a slight increase. The large reduction in the quantity of low molecular material removed when increasing the pre-feed theoretical cut position from 0.7 to 0.75 suggested that the Spherosil XOB075 packing had too large a pore diameter to make it a satisfactory packing for the fractionation of low molecular weight dextrans.

The major products for the experiments that succeeded in removing small quantities of low molecular weight fractions from the feed had concentrations of the same order of magnitude of between 10-40 g l^{-1} as the high molecular weight removal experiments inspite of having at least twice the eluent to feed ratio. This was because the major product was eluted from the fractionating section. A packing having a smaller mean pore diameter would be more effective for the fractionation of low molecular weight dextrans. This would allow lower pre-feed theoretical cut points to be used and consequently lower eluent to feed ratios, thereby improving the economical viability of the process.

8.5 CONCLUSIONS

The main conclusions for the semi-continuous chromatographic study using the SCCR5 were as follows:-

(1) the pre-feed theoretical cut position appeared to be the dominant factor in the determination of the product-split. Five experiments were performed where the pre-feed theoretical cut position was kept constant and the post-feed cut-position varied between 0.52 and 0.93, a similar product-split was obtained for the five experiments.

(2) when the pre-feed theoretical cut-position was increased, the fraction of the feed material eluted from the high molecular weight

product port increased.

(3) a large increase in the percentage of feed removed in the high molecular weight product occurred when the pre-feed theoretical cut-position was increased from 0.7 to 0.75. This suggested that for production applications in the SCCR equipment, Spherosil XOBO75 would be poor fractionating material for the removal of low molecular weight dextrans. However, because of the very sensitive response Spherosil XOBO75 could be successfully used in an analytical application to produce accurate molecular weight distributions.

(4) elevating the operating temperature of the SCCR5 changed the quantity and quality of the dextran held-up in the fractionating section, as well as a slight effect on the product split. An increase in the operating temperature resulted in a slight increase in the major product for the same theoretical cut-positions.

(5) increasing the operating temperature of the SCCR5 had a greater effect on the quantity of removal of the low molecular weight dextrans than on the high molecular weight removal experiments. This was attributed to the sensitive response of the Spherosil XOBO75 at the low molecular weight range of the feed solutions.

(6) elevating the temperature of the SCCR5 appeared to reduce the effects of concentration on g.p.c., a summary of the temperature/ concentration interactions is contained in Fig. 8.50.

(7) no significant change in the molecular weight distributions for the main product of experiments B, E and J was observed.

(8) operating the SCCR5 at 62°C enabled experiment G to produce a satisfactory fractionation of the feed material that was not possible at ambient temperatures.

(9) a reduction in the purge volume for experiments E and J compared to experiment B had no detrimental effect on the performance of the SCCR5.

8.50 Proposed temperature/concentration interactions of dextran molecules

	Increased Temperature	Increased Concentration
Effect on "large" molecules	Increase molecular size Increase diffusivity Aids intrapore diffusion	Decrease molecule size Decreases diffusivity Causes "self exclusion effect"
Effect on "small" molecules	Increases molecular size Reduces elution volume	Decreases molecular size Increases elution volume

9.0

MODELLING OF THE SCCR5

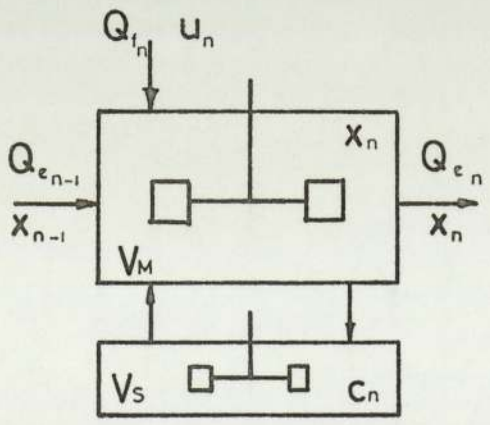
9.1 INTRODUCTION

A review of the theoretical descriptions of the g.p.c. process is contained in section 2.8. The more recent models of the g.p.c. process have concentrated upon non-equilibrium descriptions. Solutions to these models are complex and have only been successful in yielding relationships between various moments of the solute peaks. This feature together with an insufficient knowledge of the thermodynamic properties of the dextran system has prevented the use of a non-equilibrium description for the g.p.c. process. A simple linear exclusion controlled model has been used in this thesis.

9.1.1 Linear Exclusion Controlled Model

A description founded upon the "dispersion model" has been used to describe the fractionating column. This is perhaps the most widely used description for contacting devices in the chemical processes industry. Langmuir (265) as early as 1908 discussed this model. The chromatographic column was considered to consist of a series of compartments where the mobile phase leaving the compartment was in equilibrium with the stationary phase in the column. For the range of operating conditions used, correlations (266) suggest that the axial dispersion coefficient was small and so the limiting case of a plug flow model could be used.

The mass balance over a general tank, n, for a solute species is as follows;



where Q - volumetric flowrate

x - mobile phase concentrations

u - feed concentrations

c - stationary phase concentrations

V - volume of phase

subscripts e - eluent

m - mobile

s - stationary

Solute mass balance across stage n,

$$\text{Input} - \text{Output} = \text{Accumulation} \qquad (9.1)$$

Stream			
Feed	$Q_{fn} U_n$		
Mobile phase	$Q_{en-1} x_{n-1}$	$Q_{en} x_n$	
Change in mobile phase			$V_m \frac{dx_n}{dt}$
Change in stationary phase			$V_s \frac{dc_n}{dt}$

Therefore summing the components of 9.1 gives

$$Q_{fn}U_n + Q_{en-1}x_{n-1} - Q_{en}x_n = V_m \frac{dx_n}{dt} + V_s \frac{dc_n}{dt} \qquad (9.2)$$

Assuming equilibrium occurs between the mobile and stationary phase,

$$x_n = K_d C_n \qquad (9.3)$$

equation (9.2) simplifies to

$$Q_{fn}U_n + Q_{en-1}x_{n-1} - Q_{en}x_n = (V_m + K_d V_s) \frac{dx_n}{dt} \qquad (9.4)$$

Rearranging gives

$$\frac{dx_n}{dt} = a_{n-1}x_{n-1} + b_n x_n + c_n u_n \qquad (9.5)$$

where $a_{n-1} = \frac{Q_{en-1}}{V_m + K_d V_s}$

$b_n = \frac{Q_{en}}{V_m + K_d V_s}$

and, $c_n = \frac{Q_{fn}}{V_m + K_d V_s}$

If the fractionating column were to be divided into N compartments the resulting sets of equations would be:

$$x_1 = a_o x_o - b_1 x_1 + c_1 u_1$$

$$x_n = a_{n-1}x_{n-1} - b_n x_n + c_n u_n$$

$$x_N = a_{N-1}x_{N-1} - b_N x_N + c_N u_N$$

or

$$\underline{x}(t) = \underline{A}\,\underline{x}(t) + \underline{B}\,\underline{u}(t) \qquad (9.6)$$

The general solution to equation (9.6) as shown in Appendix A.2 is;

$$\underline{x}(t) = e^{\underline{A}t}\,\underline{x}_o + \int_o^t e^{\underline{A}(t-\tau)}.\underline{B}.\underline{u}(\tau)d\tau \qquad (9.7)$$

and if feed input is not time dependent the solution is

$$\underline{x}(t) = \underline{\Phi}(t).\underline{x}_o + \underline{\Delta}(t).\underline{B}.\underline{u} \qquad (9.8)$$

where $\underline{\Phi}(t)$ and $\underline{\Delta}(t)$ are matraces defined in Appendix A.2. The sequencing of the SCCR5 machine was simulated by stepping the concentration profile backwards by one column at the end of the switch time. The above description is applicable only to a single solute species and so

for a system containing i solute species a set of i matraces is required. As the experimental system was a polymer and contained many hundreds of different solute species it was necessary to choose a few components to represent key fractionating ranges. A flowsheet of the computer program used to solve equation (9.8) is contained in Fig. 9.1. A listing of the program together with a list of the variables is contained in Appendix A.2. A three integer numbering system was used to identify a computer simulation;

x - y - z

where x - indentifies variations in programme parameters

y - the number of components in the feed

z - the number in the series.

A summary of the operating conditions for all the computer simulation is contained in Appendix A.2.

9.2 SIMULATION RESULTS FOR A BINARY FEED SYSTEM

9.2.1 Variations in Programme Parameters

The accuracy of the numerical method for the simulation of the SCCR5 chromatograph was investigated using a binary feed system. For constant operating conditions the step-time incrument, and the number of terms included in the calculation of the matraces $\underline{\Phi}(t)$ and $\underline{\Delta}(t)$ were varied. Fig. 9.2 contains a summary of the product compositions at pseudo-steady state. Simulations 1-2-0, 1-2-1, 1-2-2 and 1-2-3 investigated the effect of the number of terms included in the calculation of the matraces $\underline{\Phi}(t)$ and $\underline{\Delta}(t)$. For these simulations 10 stages per column were used with the feed introduct into the 5th column, and a constant step-time of 10 second. Simulation 1-2-0 failed to converge. This indicated that more than three terms were necessary for the

Fig 9.1 Flow diagram for SCCR 5 simulation programme

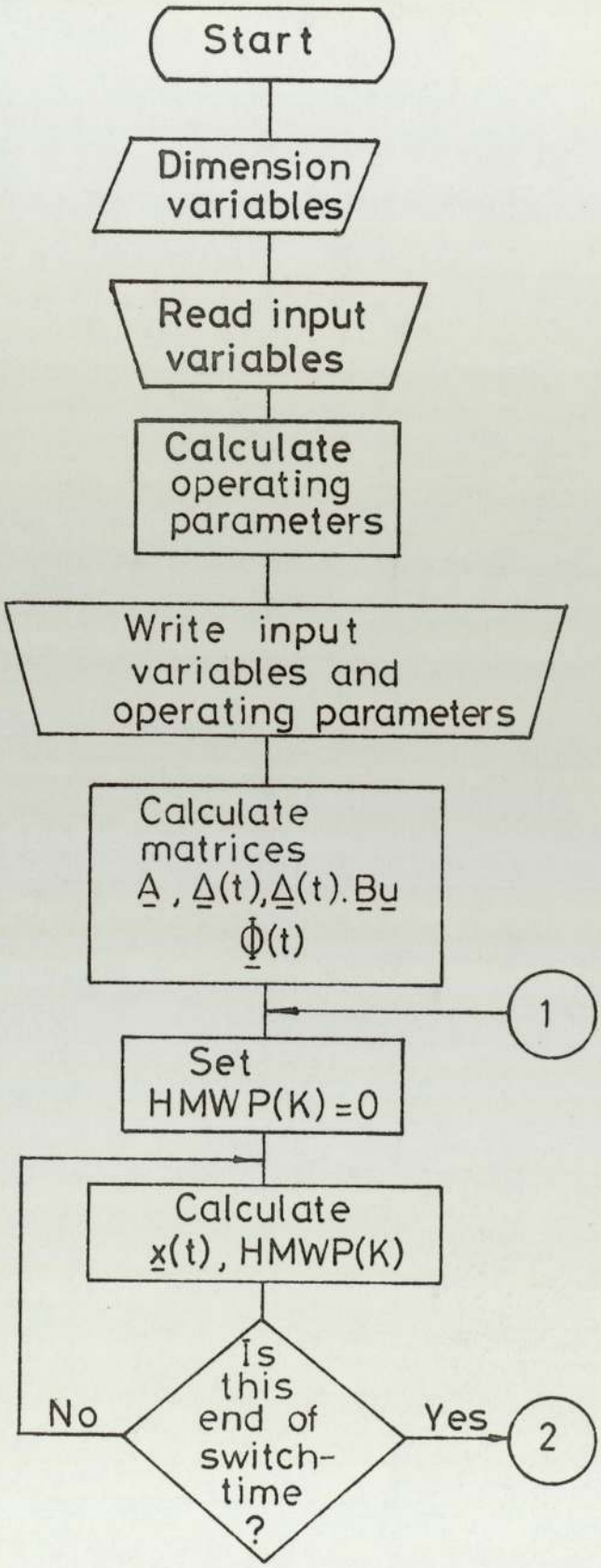


Fig 91(Cont.)

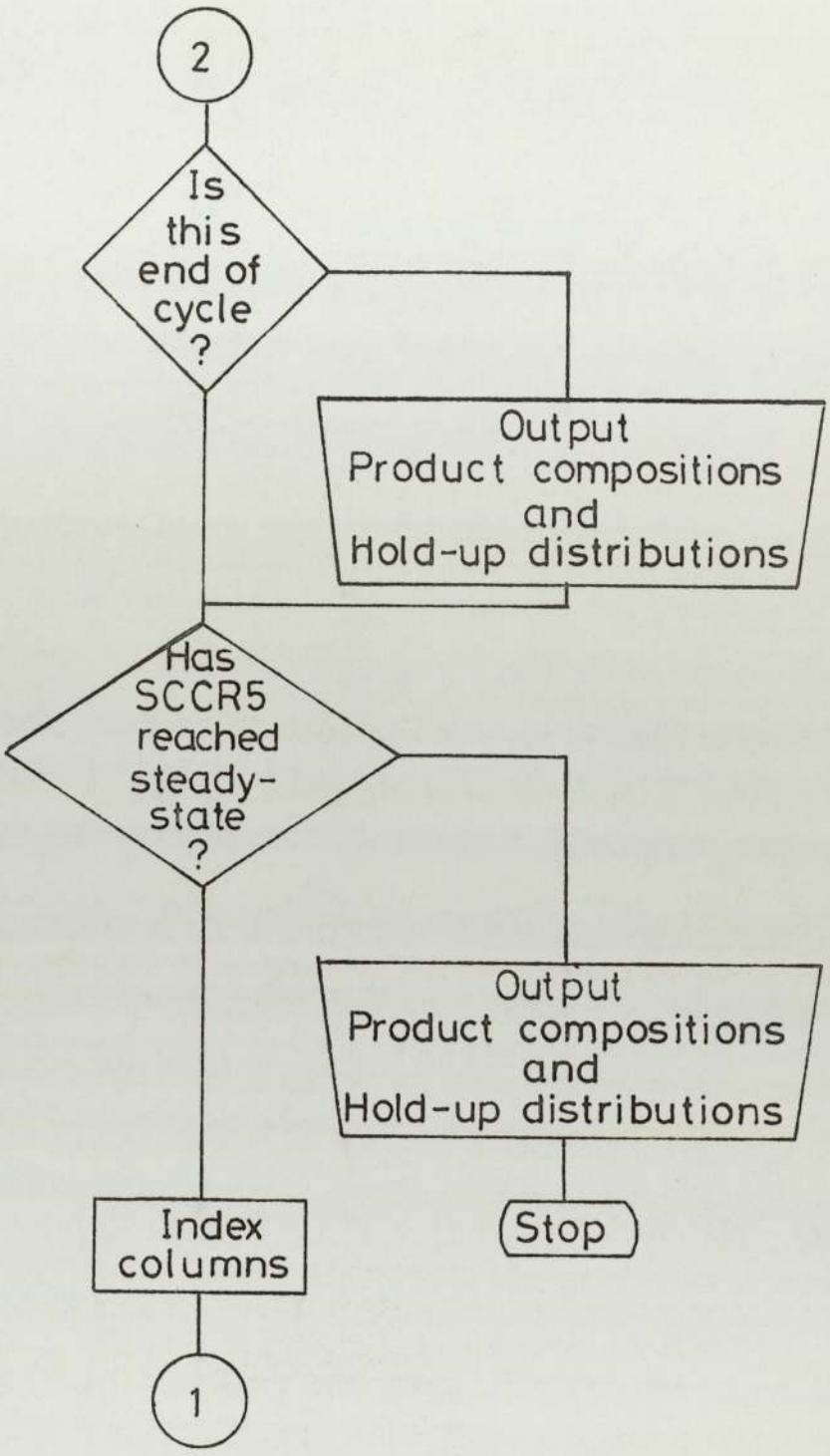

2
Is this end of cycle ?
Output Product compositions and Hold-up distributions
Has SCCR5 reached steady-state ?
Output Product compositions and Hold-up distributions
Index columns
Stop
1

Fig 9.2 Variations in product compositions.

Program parameters.

Flowrates :	eluent	1·667 ml. s^{-1}
	feed	0·333 ml. s^{-1}
Cut-positions:	pre-feed	0·286
	post-feed	0·516
switch-time		450 s
no. tanks per column		10
position of feed tank		51
step-time incrument		10 s
K_d's of components	1	0·75
	2	0·25

Mass balance during final switch.
Basis: g.

Simulation	Input mass	Purge	HMWP	No. of switches
1-2-0				
comp. 1	15	1·021 E1	2·191E-5	∞
2	15	1·074E-2	1·269E 0	
1-2-1				
comp. 1	15	1·665E1	1·797E-4	28
2	15	1·116E-2	1·325E 1	
1-2-2				
comp 1	15	1·666E 1	1·810E-4	28
2	15	1·117E-2	1·337E 1	
1-2-3				
comp 1	15	1·666E 1	1·810E-4	28
2	15	1·118E-2	1·337E 1	

calculation of $\underline{\Phi}(t)$ and $\underline{\Delta}(t)$. The concentration profiles and product compositions for simulations 1-2-2 and 1-2-3 were identical. This indicated that for a step-time increment of 10 seconds, seven terms in the calculation of $\underline{\Phi}(t)$ and $\underline{\Delta}(t)$ provided an accurate result. The maximum deviation in product compositions between simulations 1-2-1 and 1-2-2 was only 0.15%. Simulation 1-2-1 took only 77% of the time required by simulation 1-2-2. Therefore considerable savings in computer time with sufficiently accurate results were possible using only five terms to calculate $\underline{\Phi}(t)$ and $\underline{\Delta}(t)$.

The effect of the step-time increment on the accuracy of the numerical method was also investigated using the same flow conditions as the above simulations. But the number of stages per column was reduced from 10 to 7. Earlier computer programs checking the operation of the subroutines FRA DELTAT and FRA MAT showed that the numerical method was unstable if a step-time increment of greater than 20 seconds was used. Therefore a 10 second step-time increment was considered to be the maximum value that could be used. Simulations 2-2-1 and 2-2-2 investigated the effect of reducing the step-time increment from 10 to 2 seconds. Simulation 2-2-1 took only 17.5% of the time required by simulation 2-2-2. The major component in the purge and high molecular weight products changed by 1.62% and 0.34% respectively.

Therefore it was considered that for subsequent simulations sufficiently accurate results would be obtained if a 10 second step-time increment was used and the matraces $\underline{\Phi}(t)$ and $\underline{\Delta}(t)$ were evaluated to five terms.

The effect of reducing the number of stages per column between simulations 1-2-2 and 2-2-1 changed the composition of the eluted products and changed the distribution of dextran hold-up in the fractionating

column. A summary of the effect of changing the number of stages per column on the composition of the eluted products is contained in Fig. 9.3. Details of the dextran hold-up are illustrated in Figs. 9.4 to 9.7. From these diagrams the following observations were drawn;

(1) the mass of component 1 in the purifying section was reduced as the number of stages increased.

(2) the mass of component 2 in the stripping section was reduced as the number of stages increased.

(3) the greater the number of tanks the greater the resolution of the components.

The program execution time was directly related to the number of stages per column and a balance between execution time and resolution was sought. A value of 5 stages per column was chosen.

9.2.2 Effect of Increasing Feedrate

The effect of increasing the feed flowrate was investigated using a binary feed system. The increase in feed flowrate changed the post-feed theoretical cut-position which increased the migration rate of all the molecules in the purifying section. A summary of the operating conditions is contained in Fig. 9.8. Simulation 4-2-1 used similar pre-feed conditions as simulation 1-2-1, but doubling the feedrate, which increased the post-feed theoretical cut-position from 0.528 to 0.747. This caused a significant change in the distribution of component 1 in the purifying section as Figs. 9.4, 9.9 and 9.10 illustrate. This was also reflected in the composition of the high molecular weight product where the percentage of component 1 increased from 0.188 to 6.41%. The increased flowrate had little effect on the distribution of component 2 or on the mass of component 2 present in the low molecular weight product.

Fig9.3 Effect of number of tanks per column on product compositions.

Mass balance during final switch

Basis: g

Simulation	Input mass	Purge	HMWP	No. of tanks per column	No. of switches
1-2-1					
comp. 1	15	1.665E 1	1.797E-4	10	28
2	15	1.116E-2	1.325E 1		
2-2-1					
comp. 1	15	1.654E 1	3.834E-3	7	30
2	15	8.161E-2	1.316E 1		
3-2-1					
comp. 1	15	1.650E1	3.112E-2	5	33
2	15	3.278E-1	1.306E 1		
3-2-2					
comp. 1	15	1.544E1	3.879E-1	2	32
2	15	3.343E 0	1.076E 1		

Fig9.4 Concentration profile for simulation 1-2-1.

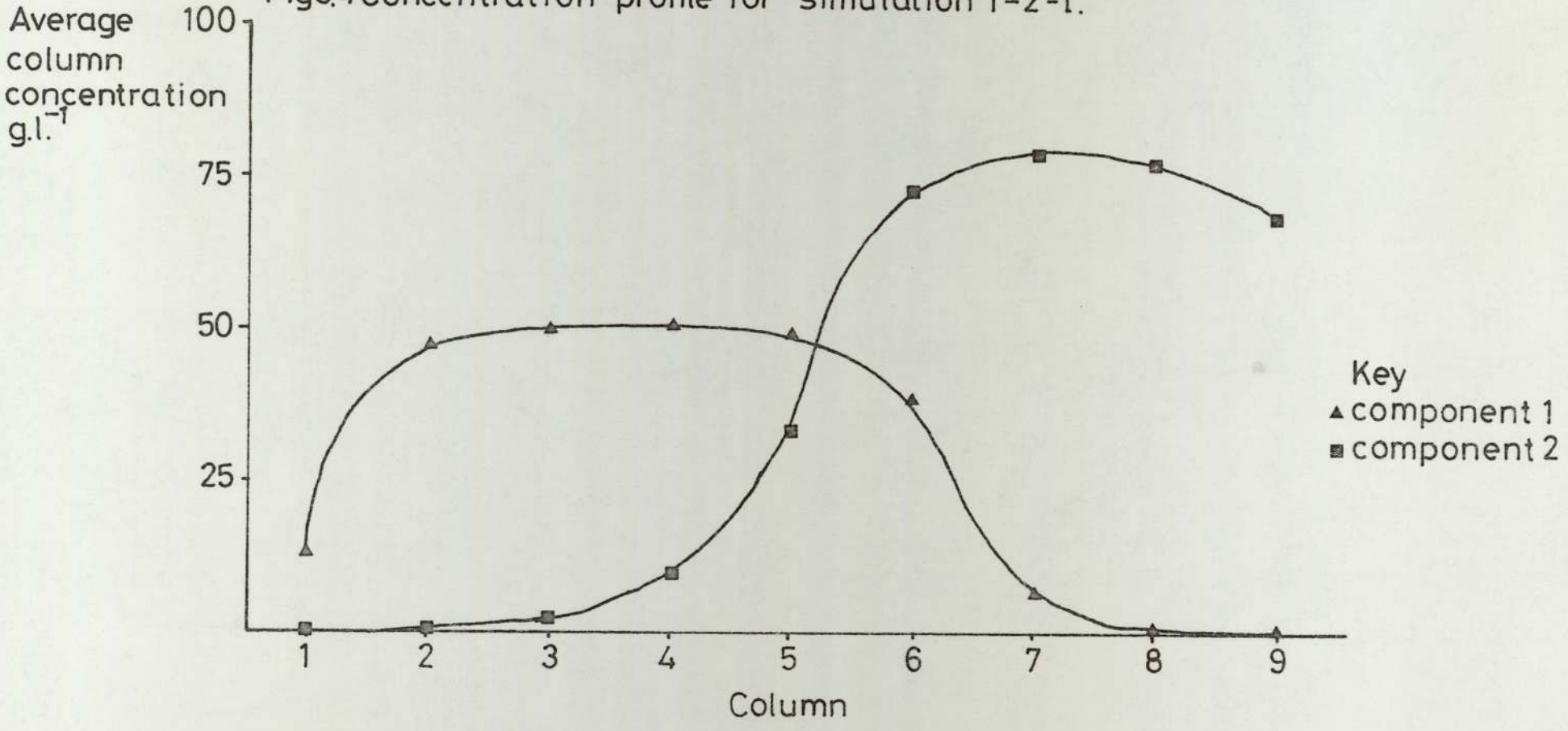


Fig9.5 Concentration profile for simulation 2-2-1.

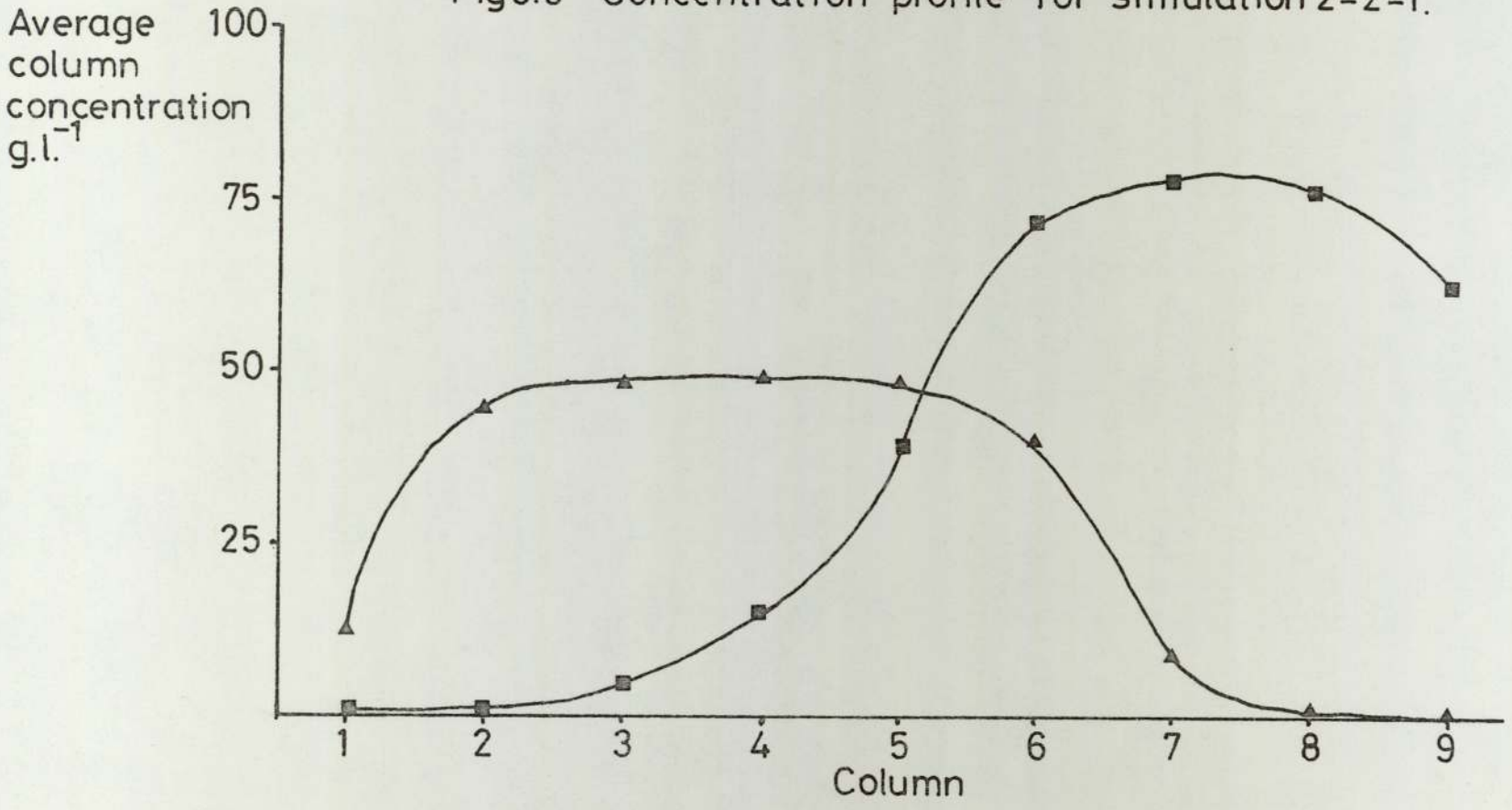


Fig 9.6 Concentration profile for simulation 3-2-1.

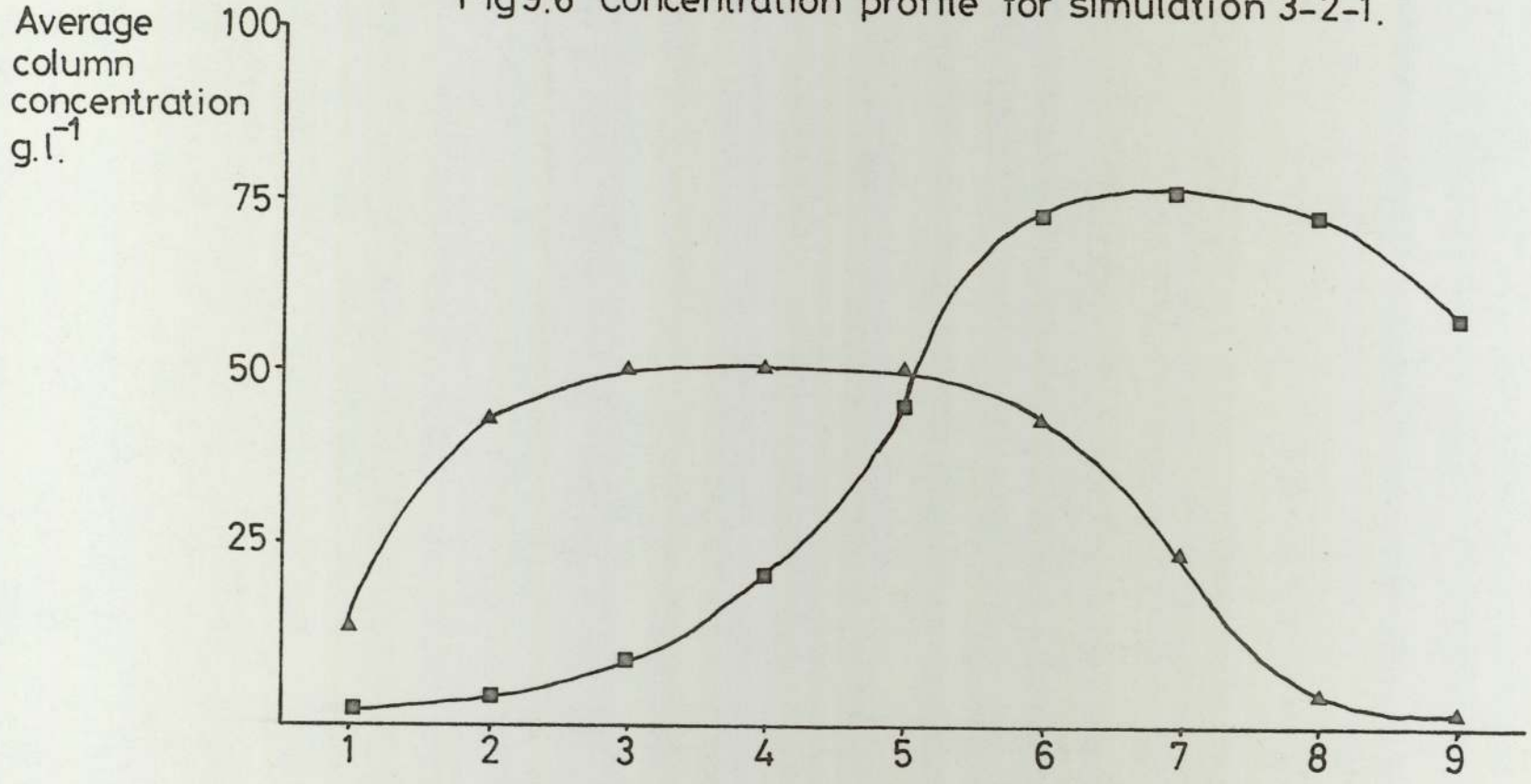


Fig9.7 Concentration profile for simulation 3-2-3.

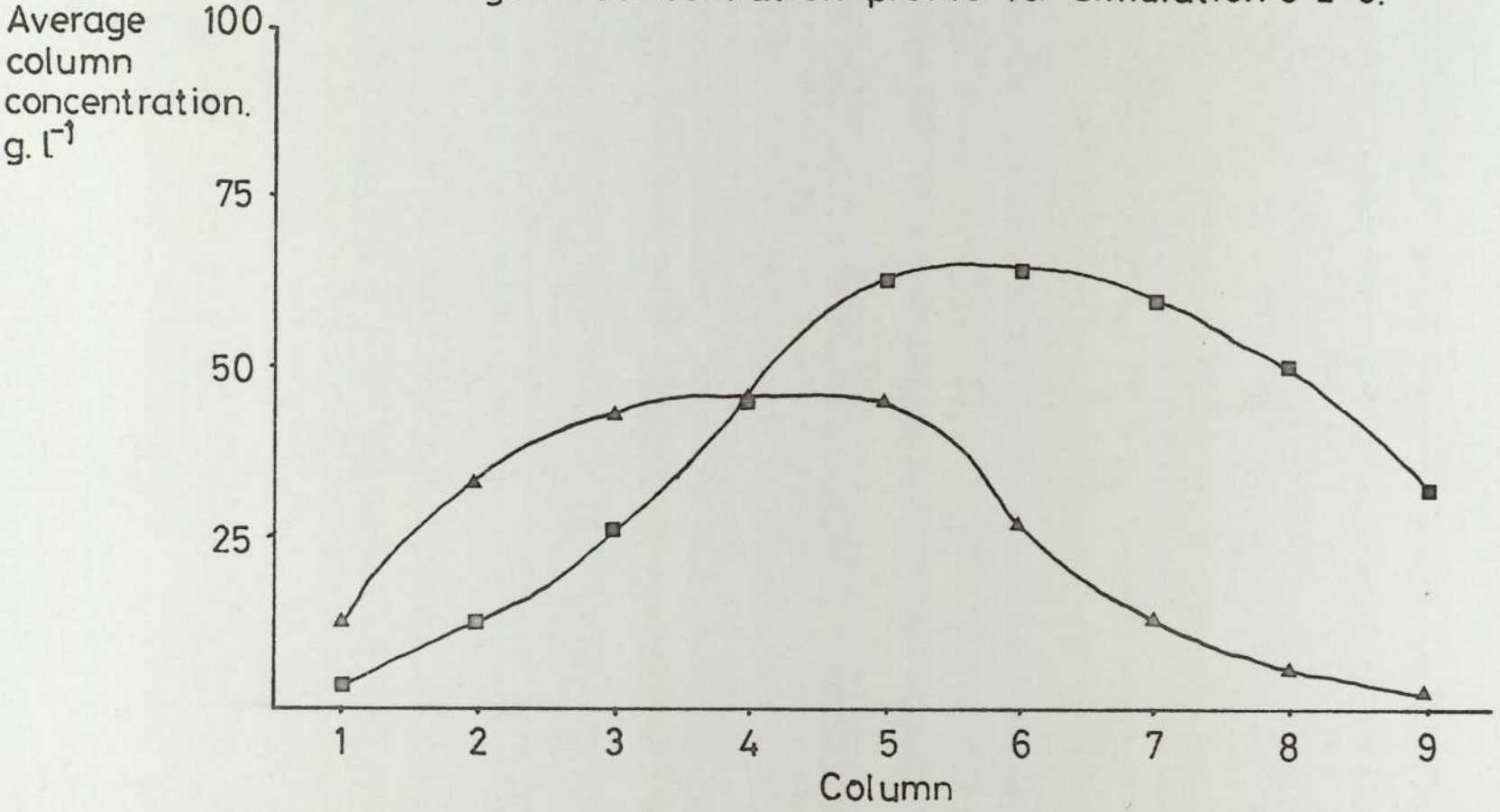


Fig 9.8 Effect of feed flowrate on product composition.

Simulation	Feed flowrate	Input mass	Purge	HMWP	No. of switches
1-2-1					
comp. 1	0.333	15	1.665E 1	1.797E-4	28
2	0.333	15	1.116E-2	1325E 1	
4-2-1					
comp 1	0.666	30	2.817E 1	1.930E 0	31
2	0.666	30	3.879E-1	2.897E 1	
4-2-2					
comp 1	0.926	41.7	3.175E 1	9.929E 0	29
2	0.926	41.7	3 789 E-1	4.087E 1	

Fig9.9 Concentration profile for simulation 4-2-1.

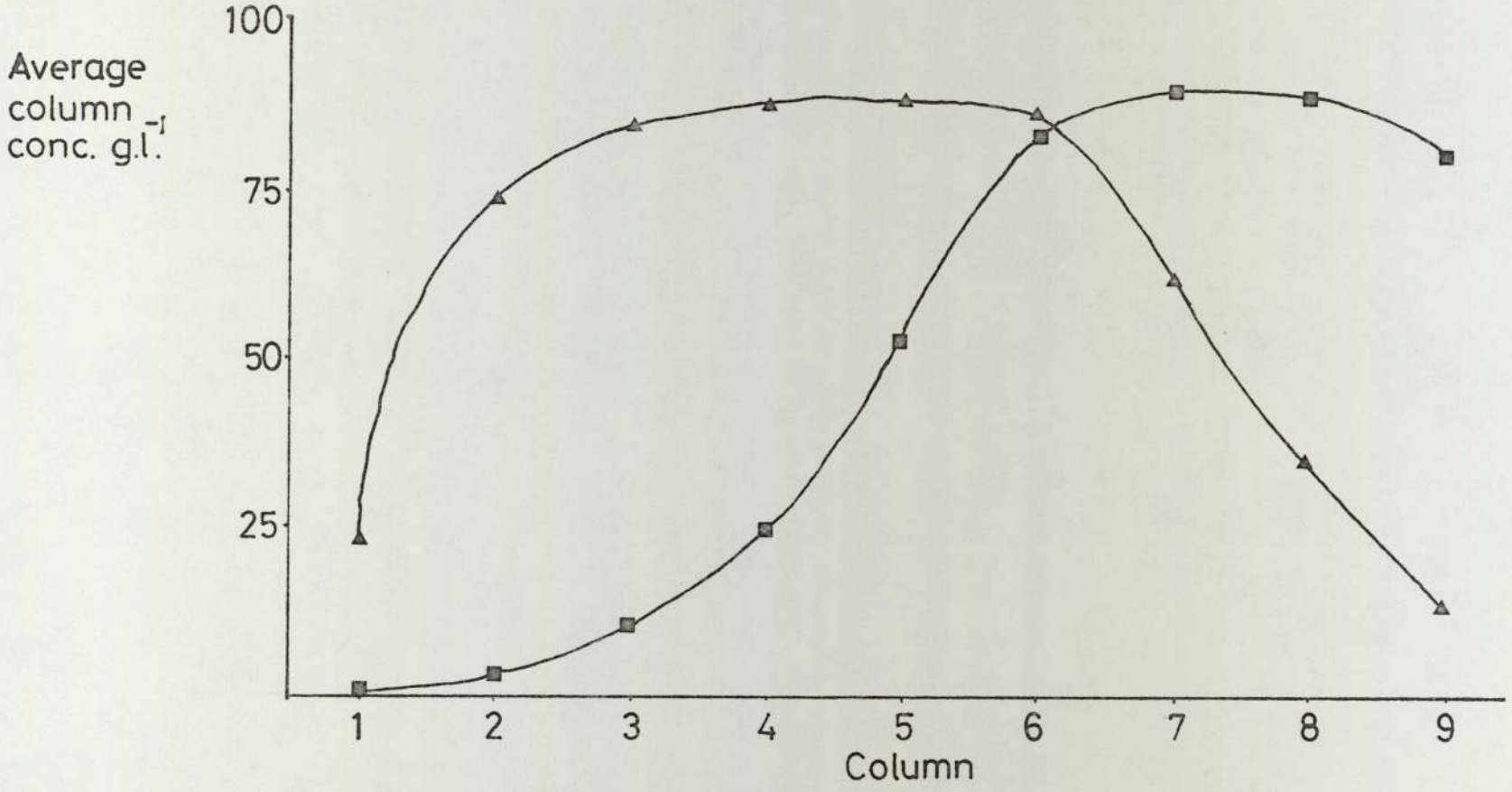
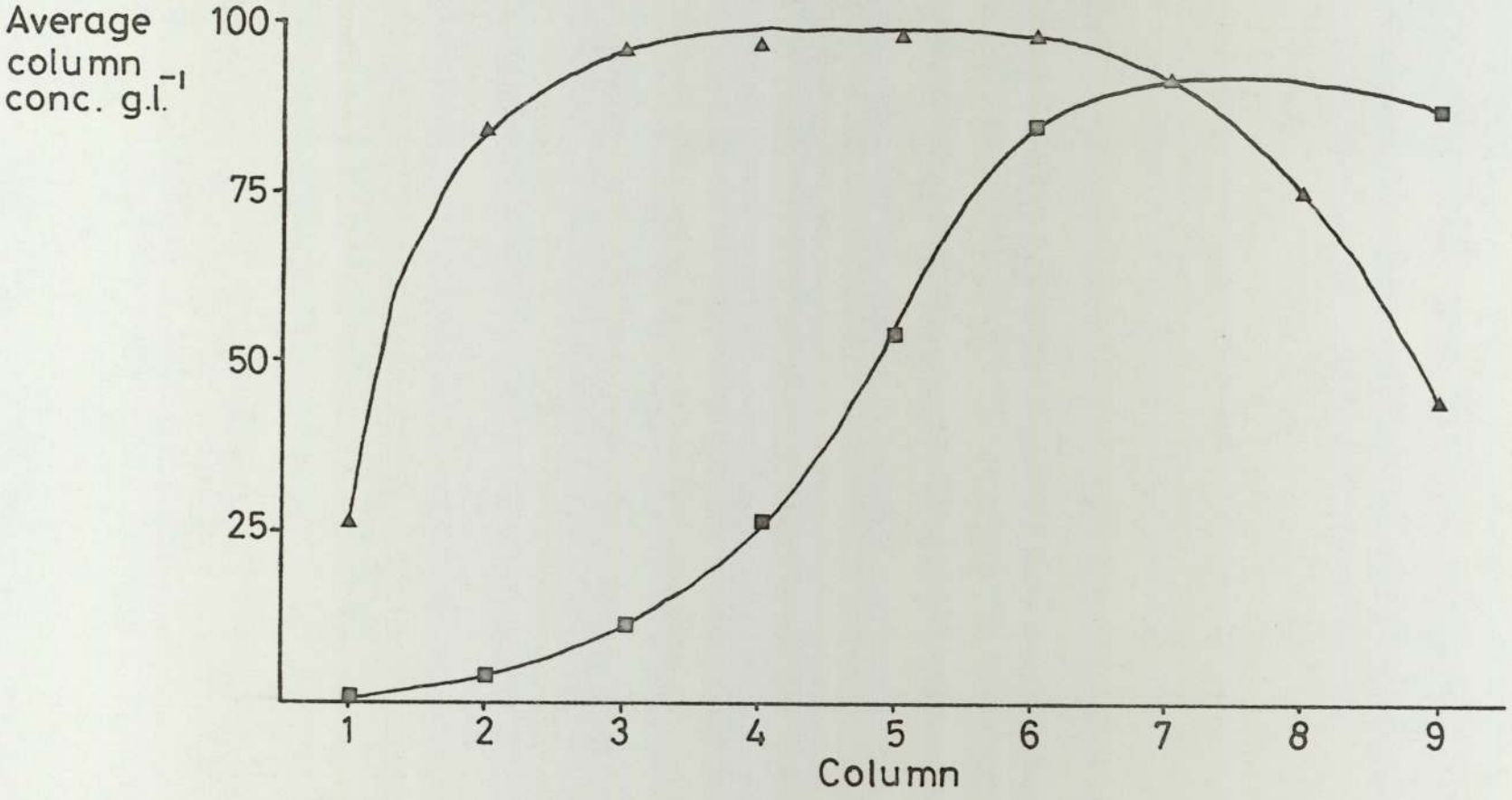


Fig 9.10 Concentration profile for simulation 4-2-2.



When the feed-rate was increased again in simulation 4-2-2, a further loss of component 1 to the high molecular weight product was observed, and the actual mass of component 2 present in the low molecular weight product remained approximately constant.

The significant flowrate dependence of the composition of the high molecular weight product was the major discrepancy between the experimental results and the simulation programme. This discrepancy is due to the simplifying assumption of the linear elution controlled method for the gel permeation process. In the SCCR5, concentration interactions caused a retardation of the migration rates of the molecules as the feed flowrate was increased. The inclusion of a concentration dependent distribution coefficient into the SCCR5 simulation programme would result in a more realistic model of the process.

9.3 SIMULATION RESULTS FOR A POLYMERIC FEED SYSTEM

Simulations of the dextran polymer system were represented by a five-component feed system. The relative concentrations of each component were obtained from an elution chromatograph of dextran BT161D on Porasil C. The elution volume between the interstitial and total liquid volumes was divided into five equal divisions and the relative concentration of each fraction calculated.

9.3.1 Effect of Increasing Feedrate

Simulations 1-5-1, 1-5-2 and 1-5-3 used similar theoretical cut-positions to those used in the high molecular weight removal experiments. A summary of the pseudo-steady state distribution profiles and the product distributions are contained in Figs. 9.11 to 9.14.

Fig 9.11 Concentration profile for simulation 1-5-1.

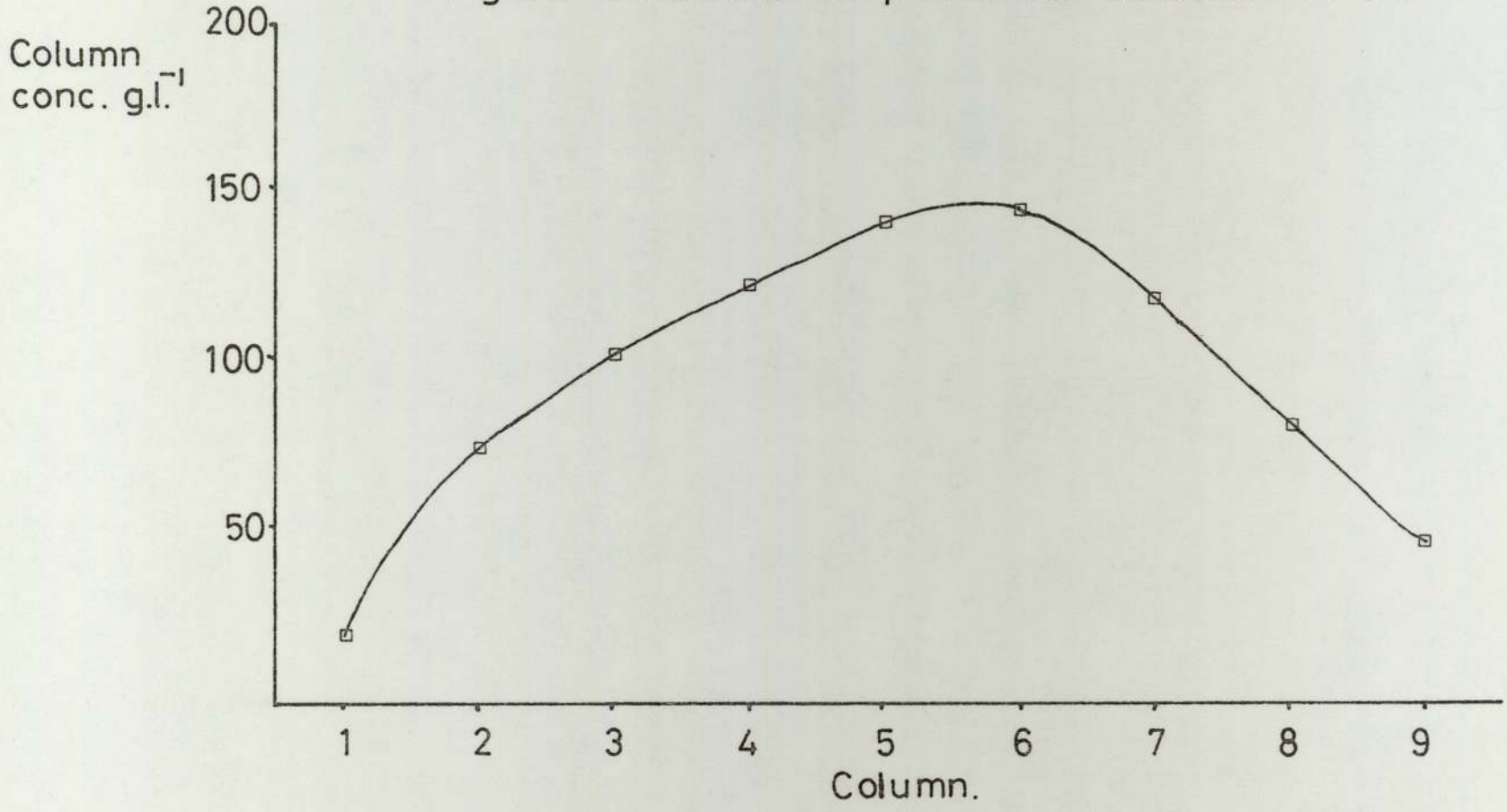


Fig 9.12 Concentration profile for simulation 1-5-2.

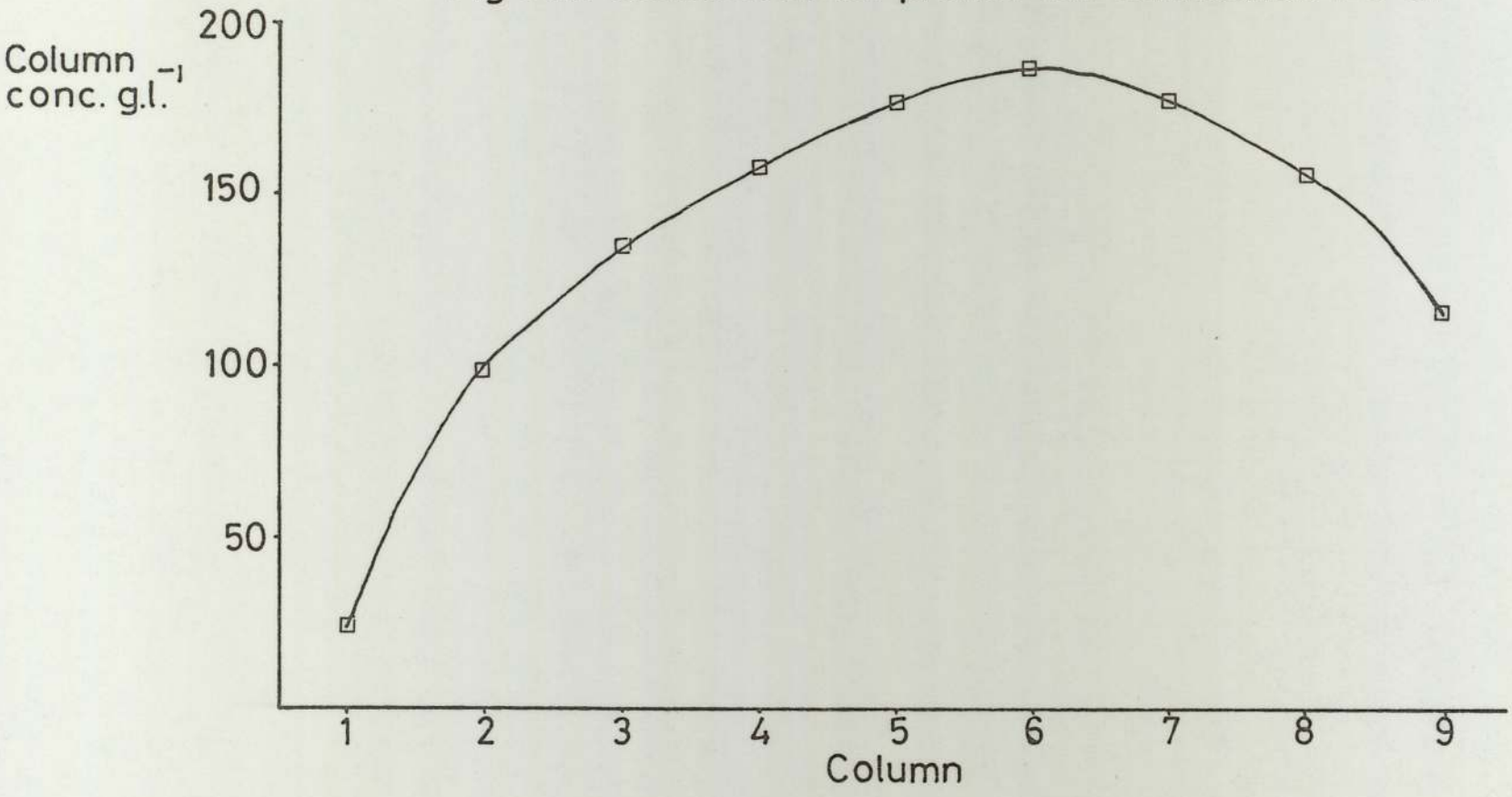


Fig 9.13 Concentration profile for simulation 1-5-3.

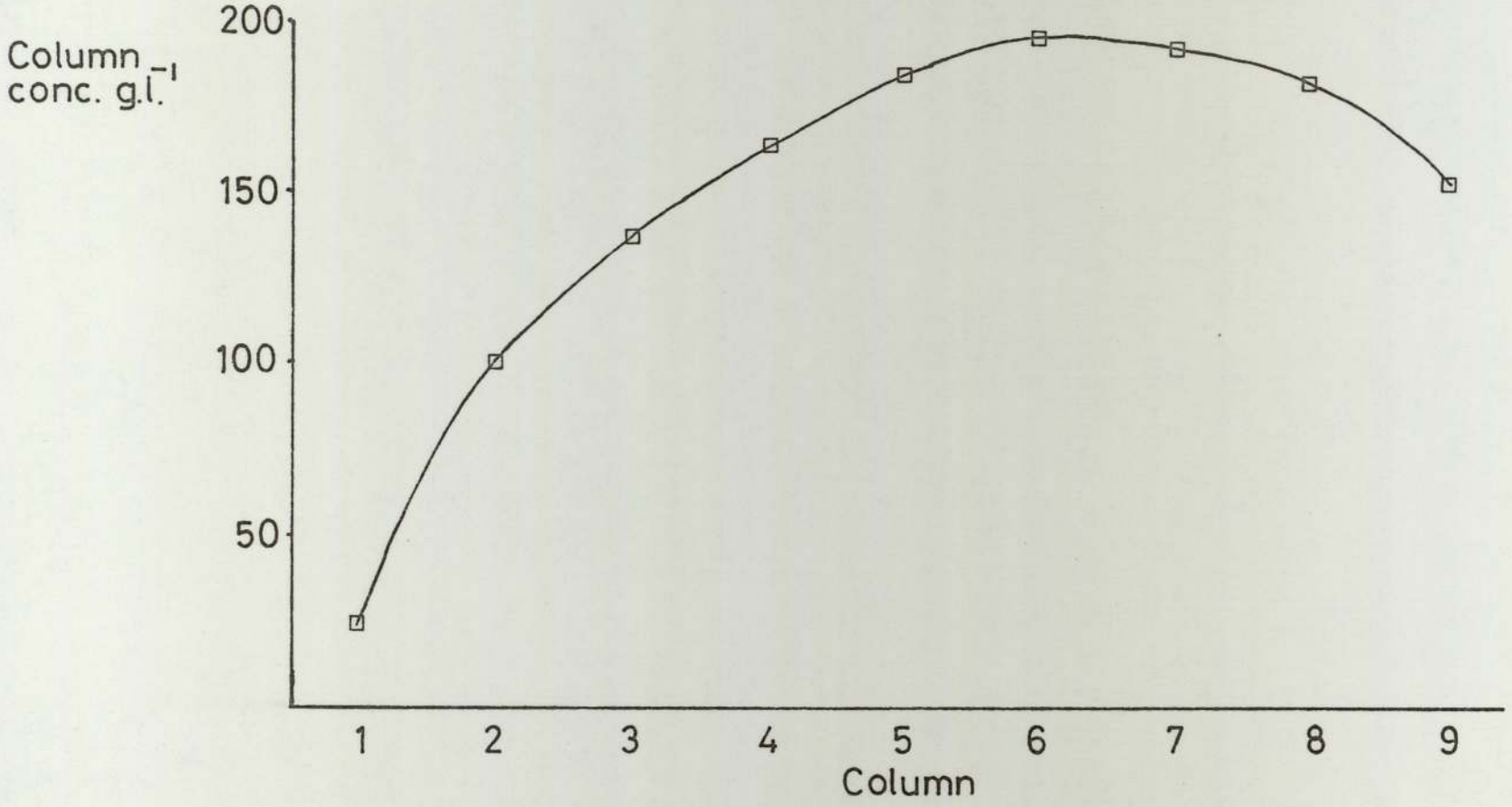


Fig 9.14 Effect of feed flowrate on product compositions.

Basis: g

Component	Simulation 1-5-1		Simulation 1-5-2		Simulation 1-5-3	
	Purge	HMWP	Purge	HMWP	Purge	HMWP
1	2·261E 0	9·863E-4	4·169E 0	9·967E-2	4·896E 0	7·548E-1
2	7·622E 0	6·577E-2	1·156E 1	2·186E 0	1·208E 1	7·126E 0
3	1·034E 1	1·766E 0	1·174E 1	1·285E 1	1·094E 1	2·328E 1
4	1·365E 0	5·347E 0	1·293E 0	1·375E 1	1·118E 0	2·003E 1
5	2·216E-3	9·331E-1	2·437E-3	1·872E 0	2·305E-3	2·597E 0

The effect of increasing the feedflow rate caused a change in the product distributions. The closest agreement for the product distribution for the theoretical model and the experimental program was for simulation 1-5-1 and experiment A where the highest eluent-to-feed ratio was used. The reason for the increase in the high molecular weight product with increased feed flowrate was that the computer simulation could not model the concentration dependence of the gel permeation process that retards the migration of the molecules that are able to penetrate the pores of the packing. Therefore the computer simulations show lower column concentrations in the stripping section and higher column concentrations in the purifying section than was obtained in the experimental programme.

Simulations 2-5-1 and 2-5-2 used a pre-feed theoretical cut point of 0.7. The concentration profiles for these simulations are illustrated in Fig. 9.15 and 9.16, and the product distributions in Fig. 9.17. Unfortunately due to the inherent nature of the model to elute a greater quantity of feed material from the high molecular weight product port than was observed in the experimental programme, these simulation only removed a small quantity, less than 5.6% of the feed material in the purge product. Therefore for subsequent low molecular weight removal simulations the pre-feed theoretical cut-position was reduced to 0.5. However, the number of switches required for simulations 2-5-1 and 2-5-2 to reach pseudo-steady state was 57 and 75, respectively. This was much greater than the high molecular weight removal simulations. This reflected an observation made in the experimental programme; low molecular weight removal experiments took longer to reach pseudo-steady state than the high molecular weight removal experiments.

Fig9.15 Concentration profile for simulation 2-5-1.

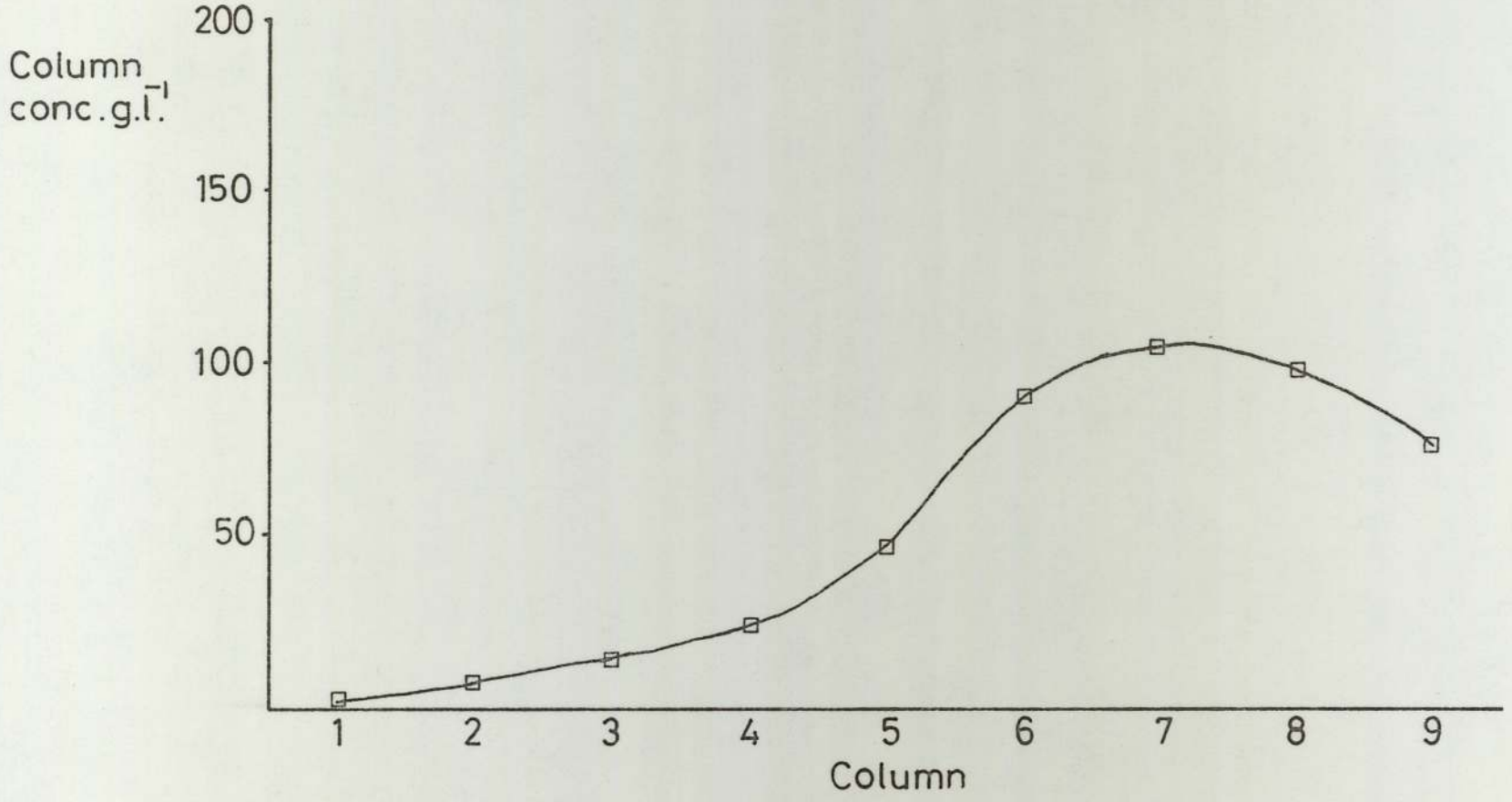


Fig 9.16 Concentration profile for simulation 2-5-2.

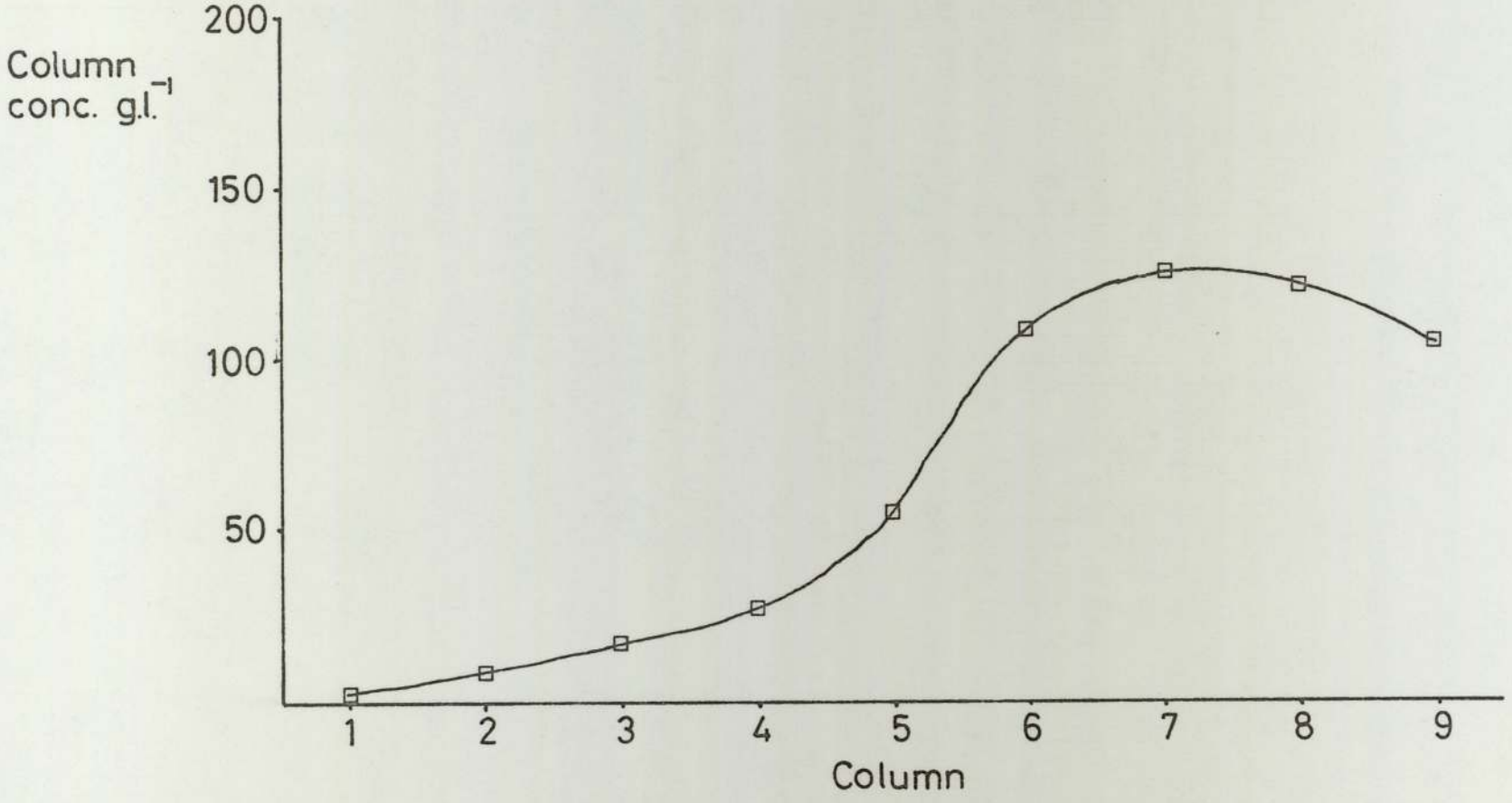


Fig 9.17 Product compositions for simulations with a pre-feed cut-position of 0.7.

Basis: g

Component	K_d	Simulation 2-5-1			Simulation 2-5-2		
		Input	Purge	HMWP	Input	Purge	HMWP
1	0.833	1.697 E0	9.589E-1	5.701 E-1	2.810 E 0	9.998E-1	1.506 E 0
2	0.667	5.469E 0	4.036E-1	4.817 E 0	9.059E 0	3.976E-1	8.315E 0
3	0.500	1.019 E 1	9.391E-3	1.030E 1	1.688E1	1.161 E-2	1.703E 1
4	0.333	6.511E 0	1.333E-5	6.580E 0	1.078 E1	1.855E-5	1.089E1
5	0.167	7.560E-1	8.569E-10	7.630E-1	1.252E0	1.253E-9	1.263E 0

Reducing the pre-feed theoretical cut-position to 0.5 increased the quantity of material removed in the low molecular weight product. Simulations 3-5-1 to 3-5-3 showed that reducing the eluent-to-feed ratio from 5 to 1.25 reduced the quantity of low molecular weight product from 32.5 to 5.8% of the product output, although the actual mass of the low molecular weight product remained approximately constant. There were no equivalent experiments performed using the pre-feed operating condition of 0.5, but the pseudo-steady state profiles for these simulations exhibit similar profiles to the low molecular weight removal experiments in Chapters 6.0 and 8.0.

9.3.2 Effect of Changing the Feedpoint Location

The effect of changing the feedpoint location for both the high and the low molecular weight simulations was investigated. For the high molecular weight removal simulations the theoretical cut-positions of simulation 1-5-1 were repeated, and for the low molecular weight removal simulations the conditions of simulation 3-5-1 were used. Changes in the pseudo-steady state distribution profiles and the composition of the products were observed when the feedpoint location was moved. These results are summarised in Figs. 9.22 to 9.25.

When the feedpoint location was moved closer to the eluent inlet port, the quantity of high molecular weight product was reduced. This aided the low molecular weight experiments to a limited extent because it reduced the quantity of unrequired low molecular weight material in the high molecular weight product. This method of operating the SCCR equipment with the feedpoint location close to the eluent port will be restricted by the loss of the required product fraction to the low molecular weight product.

Fig 9.18 Concentration profile for simulation 3-5-1.

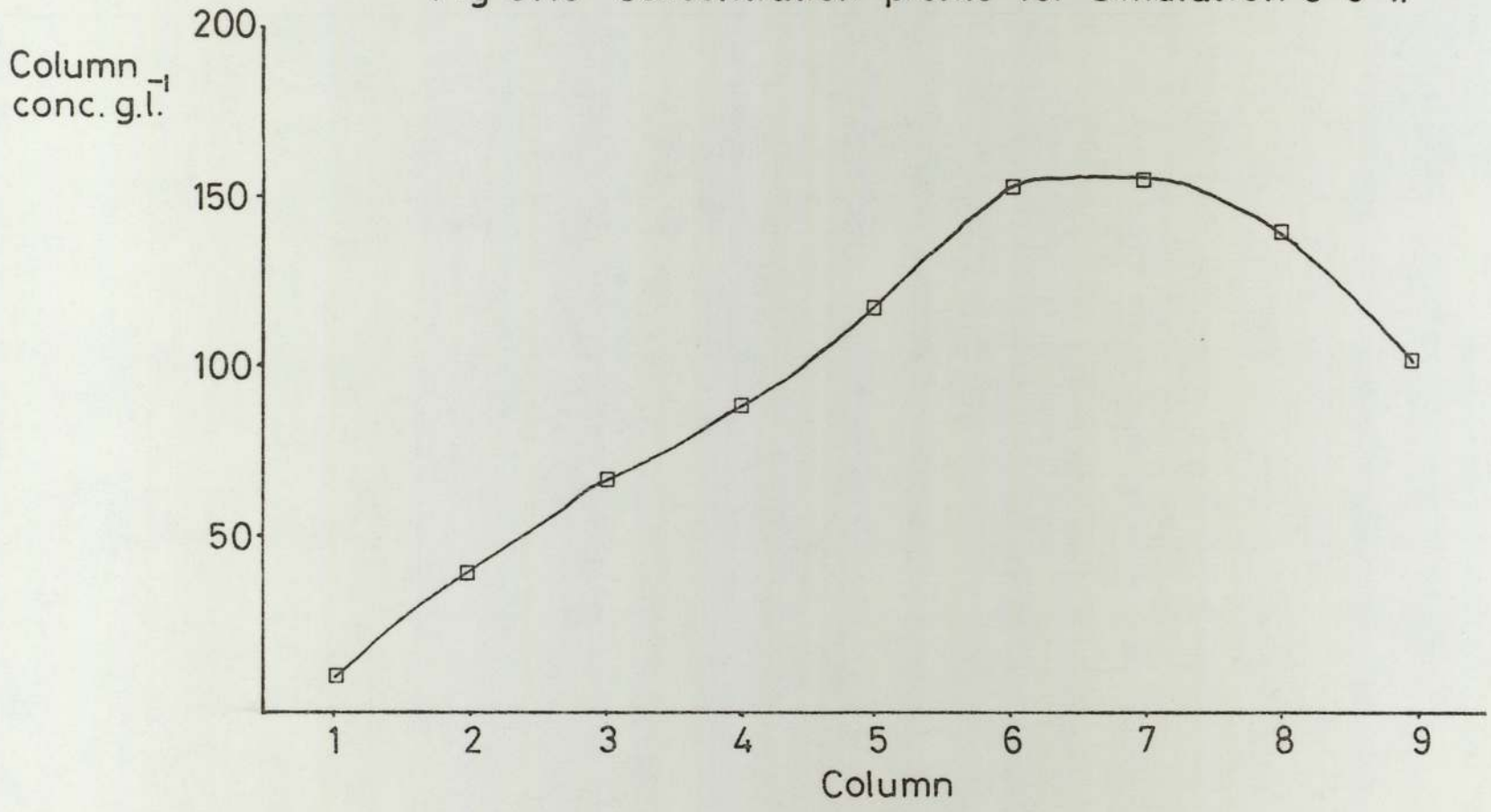


Fig 9.19 Concentration profile for simulation 3-5-2.

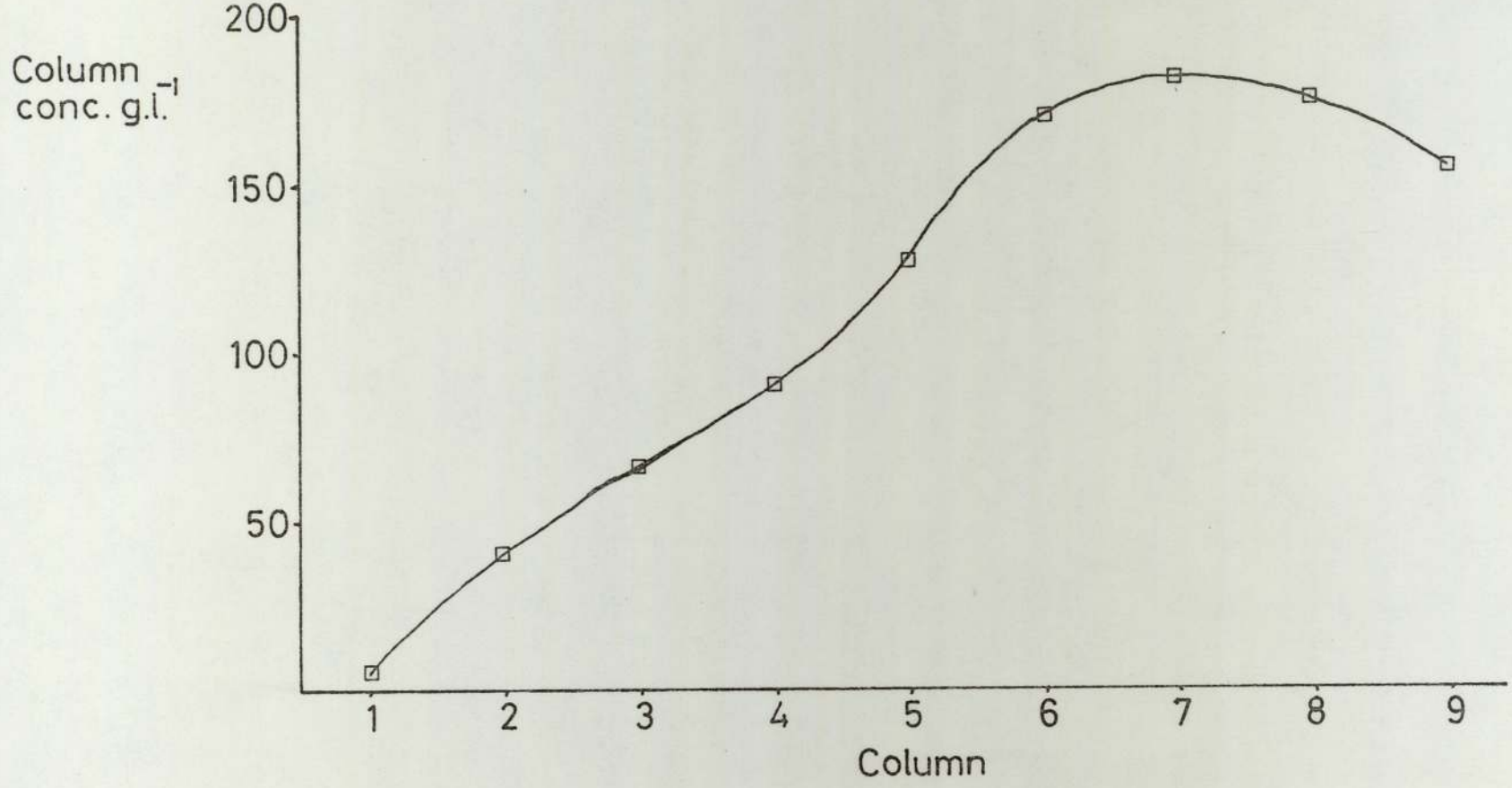


Fig 9.20 Concentration profile for simulation 3-5-3.

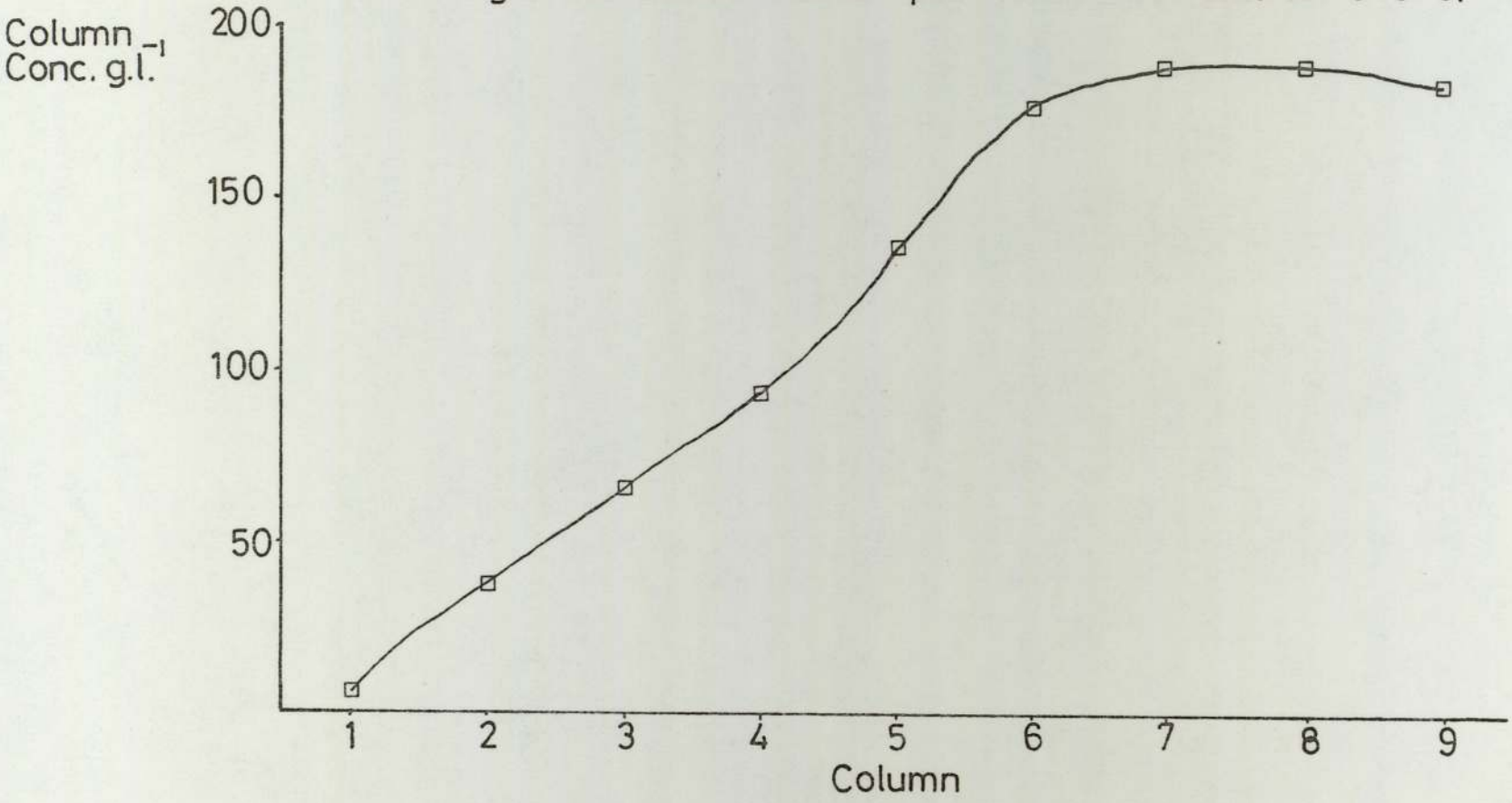


Fig 9.21 Product composition for simulations with a pre-feed cut-position of 0.5.

Basis: g

Component	K_d	Simulation 3-5-1			Simulation 3-5-2			Simulation 3-5-3		
		Input	Purge	HMWP	Input	Purge	HMWP	Input	Purge	HMWP
1	0.833	2.451E 0	2.417E 0	1.488E-1	4.903E0	3.097E 0	1.870E0	9.806E0	3.027E0	6.884E0
2	0.667	7.902E 0	4.843E 0	3.034E0	1.580E1	4.737E0	1.095E 1	3.161E 1	4.083E0	2.689E 1
3	0.500	1.472E 1	1.383 E 0	1.284E 1	2.944E 1	1.240E0	2.750E1	5.888E1	1.023E0	5.694E 1
4	0.333	9.407E 0	9.910E-3	9.493E0	1.881E1	1.181E-2	1.895E1	3.763E 1	1.229E-2	3.785E 1
5	0.167	1.092E 0	1.154E-6	1.103E 0	2.185E 0	1.712E-6	2.203E0	4.369E0	2.296E-6	4.400E0

Fig 9.22 Concentration profile for simulation 4-5-1.

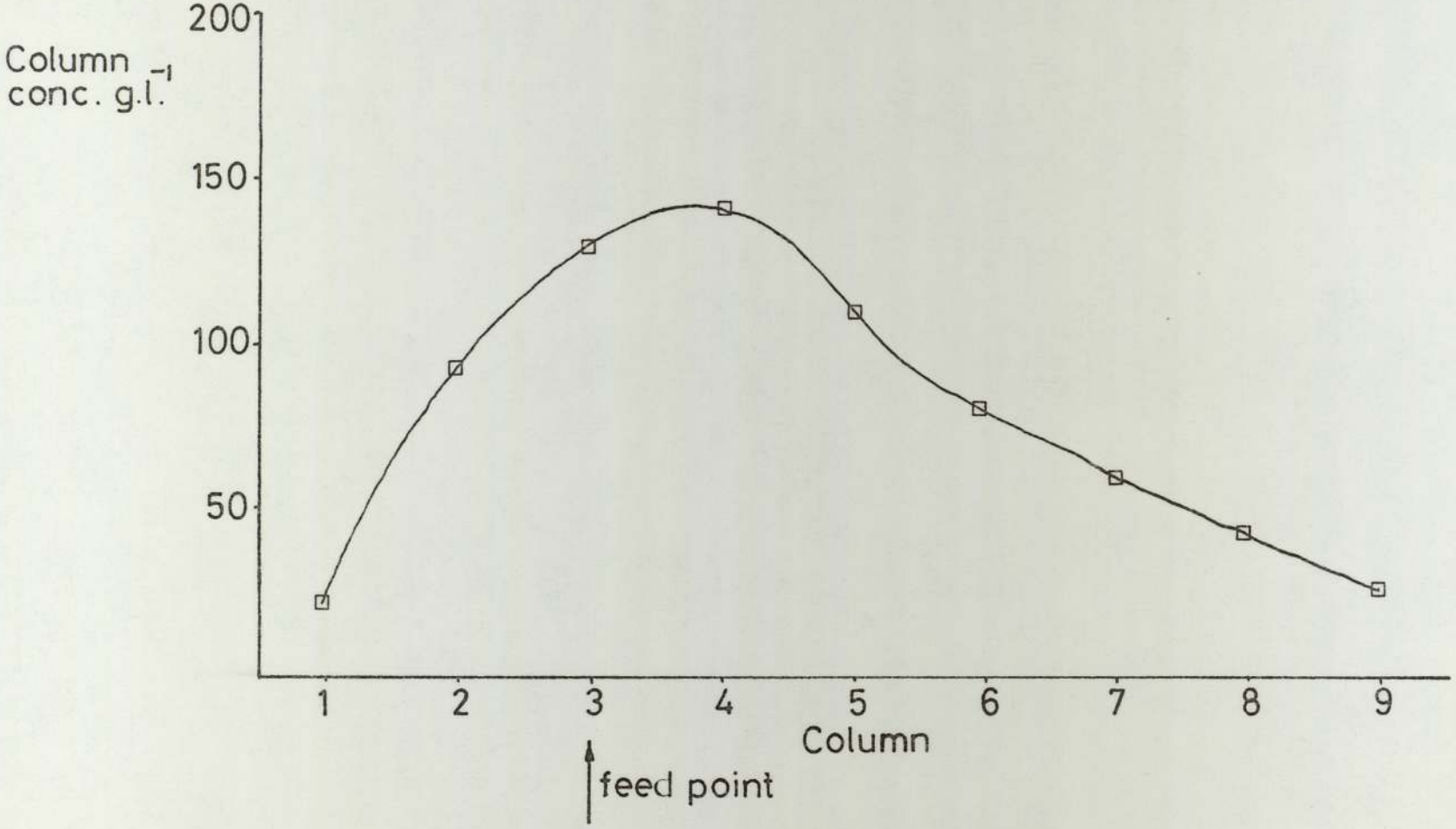


Fig 9.23 Concentration profile for simulation 4-5-2.

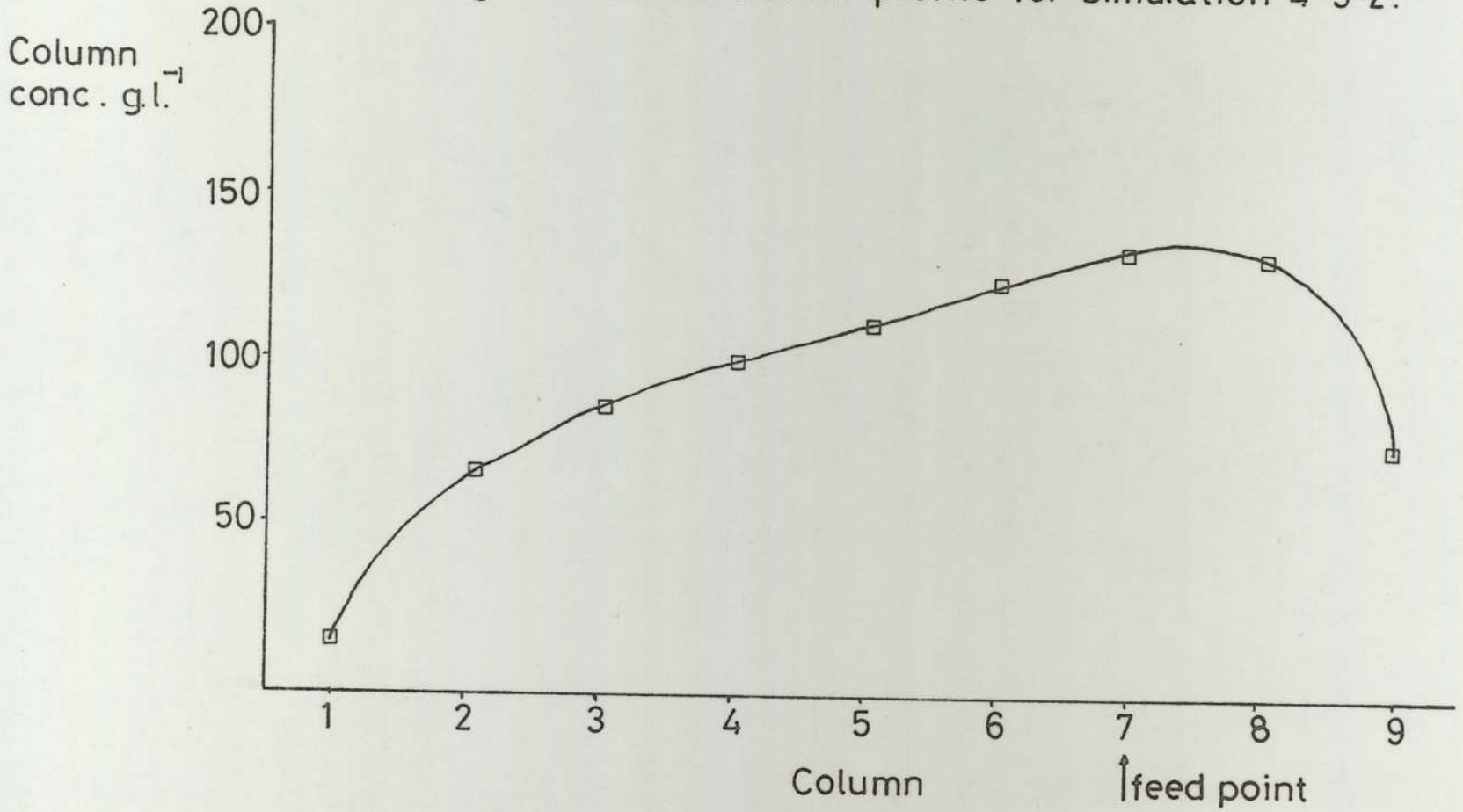


Fig 9.24 Concentration profile for simulation 4-5-3.

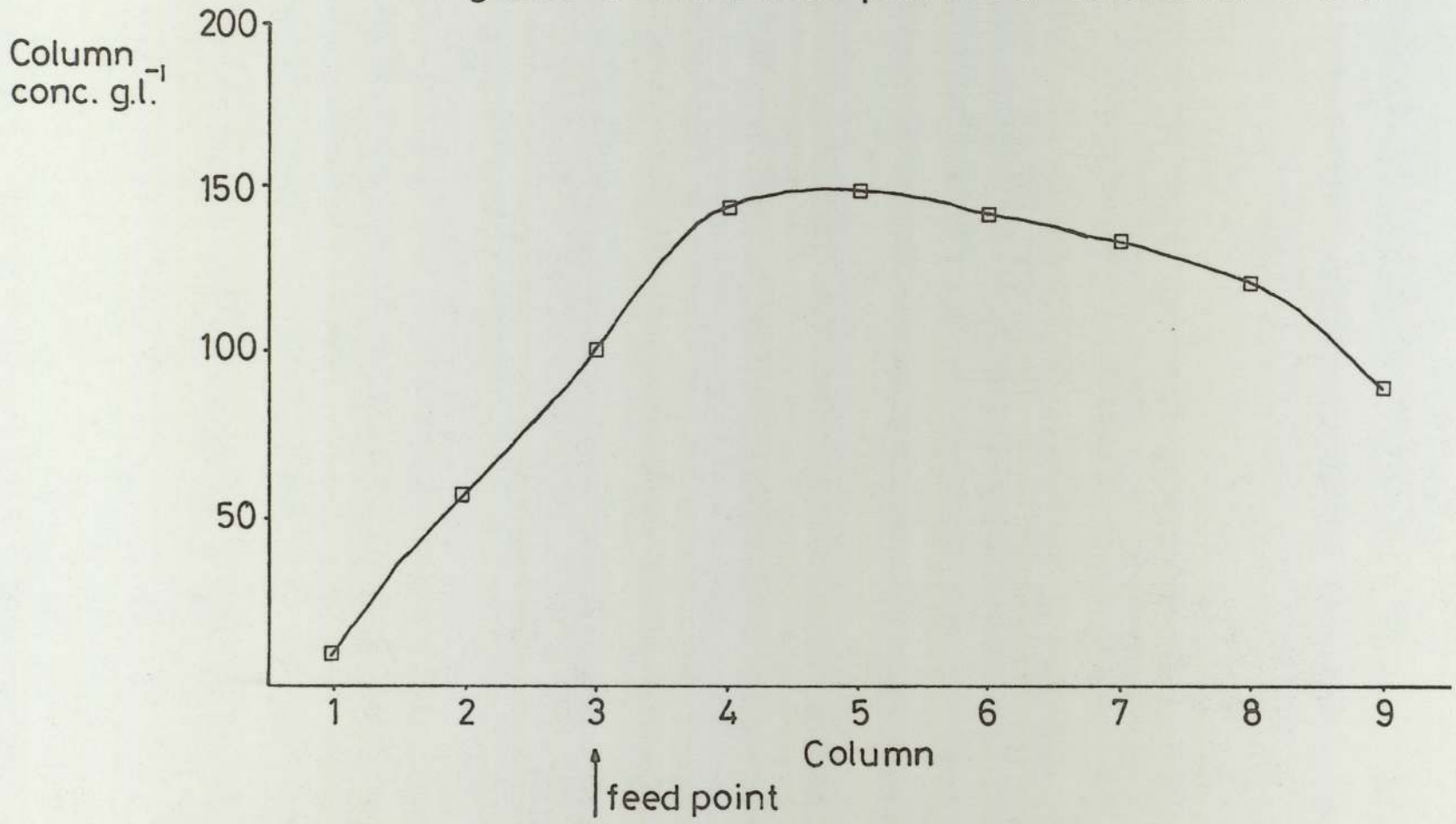
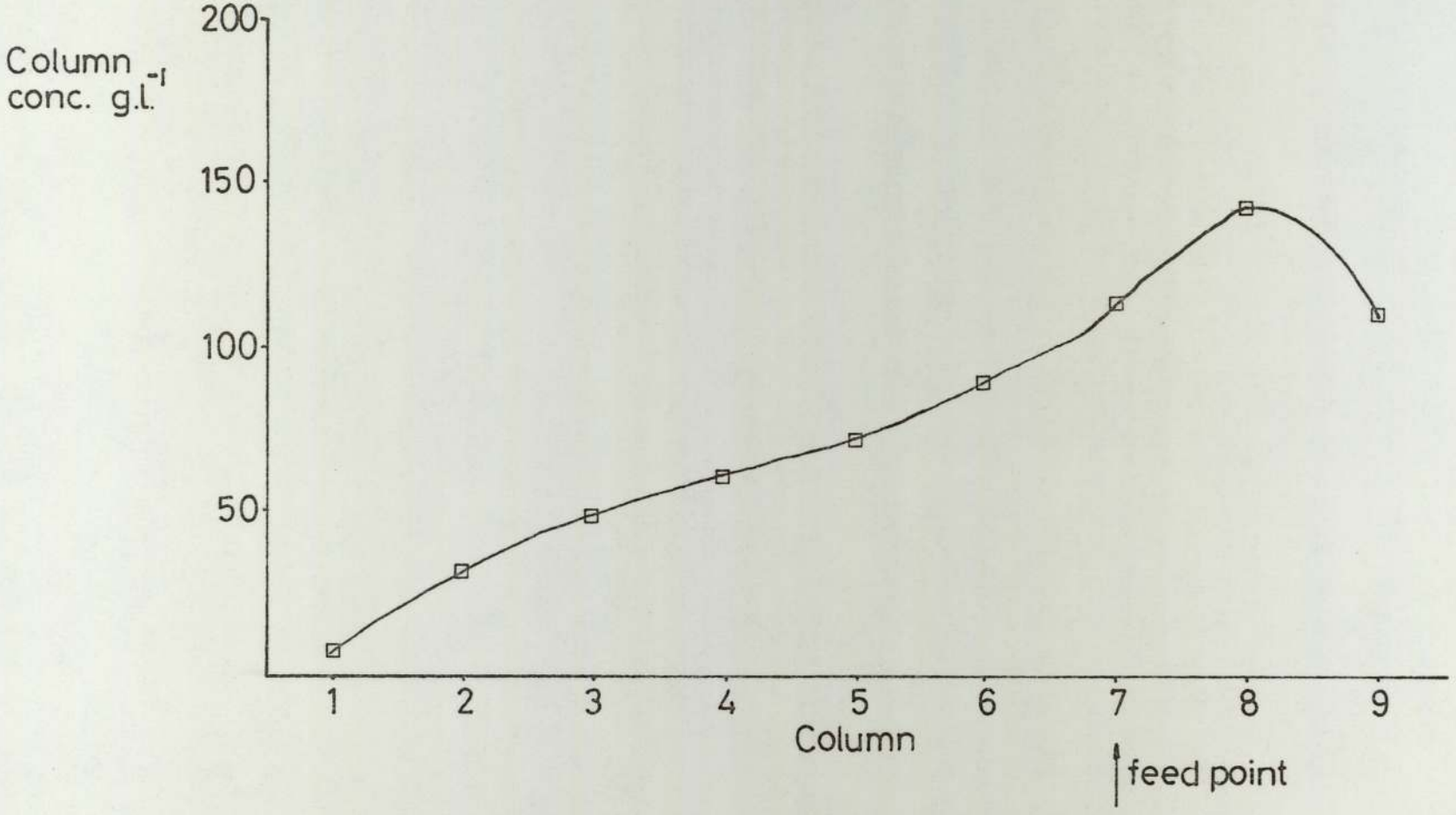


Fig 9.25 Concentration profile for simulation 4-5-4.



When the feedpoint location was moved further away from the eluent port for the same operating conditions the quantity of high molecular weight product increased. This may have a beneficial effect for high molecular weight fractionations because it reduces the quantity of high molecular weight material in the required product. Analogous to the low molecular weight fractionations the method of operating the SCCR equipment will be restricted by the loss of low molecular weight material to the high molecular weight product.

The above changes in product compositions with a change in feedpoint location indicate that an optimum feedpoint location will occur for a set of operating conditions. If the concentration interactions of the dextran molecules can be successfully modelled, then the best method of obtaining an optimum feedpoint location may be by computer simulations of the equipment.

9.3.3 Effect of Changing Column Packing

In the experimental sections it has been advocated that different packing materials are used depending upon the type of fractionation. Simulations 5-5-1 and 5-5-2 show how identical operating conditions can be used to perform both high and low molecular weight removal experiments when the characteristics of the packing are changed.

The packing material used in the experimental programme, Spherosil XOB075 had a broad fractionating range and was a satisfactory packing for the high molecular weight removal experiments. Simulation 5-5-1 indicates that it is possible to remove high molecular weight dextrans using a packing that has a narrower fractionating range. The distribution coefficients for simulation 5-5-1 were chosen so that

the low molecular weight components 1, 2 and 3 were almost totally included in the packing. Fig. 9.26 compares the compositions of the final products for simulations 1-5-3 and 5-5-1, and shows that the removal of the high molecular weight components 4 and 5 was achieved with a reduction in the loss of components 1, 2 and 3 to the high molecular weight product for simulation 5-5-1. Thus the selection of a suitable high porosity packing, such as Porasil D could help reduce the loss of clinical dextran.

For low molecular weight removal conditions an ideal packing would exclude the majoritory of the feed material and only fractionate the low molecular weight components. The distribution coefficients for simulation 5-5-2 were chosen so that only components 1 and 2 could penetrate deep into the pores of the packing. The compositions of the products of simulation 5-5-2 shown in Fig. 9.27 indicate that the required high molecular weight components can be fractionated from the unrequired components using an eluent-to-feed ratio of 1.8. The concentration of the high molecular weight product from simulation 5-5-2 would have been 57.4 g l^{-1}. This value was far greater than the observed concentrations for the high molecular weight product in the experimental programme.

9.4 CONCLUSIONS

The model describes an idealised picture of the gel permeation process taking place in the SCCR5 chromatograph. The inclusion of a concentration dependent distribution coefficient rather than the simple linear exclusion controlled distribution coefficient would result in a more realistic description of the SCCR5 chromatograph. The simplified model has, however, been able to show what effects some of the key

Fig 9.26 Effect of changing characteristics of column packing.

Basis: g

Component	Input mass	Simulation 1-5-3			Simulation 5-5-1		
		K_d	Purge	HMWP	K_d	Purge	HMWP
1	5.742E 0	0.833	4.896E 0	7.538E-1	1.00	5.321E 0	1.492E-1
2	1.851E 1	0.667	1.208E1	7.126E 0	0.900	1.672E 1	1.391E 0
3	3.448E1	0.500	1.094E 1	2.328E 1	0.800	2.887E1	6.119E 0
4	2.203E1	0.333	1.118E 0	2.003E 1	0.333	1.253E0	2.014E 1
5	2.558E0	0.167	2.305E-3	2.597E0	0.167	2.518E-3	2.600E0

Fig9.27 Simulation of a low molecular weight fractionation on a low porosity packing.

Basis: g

Component	K_d	Simulation 5-5-2		
		Input	Purge	HMWP
1	0.833	5.742E 0	4.673E0	6.796 E-1
2	0.667	1.851E 1	1.093E 1	6712 E 0
3	0.200	3.448E 1	6.695E-2	3.454E 1
4	0.100	2.203E1	1.385E-3	2 248E 1
5	0.000	2.558E0	1.460E-6	2.610E0

variables had on the chromatographic equipment.

It has been demonstrated that changing the feedpoint location changes the composition of the product fractions. The verification of the effect of changing the location of the feedpoint will be a requirement for future experimental programmes. The computer simulations also indicated that low molecular weight fractionations could be performed using a low eluent-to-feed ratio of 1.8 if the column packing material had a suitable exclusion limit.

10.0

CONCLUSIONS AND RECOMMENDATIONS

The use of g.p.c. to reduce the molecular weight range of broad dextran samples by large scale batch and semi-continuous equipment has been demonstrated. A repetitive batch chromatograph consisting of ten 5.1 cm i.d. by 70 cm long glass columns packed with Spherosil XOB075 fractionated dextran solutions of up to 265 g l^{-1} at pressures up to 520 kN M^{-2}, and at a feed throughput of 63 g h^{-1}. This method of operation was able to remove unrequired high and low molecular weight fractions from the feed material in a single operation. A similar quantity of Spherosil XOB075 was packed into stainless steel columns of identical dimensions and operated in a semi-continuous counter-current mode. This method of operation could only remove one of the unrequired fractions in a single pass, and so two operations would be required to produce a desirable product fraction. Operating at ambient temperatures dextran throughputs of up to 550 g h^{-1} at an operating pressure of 1550 kN M^{-2} have been achieved for high molecular weight removal and 240 g h^{-1} at an operating pressure of 1030 kN M^{-2} for low molecular weight removal using feed concentrations of up to 236 g l^{-1}. It was expected that under comparable operating conditions to produce similar quality products the net production rates of the repetitive batch and the semi-continuous counter-current operating methods would be 63 and 75 g h^{-1}, respectively.

Variations in the operating conditions of the SCCR5 showed that the product-split was controlled by the pre-feed cut-position. A significant change in the quantity of material removed in the low molecular weight product was observed when the pre-feed cut-position was changed from 0.7 to 0.75. This suggested that for production applications Spherosil XOB075 was a poor packing material for the removal of low molecular dextran fractions and that a packing material

having a lower exclusion limit was required. This would help to:

(1) improve stability of operation,

(2) allow a lower eluent-to-feed ratio to be used, and thereby increase feed throughput,

(3) allow increased product concentrations.

Concentration/temperature interactions were observed for the dextran molecules in the fractionating column indicated by a change in both the composition and quantity of dextran held-up. For similar operating conditions performed at 20, 45 and 62°C, the quantity of dextran hold-up was a maximum at 45°C. Increasing the operating temperature slightly increased the quantity of major product, particularly for the low molecular weight fractionation experiments. This was assigned to an inherent feature of the Spherosil XOB075 packing. For the high molecular weight removal experiments no change in the composition of the major product was observed with increased temperature. Due to a greater variation in product-split with a change operating temperature, the compositions of the products from the low molecular weight removal experiment were observed to alter. A reduction in the eluent-to-feed ratio was possible at the elevated temperature of 60°C whilst maintaining the quality of the major product. Due to the softening of the pneumatic actuating lines a lower actuating pressure had to be used. This prevented a significant increase in the hourly throughput, but the throughput per cycle was increased from 690 to 960 g which indicates that the potential hourly throughput could be 0.88 kg h^{-1} if the pressure drop limitation were increase to 1.55×10^5 N M^{-2}. A sample of nylon tubing was tested for the pneumatic actuating line and did not soften at the higher operating temperatures. Replacement of the existing pneumatic tubing with nylon tubing is

recommended before the commencement of another experimental programme.

A compartments-in-series description of the fractionating column was used in combination with a simple linear, exclusion-controlled model to describe the gel permeation process of the SCCR5. Computer simulations were performed on an ICL 1904S digital computer at the University of Aston Computer Centre. The effect of increased feedrate and feedpoint location were investigated using a binary feed system. The increase in feed flowrate had little effect on the quantity of least retained component in the low molecular weight product. The quantity of retained component in the high molecular weight product increased with increased feed flowrate. This was not observed in the experimental programme. This discrepancy between the simulated and experimentally observed results was caused by the simplifying assumption of a linear, exclusion-controlled process. The inclusion of a concentration dependent partition coefficient would result in a more realistic model of the process. The effect of changing feedpoint location for constant flow conditions resulted in a variety of product compositions. As the feedpoint was moved closer to the eluent inlet port the contamination of the preferentially retained component was reduced; however a greater loss of the least retained component to the low molecular weight product was found to occur. A reversal of these observations occurred when the feed inlet port was moved further away from the eluent port. These results signify that an optimum feedpoint location will occur for the separation of a feed mixture.

Simulations of the dextran polymer system were represented by a five-component feed. In addition to changes in feed point location and feed flowrate, the polymer-feed simulations investigated the effect of varying the pre-feed theoretical cut-position. In agreement with

the experimental programme the quantity of feed material removed in the low molecular weight product decreased as the pre-feed theoretical cut-position increased. The effect of changing the fractionating range of the column packing was also demonstrated.

Future experimental programmes should investigate alternative packing materials for use with the SCCR equipment. It has been observed on an analytical scale that silica packings were vulnerable to both physical and chemical break-down. If other silica packings were to be used, methods of strengthening the silica matrix by surface treatment could be investigated. Alternatively other inorganic packings such as alumina or titania could be used. The possible use of organic packings should also be considered. All the packing materials considered should be available in a variety of fractionating ranges. For use with "low" molecular weight fractionations macromolecular ion-exchange resins have attractive possibilities.

Further applications of the SCCR equipment should be investigated. The use of different solvents in the eluent and purge sections is a possibility. Reverse phase chromatography is an area of potential application. Reverse phase is a confusing term and requires renaming. In a reverse phase system the eluent is polar and the stationary phase is non-polar. This is the "reverse" of normal systems. Many resins used for g.p.c. and ion-exchange chromatography demonstrate considerable non-polar adsorption properties if used with the correct eluent. Thus a non-polar solute could be adsorbed by the packing in the fractionating section using a polar eluent, and purged from the purge section using a less polar eluent. The purge column could be primed with the polar eluent prior to indexing into the fractionating section. The change in mobile phase will reduce the

partition coefficient for the solute in the purge section and so reduce the purge volume required. This will increase the solute concentration in the purge stream and so improve the economic viability of the process. The technique of elution with changing solvent systems has been used with analytical and small scale laboratory preparations. But this technique could not be used as effectively on a batch column as it could in conjunction with SCCR equipment for large-scale preparative applications. A literature review should be conducted to choose future feed stocks. The use of the process as a means of isolating and producing enzymes has attractive possibilities and the purification of other feed systems in the bio-medical field should also be given consideration.

The computer simulations showed a change in the composition of the product fractions with changing feed-point location. A practical examination of this effect is recommended. The testing of the proposed purge scheme should be investigated as a method of increasing the concentration of the fractionated products. Various concentration/temperature interactions have been proposed for the dextran molecules in the fractionating column. A better understanding of the mechanism of g.p.c. under the operating conditions may be observed if a marker were added to the feed solution. A labled dextran, fluorosceinylthiocarbmoyl-dextrans (FITC-dextran) could be added and detected using ultra-violet analysis. The effect of the distribution of the FITC-dextran in the fractionating column with changes in the operating conditions could be observed. The disadvantage of this method would be that the molecular weight distribution of the FITC-dextran would vary through the fractionating column. A mono-dispersed molecule such as a protein may be used at low temperatures but thermal breakdown

may occur at higher temperatures. It would also be necessary to assign an equivalent dextran molecular weight to the protein molecule because of its more compact structure.

A more rigorous mathematical description of the SCCR equipment is also recommended, possibly using the more recent phenomenological models of the g.p.c. process.

APPENDIX A1

```
      MASTER ENGLAND
      DIMENSION X(33),F(33),W(33),P(33),SI(33)
      LOGICAL L
      L=.FALSE.
   11 CONTINUE
C
C     INPUT DATA
C
      READ(1,1000) M
      K1=6
      READ(1,1020)(X(I), I=1,M)
      READ(1,1020)(F(I), I=1,M)
      READ(1,1020)(W(I), I=1,M)
      DO 1 I=1,M
      F(I)=ALOG(F(I))
    1 CONTINUE
C
      CALL E02ABF(M,X,F,W,K1,N,SI,P,L)
C
      WRITE(2,2001)
      DO 10 I=1,K1
      WRITE(2,2002) I,P(I),SI(I)
   10 CONTINUE
      WRITE(2,2003)N
      DO 20 J=1,M
      XX=X(J)
      N1=N+1
      S=P(N1)
      DO 30 I=1,N
      I1=N1-I
      S=S*XX+P(I1)
   30 CONTINUE
      T=F(J)-S
      WRITE(2,2004) XX,F(J),S,T
   20 CONTINUE
      READ(1,1000) ISTOP
      IF(ISTOP.EQ.0) GOTO11
      STOP
 1000 FORMAT(I3)
 1020 FORMAT(4GFG.0)
 2001 FORMAT(///,10X,'PROGRAM FOR CALCULATING A LEAST SQUARES FIT FOR A
     1POLYNOMIAL APPROXIMATION',/,10X,'TO ASET OF DATA POINTS USING FOR
     2SYTHES METHOD.  LIBRAY PROGRAM E20ABF.'  ,//,7X,'COEFFICIENT',10X
     3,'COEFFICIENT',10X,'GOODNESS OF',/,10X,'NUMBER',14X,'VAL.',17X,'FI
     3T')
 2002 FORMAT(/,10X,I5,9X,1PE16.6,5X,E16.6,/)
 2003 FORMAT(///,10X,'DEGREE OF BEST FIT POLYNOMIAL IS:-',I5,///,3X,'
     1K.D.    INPUT MOLECULAR   CALCULATED        DIFFERENCE',/,4X,' VAL
     2UE           WEIGHT          MOL. WEIGHT',//)
 2004 FORMAT(/,1X,1P4E16.6)
      END
      FINISH
```

```
      MASTER MOLWTDISTRIB
      DIMENSION H(200),AMOLWT(200),WTFRAC(200),HNORM(200) ,CUMMOL(200)
    1 READ(1,1000) IUSE
      IF(IUSE.EQ.0)GOTO 9999
      READ(1,1000) INDIC1
      READ(1,1001)INDIC2,INDIC3,INDIC4
      IF(INDIC1.EQ.6)GOTO 10
C
C       INPUT  OF  DATA  FOR  PRODUCT/RECYCLE/FEED
C
      READ(1,1000) NMAX
      READ(1,1020) D1,D2,D3
      READ(1,1020) CSPEED
      READ(1,1020)CONCMA,DILSAM
      READ(1,1020)TEMP,PRESS,STROKE,RANGE,RESPON
      READ(1,1020) (H(I), I=1,NMAX)
      GOTO20
C
C     INPUT  OF  DATA  FOR  STANDARD
C
   10 READ(1,1000) NMAX
      READ(1,1020) D1,D2,D3
      READ(1,1020) CSPEED
      READ(1,1020)CONCST
      READ(1,1020) TEMP,PRESS,STROKE,RANGE,RESPON
      READ(1,1020) (H(I), I=1,NMAX)
C
C     CALCULATION  OF  STANDARD  SOLUTION
      CALL FLOW(D1,D2,RATE,CSPEED,NMAX,VI,VF,VH,D3)
      CALL SIMPSON(NMAX,VI,VF,VH,H,AREA)
      CALL NORMAL(NMAX,H,HNORM,AREA)
      RR=RANGE
       STANDA=AREA/CONCST
      CALLMWDCURVE(HNORM,AMOLWT,WTFRAC,VI,VH,NMAX,CUMMOL)
      CALL AVERAGE(NMAX,AMOLWT,WTFRAC,AVMW,AVMN,SPREAD).
       CALL      OUTPUT(INDIC1,INDIC2,INDIC3,INDIC4,NUMBER,CSPEED,RATE,T
     1EMP,PRESS,STROKE,RANGE,RESPON,CONCST,NMAX,AVMW,AVMN,SPREAD,VI,VH,H
     2,HNORM,AMOLWT,WTFRAC,CUMMOL)
      GOTO 1
   20 CONTINUE
C
C     CALCULATION  FOR  PRODUCT/RECYCLE/FEED
C
      CALL FLOW(D1,D2,RATE,CSPEED,NMAX,VI,VF,VH,D3)
      CALL SIMPSON(NMAX,VI,VF,VH,H,AREA)
      CALL NORMAL(NMAX,H,HNORM,AREA)
      R=RANGE  /RR
      CALL CONCENTRATE(CONCMA,DILSAM,AREA,STANDA,CONCEN,R)
      CALLMWDCURVE(HNORM,AMOLWT,WTFRAC,VI,VH,NMAX,CUMMOL)
      CALL AVERAGE(NMAX,AMOLWT,WTFRAC,AVMW,AVMN,SPREAD)
       CALL .    OUTPUT(INDIC1,INDIC2,INDIC3,INDIC4,NUMBER,CSPEED,RATE,T
     1EMP,PRESS,STROKE,RANGE,RESPON,CONCEN,NMAX,AVMW,AVMN,SPREAD,VI,VH,H
     2,HNORM,AMOLWT,WTFRAC,CUMMOL)
```

```
      GOTO1
 9999 STOP
 1000 FORMAT(13)
 1001 FORMAT(8I10)
 1020 FORMAT(200F0.0)
      END
      SUBROUTINE MWDCURVE(HNORM,AMOLWT,WTFRAC,VI,VH,NMAX,CUMMOL)
      DIMENSION AMOLWT(200),DIFCHT(200),HNORM(200),WTFRAC(200),CUMMOL(20
     10)
      V=VI
      DO 10 I=1,NMAX
      CUMMOL(I)=0.0
      AMOLWT(I)=CALIB(V)
      DIFCHT(I)=DIFCAL(V)
      WTFRAC(I)=HNORM(I)*DIFCHT(I)
      V=VI+VH*I
   10 CONTINUE
      C=0.
      DO 1 J=2,NMAX
      I=J-1
      A=AMOLWT(I)
      B=AMOLWT(J)
      DAB=A/B
      X=WTFRAC(I)
      Y=WTFRAC(J)
      AVXY=(X+Y)/2.
      AREA=AVXY*DAB
      CUMMOL(I)=AREA
      C=AREA+C
    1 CONTINUE
      DO 2 J=1,NMAX-1
      I=NMAX-J
      CUMMOL(I)=CUMMOL(I)/C
      IF(J.EQ.1)GOTO 2
      K=I+1
      CUMMOL(I)=CUMMOL(I)+CUMMOL(K)
    2 CONTINUE
      RETURN
      END
      SUBROUTINE CONCENTRATE(CONCMA,DILSAM,SAMPA,STANDA,ACTCON,R)
C       ACTCON-  ACTUAL CONCENTRATION OF SAMPLE
C       CONCMA-  WEIGHT OF CONCENTRATED SAMPLE
C       DILSAM-  WEIGHT OFDILUTE SAMPLE
C       SAMPA-  AREA OF SAMPLE
      REAL MODSAM
       MODSAM=(CONCMA*SAMPA)/DILSAM
      ACTCON=(MODSAM/STANDA)*R
      RETURN
      END
      SUBROUTINE AVERAGE(NMAX,AMOLWT,WTFRAC,AVMW,AVMN,SPREAD)
      DIMENSION AMOLWT(200),WTFRAC(200)
      TOP=0.
      BOTTOM=0.
```

```
      DO 10 I=1,NMAX
      TOP=WTFRAC(I)*AMOLWT(I)+TOP
      BOTTOM=WTFRAC(I)+BOTTOM
   10 CONTINUE
      AVMW=TOP/BOTTOM
      SUMNUM=0.
      DO 20 I=1,NMAX
      SUMNUM=SUMNUM+(WTFRAC(I)/AMOLWT(I))
   20 CONTINUE
      AVMN=BOTTOM/SUMNUM
      SPREAD=AVMW/AVMN
      RETURN
      END
      SUBROUTINE NORMAL(NMAX,H,HNORM,A)
      DIMENSION H(200),HNORM(200)
      DO 20 I=1,NMAX
      HNORM(I)=H(I)/A
   20 CONTINUE
      RETURN
      END
      SUBROUTINE FLOW(D1,D2,RATE,CSPEED,NMAX,VI,VF,VH,D3)
   10 RATE=(9.526*CSPEED)/(600.*D3)
      VIE=(D1*RATE*600.)/CSPEED
      VFE=(D2*RATE*600.)/CSPEED
      VI=(VIE-3.536)/5.99
      VF=(VFE-3.536)/5.99
      VH=(VF-VI)/(NMAX-1)
      RETURN
      END
      SUBROUTINE SIMPSON(NMAX,VI,VF,VH,H,AREA)
      DIMENSION H(200)
      I=NMAX/2
      PATH=(NMAX+1)/2
      ISTOP=NMAX
      AREA=0.
      IF(I.EQ.PATH)GOTO 10
      ISTOP=NMAX-1
      AREA=(H(ISTOP)*VH)/2.
   10 CONTINUE
      ODD=0.
      EVEN=0.
      DO 20 I=2,ISTOP-1,2
   20 EVEN=EVEN+H(I)
      DO 30 I=3,ISTOP-2,2
   30 ODD=ODD+H(I)
      AREA=AREA+(VH/3.)*(H(1)+4.*EVEN+2.*ODD+H(ISTOP))
      RETURN
      END
      FUNCTION CALIB(V)
      DATA A/14.45445/, B/-14.04179/, C/35.97016/, D/-99.59339/,
     1 E/138.7001/,  F/-70.4618/
      HIGH=1.0
      ALOW=0.0
```

```
      IF(V.GT.HIGH)V=1.0
      IF(V.LE.ALOW)  V=0.0
      X=A+V*(B+V*(C+V*(D+V*(E+V*F))))
      CALIB=EXP(X)
      RETURN
      END
      FUNCTION DIFCAL(R)
      ALIM=0.1
      IF((ABS(R)).GE.ALIM)GO TO 10
      X=0.01
      GOTO 20
   10 X=R/100.
   20 CONTINUE
      X1=R+X
      X2=R-X
      DIFR=X2-X1
      XHIGH=CALIB(X1)
      XLOW=CALIB(X2)
      IF(XHIGH.EQ.XLOW) GOTO 30
       A=ALOG((XHIGH/XLOW))
      DIFCAL=ABS(DIFR/A)
      GOTO 40
   30 DIFCAL=0.001
   40 CONTINUE
      RETURN
      END
      SUBROUTINE OUTPUT(INDIC1,INDIC2,INDIC3,INDIC4,NUMBER,CSPEED,RATE,T
     1EMP,PRESS,STROKE,RANGE,RESPON,CONCEN,NMAX,AVMW,AVMN,SPREAD,VI,VH,H
     2,HNORM,AMOLWT,WTFRAC,CUMMOL)
      DIMENSION H(200),HNORM(200),AMOLWT(200),WTFRAC(200),CUMMOL(200)
      IF(INDIC1.EQ.1) WRITE(2,2001)
      IF(INDIC1.EQ.2) WRITE(2,2002)
      IF(INDIC1.EQ.3) WRITE(2,2003)
      IF(INDIC1.EQ.4) WRITE(2,2004)
      IF(INDIC1.EQ.5) WRITE(2,2005)
      IF(INDIC1.EQ.6) WRITE(2,2006)
      IF(INDIC1.EQ.6)GOTO 10
      WRITE(2,2010) INDIC2,INDIC3,INDIC4
      GOTO 20
   10 WRITE(2,2011)INDIC2,INDIC3,INDIC4
   20 CONTINUE
      WRITE(2,2020) RATE,STROKE,TEMP,PRESS,CSPEED,RANGE,RESPON,CONCEN
      WRITE(2,2030) AVMW,AVMN,SPREAD
 2035 WRITE(2,2035)
      V=VI
      DO 30 I=1,NMAX
      WRITE(2,2040)I,V,AMOLWT(I),H(I),HNORM(I)
      V=V+VH
   30 CONTINUE
      WRITE(2,2045)
      DO 40 I=1,NMAX
      J=NMAX+1-I
      WRITE(2,2046) J,AMOLWT(J),HNORM(J),WTFRAC(J)
```

```
      IF((CUMMOL(J).LE.0.98).AND.(CUMMOL(J).GT.0.02)) WRITE(2,2047)
     1CUMMOL(J)
   40 CONTINUE
      RETURN
 2001 FORMAT(1H1,///,10X,'THE ANALYSIS FOR THE FEED OF RUN NO.')
 2002 FORMAT(1H1,///,10X,'THE ANALYSIS OF PRODUCT ONE FOR RUN NO.')
 2003 FORMAT(1H1,///,10X,'THE ANALYSIS OF PRODUCT TWO FOR RUN NO.')
 2004 FORMAT(1H1,///,10X,'THE ANALYSIS OF RECYCLE ONE FOR RUN NO.')
 2005 FORMAT(1H1,///,10X,'THE ANALYSIS OF RECYCLE TWO FOR RUN NO.')
 2006 FORMAT(1H1,///,10X,'***STANDARD SOLUTION***',//,10X,'THE DATE  OF
     1 THE ANALYSIS IS :--')
 2010 FORMAT(42X,I4,'-',I4,'-',I4)
 2011 FORMAT('+',42X,I5,'-',I5,'-',I5)
 2020 FORMAT(//,10X,'THE CONDITIONS  WERE  :-',//,10X,'FLOWRATE, ML/MIN
     1=',F10.6,'  ;     PERCENTAGE  STROKE ',F10.2,//,10X,'TEMPERATURE
     2DEG.C ',F10.2,'    ;   PRESSURE  LBS/SQ.IN.  ',F10.2,//,10X,'CHART
     3-SPEED ',F10.1,'  ;    RANGE  SETTING',F10.1,//,10X,'RESPONSE   MI
     4LLI-VOLTS',F10.1,'  ;  CONCENTRATION ;GMS/100 ML',F10.6)
 2030 FORMAT(//,10X,'WEIGHT AVERAGE MOL. WT. ',  F20.0,/,10X,'NUMBER
     1AVERAGE MOL. WT.',F20.0,/,16X,'MW/MN RATIO',OPF10.6)
 2035 FORMAT(//,15X,'INPUT  DATA',                          ///,5X,'POINT',1
     20X,' K.D. VALUE  ',12X,'MOL.WT.    ',9X,'CHROMATOGRAM',6X,'NORMALI
     3SED'                    ,          /,68X,'HEIGHT',10X,'FRACTION')
 2040 FORMAT(I10,5X,F15.4,5X,F20.0,9X,F10.5,1X,1P2E20.6)
 2045 FORMAT(//,15X,'CALCULATED DATA',///,5X,'POINT',13X,'MOL. WT.',9X
     1,'NORMALISED',9X,'WT. FRAC. ',9X,'CUMULATIVE',/,40X,'HEIGHT',9X,
     2'PER LOG MOL. WT.',4X,'WEIGHT FRACTION')
 2046 FORMAT(  I10,F20.0,1P2E20.6)
 2047 FORMAT(  70X,1PE20.6 )
      END
      FINISH
```

Input variables in programme ENGLAND

F	molecular weight of sample
M	no. of sample points
W	weighting, set = 1.
X	elution volume

Input variables in programme MWDC

CONCMA	mass of concentrated sample
CONCST	conc. of standard
CSPEED	chart speed
DILSAM	mass of dilute sample
D1	initial elution point
D2	final elution point
D3	elution point of glucose
H	height of chromatograph
INDIC1,4	numbering integers
NMAX	no. of sample points
PRESS	column pressure
RANGE	detector range
RESPON	detector response
STROKE	pump stroke
TEMP	column temperature

APPENDIX A2

Solution of general state equation

General equation,

$$\dot{\underline{x}}(t) = \underline{A}\,\underline{x}(t) + \underline{B}\,\underline{u}(t) \qquad \underline{x}(o) = \underline{x}_o$$

Taking Laplace Transforms,

$$s\underline{X}(s) - x_o = \underline{A}\,\underline{X}(s) + \underline{B}\,\underline{U}(s)$$

$$s\underline{I}\,\underline{X}(s) - \underline{A}\,\underline{X}(s) = x_o + \underline{B}\,\underline{U}(s)$$

$$\underline{X}(s) = (s\underline{I} - \underline{A})^{-1} x_o + (s\underline{I} - \underline{A})^{-1}\underline{B}\,\underline{U}(s)$$

Inverting,

$$\underline{x}(t) = L^{-1}\left[(s\underline{I} - \underline{A})^{-1}.\, x_o\right] + L^{-1}\left[(s\underline{I} - \underline{A})^{-1}.\underline{B}\,\underline{U}(s)\right]$$

Now,

$$L^{-1}\left[(s\underline{I} - \underline{A})^{-1}\right] x_o = \exp(\underline{A}\,t).\, x_o$$

from standard transform tables, and using the convolution theorem

$$L^{-1}\left[(s\underline{I} - \underline{A})\ \underline{B}\,\underline{U}(s)\right] = \int_o^t \exp(\underline{A}\tau).\,\underline{B}\,\underline{u}\,(t-\tau)\,d\tau$$

$$= \int_o^t \exp(\underline{A}(t-\tau)).\,\underline{B}\,\underline{u}(\tau)\,d\tau$$

So,

$$\underline{x}(t) = \exp(\underline{A}\,t).\,\underline{x}_o + \int_o^t \exp(\underline{A}(t-\tau)).\,\underline{B}\,\underline{u}(\tau)\,d\tau$$

If $\underline{B}\,\underline{u} \neq g(t)$, then

$$\int_o^t \exp(\underline{A}(t-\tau)).\,\underline{B}\,\underline{u}(\tau)\,d\tau = \left[-\underline{A}^{-1}\exp(\underline{A}(t-\tau))\right]_o^t.\,\underline{B}\,\underline{u}$$

$$= \underline{A}^{-1}.\left[\exp(\underline{A}t) - I\right].\,\underline{B}\,\underline{u}$$

Now, $\exp(\underline{A}t)$ can be found by the series

$$\exp(\underline{A}t) = \underline{\Phi}(t) = I + \underline{A}^1\frac{\underline{t}^1}{1!} + \underline{A}^2\frac{\underline{t}^2}{2!} + \underline{A}^3\frac{\underline{t}^3}{3!} + \ldots$$

and,

$$\underline{A}^{-1}\left[\underline{\Phi}(t) - \underline{I}\right] = \underline{\Delta}(t) = t\left[\underline{I} + \underline{A}^1\frac{\underline{t}^1}{2!} + \underline{A}^2\frac{\underline{t}^2}{3!} + \underline{A}^3\frac{\underline{t}^3}{4!} + \ldots\right]$$

Therefore solution procedure is,

(i) generate $\underline{\Delta}(t)$

(ii) evaluate $\underline{\Delta}(t).\underline{B}\,\underline{u}$

(iii) calculate $\underline{\Phi}(t) = \underline{I} + \underline{A}.\underline{\Delta}(t)$

thus,

(iv) solve $\underline{x}(t) = \underline{\Phi}(t).\underline{x}_o + \underline{\Delta}(t).\underline{B}\underline{u}$

```
      TRACE 0
      MASTER SCCR5MODEL
      DIMENSION XFEED(5),BU(90,5),QVPREFD(5),QVPOSFD(5)
      DIMENSION
     2DELTFRAC(90,10,5),TPHIFRAC(90,10,5),DELTABU(90,5),HMWP(5),XO(90,5)
      DIMENSION
     3AFRAC(90,2,5),XT(90,5),TPHIXO(90,5),XP(10,5),RKD(5)
      DIMENSION
     4 COLMAS(9,5),SUMPURG(5),TOTCOL(9),AVCONC(9)
      EQUIVALENCE (DELTFRAC(90,10,5), TPHIFRAC(90,10,5))
      LOGICAL ICONC
      LOGICAL ISSTEADY
      ISSTEADY=.FALSE.

      AAA=0.5
      ERRORPG=0.1
      VO=564.0
      VI=651.0
      INDEXNO=0



  200 CONTINUE
      READ(1,8000)NUMTKSFR,NUMTKSPG
      READ(1,8000)KOMPON,NFEEDPLT
      READ(1,8000)ITIME,LIMITSWT
      READ(1,8000) JMAX,NOOFCOLS
      READ(1,8001)DELTAT
      READ(1,8001)(XFEED(K), K=1,KOMPON)
      READ(1,8001)QELUENT,QFEED
      READ(1,8001)DAY,DATE,YEAR
      READ(1,8001)(RKD(K), K=1,KOMPON)

C     CALCULATING  CUT-POINTS
      ITIMESTP=ITIME/DELTAT
      VO=VO/NUMTKSPG
      VI=VI/NUMTKSPG
      CUTPRE=((QELUENT*DELTAT*ITIMESTP)-(VO*NUMTKSPG))/(VI*NUMTKSPG)
      CUTPOS=((QELUENT+QFEED)*DELTAT*ITIMESTP-VO*NUMTKSPG)/(VI*NUMTKSPG)

      WRITE(2,9200)DAY,DATE,YEAR
      WRITE(2,9201)QELUENT,QFEED
      WRITE(2,9202) CUTPRE,CUTPOS
      WRITE(2,9203) ITIME,NUMTKSPG,NUMTKSFR,NFEEDPLT,DELTAT
      WRITE(2,9204)
      DO 201 K=1,KOMPON
  201 WRITE(2,9205) K,RKD(K)
C    SECTION THREE
C                    SETTING INITIAL ARRAYS


C    CALCULATING CONSTANTS FROM INPUT VARIABLES
```

```
      DO 351 K=1,KOMPON
      QVPREFD(K)=QELUENT/(VO+RKD(K)*VI)
      QVPOSFD(K)=(QELUENT+QFEED)/(VO+VI*RKD(K))
  351 CONTINUE

C     SETTING INITIAL ARRAYS

      DO 310 K=1,KOMPON
      DO 310 I=1,NUMTKSFR
      BU(I,K)=0.0
      IF(I.EQ.NFEEDPLT)BU(I,K)=XFEED(K)*QFEED/(VO+RKD(K)*VI)
  310 CONTINUE
      DO 311 K=1,KOMPON
      DO 311 I=1,NUMTKSFR
      DO 311 J=1,2
      IF((I.EQ.1).AND.(J.EQ.1))GOTO 319
      IF(I.GE.NFEEDPLT)GOTO 318
      AFRAC(I,J,K)=QVPREFD(K)
      IF(J.EQ.2) AFRAC(I,J,K)=-1.0*AFRAC(I,J,K)

      GOTO 311
  318 AFRAC(I,J,K)=QVPOSFD(K)
      IF((J.EQ.1).AND.(I.EQ.NFEEDPLT))AFRAC(I,J,K)=QVPREFD(K)
      IF(J.EQ.2) AFRAC(I,J,K)=-1.0*AFRAC(I,J,K)
      GOTO 311
  319 AFRAC(I,J,K)=0.0
  311 CONTINUE

C     CALCULATION OF DELTA(T)FOR FRACTIONATING SECTION

      CALL FRADELTAT(AFRAC,DELTAT,NUMTKSFR,JMAX,DELTFRAC,KOMPON)


C     CALCULATING  DELTA(T).BU.

      DO 420 K=1,KOMPON
      NTIMES=JMAX
      DO 420 I=1,NUMTKSFR
      II=NUMTKSFR-I+1
      L=NUMTKSFR-I+1
      IF(L-JMAX) 421,422,422
  421 NTIMES=NTIMES-1
  422 DO 430 N=1,NTIMES
      M=JMAX-N+1
      DELTABU(L,K)=DELTABU(L,K)+DELTFRAC(L,M,K)*BU((II),K)
  430 II=II-1
  420 CONTINUE

C     CALCULATION OF PHI(T) FOR FRACTIONATING SECTION

      DO 439 K=1,KOMPON
```

```
      CALL FRAXMAT(DELTFRAC,AFRAC,NUMTKSFR,JMAX,K)
  439 CONTINUE
      DO 440 K=1,KOMPON
      DO 440 I=1,NUMTKSFR
      TPHIFRAC(I,JMAX,K)=TPHIFRAC(I,JMAX,K)+1.0
  440 CONTINUE

  500 CONTINUE
C
C   CALCULATE X(T)
      DO 510 K=1,KOMPON
      HMWP(K)=0.0
      DO 510 ISTEP=1,ITIMESTP
C   CALCULATE PHI(T).XO
      NTIMES=JMAX
      DO 501 I=1,NUMTKSFR
      II=NUMTKSFR+1-I
      L=NUMTKSFR-I+1
      IF(L-JMAX) 502,503,503
  502 NTIMES=NTIMES-1
  503 DO 504 N=1,NTIMES
      M=JMAX-N+1
      TPHIXO(L,K)=TPHIFRAC(L,M,K)*XO((II),K)+TPHIXO(L,K)
      II=II-1
  504 CONTINUE
  501 CONTINUE

C   CALCULATE  X(T)=PHI(T).XO+DELTA(T).BU

      DO 520 N=1,NUMTKSFR
      XT(N,K)=TPHIXO(N,K)+DELTABU(N,K)
  520 TPHIXO(N,K)=0.0

C   CALCULATE H.M.W.P.

      HMWP(K)=HMWP(K)+XT(NUMTKSFR,K)*0.001*DELTAT*(QELUENT+QFEED)

C  IS THIS END OF SWITCH

      IF((ISTEP.EQ.ITIMESTP).AND.(K.EQ.KOMPON))GOTO 600
      DO 535 N=1,NUMTKSFR
      XO(N,K)=XT(N,K)
  535 CONTINUE
  510 CONTINUE

C    SECTION SIX

  600 CONTINUE
      NUMSWITCH=NUMSWITCH+1
      INDEXNO=INDEXNO+1
      IF(INDEXNO.EQ.NOOFCOLS)GOTO 610
  609 CONTINUE
```

```
C  CHECKING STEADY-STATE BY INPUT-OUTPUT<ERROR

      IF(NUMSWITCH.EQ.1)GOTO 750
      AOUTPUT=0.0
      DO 710 K=1,KOMPON
      SUMPURG(K)=0.0
      DO 710 I=1,NUMTKSPG
  710 SUMPURG(K)=SUMPURG(K)+XP(I,K)*(VO+VI)*0.001
      DO 751 K=1,KOMPON
  751 AOUTPUT=AOUTPUT+SUMPURG(K)+HMWP(K)
      IF(AOUTPUT.GT.(AAA*AINPUT))GOTO 720
      GOTO 611
  720 IF(AAA.EQ.0.5)GOTO 721
      IF(AAA.EQ.0.75)GOTO 722
      IF(AAA.EQ.0.9)GOTO 723
      IF(AAA.EQ.0.95)GOTO 724
      IF(AAA.EQ.0.99)GOTO 725
  721 WRITE(2,9721) AAA,NUMSWITCH
      AAA=0.75
      GOTO 611
  722 WRITE(2,9721) AAA,NUMSWITCH
      AAA=0.9
      GOTO 611
  723 WRITE(2,9721) AAA,NUMSWITCH
      AAA=0.95
      GOTO 611
  724 WRITE(2,9721) AAA,NUMSWITCH
      AAA=0.99
      GOTO 611
  725 WRITE(2,9721) AAA,NUMSWITCH
      ISSTEADY=.TRUE.
      GOTO 800
  750 CONTINUE
      DO 756 K=1,KOMPON
  756 AINPUT=XFEED(K)*QFEED*DELTAT*ITIMESTP*0.001+AINPUT
      WRITE(2,9756) AINPUT
      DO 757 K=1,KOMPON
      XFO=XFEED(K)*0.001*NOOFCOLS*DELTAT*ITIMESTP*QFEED
  757 WRITE(2,9757) K,XFEED(K),XFO


  611 CONTINUE

C  INDEXING COLUMN

      DO 623 K=1,KOMPON
      DO 620 I=1,NUMTKSPG
  620 XP(I,K)=XT(I,K)
      DO 621 I=1,NUMTKSFR-NUMTKSPG
      XO(I,K)=XT((I+NUMTKSPG),K)
  621 CONTINUE
      DO 622 I=NUMTKSFR-NUMTKSPG+1,NUMTKSFR
  622 XO(I,K)=0.0
```

```
  623 CONTINUE
      GOTO 1000

C    END  OF  CYCLE  OUTPUT  RESULTS

  610 CONTINUE


      INDEXNO=0
      DO 612 K=1,KOMPON
      SUMPURG(K)=0.0
      DO 612 I=1,NUMTKSPG
  612 SUMPURG(K)=SUMPURG(K)+XP(I,K)*(VO+VI)*0.001
      DO 629 I=1,NOOFCOLS-1
      DO 629 K=1,KOMPON
  629 COLMAS(I,K)=0.0
      III=1
      DO 630 I=1,NOOFCOLS-1
      DO 631 K=1,KOMPON
      DO 631 II=III,NUMTKSPG+III-1
  631 COLMAS(I,K)=COLMAS(I,K)+XT(II,K)*(VO+VI)*0.001
      III=III+NUMTKSPG
  630 CONTINUE
      NN=NUMSWITCH/NOOFCOLS
      WRITE(2,9631) NN,(I, I=1,NOOFCOLS-1)
      DO 632 K=1,KOMPON
      DO 640 I=1,NOOFCOLS-1
      TOTCOL(I)=0.0
      DO 641 KK=1,KOMPON
  641 TOTCOL(I)=TOTCOL(I)+COLMAS(I,KK)
  640 CONTINUE
  632 WRITE(2,9632) K,(COLMAS(I,K), I=1,NOOFCOLS-1)
      DO 642 I=1,NOOFCOLS-1
  642 AVCONC(I)=(TOTCOL(I))/((VO+VI)*NUMTKSPG*0.001)
      WRITE(2,9843)
      WRITE(2,9844)(TOTCOL(I), I=1,NOOFCOLS-1)
      WRITE(2,9845)
      WRITE(2,9846) (AVCONC(I), I=1,NOOFCOLS-1)
      WRITE(2,9847)
      DO 643 K=1,KOMPON
  643 WRITE(2,9848) K,SUMPURG(K),HMWP(K)
      GOTO 609

 1000 CONTINUE

C   CHECK FOR PSEUDO-STEADY STATE

      IF(ISSTEADY )GOTO 800
      IF(NUMSWITCH.EQ.LIMITSWT) GOTO 1099
      GOTO 500
```

```
 1099 WRITE(2,9999)

  800 CONTINUE
C                OUTPUT RESULTS
      WRITE(2,9801) NUMSWITCH
      WRITE(2,9802)(I, I=1,NOOFCOLS-1)
      DO 829 I=1,NOOFCOLS
      DO 829 K=1,KOMPON
  829 COLMAS(I,K)=0.0
      III=1
      DO 830 I=1,NOOFCOLS
      DO 831 K=1,KOMPON
      DO 831 II=III,NUMTKSPG+III-1
  831 COLMAS(I,K)=COLMAS(I,K)+XT(II,K)*(V0+VI)*0.001
      III=III+NUMTKSPG
  830 CONTINUE

C       OUTPUT PSEUDO-STEADY STATE PROFILE

      DO 832 K=1,KOMPON
  832 WRITE(2,9632) K,(COLMAS(I,K), I=1,NOOFCOLS-1)
      DO 840 I=1,NOOFCOLS-1
      TOTCOL(I)=0.0
      DO 841 K=1,KOMPON
  841 TOTCOL(I)=TOTCOL(I)+COLMAS(I,K)
  840 CONTINUE

C  AVERAGE COLUMN CONCENTRATIONS

      DO 842 I=1,NOOFCOLS-1
  842 AVCONC(I)=(TOTCOL(I))/((V0+VI)*0.001*NUMTKSPG)
        WRITE(2,9843)
      WRITE(2,9844)(TOTCOL(I), I=1,NOOFCOLS-1)
      WRITE(2,9845)
      WRITE(2,9846)(AVCONC(I), I=1,NOOFCOLS-1)
      WRITE(2,9847)
      DO 848 K=1,KOMPON
  848 WRITE(2,9848) K,SUMPURG(K),HMWP(K)
      STOP

C   FORMAT  STATEMENTS


 9200 FORMAT(////,5X,'MODEL  FOR  S.C.C.R.5.',/,
     15X,'INPUT  INFORMMATION  :-',/,
     225X,'COMPUTER  RUN  NUMBER :-',3F10.0,///)
 9201 FORMAT(19X,'THE  FLOWRATES  WERE :   ELUENT',F15.5,3X,'ML S-1',/,
     1                                  44X,'FEED  ',F15.5,3X,'ML S-1')
 9202 FORMAT(19X,'THE THEORETICAL  CUT POSITIONS  WERE:',/,
     144X,'PRE-FEED',F12.5,/,
     244X,'POST-FEED',F11.5)
 9203 FORMAT(20X,'SWITCH-TIME                          ',5X,I8,8X,'S',/,
     116X,'NO. OF TANKS PER COLUMN',20X,I5,/,
```

```
     216X,'TOTAL NO. OF TANKS IN FRACTIONATING SECTION',I5,/,
     316X,'POSITION OF FEED TANK,TANK NO.',13X,I5,/,
     416X,'STEP TIME INCRUMENT',25X,F8.3,'    S')
 9204 FORMAT(//,20X,'THE KDS OF THE COMPONENTS ARE:-',
     1/26X,'COMPONENT   KD')
 9205 FORMAT(/,30X,I5,5X,F5.3)
 9631 FORMAT(1H1,//,7X,'THE DISTRIBUTION OF DEXTRAN FOR CYCLE',I4,2X
     1,'  WAS:',//,15X,'COLUMN    COLUMN    COLUMN    COLUMN    COLUMN',
     27X,'COLUMN  COLUMN   COLUMN    COLUMN',//,
     39X,9I10,//,2X,'COMPONENT')
 9632 FORMAT(/,I7,8X,1P9E10.3)
 9721 FORMAT(////,5X,'THE  OUTPUT  MASS  HAS  REACHED ',2X,F5.3,2X,' AS
     1FACTION  OF  THE  INPUT  MASS  DURING  SWITCH  NUMBER ',I5)
 9801 FORMAT(1H1,//,5X,'****  STEADY-STATE  ****',/,
     15X,'STEADY-STATE HAS BEEN REACHED AFTER ',I5,' SWITCHES',///,
     27X,'THE DISTRIBUTION OF THE DEXTRAN IN THE S.C.C.R.5. MACHINE IS',
     3//,15X,'COLUMN    COLUMN    COLUMN    COLUMN    COLUMN    COLUMN
     4    COLUMN    COLUMN    COLUMN')
 9802 FORMAT(1HO,9X,9I10,/,2X,'COMPONENT',/)
 9843 FORMAT(//,4X,'TOTAL',/5X,'MASS',/,5X,'GMS.')
 9844 FORMAT(12X,1P9E10.3)
 9845 FORMAT(/,4X,'AVERAGE',/,4X,'COLUMN',/,5X,'CONC.',/,7X,'-1',/,4X,'G
     1.L.')
 9846 FORMAT(12X,1P9E10.3)
 9847 FORMAT(///,5X,'THE MASS OF DEXTRAN ELUTED FROM THE PURGE',
     1/,5X,'AND THE HIGH MOLECULAR WEIGHT OUTLET PORTS',
     2/,5X,'DURING THE FINAL SWITCH WERE AS FOLLOWS:',
     3//,5X,'COMPONENT',8X,'PURGE',6X,'H.M.W.P.')
 9848 FORMAT(/,8X,I2,3X,1PE12.4,3X,E12.4)
 9999 FORMAT(///,10X,'****  N.B. STEADY-STATE HAS NOT BEEN REACHED ****
     1',/, 10X,'THE NUMBER OF SWITCHES HAS REACHED THE SET MAXIMUM')
 9915 FORMAT(1HO,5X,'THE MASS OF SOLUTE REMAINING IN THE PURGE',1X,
     1'SECTION IS  ',1PE10.3,' GMS.')
 9756 FORMAT(///,5X,'THE TOTAL INPUT PER SWITCH  IS  ',1PE10.3,' GMS.',
     1//,5X,'THE INPUT OF EACH COMPONENT PER CYCLE',/
     4,5X,'IS AS FOLLOWS',/,
     25X,'COMPONENT',5X,'CONCENTRATION',5X,'MASS',/,
     332X,'-1',/,28X,'GMS. L.',8X,'GMS.')
 9757 FORMAT(9X,I3,8X,1P2E10.3)
 8000 FORMAT(2I10)
 8001 FORMAT(10F0.0)
      END

C  SUBROUTINES  FOR   THE  CALCULATION  OF
C          DELTA(T)  IN  THE  STATE  EQUATION


      SUBROUTINE FRADELTAT(A,DT,IMAX,JMAX,DELTA,KOMPON)
      DIMENSION A(90,2,5),AMOD(90,10,5),DELTA(90,10,5)
C
C
C
      DO 43 K=1,KOMPON
```

```
      DO 22 I=1,IMAX
      DO 22 J=1,JMAX
      AMOD(I,J,K)=0.0
      IF(J.EQ.JMAX) AMOD(I,J,K)=1.0
   22 CONTINUE


C
C
      DO 30 N=1,JMAX
      IF(N.EQ.1)GOTO 32
C
C
      CALL FRAXMAT(AMOD,A,IMAX,JMAX,K)

C
   32 CONTINUE
      DO 33 I=1,IMAX
      DO 33 J=1,JMAX
      AMOD(I,J,K)=AMOD(I,J,K)*DT/N
   33 DELTA(I,J,K)=DELTA(I,J,K)+AMOD(I,J,K)
   30 CONTINUE
      DO 42 I=1,IMAX
      L=JMAX-I
      IF(L) 42,42,40
   40 CONTINUE
      DO 41 J=1,L
   41 DELTA(I,J,K)=0.0
   42 CONTINUE
   43 CONTINUE
      RETURN
      END
C  SUBROUTINE  FOR  THE  MULTIPLICATION  OF
C   TWO  MATRACIES  ONE  BEING  N BY 2


      SUBROUTINE FRAXMAT(AA,A,IMAX,JMAX,K)
      DIMENSION AA(90,10,5),A(90,2,5),AAOLD(90,10,5)
C
C
C   MULTIPLY MATRIX
C
C
C
      DO 1 I=1,IMAX
      DO 1 J=1,JMAX
    1 AAOLD(I,J,K)=AA(I,J,K)
C
      NT=JMAX
      DO 35 I=1,IMAX
      II=IMAX+1-I
      IJ=II
      IF (II-JMAX) 31,32,32
```

```
   31 NT=NT-1
   32 DO 35 N=1,NT
      NN=JMAX+1-N
      IF(NN.EQ.JMAX)GOTO 34
      AA(II,NN,K)=AAOLD(II,NN,K)*A(IJ,2,K)
     1+AAOLD(II,NN+1,K)*A(IJ+1,1,K)
      GOTO 33
   34 AA(II,NN,K)=AA(II,NN,K)*A(IJ,2,K)
   33 CONTINUE
      IJ=IJ-1
   35 CONTINUE

      RETURN
      END
      FINISH
****
```

Input variables in programme SCCR5MODEL

DATE	month of analysis
DAY	day of analysis
DELTAT	time increment
ITIME	switchtime
JMAX	terms in Δ matrix
KOMPON	no. of components in feed
LIMITSWT	maximum no. of switches
NFEEDPLT	feedpoint stage
NUMTKSFR	total no. of stages
NUMTKSPG	no. of stages per column
QELUENT	eluent flowrate
QFEED	feed flowrate
RKD	K_d of components
XFEED	concentration of feed
YEAR	year of analysis

Summary of simulation conditions

Simulation No	Eluent rate ml. s^{-1}	Feed rate ml.s^{-1}	Switch time s.	No. of stage per column	No. of feedpoint	No. of terms in $\underline{\Delta}$ matrix.
1-2-0	1·667	0·333	450	10	51	3
1-2-1	1·667	0·333	450	10	51	5
1-2-2	1·667	0·333	450	10	51	7
1-2-3	1·667	0·333	450	10	51	10
2-2-1	1·667	0·333	450	7	36	7
2-2-2	1·667	0·333	450	7	36	7
3-2-1	1·667	0·333	450	5	26	5
3-2-2	1·667	0·333	450	2	11	5
3-2-3	1·667	0·333	450	1	5	5
4-2-1	1·667	0·666	450	5	26	5
4-2-2	1·667	0·926	450	5	26	5

Simulation No	Eluent rate ml. s^{-1}	Feed rate ml.s^{-1}	Switch time s.	No. of stage per column	No. of feedpoint	No. of terms in Δ matrix.
1-5-1	1.667	0.333	450	5	26	5
1-5-2	1.667	0.667	450	5	26	5
1-5-3	1.667	0.926	450	5	26	5
2-5-1	0.845	0.103	1200	5	26	5
2-5-2	0.845	0.170	1200	5	26	5
3-5-1	0.741	0.148	1200	5	26	5
3-5-2	0.741	0.296	1200	5	26	5
3-5-3	0.741	0.592	1200	5	26	5
4-5-1	1.667	0.333	450	5	16	5
4-5-2	1.667	0.333	450	5	36	5
4-5-3	0.741	0.148	1200	5	16	5

Simulation No	Eluent rate ml. s^{-1}	Feed rate ml.s^{-1}	Switch time s.	No. of stage per column	No. of feedpoint	No. of terms in $\underline{\Delta}$ matrix.
4-5-4	0.741	0.148	1200	5	36	5
5-5-1	1.667	0.926	450	5	26	5
5-5-2	1 667	0.926	450	5	26	5

APPENDIX A3

Calculation of Theoretical Cut-positions

e.g.

Average conditions for experiment A.

Flowrate;	Eluent	99.6 cm^3 min^{-1}
	Feed	20.1 cm^3 min^{-1}
	Purge	455.0 cm^3 min^{-1}
Switch time		7.5 min
Average v_o' for SCCR5		564 cm^3/column
Average v_i' for SCCR5		651 cm^3/column

(a) Pre-feed Cut Position,

from,

$$K_d = \frac{v_e - v_o'}{v_i'}$$

$$= \frac{99.6 - \frac{564}{7.5}}{\frac{651}{7.5}}$$

$$= \underline{0.286}$$

(b) Post-feed Cut Position,

$$= \frac{(99.6 + 20.1) - \frac{564}{7.5}}{\frac{651}{7.5}}$$

$$= \underline{0.516}$$

NOMENCLATURE

a	exponent in Mark-Houwink equation
A^+	interaction coefficient
$\underline{A}$	distribution matrix in state equation
$\underline{B}$	feed flowrate vector
c	solute concentration
c_m	concentration in mobile phase
c_s	concentration in stationary phase
c_s'	concentration of sephadex gel
c^+	solute concentration at equilibrium
d_p	mean particle diameter
D	polydispersity of polymer sample
D_e	eddy diffusion coefficient
D_i	intraparticle diffusion coefficient
D_m	solute diffusion coefficient in mobile phase
D_p	longitudinal dispersion coefficient
D_s	solute diffusion coefficient in stationary phase
E_p	dimensionless voidage parameter
ΔF	total free energy change
ΔF_d	energy change due to dissolution in gel phase
ΔF_s	energy change due to entropic variation
g_m	gradient of calibration curve
g_1, g_2	geometric factors
h	reduced plate height
h_i	height of chromatograph above baseline
H	height equivalent to a theoretical plate
HMWP	high molecular weight product

ΔH_o	standard enthalpy change
K	equilibrium distribution coefficient
K_d	Wheaton-Bauman distribution coefficient
$K_{g.p.c.}$	experimental distribution coefficient
L	length of column
m	number of moles of solute
M	molecular weight of solute
M_{AUX}	molecular weight of auxillary polymer sample
$\bar{M}_n$	number average molecular weight
M_{peak}	molecular weight at peak of elution curve
M_{pol}	molecular weight of required solute species
$\bar{M}_w$	weight average molecular weight
$\bar{M}_\eta$	viscosity average molecular weight
$\bar{M}_z$	z average molecular weight
n	total number of molecules in mobile and stationary phases
n_i	number of molecules of molecular weight M_i
n_m	number of moles in mobile phase
n_s	number of moles in stationary phase
N	number of theoretical plates in column
P_c	permeation constant
Q	volumetric flowrate
r	distance from centre of particle
r_m	mean hydrodynamic radius of molecule
r_p	mean radius of pore
R	retention parameter
R_c	column radius
R_s	resolution
R_{sp}	specific resolution

ΔS	standard entropy change
t	time
t_o	retention time of non-retained component
t_r	retention time
u	concentration of feed solution
$\underline{U}$	vector for concentrations of feed solutions
v	average linear velocity of mobile phase
v_R	reduced velocity
v_s	average linear velocity of solute
V_a	volume of column accessable to solute
V_e	elution volume of solute
V_i	interstitial volume of column
V_o	void volume of column
V_p	volume of packing in column
V_R	retention volume of solute
V_S	volume of stationary phase in column
w_f	weight fraction of solute
W	width of eluted solute peak
W_s	weight of eluted sample
x	concentration of mobile phase in model
$\underline{x}(t)$	vector for solute concentrations in model
$\underline{x}_o$	vector for initial concentrations in model
$\underline{\dot{x}}$	vector for rate of change of solute concentrations
z	distance along column
α	external voidage of column
β	internal voidage of packing
γ'	tortuosity factor
ε	departure from equilibrium

η	intrinsic viscosity
λ_1	rate of entrapment of solute
λ_2	rate of escapment of solute
σ	variance of solute peak
Δ	difference

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