DEXTRAN POLYMER FRACTIONATION

BY

PRODUCTION SCALE CHROMATOGRAPHY

AND

ULTRAFILTRATION

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SUMMARY

Dextran Polymer Fractionation by Production Scale Chromatography and Ultrafiltration

Reviews of gel permeation chromatography (GPC) and of ultrafiltration (UF) theory have been made.

The aims of the project were to fractionate dextran, a polyglucose, on an existing chromatograph to achieve more optimum conditions of operation and to eliminate the silica dissolved from the chromatographic packing and present in the dextran products. Also to modify an existing mathematical model of the GPC process to take into account concentration effects on the chromatograph when fractionating dextran.

The semi-continuous chromatograph (SCCR5) used for dextran fractionations consisted of ten stainless steel columns of 5.1 cm I.D. by 70 cm length, packed initially with Spherosil XOB 075 that has been later replaced with the XOB 030 grade in an attempt to improve the GPC fractionation.

Ultrafiltration (UF) has been tried for removing silica from dextran solutions, and it was found that UF was not only useful in removing silica but can be also used for the fractionation and concentration of dextran solutions. The UF has been carried out on a 402A Amicon stirred cell system, on a DC2A Amicon hollow fibre cartridge system, a Patterson Candy International reverse osmosis system and an Amicon DC30 system.

Ion exchange resins have been used for the complete removal of silica from dextran solutions.

GPC, UF and ion exchnage have been combined into a novel process that produces clinical dextran 40 from dextran hydrolysate with a satisfactory yield. The GPC process removed the very high molecular weight material. The UF process removed the very low molecular weight dextran and most of the silica and concentrated the final solution. Then this solution was passed through an ion exchange cartridge to produce a silica-free product. This process could offer a more satisfactory alternative to dextran fractionation using ethanol-water solutions, although the economics of the process have still to be established.

A description of the effects of the operating conditions on the fractionation performance of the GPC on the SCCR5 have been discussed.

The existing simulation program for GPC fractionations on the SCCR5 unit and, based on the linear exclusion model, has been modified to take into account the concentration and temperature changes that affect the fractionation.

A good agreement between the computer simulations and experimental results has been achieved, but the model can be further improved.

Key Words: Gel permeation chromatography, semi-continuous, ultrafiltration, ion exchange, dextran

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To my parents for their support and encouragement.

Finally and above all to Maria, my wife, for her love and help.

DEDICATION

To: My Son, John

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1.0 INTRODUCTION

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Dextran is a glucose polymer and for many years it has been used in the field of medicine, as a blood plasma volume expander, and for the production of iron dextran which is used in the treatment of anaemia and other clinical uses (1-3).

The method of manufacture involves fermentation of sucrose to produce native dextran of several million daltons (approximately 50,000,000). Then the native dextran is hydrolysed to produce dextran hydrolysate having a molecular weight range from 180 daltons to 3,000,000 daltons.

Before the dextran can be used in its several clinical applications, it is necessary to reduce its molecular weight range by removing most of the larger and smaller molecules (Fig. 1.1).

The present industrial process involves fractional precipitation of dextran hydrolysate from aqueous solutions using ethanol, but the ethanol is a fire hazard and very expensive to recover.

Therefore a process that involves only aqueous solutions is preferable. Gel permeation chromatography (GPC) and ultrafiltration (UF) are two of these processes.

GPC is a form of liquid chromatography based on the unique properties of the gels (porous silica, agarose, etc.) for separating polymers, primarily on the basis of molecular size. Since dextran molecules are of different sizes, GPC is useful for fractionating dextran.

GPC has many applications ranging from analytical separations of microliter sample volumes to large scale industrial purifications (4-27).

Ultrafiltration (UF) is another process that separates/fractionates molecules according to their molecular size and utilises porous membranes as a separation means. UF is a useful technique in the separation, fractionation, purification and concentration of macromolecules in aqueous solutions (28-43).

Barker and co-workers (7,44-48) have been using continuous, counter-current GPC for dextran fractionation for the past ten years. The aims of this project and the differences with earlier work were as follows:

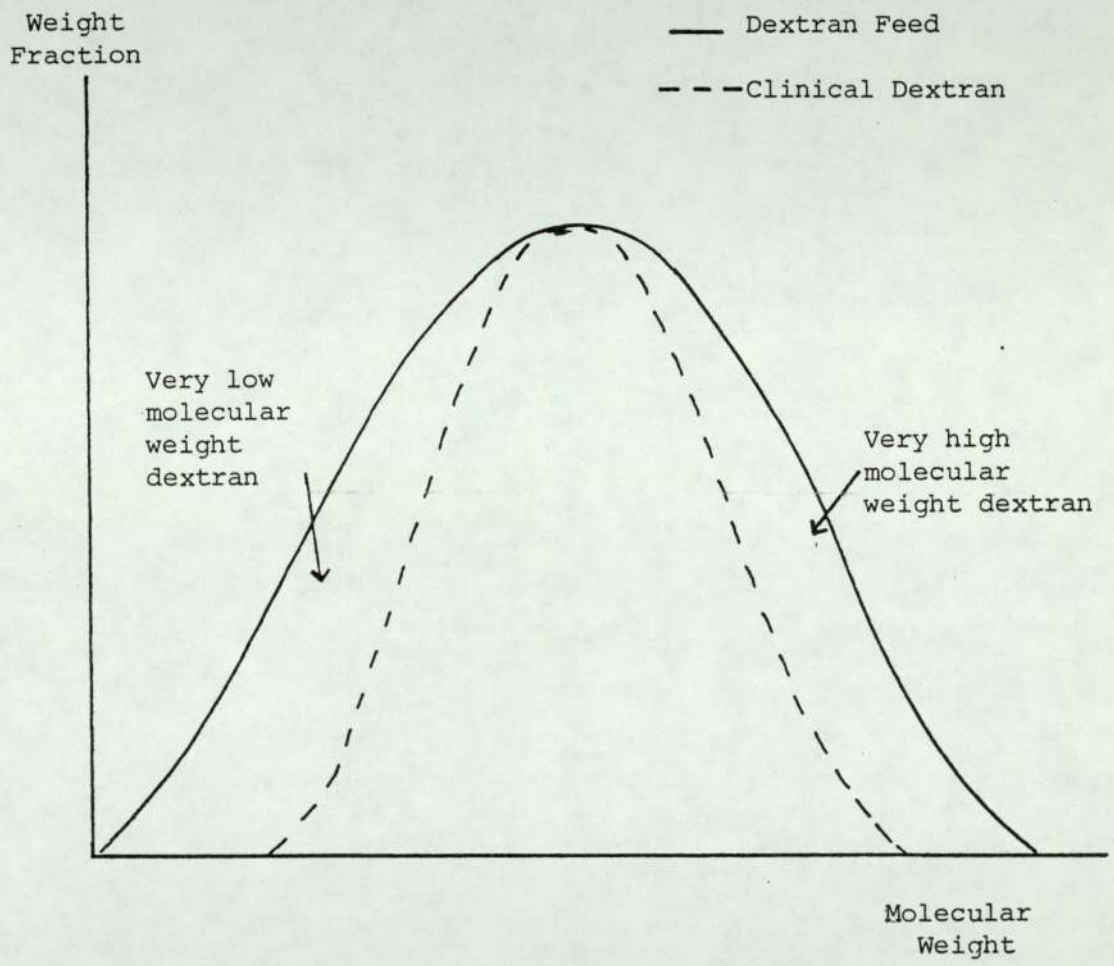
(a) To achieve better operating conditions than my predecessors using an existing semi-continuous chromatographic refiner for dextran fractionations.

(b) Because the silica packing in the chromatographic refiner was dissolved and the final products were contaminated with silica an alternative packing was to be considered, or if the same packing was to be used a way of removing the silica from the final products was to be found.

(c) The existing mathematical model for the dextran fractionation on the chromatographic refiner did not take into account concentration effects that affected the separations, hence this mathematical model was to be modified so that it would consider the concentration effects.

(d) UF was to be investigated to see if it could be used as an alternative process to GPC for dextran fractionation.

Fig. 1.1 Reduction of Dextran Hydrolysate to Clinical Dextran Fraction



Specification of Clinical Dextran 40

Molecular Weight 12,000<85% of Dextran <98,000 Molecular Weight

2.0 LITERATURE SURVEY

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2.1 LIQUID CHROMATOGRAPHY

2.1.1 LIQUID CHROMATOGRAPHY

Chromatography with a liquid mobile phase can be traced back to the work of the Russian botanist, M. Tswett (49). However, in the past few years liquid chromatography has taken its modern form, with the use of sensitive detectors and the development of very fine (5-15 μm) and rigid packings. Nowadays liquid chromatography is a much-used analytical technique and a preparative tool.

Liquid chromatography is divided into four categories and the names assigned to various liquid chromatography techniques depends on the retention mechanisms. The four categories are:

(i) Partition chromatography

(ii) Adsorption chromatography

(iii) Ion exchange chromatography

(iv) Exclusion chromatography

Partition chromatography relies on the adsorption of solutes by an inert solid support coated with a liquid stationary phase, whereas adsorption chromatography depends on the solute associating with active sites on the stationary phase. Nowadays, appropriately charged ion exchange resins have been used as a stationary phase for adsorption chromatography, although ion exchange is not related to this retention mechanism.

Ion exchange chromatography relies on the reversible exchange of ions between the stationary phase and the mobile phase.

Finally exclusion chromatography is the separation of molecules according to their molecular size. Very large molecules that cannot penetrate the pores of the stationary phase are eluted first and very small molecules that can penetrate all the pores are retained and eluted last. The exclusion mechanism which forms the basis for gel permeation chromatography (GPC) will be discussed in detail in Section 2.2.

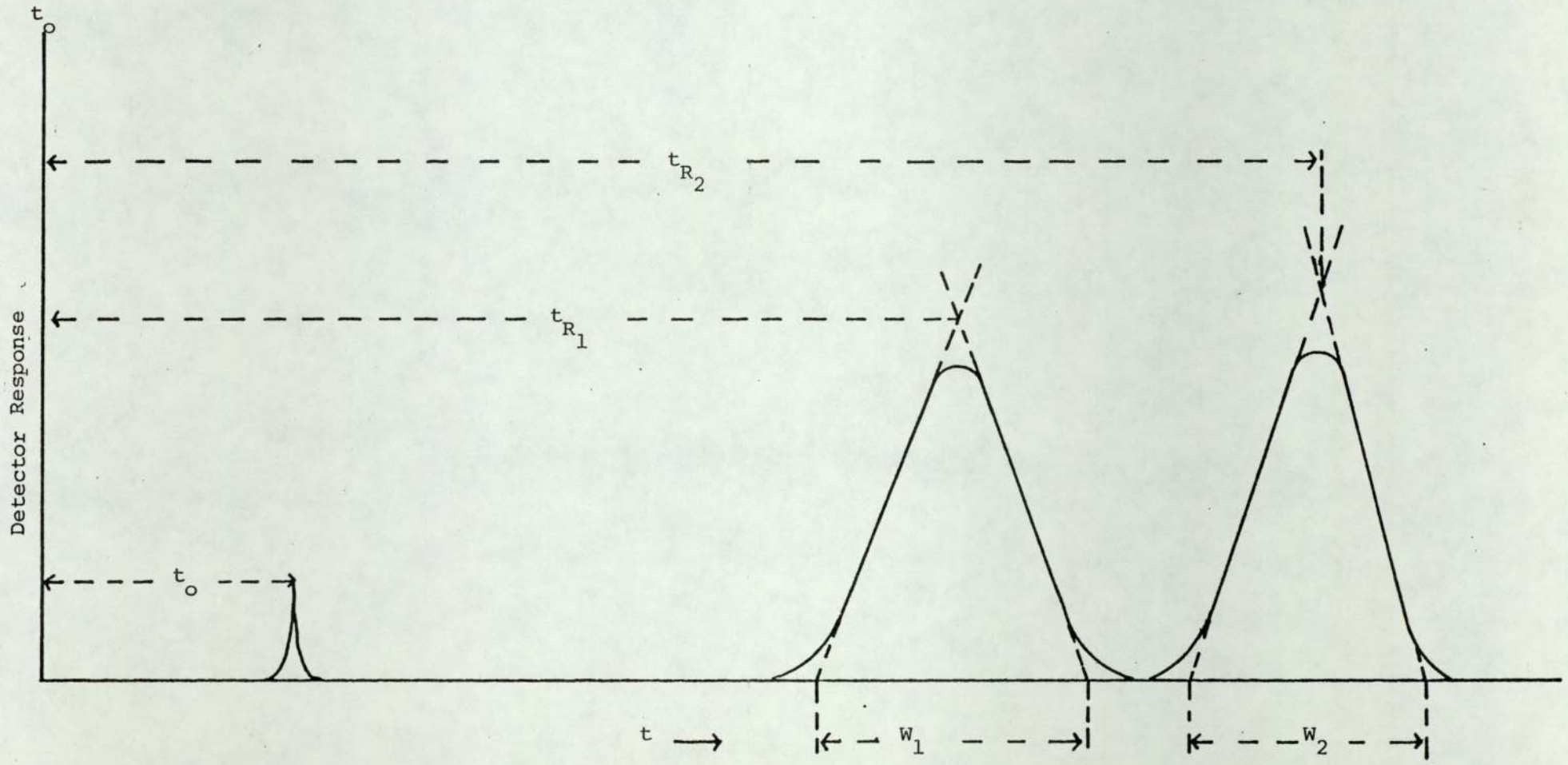
2.1.2 THEORY OF LIQUID CHROMATOGRAPHY

The chromatographic separation of a sample into its constituents is achieved by differences in equilibrium distribution of the constituents between two phases. One phase is called the stationary phase (packing material) and the other which flows past the stationary phase is called the mobile phase (eluent).

A component that is in the stationary phase is retarded, and it only travels through the column when it is in the mobile phase. Its migration rate, R, through the column is therefore given by the fraction of time it spends in the mobile phase.

The migration rate, R, depends on the equilibrium distribution of the component between the two phases, components having higher affinity for the mobile phase being eluted first and those favouring the stationary phase are eluted last.

Fig. 2.1 Illustration of Important Parameters for the Characterisation of Separations



A typical separation of two components is shown in Fig. 2.1. From this elution chromatogram, important information may be obtained.

(a) Elution volumes and partition coefficient

The elution volume of a component, V_{R_1}, a fundamental retention parameter in column chromatography, may be obtained directly by measuring the retention time, t_{R_1}, of that component and multiplying it by the volumetric flow rate, Q. Thus

$$V_{R_1} = t_{R_1}.Q \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.1)$$

where Q = volumetric column flow rate, and t_{R_1} retention time of component 1.

Similarly,

$$V_{R_2} = t_{R_2}.Q \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.2)$$

Also the elution volume of an unretained solute, V_o, that passes through the column at the same rate, at which the mobile phase is flowing is

$$V_o = t_o.Q \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.3)$$

and is a measure of the total volume of the mobile phase contained within the column; it is often called void volume.

The net retention volume, V_N, is

$$V_N = V_R - V_o \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.4)$$

and it is proportional to the stationary phase volume V_s. The proportionality factor is the thermodynamic partition coefficient K.

Then

$$V_N = V_R - V_o = K.V_s \quad \ldots\ldots\ldots\ldots \quad (2.5)$$

or

$$V_R = V_o + KV_s \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.6)$$

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

$$V_R = V_o + K_d V_i \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.7)$$

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 is equal to V_i plus the volume of the packing V_p. But since V_p does not participate in gel permeation chromatography, V_s is replaced by V_i and K by the g.p.c. coefficient K_d.

(b) Capacity and separation factors

The capacity factor (K') of a column system is a measure of sample retention by the column in column volumes. It is simply the ratio of the component elution volume to the void volume expressed as:

$$K' = \frac{V_R - V_o}{V_o} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.8)$$

The ratio of the capacity factors of the two components is designated as the separation factor, α:

$$\alpha = \frac{V_{R2} - V_o}{V_{R1} - V_o} = \frac{K'_2}{K'_1} \quad \ldots\ldots\ldots\ldots\ldots \quad (2.9)$$

The separation factor is a measure of the selectivity of the separation system, and by convention is always expressed as greater than or equal to unity. The more selectively a stationary phase retains one of two components, the greater is the separation factor of these components. If $\alpha = 1$, there are no thermodynamic differences between the two components in a given system and they cannot be separated.

(c) Resolution and Efficiency

In all chromatography systems, axial spreading of 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. Resolution is the term quantifying the degree of separation in a chromatographic system and for a binary system is defined by:

$$R_S = \frac{2(V_{R2} - V_{R1})}{W_1 + W_2} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.10)$$

where W_1 and W_2 are the width of the peaks as defined in Fig. 2.1.

More practical relationships between resolution and experimental variables are suggested by Purnell and others (50,51).

For good resolution, narrow baseline widths (minimum band spreading) are certainly desirable, particularly when the separation factor is small. An empirical measure of column efficiency is the Number of Theoretical Plates, N, in which

$$N = 16\left(\frac{V_R}{W}\right)^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.11)$$

The narrower the peak, the higher N and the more efficient the column.

Giddings (52) 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:

$$\phi = 1 + 0.2N^{\frac{1}{2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.12)$$

Christopher (53) suggested a suitable measure for the performance of a gel permeation column was its ability to give molecular weight averages that agree with their theoretical values; the percentage difference between the measured and theoretical values was a measure of the column efficiency. Cooper (54) proposed that column efficiencies could be compared by measuring the polydispersity value of a narrow polymer distribution eluted from a column.

Knox and McLean (55) showed that the true number of theoretical plates cannot generally be obtained from the elution peak of a polymer whose polydispersity is much larger than 1.01.

Metzger et al (56,57) suggested that the impurity of a component on mass or mole basis can be used as a measure of the separation effectiveness.

2.2 GEL PERMEATION CHROMATOGRAPHY

2.2.1 INTRODUCTION TO GEL PERMEATION CHROMATOGRAPHY

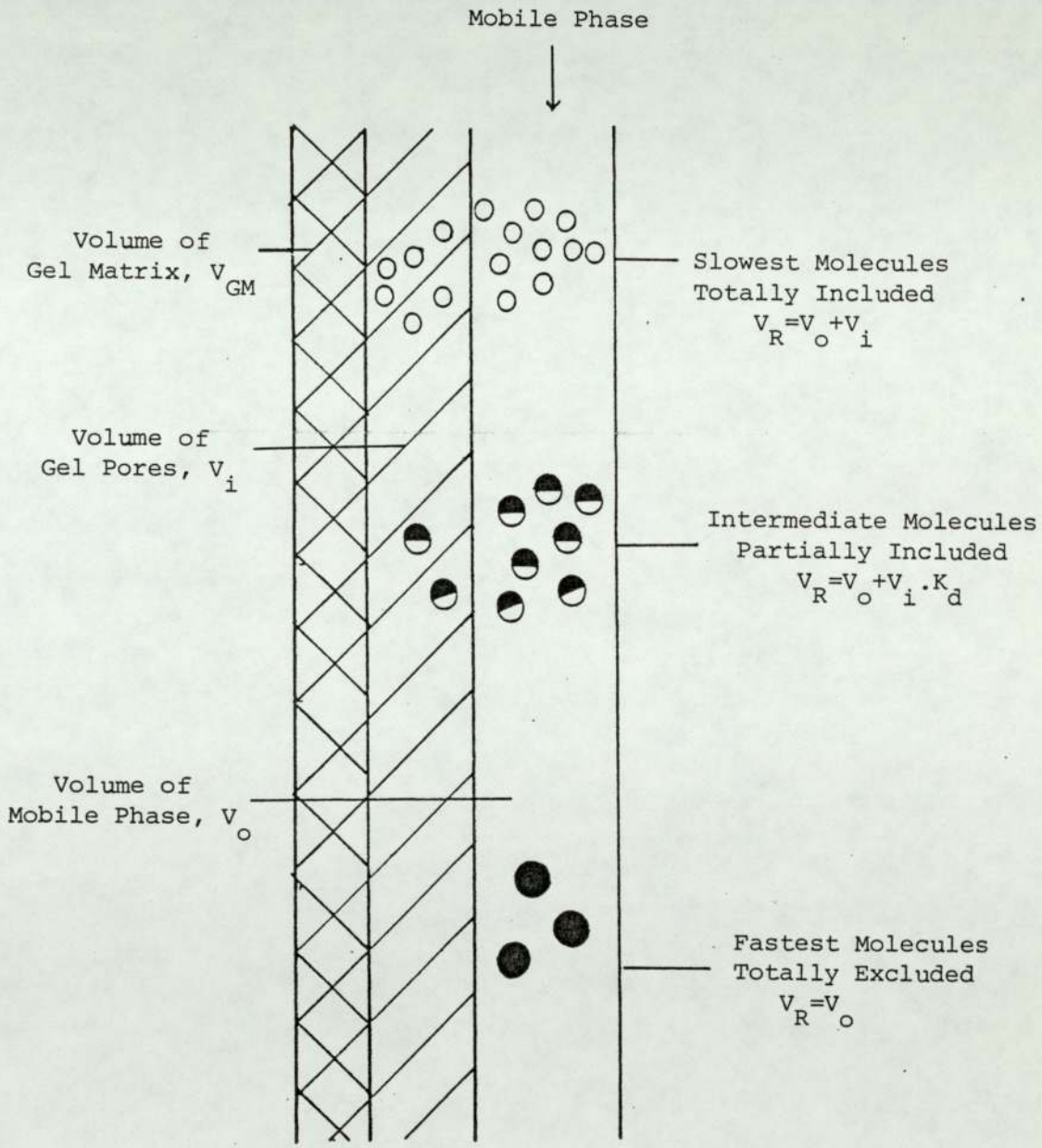
GPC is a form of liquid chromatography where solute molecules are retarded by their ability to permeate into the solvent filled pores of the packing material. Thus GPC is really a special case of liquid chromatography. The separation is found to be dependent on molecular size, and speculation as to the exact mechanism involved has led to various alternative names, e.g. gel chromatography, gel filtration, exclusion chromatography, molecular sieve chromatography and restricted diffusion chromatography.

Historically, it is difficult to decide who performed the first separation based on molecular size, but biopolymers were certainly being separated by the early 1950s using aqueous solvents and cross-linked gels. An important step in the development of the technique was the introduction of cross-linked dextrans, trade name Sephadex (58). A short time after this, rigid packings were developed and commercial automated instruments came onto the market to give GPC its modern form.

2.2.2 THE PRINCIPLE OF GEL PERMEATION CHROMATOGRAPHY

In a GPC system the stationary phase consists of column(s) packed with small gel particles, which have pores of variable size. Through the gel particles flows a solvent which is the mobile phase. The same solvent

Fig. 2.2 Illustration of the GPC Fractionation Process



also fills all the pores of the gel and is used to dissolve the sample, before introducing it into the column.

The mobile phase flows through the interstitial regions and the solute molecules travel only through the column when they are in these regions. Solute molecules permeating into the pores are retarded, and the more time molecules spend in the pores, the more they are retarded.

Large molecules that are completely excluded are thus eluted first, and small molecules which can completely penetrate the pores are eluted last (Fig. 2.2).

A species is eluted at a volume equal to the volume available to it in the column, which in the case of totally excluded molecules is V_o, the void volume; and for completely included molecules is V_o+V_i, where V_i is the pore volume. For intermediate molecules the elution volume is dependent on the pore volume accessible to the species, V_{iACC} and is given by:

$$V_R = V_o + K_d V_i \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.7)$$

where K_d is the distribution coefficient, that is defined as:

$$K_d = \frac{V_{iACC}}{V_i} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.13)$$

The values of K_d for GPC are always equal to or between 0 and 1.

2.3 COLUMN PACKINGS AND PACKING TECHNIQUES

The heart of the GPC instrument is the column bed, since it is within the column that the separation takes

place. A variety of porous packing materials and packing techniques are available for GPC.

The packings can be classified by the rigidity of the material, rigid, semirigid and soft gels, or they can be divided according to the material from which they are made, organic and inorganic gels.

Finally the GPC gels can be used as an analytical packing, if they have a particle diameter of less than 70 μm or for preparative work and large scale equipment if they are larger.

2.3.1 CONVENTIONAL ORGANIC-BASED PACKINGS

(a) Soft Gels (Aerogels)

Cross-linked dextran gels - Sephadex was the first gel available commercially. Now it is marketed by Pharmacia Fine Chemicals in eight different pore sizes, having a molecular weight exclusion limit of around 200,000. This gel swells in water and is stable in the pH range 2-10. It is relatively soft and low flowrates must be employed. It is used to fractionate polysaccharides (59,60).

Enzacryl gels - Cross-linked gels of poly(acryloylmorpholines), have the interesting property of being compatible with both aqueous and organic phases. However, in aqueous mobile phases, the gels are soft and low flowrates must be used. In aqueous GPC, applications have included polyethylene glycols, oligometric diols and polysaccharides (61). The exclusion limit of these gels is up to 100,000 daltons.

Sephacryl - This gel is produced by copolymerizing allyl dextran with N,N'-methylenebisacrylamide. It is available in two pore sizes. Sephacryl S-200 is comparable to Sephadex G-200 (superfine). Since Sephacryl is produced in smaller particle sizes than Sephadex, its column efficiency is 2-3 times higher. Also because of its rigidity, higher flowrates can be employed compared to Sephadex. But Sephacryl has unusual adsorption properties (62).

Agarose gels - These materials are stable in the pH range 4-9, but since they are not cross-linked they cannot be dried or operated outside the temperature range 0-40^{o}C.

Sepharose (63) has been cross-linked by reaction with 2,3-dibromopropanol. This produces a gel with similar porosity to the parent gel, but with greatly increased thermal and chemical stability. It is softer compared to Sephacryl.

Bio-gel - This is a cross-linked polyacrylamide gel. This gel is not subject to attack by micro-organisms and is available in ten pore sizes (64). The exclusion limit for polysaccharides is 1.5×10^{5} daltons.

Ultragel - These composite packings were introduced in 1975 and consist of both polyacrylamide and agarose. Therefore these gels have a fractionating range greater than the polyacrylamide gels and they are more rigid than agarose. Although they have good flow properties and wide pH stability, the maximum operating temperature is 36^{o}C because of the low melting point of agarose (63,65).

Aquapak - This is a polystyrene gel cross-linked with divinylbenzene. Fractionations of dextrans and sodium lignin sulfonates were demonstrated (66).

Bio-Beads is a similar gel to Aquapak.

Cross-linked cellulose gels - The preparation of these gels and their performance compared with the performance of other soft gels are described by Sikuga (67).

(b) Semi-Rigid and Rigid Gels

Hydrogel - This is reported to be a highly cross-linked ethylene glycol dimenthacrylate polymer packing. Three pore sizes are available with a molecular weight exclusion limit greater than $1x10^6$ for dextran solutions. The gel can withstand pressures of up to 200 atmospheres. It was reported that no deterioration in efficiency or change in calibration was observed, over a long period (1 year), using Hydrogel packed columns for dextran analysis (46,67-70). Unfortunately, the Hydrogel range of GPC packings was withdrawn from the market due to poor batch reproducibility.

Spheron - This is a similar packing to Hydrogel, but has a higher efficiency and lower exclusion limit of ca. $5x10^5$ daltons. Columns packed with Spheron were used for dextran analysis (46,68,69,71,72). Their packed bed was found to compress and the efficiency was rapidly reduced (46,68).

Toyopearl - This a semi-rigid, spherical hydrophilic polymer (polyvinyl) gel. It has good efficiency and

resolution and a high exclusion limit (ca. $1x10^7$), but it is a relatively soft packing and thus low flowrates must be used (73-75).

Shodex Oh pak - Although the exact structure of this packing is not known, it is assumed to be composed of a methacrylate glycerol copolymer. Presently, only one pore size is available which has a fractionation range $<4x10^5$. The packing is stable within pH range 4-12 and has been used for the GPC of polysaccharides, peptides and sodium alkybenzene sulfonates (76).

Shodex Ion pak - These sulphonated poly(styrene divinylbenzene) gels are available in a wide variety of pore sizes. Since they are ionic, it is apparent that a salt or a buffer must be added to the mobile phase in order to supress ionic exclusion and ion exchange mechanisms. Because of the hydrophobic structure of the polystyrene divinylbenzene, one might also assume that hydrophobic interactions could occur. The major applications of these packings are the GPC of carbohydrates, oligosaccharides, polysaccharides and polyethylene glycols (77-79).

TSK-PW - The exact structure of this high-performance polymeric gel has not been published; however it does contain $-CH_2.CHOH.CH_2O-$ groups. TSK is available in many pore sizes, although only a few can be commercially obtained at this time. These packings have wide GPC applications (80-85). They were found to be very stable over a period of two years.

2.3.2 INORGANIC PACKINGS

Almost all the inorganic packings used for aqueous GPC are made from silica. They are rigid and can withstand very high pressures.

In aqueous GPC, adsorption can arise from hydrogen bonding, hydrophobic and ionic interactions. For high molecular weight compounds, adsorption can be quite severe when silica gels are used. In addition, with long term use, silica slowly dissolves in aqueous mobile phases, especially at high pH values. The major thrust in column technology has been to develop deactivated silica packings or rigid cross-linked gels to prevent non-size exclusion effects from occurring.

(a) Unmodified Silica Packings

Barker et al (46,86) have used DuPont's SE and Zorbax PSM packings for dextran analysis. They noticed a significant drop in column efficiency after a few weeks and assumed that this might have been caused by dissolution of silica.

Barford et al (87) have used DuPont's SE and glycerylpropyl-CPG packings for the GPC of proteins, but noted that adsorption occurred and long-term column stability was a problem.

Lichrospher was used by Buytenhuys (88) on polysaccharides and exhibited no anomalous chromatographic effects, providing that the mobile phase was of sufficient ionic strength.

Porasil and Spherosil have been tried by Barker and others (7,44-48,88-94) as analytical and preparative packings and it was reported that they slowly dissolve in water. For the preparative work the rate of dissolution was sufficiently low, compared to the volume of the columns used, to avoid creating any significant problems.

CPG packings are widely used for dextran analysis and fractionation (95-102) and they seem to behave similarly to Spherosil and Porasil gels.

(b) Modified Silica Packings

Glycophases - These packings contain silyl-propylglycerol groups chemically bonded onto silica packings. These groups deactivate the silica, as well as provide a thin neutral layer that excludes sensitive compounds from contacting the silica surface. The first commercially available packing of this type was SynChropak, which is also marketed under the names of Aquapore and Bio Sil GFC. The packings have been used for the GPC of a wide variety of water soluble polymers including polysaccharides (103).

Another such packing is glycerylpropyl-CPG.

More recently, Lichrosorb DIOL has been used for the GPC of proteins (104).

Bondagel - These packings consist of a monomolecular layer of polyether chemically bonded onto silica. Bondagel has been used to characterize a number of water soluble polymers including polysaccharides (105,106).

TSK-SW Columns - The composition of these high performance silica-based packings has not been reported; however they do contain hydroxyl groups. They have a wide variety of GPC characterization applications (107-109)

2.3.3 COLUMN PACKING TECHNIQUES

There is none best column-packing method for all packings, since the optimum procedure is determined by the particle size and the nature of the material. The primary purpose is to pack the column uniformly without channels or particle sizing within the column. Rigid solids and semi-rigid, hard gels generally are packed as densely as possible without fracturing. Columns of rigid solids may be made by dry-packing or slurry packing techniques, depending on particle size; dry packing is normally used with particles of >20 μm. Small particles tend to form larger aggregates, and porous particles, smaller than ca. 20 μm, cannot be dry packed easily into homogeneous beds. Soft gels can only be slurry-packed.

(a) Slurry Packing

Columns of <20 μm rigid particles (e.g. silica) are best prepared by high-pressure slurry packing techniques.

Slurries of 5-15% w/w of packing are mainly used. The slurry must be degassed and dispersed by vigorous shaking. Particle segregation is minimised by rapidly forcing a homogeneous slurry into the column with sufficient force to make the resulting bed compact and stable. The

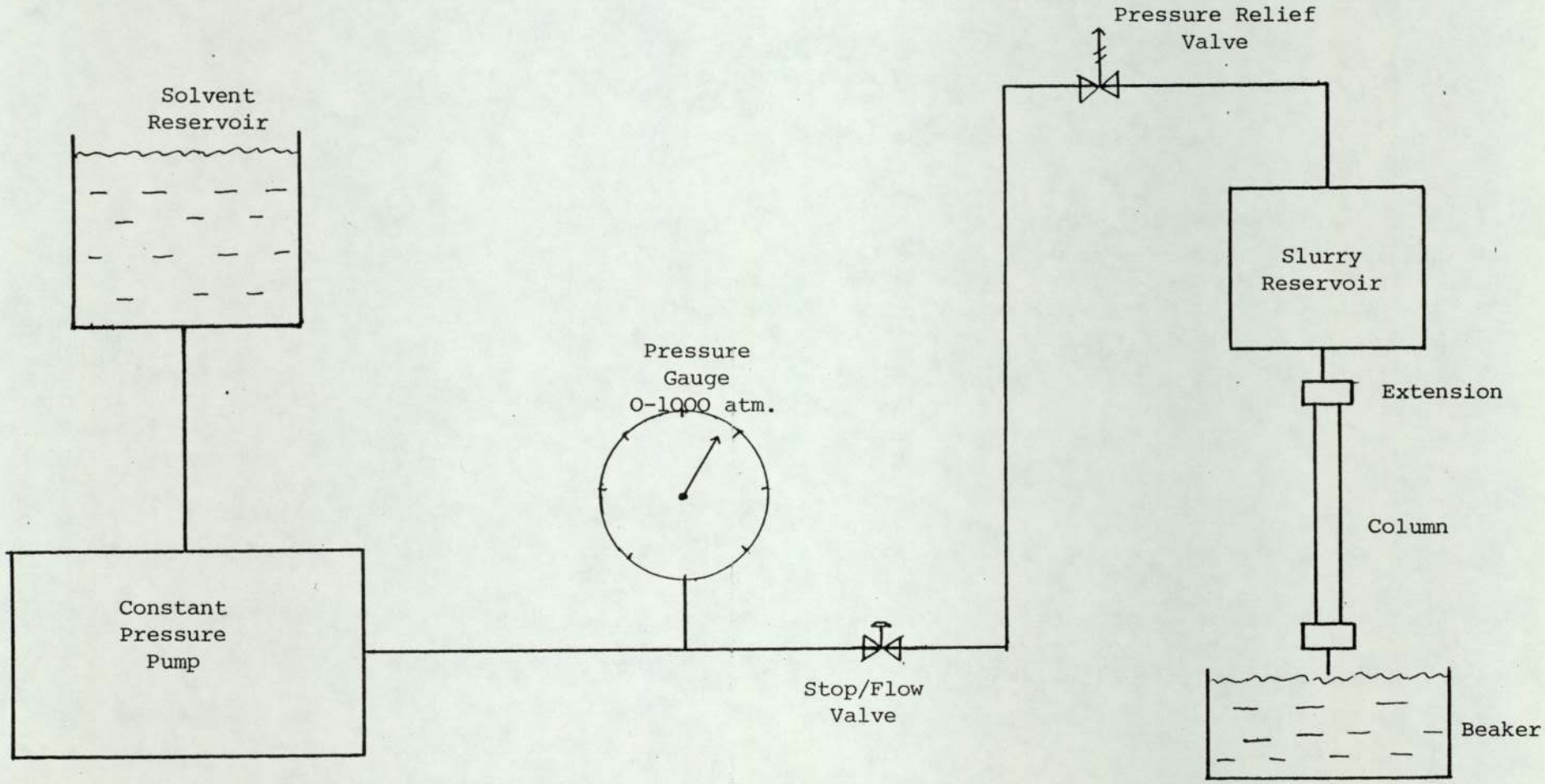
homogeneity and compactness of the formed column bed are increased by using very high flow rates to pump the slurry into the column. The long-term mechanical stability of the packed bed is markedly enhanced by a series of sudden repressurisation processes at high pressures to further consolidate the packing. Columns formed by this approach have been used for many months without evidence of packed bed settling.

A typical apparatus for preparing columns by the slurry-packing technique is shown in Fig.2.3. The apparatus uses a constant-pressure, pneumatic-amplifier pump to force the slurry rapidly into the empty column. The velocity of the slurry is proportional to the pressure used, and this velocity decreases as the packed bed is formed. Best performance is obtained when the maximum pressure possible is used. Detailed description of this packing procedure is reported in several chromatography books and papers (110-115).

Columns of semi-rigid organic gels are generally packed by the slurry-packing technique described above, with some modification. Since these polymeric gels are less dense than silica, the actual slurry packing procedure is carried out with a lower density solvent.

Softer gels are first allowed to swell in a suitable organic solvent. The swollen gel is then stirred and packed into the appropriate column, by introducing the slurry in several increments while allowing the particles to settle. Further compaction of this column bed at the operating pressure is desired before use.

Fig. 2.3 Typical Apparatus Used in the High Pressure Slurry Packing Technique



(b) Dry-Packing Technique

This packing method is used with rigid particles of greater than 20 μm. The 'tap-fill' method for dry-packing columns of rigid gels has been widely used for preparing preparative columns for many years (116).

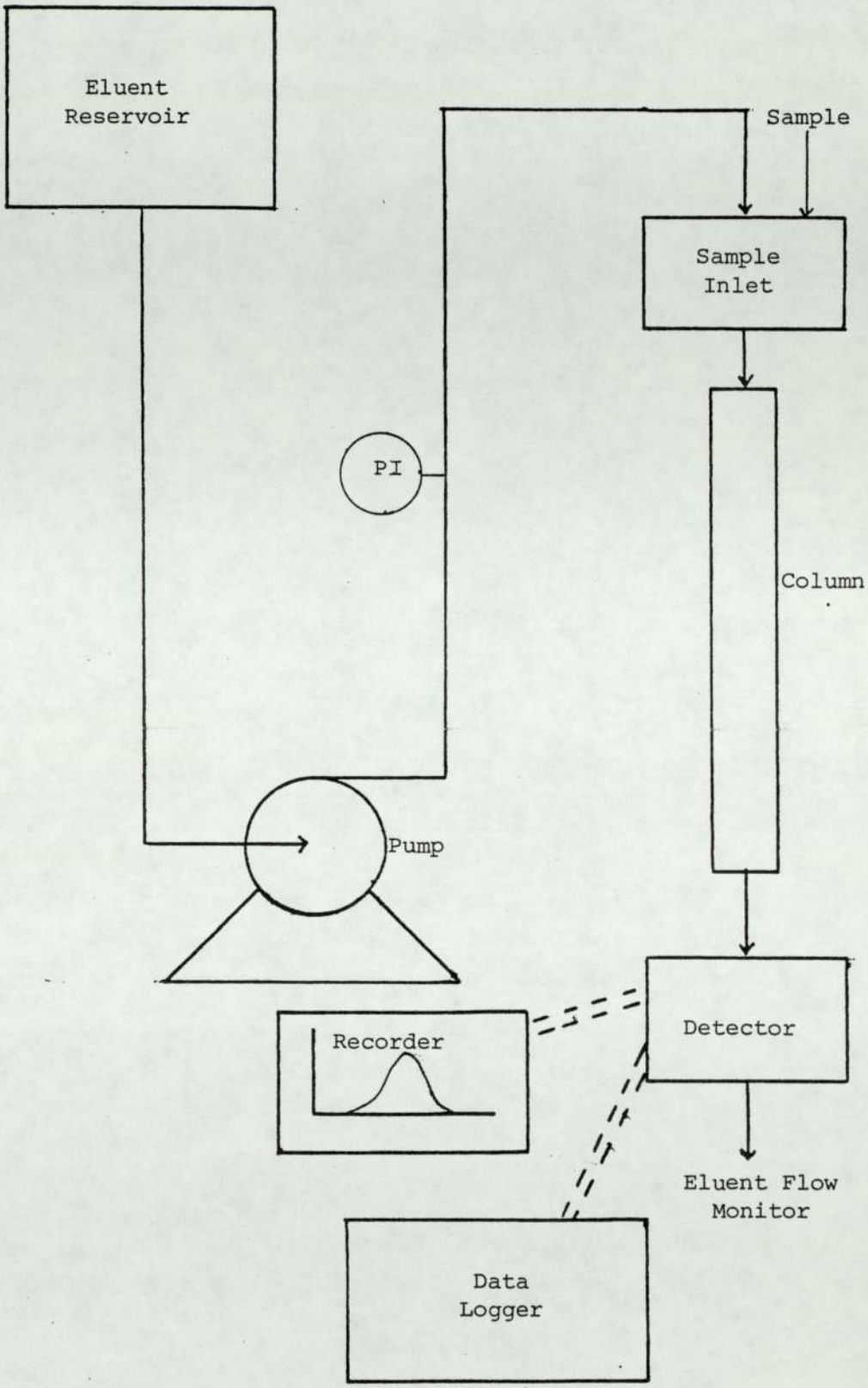
The clear empty column with a porous plug-end fitting attached is held vertically and a small quantity of packing is added through a funnel. The column is tapped firmly on the floor about 80-100 times while lightly rapping the side and rotating the column slowly. The column should then be very gently tapped vertically without rapping the side. Another increment of packing is added and the procedure is repeated. After the column is filled very gentle tapping should be continued for 3-5 minutes.

2.4 ANALYTICAL GEL PERMEATION CHROMATOGRAPHY

2.4.1 EQUIPMENT

Gel permeation chromatography is normally employed both as an analytical method and as a preparative technique. The experimental arrangement commonly used for analytical work is essentially that given in Fig.2.4. The equipment consists of an eluent reservoir, a pump, a pressure indicator, a sample loading device, a packed column, a detector, a recorder and an eluent flowrate monitoring device.

Fig. 2.4 Line Diagram of an Analytical GPC System



In practice a sample of the mixture to be analysed is loaded on to the top of the column, and eluent is pumped through it. For most chromatographic systems, the injection of the sample into the column is either by syringe or injection valve. Bristow (113) summarised the advantages and disadvantages of each injection method. Pumps are carefully chosen to ensure constant flowrate. In aqueous systems peristaltic pumps may be used for low pressure systems, but piston pumps are commonly employed.

Eluent, which has passed through the column, is monitored by a detector that responds to the different solutes in the sample as they emerge at different times from the column. The detectors that are most commonly used are refractive index detectors and ultraviolet detectors, although other less common detectors have been used and have been reviewed in the literature (110-113,117,118). Unfortunately there has been no 'ideal' detectors developed as yet that can be used for all GPC applications; often it is necessary to select more than one detector depending on the problems.

In GPC the most widely used detector is the differential refractometer (or refractive index detector). It is however one of the least sensitive detectors and subject to baseline fluctuations by changes in temperature, pressure and flowrate.

Finally the signal from the detector is transmitted to the recorder that produces a chromatogram.

Modern GPC systems (119-125) also have a data logger connected to the detector, so that the data can be stored in a computer for later calculations without any manipulation.

2.4.2 POLYMER CHARACTERIZATION

Polymer samples consist of various molecular-chain lengths and therefore have a distribution of molecular weights. Since many of the physical properties of the polymer system are affected by the molecular weight distribution (MWD), a knowledge of this data is essential in explaining or predicting the behaviour of the polymeric system. One method of obtaining this data is GPC.

The molecular weight distribution may be represented in the form of a histogram (Fig.2.5), which truly represents the discrete distribution of the system. However, as the polymer usually contains a very large number of different molecular weights, it is often more convenient to treat the distribution as continuous and to represent it as either a differential or integral distribution curve (Figs.2.6 and 2.7).

Since no single number can adequately characterize the molecular weight of a polymer, various averages are used. Of the various molecular weight averages the most common are:

Molecular Weight Distribution Curves

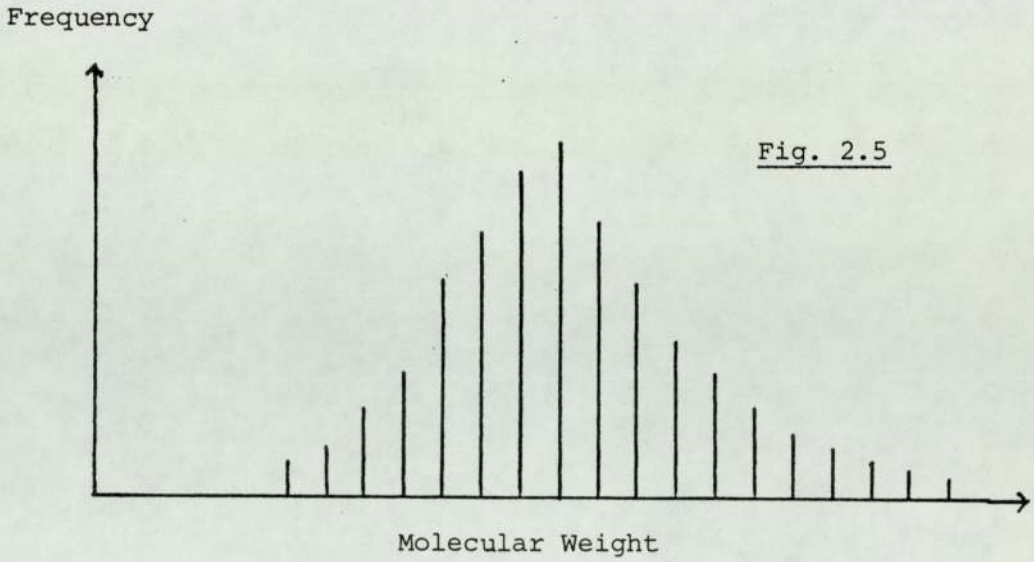


Fig. 2.5

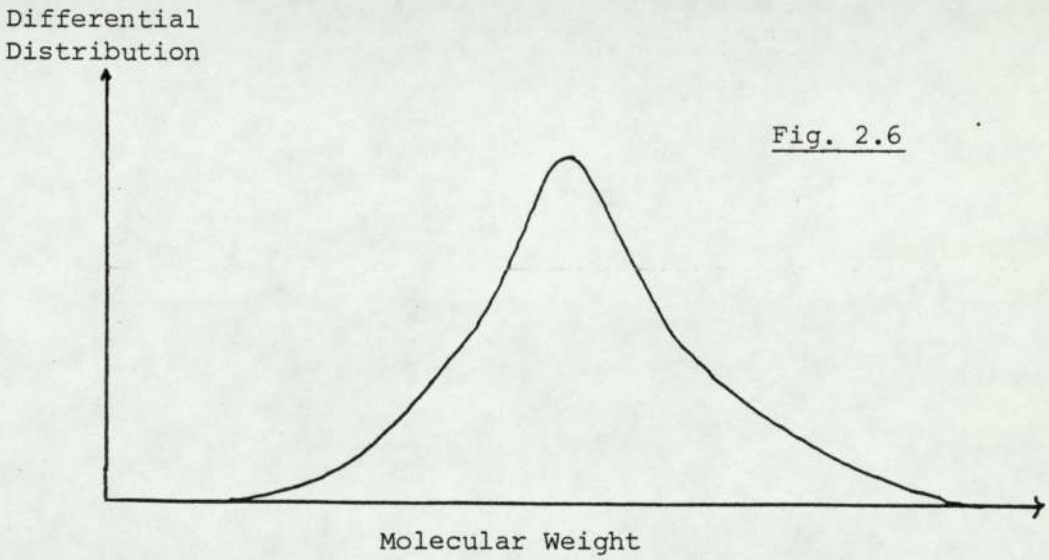


Fig. 2.6

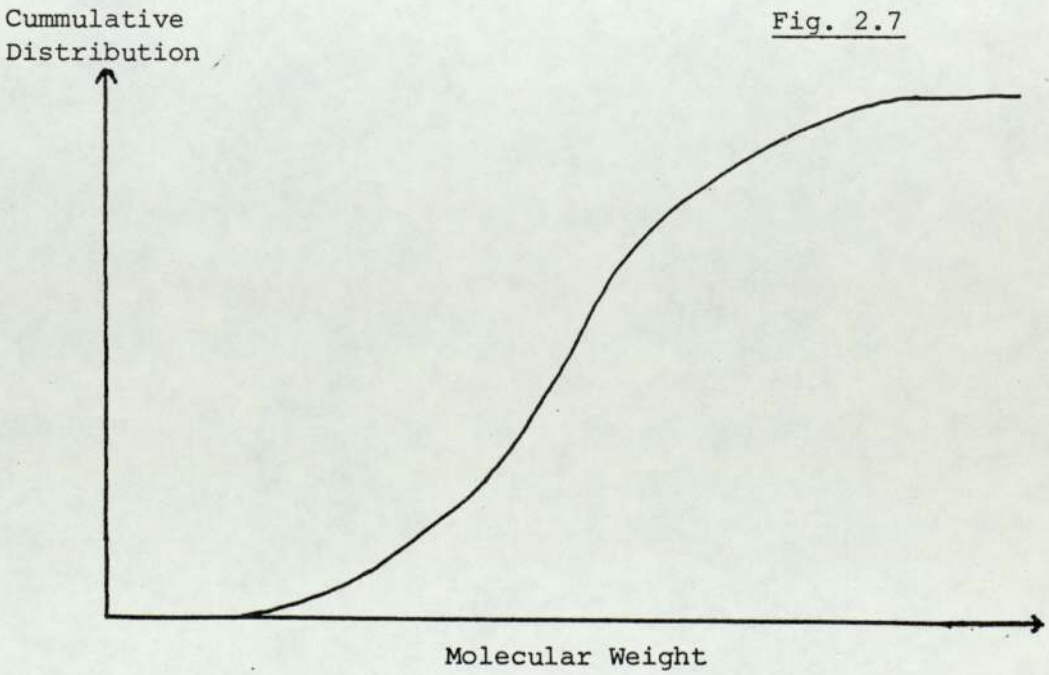


Fig. 2.7

$$\text{Number Average, } \bar{M}_N = \frac{\Sigma n_i M_i}{\Sigma n_i} \quad \ldots\ldots\ldots\ldots\ldots \quad (2.14)$$

$$\text{Weight Average, } \bar{M}_W = \frac{\Sigma n_i M_i^2}{\Sigma n_i M_i} \quad \ldots\ldots\ldots\ldots\ldots \quad (2.15)$$

where n_i = number of molecules of molecular weight M_i

The term polydispersity is often used to describe the width of a molecular weight distribution and is defined as:

$$\text{Polydispersity, } \bar{D} = \frac{\bar{M}_W}{\bar{M}_N} \quad \ldots\ldots\ldots\ldots\ldots \quad (2.16)$$

This ratio is always greater than one, since $\bar{M}_W$ is always greater than $\bar{M}_N$, except in the case of a single species where the ratio is unity.

2.4.3 COLUMN CALIBRATION

The complex part of GPC is the production of the final molecular weight distribution curve and the weight average and number average molecular weights from the chromatographic data.

The raw data consists of an elution profile of detector response against elution volume. The detector response is proportional to weight of polymer present, and the molecular weight of the polymer is a logarithmic function of K_d, a function of the elution volume (Equation 2.7).

In order to relate the elution volume (through K_d) to the molecular weight, calibration of the GPC column is necessary. This is done by chromatographing standard samples of known molecular weight. Ideally these should

be as near monodisperse as possible and should also consist of the same polymer as the one being characterized. The former simplifies the procedure and the latter ensures that no doubt can be expressed concerning the validity of the calibration.

In GPC with organic eluents, polystyrene standards of this type are available and are widely used as calibrants for a variety of polymers in addition to polystyrene. This requires the use of the universal calibration (46,126-129), a concept derived from studies on the properties of polymers in dilute solution, which have also thrown light on the mechanism of GPC. The separation achieved by GPC is dependent on the effective volume of the polymer molecule in solution. For a monodisperse polymer this hydrodynamic volume is proportional to the product of the intrinsic viscosity (η) and its molecular weight (M). Thus a plot of log (ηM) against elution volume has provided a universal calibration curve found to be true for many polymers.

However, polystyrene standards are not suitable for aqueous GPC and dextran is the only satisfactory molecule for calibration. Dextran fractions of low polydispersity are not readily available and have to be produced by fractional precipitation or preparative GPC, normally in the laboratory requiring them. Lansing and Kraemer (97,130) developed a method in which standards with polydispersity less than 1.1 are first characterised by measuring their $\bar{M}_W$ values by light scattering and their $\bar{M}_N$ values by end group analysis.

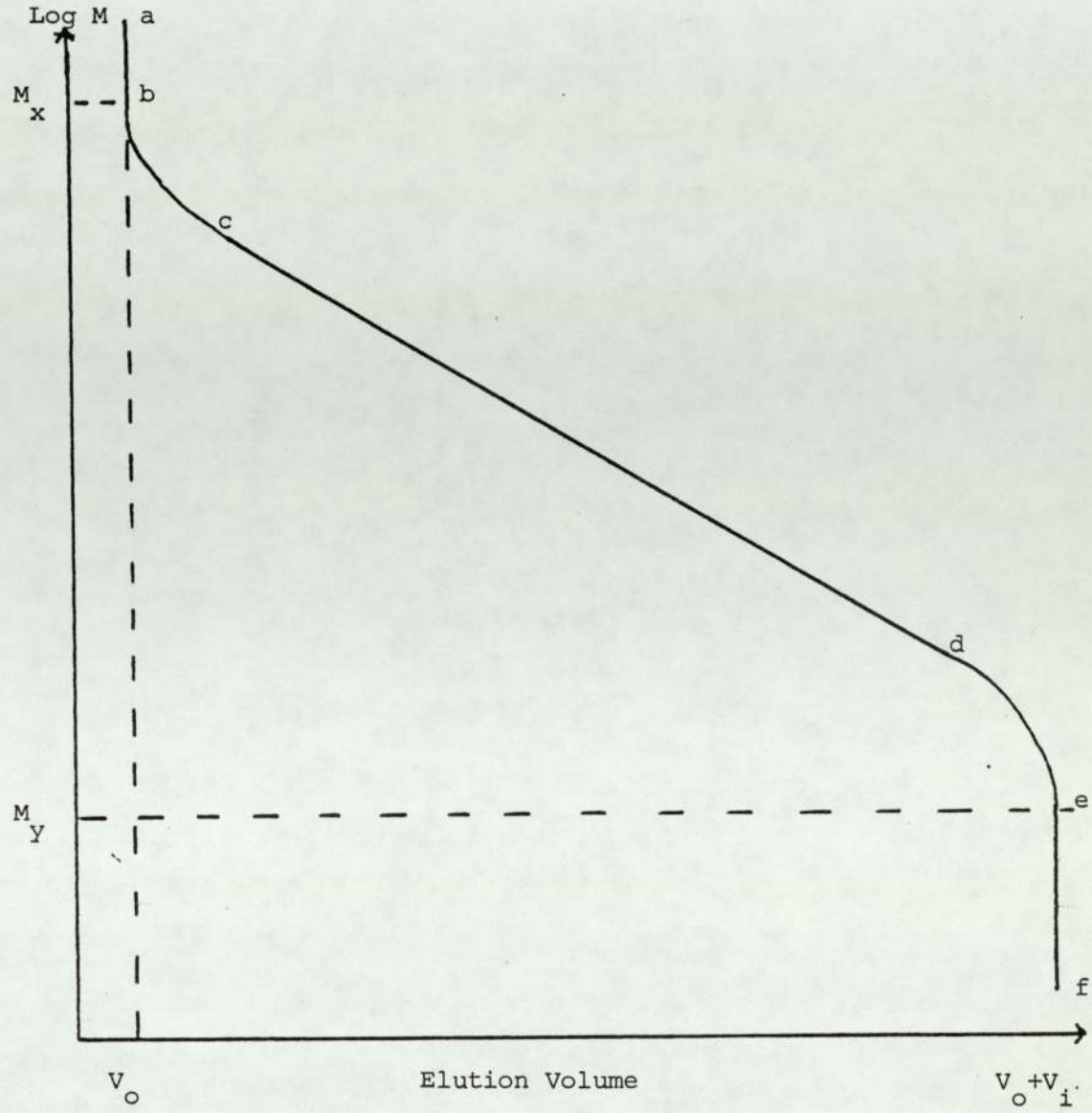
The fractions are then assumed to have a Gaussian distribution (weight vs. log molecular weight) and by using their derived equation the theoretical molecular weight distribution is calculated. The samples are then chromatographed and the characteristics thus obtained equated with those from the calculated molecular weight distribution. By this means a series of suitable standards may be used to produce the calibration curve (Fig.2.8).

Alternatively, broader fractions of polymer can be used. This necessitates a different approach, as was used by Nilsson and Nilsson (131). In this case 'broad' fractions are analysed by GPC, their $\bar{M}_W$ values having been obtained by light scattering measurements. Each elution curve is divided into at least twenty vertical sections, the areas and elution volumes measured, and the elution volume (V_R) converted to a K_d value. Nilsson and Nilsson then assumed that the molecular weight (M_i) and the K_d value were related by the following formula:

$$M_i = b_5 + \exp\{b_4 + b_1(K_d) + b_2(K_d)^2 + b(K_d)^3\} \quad \ldots \quad (2.17)$$

Values of the constants b_1 to b_5, which give the optimum agreement between the actual values of $\bar{M}_W$ measured by light scattering for each standard fraction and the calculated values obtained from the elution profile using equations (2.15) and (2.17), and where i refers to the ith vertical section, are then determined by computation. This minimisation of the difference is carried out using Hartley's modification of the

Fig. 2.8 A Typical Calibration Curve for a GPC Column



a-b molecules are too large to enter the pores

b-e fractionation takes place of molecules with molecular weight between M_y and M_x

e-f molecules are too small, i.e. they are totally included in the pores

c-d linear calibration range

Gauss-Newton method (132). The form of equation (2.17) thus derived is used to calculate the molecular weight corresponding to each value of K_d and a calibration curve drawn (Fig.2.8).

This is the preferred calibration procedure since any one set of (K_d,M) coordinates is estimated from the several overlapping profiles of the dextran standards. Also this calibration can be easily incorporated into an automated calculation.

The various calibration methods are described in detail in several papers and liquid chromatography books (46,111,125-131,133-137).

2.4.4 DATA TREATMENT

The chromatogram (elution curve) of the sample is given by plotting the detector response h' against elution volume, V_R and it may be used to construct the full molecular weight distribution (MWD) curve, provided that the calibration (log M against V_R) for the analytical GPC columns is determined (46,110-112,121,129).

The required MWD curve is represented by a graph of dw/dM against M, where w is the weight fraction of the sample with molecular weight below M. The term dw/dM may be expanded to give:

$$\frac{dw}{dM} = \frac{dw}{dV_R} \cdot \frac{dV_R}{d(\log M)} \cdot \frac{d(\log M)}{dM} \quad \ldots\ldots\ldots\ldots \quad (2.18)$$

$$= \frac{dw}{dV_R} \cdot \frac{dV_R}{d(\log M)} \cdot \frac{1}{M} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.19)$$

Fig. 2.9 Summary of Steps in the Conversion of a GPC Chromatogram to a MWD Curve

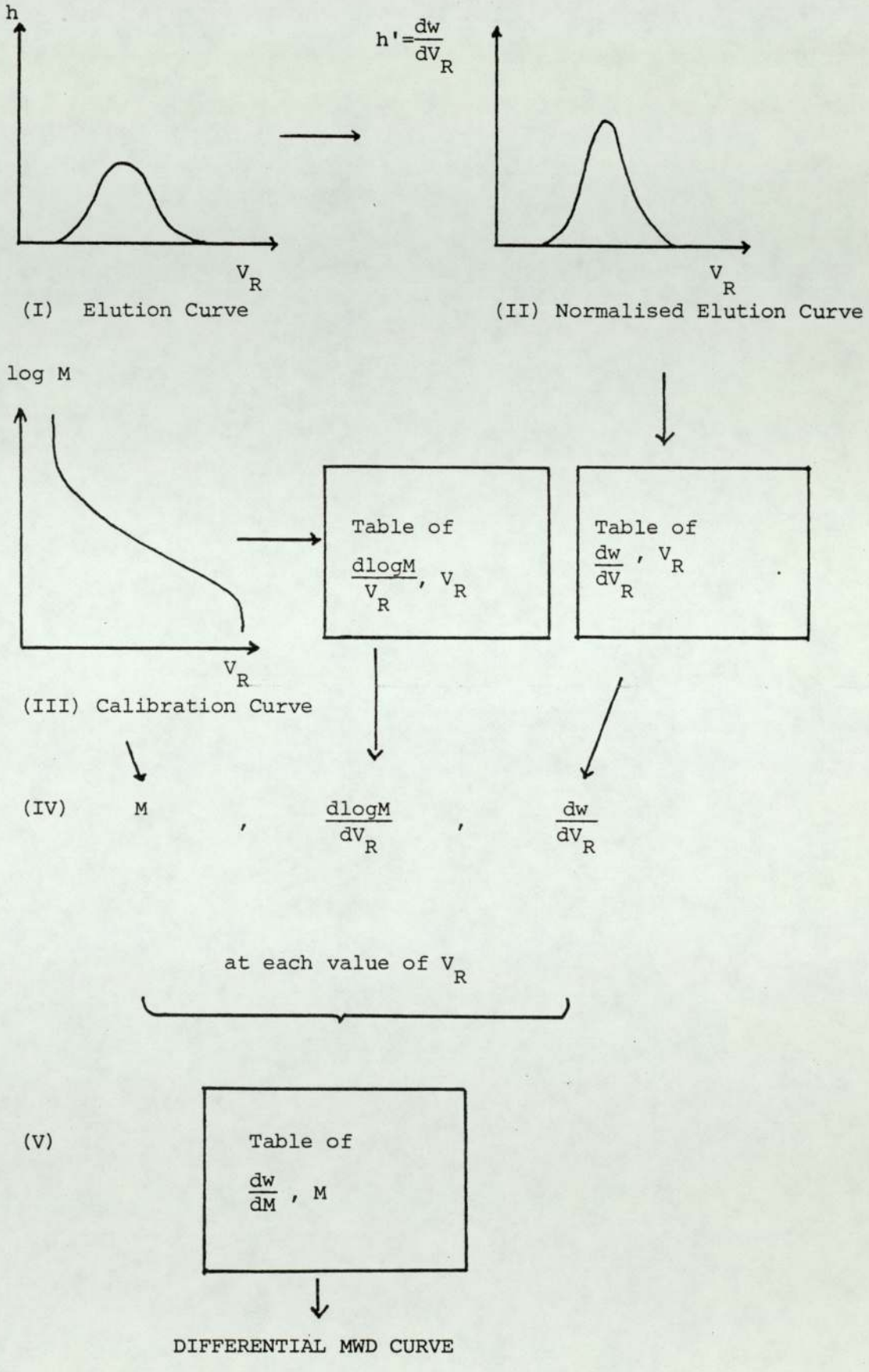


Fig.2.9 illustrates the process of data treatment. The GPC chromatogram (I) is normalised to give a unit area curve. Heights h are measured at various regular intervals along the V_R-axis and each value is divided by the total area under the curve. The normalised ordinate values $h' = \frac{dw}{dV_R}$ are then plotted against V_R to give the normalised elution curve (II) so the value of $\frac{dw}{dV_R}$ can be generated for any value of V_R. Likewise the calibration curve (III) will give a range of values of dw/d(logM) for the same V_R values, and a corresponding range of values for M (IV). Then the values of dw/dM can be calculated from equation (2.19) and the normalised weight differential MWD curve can be constructed (V).

The $\bar{M}_W$ and $\bar{M}_N$ can be calculated from equations

$$\bar{M}_W = \frac{\Sigma n_i M_i^2}{\Sigma n_i M_i} = \frac{\Sigma W_i M_i}{\Sigma W_i} = \frac{\Sigma h_i' M_i}{\Sigma h_i'} \quad \ldots\ldots\ldots\ldots\ldots \quad (2.20)$$

$$\bar{M}_N = \frac{\Sigma n_i M_i}{\Sigma n_i} = \frac{\Sigma W_i}{\Sigma (W_i/M_i)} = \frac{\Sigma h_i'}{(\Sigma (h_i'/M_i)} \quad \ldots\ldots\ldots \quad (2.21)$$

Here W_i and n_i are the weight and number of molecules of molecular weight M_i respectively.

The method described above for determining the MWD curve and average molecular weights of samples assumes that no band-spreading occurs and all the molecules of a particular size elute together at the same elution volume, V_R. Many researchers prefer to create the most efficient GPC system that they can and then to assume no band spreading in the data treatment (139). For most purposes this approach is quite sufficient and

for broad samples ($\bar{M}_W/\bar{M}_N$>2) the error is small. However, all data can be improved by correction for band-spreading, especially that for narrow MWD samples, and there have been many attempts to correct for this inefficiency in the treatment of GPC data (110-112,121,129,137,139-155). The usual approach has been based on the expression

$$F(V_R) = \int_0^{+\infty} W(y) \cdot G(V_R - y) \cdot dy \quad \ldots\ldots\ldots\ldots \quad (2.22)$$

first suggested by Tung (155). This integral, which has the form of the convolution integral, relates the experimental chromatogram $F(V_R)$, the true chromatogram $W(y)$, and the instrumental spreading function $G(V_R-y)$ which describes the weight fraction of solute that should have been eluted at the retention volume y but actually dispersed and detected at the retention volume, V_R.

2.5 RETENTION MECHANISMS

Many theoretical models have been proposed for the size separation of polymers with a porous column packing in GPC (46-48,111,156-162). The theories are conveniently classified under two headings - equilibrium models and non-equilibrium models.

2.5.1 EQUILIBRIUM MODELS

Equilibrium models assume that the time taken by a molecule to get in and out of the pores of the stationary

phase is small compared to its residence time in the column, and so almost instantaneous equilibrium between the two phases is achieved. Then at equilibrium the concentration between the phases is related by a distribution coefficient, K_d:

$$C_s = K_d C_m \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.23)$$

where C_s and C_m are the concentrations in the stationary and mobile phase respectively. The general equation for the retention volume of a solute is:

$$V_R = V_o + K_d V_i \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.13)$$

This equation relates the distribution coefficient, K_d to an accessible pore volume, but it provides no information about the molecular size or the pore structure. This is why GPC is still only an empirical science.

Three forms of equilibrium models exist.

(a) Steric Exclusion Models

Steric exclusion may be considered as a mechanism where the chromatographed solute molecules are excluded from a part of the porous volume, due to the structure of the porous particles and the distribution of pore sizes. The size of the excluded volume increases with the molecular size of the solute. In attempts to elucidate the theory, various research workers (163-165) have assigned to the gel a structure and then attempted to correlate the exclusion of various molecules from

the structure with experimental data.

(b) Stochastic Models

This model was developed by Carmichael (166) in an attempt to generate a solute/time elution profile from GPC. It was assumed that the solute retention is described in terms of the rate constants for the transitions from the mobile phase to the stationary phase and vice-versa. This model applies only in dilute systems and where low flowrates are used.

(c) Thermodynamic Models

A more rigorous treatment than the models discussed in the previous section has been given by Casassa and Tagani (167,168) for linear and branched polymer chains in various simple pores. They consider that molecular configuration can be described more satisfactorily than pore geometry, and consequently a comparison between different molecules in simple pore structures is undertaken, the assumption being that similar behavioural differences will exist in more complex pore geometries.

Casassa concludes that the hydrodynamic radius is the determining parameter for the equilibrium distribution of a solute molecule between the mobile and the stationary phase.

Other thermodynamic models have been proposed by Giddings (169) and Van Kreveld (170).

2.5.2 MON-EQUILIBRIUM MODELS

In GPC where the mobility of the macromolecules in solution is very low, non-equilibrium is more likely to occur. Non-equilibrium models seek to introduce terms that account for departure from equilibrium partitioning under certain conditions.

Models based on mechanisms other than equilibrium partitioning are:

(a) Flow Model Separation

In this model it was assumed as isolated solute molecule undergoing Brownian motion inside a thin capillary, will have an average velocity greater than that of the solvent down the capillary 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 at the wall. As the large solute molecules experience only the higher velocities they migrate through the bed quicker than the small solute molecules. A good description of this separation mechanism was given by Dimarzio and Guttman (171-173).

(b) Diffusion-Controlled Models

The theory behind the diffusion-controlled model often required the solution to a partial differential equation which has complex initial and boundary

conditions such that no closed-form general solution can be obtained.

Yau and Malone (174) 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 operate in GPC separations and that a theoretical model including both the mechanisms was necessary.

This mechanism was also suggested by Hermans (175).

(c) Phenomenological Models

More recently suggestions have been made that the chromatographic conditions occurring in practical GPC 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. This class of model was proposed by Ouano and Baker (176) and is called the phenomenological model.

Kubin (177) 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.

2.6 ZONE BROADENING

Separation in chromatography is achieved through differences of relative migration rates of solutes,

which is governed by thermodynamic equilibrium. However, the effectiveness of any separation is also dependent on the degree of overlap of the solute zones, which is governed by the column dynamics. It is obviously desirable to keep zone dispersion to a minimum, as narrower solute zones lead to the achievement of better resolution. In common with most unit operations there are two theories that account for zone broadening, the plate theory and the rate theory.

2.6.1 PLATE THEORY

Martin and Synge (178) 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 plates where the mobile phase leaving was in equilibrium with stationary phase throughout the plate and by 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, was defined by:

$$H = \frac{d\sigma_z^2}{dZ} = \frac{L}{N} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.24)$$

where σ_z and Z are the length based second moment and the distance along the column; L is the length of the column and N is the number of plates in the column.

Although the plate height is a useful index having almost a universal acceptance for the comparison of the efficiencies of chromatographic columns, it fails to account for the kinematic processes occurring in the bed.

2.6.2 RATE THEORIES

Rate theories attempt to account for kinematic processes occurring in the spreading of a solute band. Lapidus and Amudson (179) developed a model incorporating mass transfer and longitudinal diffusion terms. This model was extended by Van Deemter et al (180) to include contributions from axial diffusion and finite rates of mass transfer. The form of their equation is as follows:

$$H = A + \frac{B}{V} + C'_m V + C'_s V \quad \ldots\ldots\ldots\ldots \quad (2.25)$$

where

A = eddy diffusion term

B = longitudinal diffusion term

C'_m, C'_s = resistance to mass tranfser in the mobile and stationary phase respectively

Equation (2.25) implies that the contributions to the plate height are independent of one another. Giddings (181) 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:

$$H = \frac{B}{V} + C_s'V + \frac{1}{\frac{1}{A} + \frac{1}{C_m'V}} \quad \ldots\ldots\ldots\ldots\ldots \quad (2.26)$$

One of the more rigorous theories, "the generalised non-equilibrium theory" developed by Giddings (181) 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. Slow mass transfer rates between the two phases are accountable for the non-equilibrium situation.

This theory has been applied by Giddings and Mallik (182) specifically to GPC where they obtained the following expression:

$$H = \frac{4D_m}{4Rv} + \frac{R(1-R)}{20}\,\frac{d_p^2 v}{D_m} + \{2g_1 d_p + \frac{D_m}{g_2 d_p^2 v}\}^{-1} \quad .. \quad (2.27)$$

Yau's (174) interpretation of GPC that the retardation of a solute molecule is the combination of steric exclusion and restricted diffusion has led Billmeyer, Johnson and Kelley (183-185) to propose 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 include a velocity profile effect. The resulting equation was:

$$H = \{2\gamma'\frac{D_m}{v} + 2\lambda_e d_p + \frac{2v'R_c^2}{\frac{\gamma' D_m}{v}+\lambda_e d_p}\} + P_c v \quad \ldots\ldots\ldots \quad (2.28)$$

Several other models have been proposed by other research workers and they are reviewed by England (46), Ellison (47), Williams (48) and Yau, Kirkland and Bly (111).

The plate height equation can be expressed in terms of dimensionless quantities, reduced plate height h, and reduced velocity u:

$$h = \frac{H}{d_p} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.29)$$

and

$$u = \frac{vd_p}{D_c} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.30)$$

2.6.3 FACTORS AFFECTING GPC

The experimental parameters that can affect peak dispersion in GPC are (1) column parameters: V_i/V_o, K_d', R_c and d_p; (2) kinetic factors: v and D_m; and (3) other experimental parameters: temperature, viscosity, concentration and dead volume. From the existing band broadening theories and the experimental evaluations on these theories, their effects in GPC are discussed below.

(a) Column Parameters

The column parameter V_i/V_o, the ratio of pore volume to void volume is basically a GPC retention parameter which is directly proportional to the porosity of the GPC packings. Although the plate height increases

with V_i/V_o (111), GPC column packings with large porosity are preferred because of their separating ability. Large V_i/V_o values mean more useful pore volume available for the molecular weight separation and better overall GPC resolution.

The quantity K_d, the GPC distribution coefficient, is also a retention parameter that is dependent on the size of the solute molecules relative to the packing pore size. Like V_i/V_o, K_d is usually optimised for molecular weight selectivity and GPC resolution rather than peak broadening.

It was theoretically and experimentally predicted (177) that as K_d decreases the plate height increases and only when K_d approaches zero the plate height reaches a maximum and then starts to decrease as K_d decreases further.

The radius of the column is usually selected according to the scale of separation required, and it was predicted that the plate height increases as the diameter of the column increases (47,183,185).

Packing particle diameter d_p is the most influential of all experimentally adjustable parameters affecting chromatographic band broadening. The column efficiency increases as the diameter of the packing material falls. It was verified experimentally that the plate height varies with the square of the particle diameter (186).

(b) Kinetic Factors

D_m, the solute diffusion coefficient in the mobile phase, is the main cause for the molecular weight dependence of the GPC plate height. As the solute size increases, with molecular weight, it causes D_m to decrease. And as the solute diffusion coefficient D_m decreases the plate height increases (111).

Another very important parameter that affects band broadening is the solvent velocity (v). The relationship between plate height and solvent velocity can be described by the van Deemter equation. This shows that the plate height increases as the flowrate increases at high velocities, but at low flowrates the plate height decreases with flowrate increasing.

(c) Other Factors

As a consequence of the low diffusion coefficients of sample molecules in the liquid mobile phase, considerable contribution to band spreading results from the dead volume in the injection valve, in the connections between the column and the detector and from the detector volume itself. The greater the volume of the connections and the detector cell in relation to the sample retention volume, the more troublesome is the band broadening (111).

The diffusion coefficient D_m increases in solvents of low viscosity and poor solvents to cause the plate height to decrease. However, solvent choices for many important polymers are often limited (111).

Relatively very few papers have considered the effect of temperature on GPC. It has been demonstrated experimentally that the number of theoretical plates for a column increases as the temperature increases (187). This effect must be due to the changes in diffusivity with temperature.

Also the elution volume of a solute molecule decreases as the temperature rises (187) due to the thermal expansion of molecules.

The dependence of the elution volume and the peak dispersion on the column concentration will be discussed in Section 2.6.4.

2.6.4 CONCENTRATION EFFECTS

Concentration effects, i.e. the dependence of the elution volume and of the width of the elution curve on concentration and overall amount of injected polymer solution in GPC have been observed by many workers. Waters (188) supposed the increase in elution volume with increasing concentration to be due to higher viscosity of injected solution. Boni et al (189) observed that the change in elution volume with a change in concentration was a linear function of the logarithm of molecular weight or of intrinsic viscosity. In the latter case they obtained a simple linear dependence for various polymers. The hypothesis of viscosity phenomena was supported by Goetze et al (190), who injected a polymer solution in a solvent whose relative

viscosity was higher than that of the solvent used as the mobile phase. According to them, viscosity phenomena cause a change in the elution volume, but not the whole change can be assigned to these phenomena. Moore (191) explained the viscosity phenomena as "viscous fingering". Ouano (192) stressed the effect of overloading of the column in the injection of solutions of mixtures of standard polymers having different molecular weights and high concentrations. Rudin (193) showed that the effective hydrodynamic volume of macromolecules in solution decreased with increasing concentration and that this effect must be taken into consideration in constructing a universal calibration graph. The hypothesis concerning the effect of concentration on the elution volume was supported also by other research workers (194,195) who observed that the effect of concentration on the elution volume in a thermodynamically poor solvent was weaker. Using the latter observations, it was suggested that the thermodynamic quality of the solvent should be estimated from concentration effects (196). It was also observed that the mutual arrangement of the individual columns affected the concentration dependence of the elution volume (197) and that with increasing flow of the solvent, the concentration dependence of the elution volume decreased (198). An increase in the width of the elution curve with increasing concentration and volume of the injected polymer solution was observed by several authors (199-201).

Janca and Pokorny (202) reported the concentration effects are even more pronounced with increasing column efficiency.

Hellsing (203) investigated the effect of concentration of the polymer present in the mobile phase on the elution volume of natural macromolecules. Batrick and Johnson (204) outlined the possibility of using differential GPC in the study of concentration effects, while Bakos et al (205) utilised the same method in the study of incompatibility of various polymers and concentration effects under such conditions. In some papers (206,207), concentration effects were interpreted as a consequence of the osmotic pressure at the boundary of the mobile and stationary phase, leading to shrinkage of the gel in the eluting zone (206) and/or redistribution of macromolecules of various sizes in the polydisperse sample (207). Cantow et al (208) observed exceptionally a stronger effect of concentration with samples having a broad distribution compared to those with a narrow distribution. Altgelt (209) assigns concentration effects at particularly high concentrations to secondary exclusion. Janca (210) and Holding (187) proposed that the change in elution volume following a change in the concentration of the injected polymer solution is due to many contributing processes. But the more significant are: the change in the effective size of permeating molecules and thus a change in the distribution coefficient according to the respective calibration

curve; viscosity phenomena in the interstitial volume; and finally secondary exclusion. The first two contributions lead to an increase of elution volume, while the last secondary exclusion causes a reduction in elution volumes with increasing concentration.

2.7 PRODUCTION SCALE CHROMATOGRAPHY

2.7.1 CLASSIFICATION OF EQUIPMENT

The utilisation of chromatography in production scale separations or fractionations is one of increasing importance in recent years.

For the last quarter of this century, several pilot plant and commercial chromatographs have been constructed. For the construction of these chromatographs two principle approaches have been used:

(I) direct scale-up of analytical equipment by automated repetitive operation of large diameter columns (batch chromatography)

(II) the development of novel designed equipment that permit continuous introduction of feed (continuous chromatography)

The continuous equipment can be further divided into:

(1) Cross-current systems

(2) Co-current systems

(3) Counter-current systems

Counter-current chromatography has been developed in three stages:

(a) Moving bed

(b) Moving column

(c) Simulated moving bed

2.7.2 BATCH CHROMATOGRAPHY

In the batch chromatography direct scale-up of the conventional analytical system has been attempted. The problems associated with scaling-up batch systems with particular reference to liquid-liquid chromatography are discussed by Ellison (47) and Charm et al (211). Essentially, scaling-up the analytical process has involved the use of larger diameter packed beds, some of which have incorporated baffling systems (212) to promote radial mixing and reduce longitudinal dispersion.

A small preparative system for liquid chromatography is the Prep LC/System 500 marketed by Water Assoc., Massachusetts, USA (213). It is a completely self contained unit, for the production of gram quantities of material in a laboratory, comprising variable solvent inputs, pump, sample introduction, columns, detection and recording systems. The columns are 5.7 cm diameter by 30 cm long prepacked with radially compressed silica cartridges.

The literature provides a number of references to the large scale application of liquid-liquid chromatography.

Pharmacia Fine Chemicals Co., Uppsala, Sweden commercially produce a piece of process equipment, "The Sephamatic Gel Filter", which is designed for high capacities and for use with semi-rigid gels. The applications of this system include industrial separation of the large and small molecular weight biological components from milk and whey (22,23); it has also been applied to the purification of proteins and enzymes (16). Samuelson et al (17) have reported using 20 cm diameter columns to produce protein enriched milk. Columns of 30 cm have been used by Janson (10) to separate rape seed proteins and to purify insulin. The industrial purification of insulin using batch chromatography is now applied by Vitrum A.B. (Stockholm, Sweden), using a battery of six 45 cm internal diameter by 15 cm deep columns packed with Sephadex G-50 (10). It is also reported that large scale chromatographic equipment are used in the sugar industry for the desugarisation of cane molasses (214,215).

Several workers (24,215) have examined the cost effectiveness of using liquid chromatography as a unit operation.

Conder (216) has published a review discussing the criteria that can be applied in the selection of large columns for repeated batch chromatography over more conventional separation techniques.

2.7.3 CONTINUOUS CHROMATOGRAPHY

2.7.3.1 CROSS-CURRENT SYSTEMS

Cross-current flow processes involve the movement of the chromatographic bed, laterally to the movement, within the bed, of the mobile phase. The development of this type of moving bed system has taken two main forms. In one case the development has been concerned with helical flow through annular columns, or tube bundles arranged in an annular shape, and in the second case with radial flow across annular columns or between parallel discs.

Several chromatographic researchers built cross-current units based on the principle of helical flow, where the rotating annulus has been constructed in the form of a packed bed (217), and a series of packed columns arranged in a circle (218-220). Fox (218-220) and Dunhill and Lilley (8) have reported the use of such a device for GPC separations. The principle of radial flow has been used by Sussman and co-worker (221-222) to construct a chromatographic system where the stationary phase is coated on to two parallel rotating discs.

The main advantage of the cross-current systems compared to other forms of continuous chromatographic equipment is the ability to continuously resolve a multicomponent mixture in a single stage operation. However, the degree of precision in the construction of

this type of equipment is likely to restrict the large scale uses of the proposed chromatographs to laboratory scale applications.

2.7.3.2 CO-CURRENT SYSTEMS

In recent years several cyclic separation processes have been developed that operate under variable conditions in order to enhance the separation. Process variables such as feed flow, temperature, pH and pressure have been varied (223-226). However, extensive development work is required before any of these schemes could be applied to an industrial use.

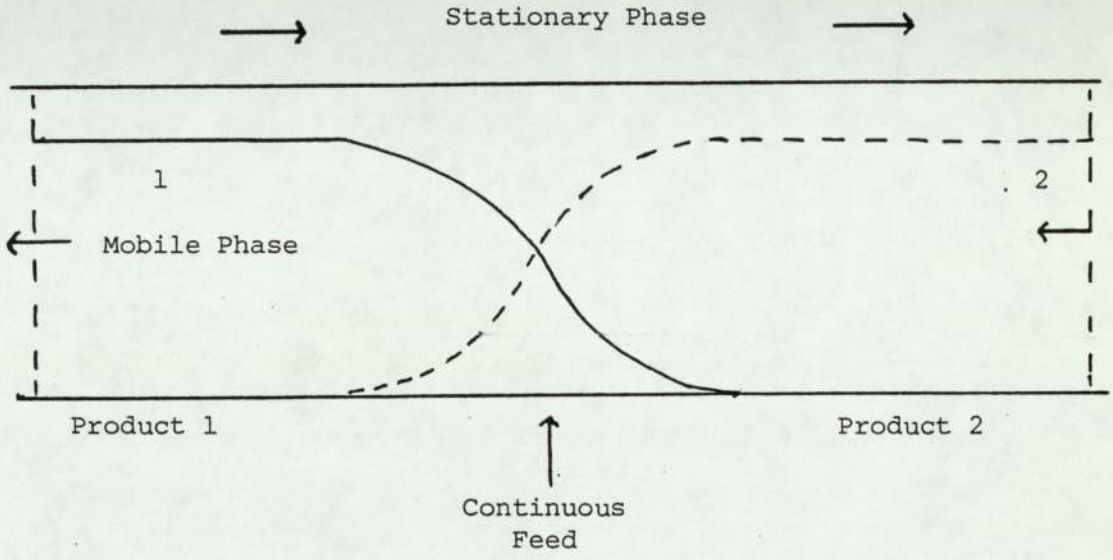
2.7.3.3 COUNTER-CURRENT SYSTEMS

The counter-current movement of the stationary phase and mobile phase is perhaps the most obvious method of obtaining continuous operation of a chromatographic column (Fig.2.10).

(a) Moving Packing

In this case the chromatographic packing flows under gravity counter-current to a stream of inert mobile phase in a vertical column. When the feed mixture is pumped into the centre of the column, the fraction having greater affinity for the mobile phase is carried upward, while the fraction that has higher affinity for the stationary phase is carried down

Fig. 2.10 Binary Mixture Concentration Profile, Counter-Current Flow Process



Component 1 = Least Retarded Component

Component 2 = More Retarded Component

$(K_2 > K_1)$

the column where it is purged from the packing.

Typical small scale moving bed chromatographs have been reported by Barker and others (227,228).

An industrial scale moving bed chromatograph has been used for the recovery of ethylene from a mixture of hydrocarbon gases.

The future of the moving-bed chromatographic column is thought to be limited because of the following inherent disadvantages:-

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

(ii) Attrition of the packing due to movement.

(iii) Unevenly packed columns are resulting in low efficiencies.

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

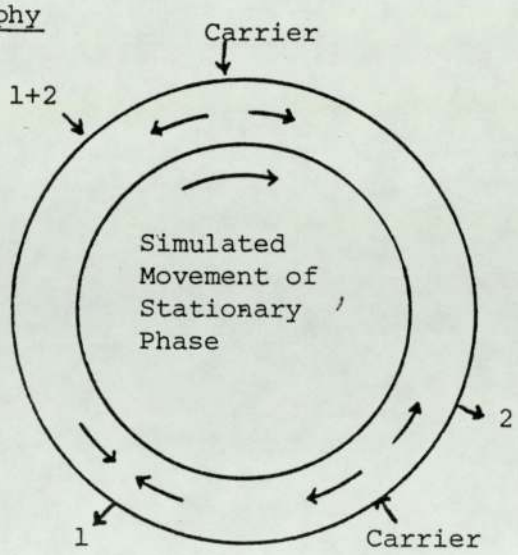
(b) Moving Column

To overcome the above problems various designs of equipment have been proposed based on the rotation of a closed circular column, packed with chromatographic packing and rotated past fixed inlet and outlet ports. The object was to make the packing and its metal surround move as one unit, thus avoiding the attrition problem and the necessity of providing air lifts to carry the solids back to the top of the column.

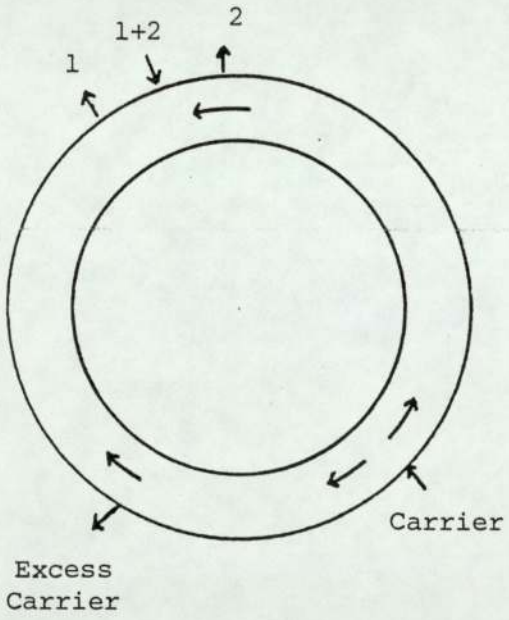
Three basic schemes have been proposed (229-232) and are illustrated diagramatically in FIg.2.11. The schemes proposed by Pichler Glasser and Luft require

Fig. 2.11 Proposed Schemes for Moving Column Counter-Current Chromatography

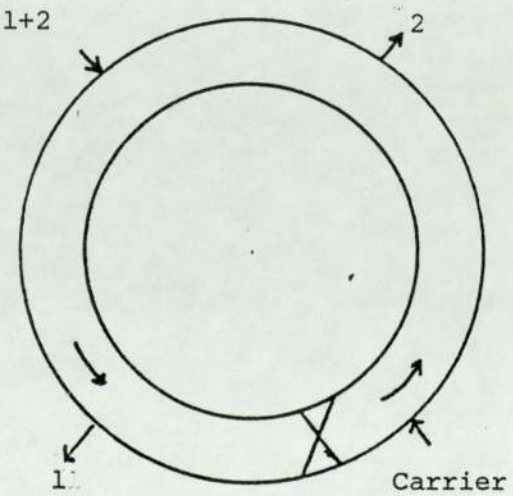
(a) Pichler Glasser



(b) Luft



(c) Barker



that the mobile phase rates 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 of it passed counter-currently against the rotation of the column. The inclusion by Barker of a cam-operated valve, between the mobile phase inlet port and the product 1 outlet port, insured that the mobile phase flow was unidirectional. Further developments of the design were reported (7,48,233).

The feasibility of large scale production equipment with moving columns is thought unlikely and that the main application of moving column system would be in laboratory scale production of fine chemicals.

(c) Simulated Moving Bed

To avoid the problems of moving bed designs and the limited size of the moving columns, Universal Oil Products, USA, developed a simulated moving bed system, known as the Sorbex Technique (234,235).

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.

The Sorbex process has a wide use of applications in oil industry in the separation of isomers (234-236).

Szepesy et al (237) constructed a chromatographic system similar to Sorbex process. The equipment consists of twelve columns connected to each other by means of a rotary valve. Rotation of the valve alters the inlet and outlet ports in such a manner that the counter-current bed movement is simulated.

Barker and co-workers (46,47,238) have also developed a simulated bed chromatograph based on 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.

Of all the continuous chromatographic designs, the simulated moving bed process has the most industrial interest.

Reviews covering all the designs of large scale chromatographs in detail have been presented by Barker (239) and Williams (48).

2.8 ULTRAFILTRATION (UF)

2.8.1 INTRODUCTION

Ultrafiltration was essentially a laboratory curiosity before 1960 but recently it has been rapidly gaining prominence as a practical process for the concentration and purification of macromolecular and colloidal species in solution. Increased interest in

the process has been closely tied to the development of finely produced, high flux membranes capable of distinguishing among molecular species in a 1 nm to 10 μm size range and of equipment capabale of efficiently utilizing the properties of these membranes. Almost every polymeric material can be used for the construction of membranes.

Theoretically, and now in practice, ultrafiltration offers an attractive alternative to a number of unit operations in the food processing, chemical processing, pharmaceutical and medical industries for the concentration, purification and sterilisation of macromolecular and colloidal solutions on both laboratory and industrial scales.

The initial applications for ultrafiltration membranes were in the experimental, clinical and biological laboratories, with emphasis on protein concentration, purification and fractionation on a small scale. The success of the process in replacing conventional precipitation, dialysis and GPC procedures for many laboratory experiments has been well documented (240-242).

One of the major applications of interest on the industrial scale is the replacement of conventional heat drying and even vacuum evaporation by UF. The UF process is athermal and permits removal of up to 90% of the water at ambient temperature avoiding thermal and oxidative degradation of the products. Also the absence of phase change results in lower energy costs, and the

expensive heat generating or heat transfer equipments required for overcoming the latent heat in evaporation or freezing are not required in UF.

2.8.2 THE PRINCIPLE OF ULTRAFILTRATION

The basic principle of operation of UF is simple, as illustrated in Fig. 2.12. Flowing by the membrane is a solution containing two solutes: one of the molecular size too large to pass through the pores of membrane, and the other small enough to pass through. A hydrostatic pressure is applied to the upstream side of the supported membrane, and solvent plus small-molecule solutes pass through the membrane, while the large-molecule solutes are retained (rejected) by the membrane. A fluid concentrated in the retained solute is collected from the upstream side of the membrane, and a solution of small-molecule solutes is collected from the downstream side. Where only a single solute is present and is rejected by the membrane, the liquid collected downstream is pure solvent.

Retained particle size is one characteristic distinguishing UF from other filtration processes. Viewed on a spectrum of membrane separation processes, UF is only one of a series of membrane methods that can be used for macromolecular separations. Reverse osmosis (RO) is used to separate smaller molecules. Microporous filters are capable of virous and bacteria retention and finally conventional industrial

Fig. 2.12 Schematic Diagram of Membrane UF Process

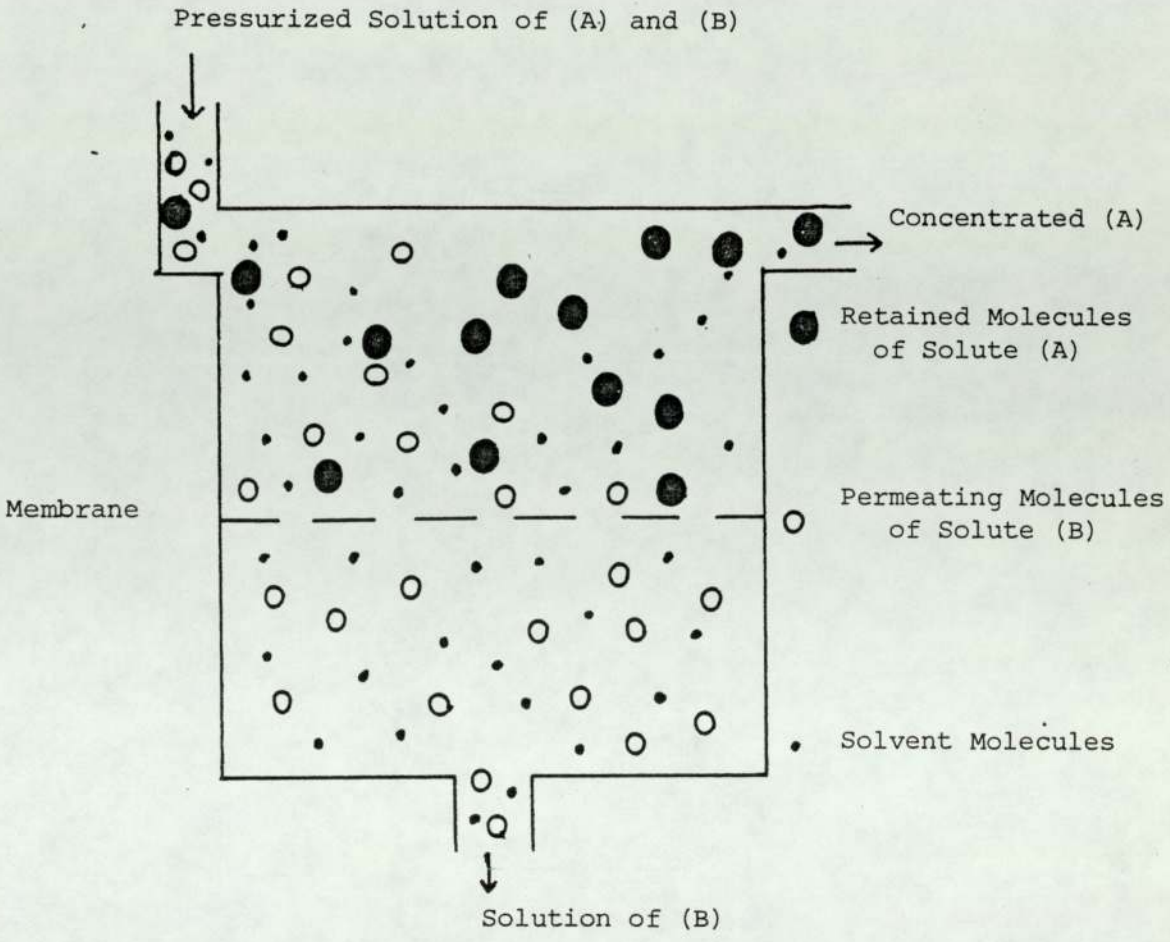
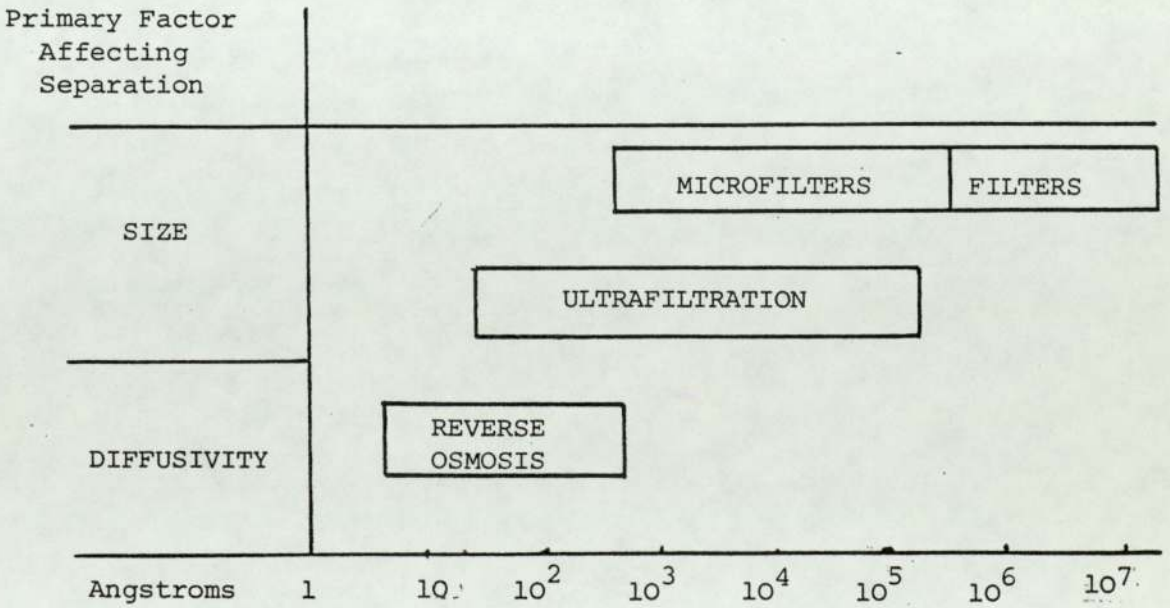


Fig. 2.13 Useful Ranges of Various Membrane Processes



filters retain normal particulate materials (see Fig.2.13).

2.8.3 IDEAL SYSTEMS

2.8.3.1 RETENTIVITY

The primary characteristics of any membrane are its pore size distribution which defines selectivity, and solvent flow, which depicts performance.

Conventionally, performance is derived from the rejection coefficient (σ) obtained from examination of a series of compounds of progressively increasing molecular weight. During evaluation, the ultrafiltration unit is charged with a test solution of a well-characterized solute and pressurized. After some volume reduction, the content of the unit and the ultrafiltrate are analysed. Since solute permeation is an exponential function of the decreasing volume of solution in the unit (31), expressions for the rejection, derived from either retentate or ultrafiltrate content are:

$$\sigma_{ret} = \frac{\ln(C_t/C_o)}{\ln(V_o/V_t)} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.31)$$

$$\sigma_{uf} = \{\frac{V_o}{V_f} - \frac{C_f}{C_o}(\frac{V_o}{V_f} - 1)\}/\ln(\frac{V_o}{V_f}) \quad \ldots\ldots \quad (2.32)$$

where C_o and V_o are the initial concentration and volume, respectively, C_t and V_t are the final concentration

and volume of the retentate, and C_f and V_f are the final concentration and volume of the ultrafiltrate.

Dilute solutions (<0.25%) must be employed to minimise the effects of solute polarization (to be discussed later). Typical rejection (retention) curves obtained from the above evaluation are shown in Fig.2.14.

A further operational definition of solute permeation often used refers to membranes as possessing either 'sharp' or 'diffuse' cut-off levels. Fig.2.14 also illustrates this aspect of performance plotted with the usual rejection versus log molecular weight convention. Theoretically, an ideal membrane with narrow distribution of pore diameters would display the more vertical inflection (sharp).

In general, membranes tend to display more 'diffuse' cut-offs under actual operating conditions than when used with very dilute solutions. This is due to the phenomenon of 'concentration polarization', which is an accumulation of macromolecules at the membrane interface. It restricts solvent flow and may impair solute permeation.

2.8.3.2 FLOW AND PERMEATION IN IDEAL SYSTEMS

Consider a system operating at steady-state under isothermal conditions, with a semi-permeable membrane separating two fluid compartments, such as that illustrated in Fig.2.15. An aqueous solution containing

Fig. 2.14 Solute Retentivity by 'Sharp' and 'Diffuse' Cut-Off UF Membranes

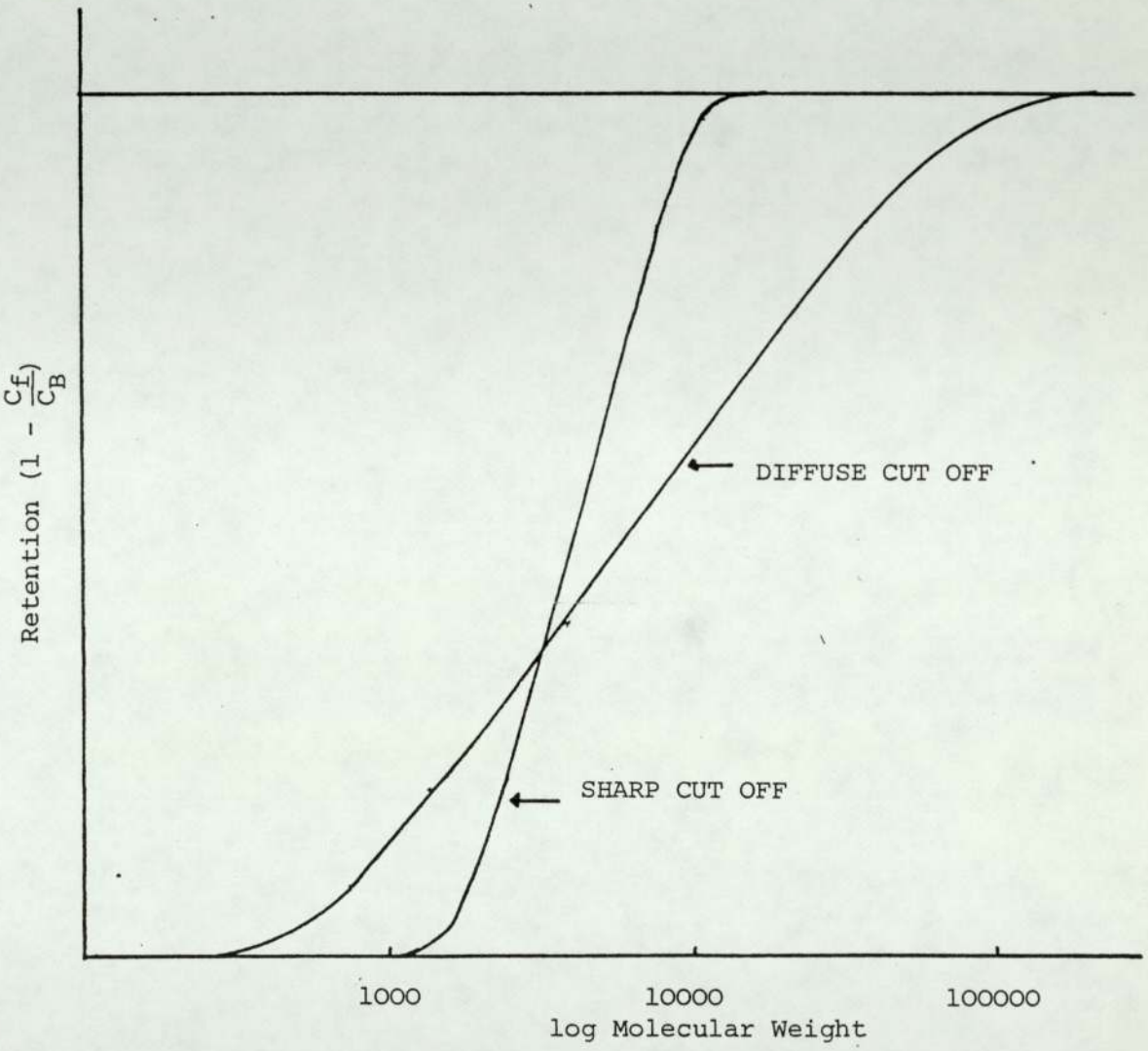
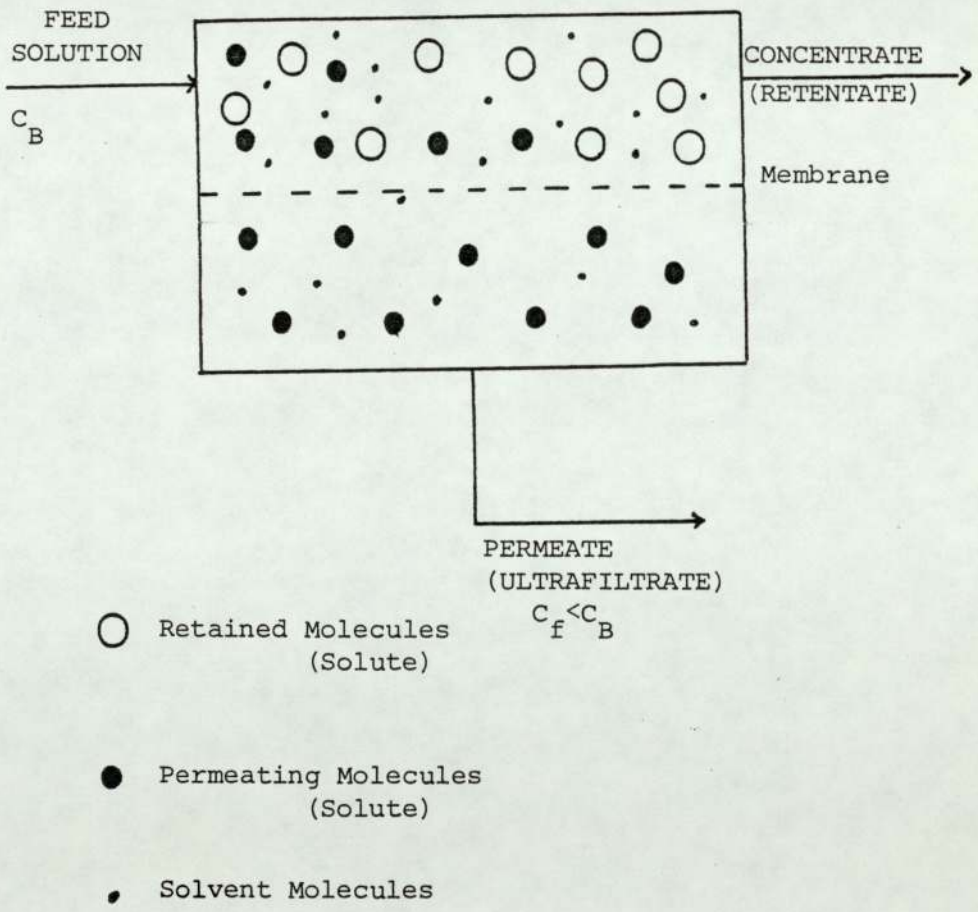


Fig. 2.15 Schematic Flow Diagram of UF Operation



a single solute concentration C_B and elevated pressure P_B flows continuously by the upstream surface of the membrane. The membrane is permeable to the solvent but only partially permeable to the solute, so that the solute concentration C_f in the permeate is less than C_B. Permeate flowing at a membrane flux J_1 is continuously removed from the downstream compartment at a lower pressure P_f. The flow of solution across the upstream face of the membrane is high relative to the permeation flux J_1, so that the concentration change across the upstream compartment is negligible. Under these conditions, the flux J, can be described by the relationship:

$$J_1 = K_m\{(P_B-P_f) - (\Pi_B-\Pi_f)\} = K_m\{\Delta P-\Delta\Pi\} \quad .... \quad (2.33)$$

where Π_B and Π_f are the solution osmotic pressures corresponding to the concentrations C_B and C_f, respectively, and K_m is a constant over a wide range of pressures and concentrations.

When a membrane is selective to large molecules only and freely transmits low molecular-weight solutes, the concentration of small molecules on both sides of the membrane will be equal, and the transmembrane osmotic pressure difference will be negligible relative to the applied pressure.

Therefore, for UF process equation (2.33) is:

$$J_1 = K_m\Delta P \quad ..... \quad (2.34)$$

and

$$J_2 = C_B(1-\sigma)J_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.35)$$

where J_2 is the solute flux, and $(1-\sigma)$ is the fraction of the solvent flux carried by pores large enough to pass the solute.

Since mass balance dictates that

$$J_2 = J_1 C_f \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.36)$$

then

$$\sigma = 1 - \frac{C_f}{C_B} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.37)$$

With systems of this type, flux is linearly related to hydraulic pressure gradient and the rejection coefficient is essentially constant and pressure independent. It should be noted, however, that with concentrated solutions, viscous drag can reduce flow in the pores which pass the solute and hence increase rejection.

2.8.4 CONCENTRATION POLARIZATION (NON-IDEAL SYSTEMS)

2.8.4.1 FLOW EFFECTS

The accumulation of gel-like layers of macrosolute at the membrane surface will substantially influence solvent throughput in UF systems. It is possible to calculate solvent flux on the basis of mass transfer of membrane-retained species back into the bulk solution if it is assumed that the gel layer has a fixed gel

concentration, but can vary in thickness or porosity (243). Flux is independent of driving force since the resistance to flow of the gel layer will adjust itself until convective transport (J_1C) by the solvent is equal to the back-diffusive transport $D_s(dc/dx)$. At a steady state

$$J_1C = D_s(dc/dx) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.38)$$

where c is the local concentration of membrane-retained solutes and dc/dx is the concentration gradient.

Assuming a highly selective membrane, i.e. where the permeate concentration of solute c_f is negligible, equation (2.38) can be integrated across the boundary layer to give

$$J_1 = D_s \ln(C_g/C_B)/\delta \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.39)$$

where δ is the thickness of the boundary layer. To sustain or improve flux, fluid management techniques must be directed towards decreasing the boundary layer thickness and thus increasing the mass transfer coefficient which is given by

$$K = D_s/\delta \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.40)$$

Hence

$$J_1 = K \ln(C_g/C_B) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.41)$$

The gel layer does not accumulate as a filter cake would because of mass transfer of solute back from the gel into the bulk of fluid stream. After operating at steady state at a given pressure, if the

pressure is increased, a transient increase in flux is observed which ultimately decreases to the same value observed at the prior steady state. During that transient period, solute is brought to the gel layer at a more rapid rate because of the pressure increase. At the same time solute deposition in the gel increases until the hydraulic resistance of the gel slows solution transport just to the point where the solute mass balance in equation (2.38) is satisfied.

In the UF of some solutions, such as certain protein solutions, a significant osmotic pressure can prevail at the membrane surface when concentration polarization is high. Under these circumstances, even without gel formation, there is a significant decrease in flux relative to that observed with water at the same pressure (244).

For any system where c_f is not negligible equation (2.41) becomes

$$J_1 = K \ln\left(\frac{C_g - C_f}{C_B - C_f}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.42)$$

Equation (2.42) implies that flux will go to zero when the bulk concentration reaches the value of C_g. Evidence at these high concentrations are scanty, but in some cases flux appears to approach zero assymptotically as concentrations in excess of C_g are reached.

2.8.4.2 SOLUTE PERMEATION EFFECTS

With respect to solute permeation in idealised systems, equation (2.37) shows that σ is given by $(C_B-C_f)/C_B$ only in cases where the concentration polarization modulus is insignificant and σ is a function solely of the membrane properties. However, with the interposition of a secondary membrane of accumulated macrosolute considerable alteration in permeation will be observed when examining membranes that initially display some degree of permeation for the solute. Consider a non-aggregating system in which the gel layer is mobile and finite rejection is caused by a distribution of pore size within the membrane. If fraction a of the total solvent passes through the solute-rejecting pores while a fraction (1-a) carries solute at concentration C_B through the membrane, the rejection coefficient at infinite dilution is given by

$$\sigma = 1 - \frac{(1-a)C_B}{C_B} = a \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.43)$$

and the polarization modulus C_W/C_B is unity. As the concentration of the solute at the membrane surface, C_W, increases due to polarization, the solute carried through the membrane is at a higher concentration, C_{pore}. In fact, $C_W > C_{pore} > C_B$ and C_{pore} increases with C_W. The rejection coefficient is then expressed by

$$\sigma = 1 - \frac{(1-a)C_{pore}}{C_B} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.44)$$

Thus σ decreases as C_W, and therefore the concentration polarization increases (31).

Fig. 2.16 Concentration Profile in the Boundary Layer for Well-Developed Turbulent Flow

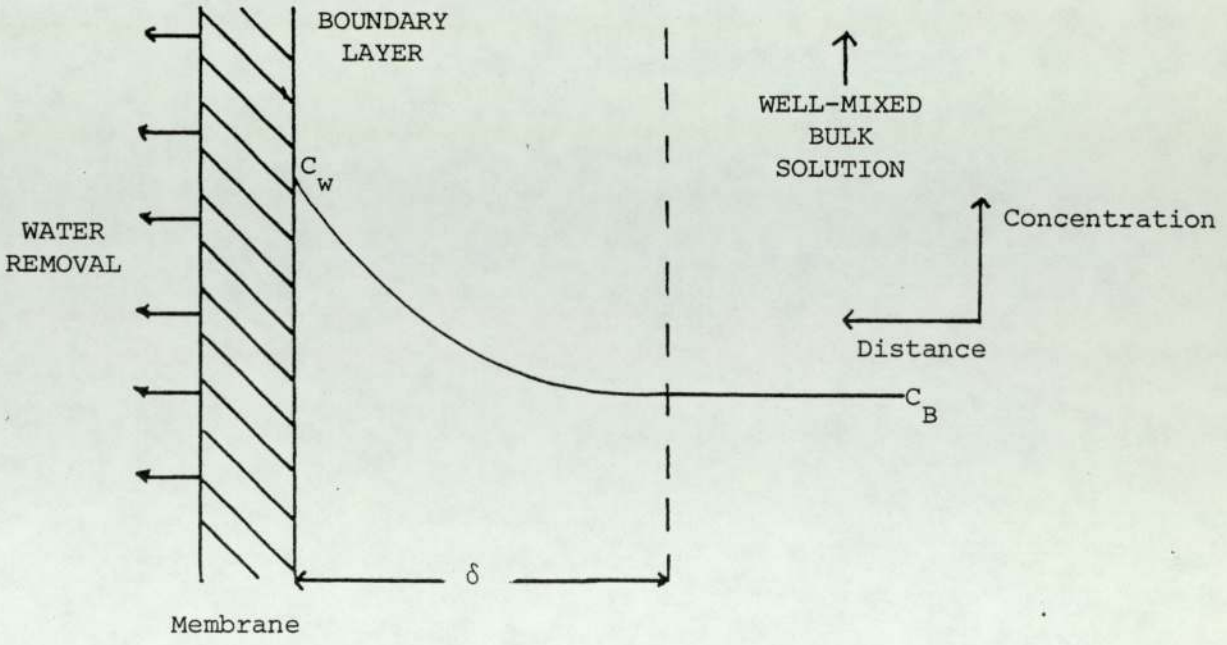
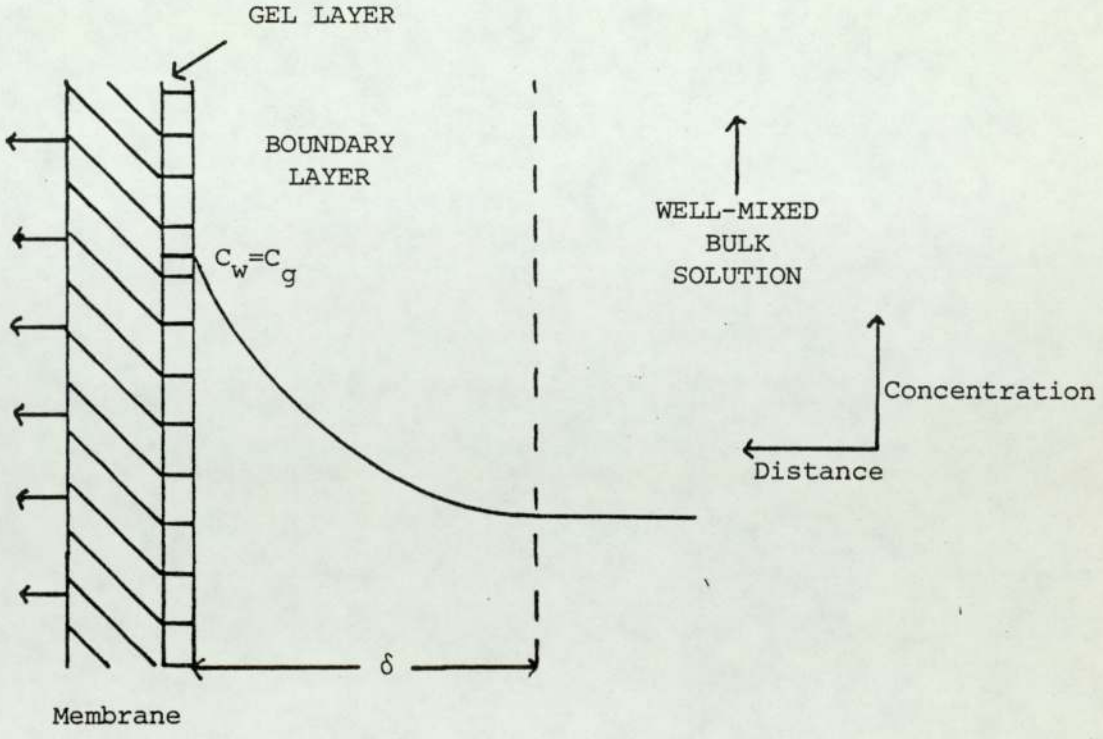


Fig. 2.17 Gel Formation in Concentration Polarization



2.8.4.3 MATHEMATICAL MODELLING FOR POLARIZATION CONTROL

The presence of a fluid-phase mass-transfer coefficient, K, in the flux equation (2.42) indicates that control of the fluid properties and flow conditions has an important effect in UF system design. Known mass transfer relationship can be used at least for purposes of data correlation. For example in turbulent flow the mass transfer coefficient can be related to fluid properties and conditions by equation (245)

$$\frac{Kd}{D_s} = (\text{constant})_1 (Sc)^{0.3} (Re)^{0.9} \quad \ldots\ldots \quad (2.45)$$

where d is the flow channel height, Sc the Schmidt number, and Re the Reynolds number. If UF flux is directly proportional to the mass transfer coefficient for a fixed polarization ratio $(C_g - C_f / C_B - C_f)$ the relationship between flux and Reynolds number should also conform to this same functionality (246).

In laminar flow, K can be related to flow condition as follows (247)

$$\frac{Kd}{D_s} = (\text{constant})_2 (Re.Sc.\frac{d}{L})^{1/3} \quad \ldots\ldots\ldots \quad (2.46)$$

where L is the channel length.

Combining equation (2.42) and (2.46) then,

$$J_1 = (\text{constant}) \{(\dot{\gamma})_{y=o} \frac{D_s^2}{L}\}^{1/3} \ln(\frac{C_g - C_f}{C_B - C_f}) \quad \ldots \quad (2.47)$$

where $(\dot{\gamma})_{y=o}$ is the shear rate at membrane surface (sec^{-1}), (y is the distance from the membrane).

From this relationship it would be expected that the water flux would vary directly as the cube root of the wall shear rate and inversely as the cube root of the channel length, for fixed polarization ratio.

Thus, convection promotion devices placed in the flow channels lead to enhanced fluxes (248). The fluxes are also increased when high velocities are applied, and thin and short channels are used.

Similar relationships to equation (2.47) are available for turbulent flow of solutions in channels and stirred cells (243,249). As in the laminar flow cases, the relationships are based on conventional chemical engineering correlations for mass transfer from a wall of constant concentration. In the turbulent flow cases the relationships are based on empirical correlations rather than exact mathematical solutions, since the latter are not available.

2.8.5 ULTRAFILTRATION MODES

2.8.5.1 CONCENTRATION MODE

A UF system can be used to concentrate a macromolecular solution by removing solvent and some low molecular weight material. As the concentration of macromolecules in the feed/retentate solution increases its volume decreases (Fig.2.18).

2.8.5.2 DIAFILTRATION MODE

As the concentration polarization increases the membrane selectivity decreases. When microsolute removal and especially macrosolute fractionation is taking place it is necessary to have high membrane selectivity because of the difficulty of separation. Thus dilute solutions must be used all the time to achieve better fractionation. This is achieved by keeping the feed/retentate volume constant by replacing the amount of solvent (and solute) that passed through the membrane with fresh solvent (Fig.2.19).

2.8.5.3 DIALYSIS MODE

A UF system working in dialysis mode has the feed solution flowing across the membrane in the high pressure section and fresh solvent flowing across the other side of the membrane. Rapid removal of microsolutes from process fluids can be accomplished in this mode, because microsolutes are removed from the process solution in two ways: (1) microsolute diffuses into the dialysate liquid through the membrane walls; (2) liquid with microsolute is convectively transported through the membrane walls as ultrafiltrate (Fig.2.20).

FIG. 2.18 UF UNIT IN CONCENTRATION MODE OPERATION

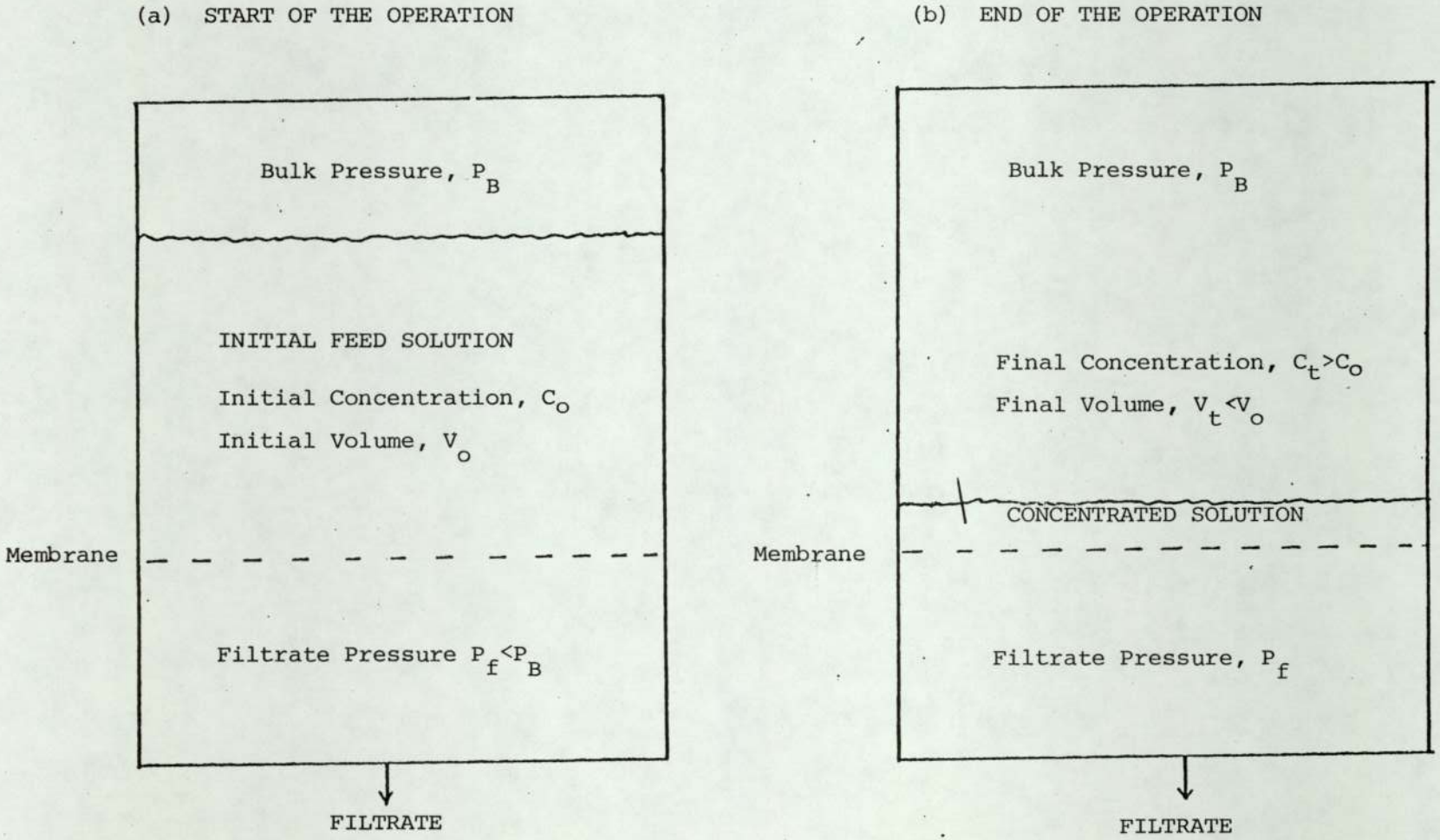


Fig. 2.19 UF Unit in Diafiltration Mode Operation

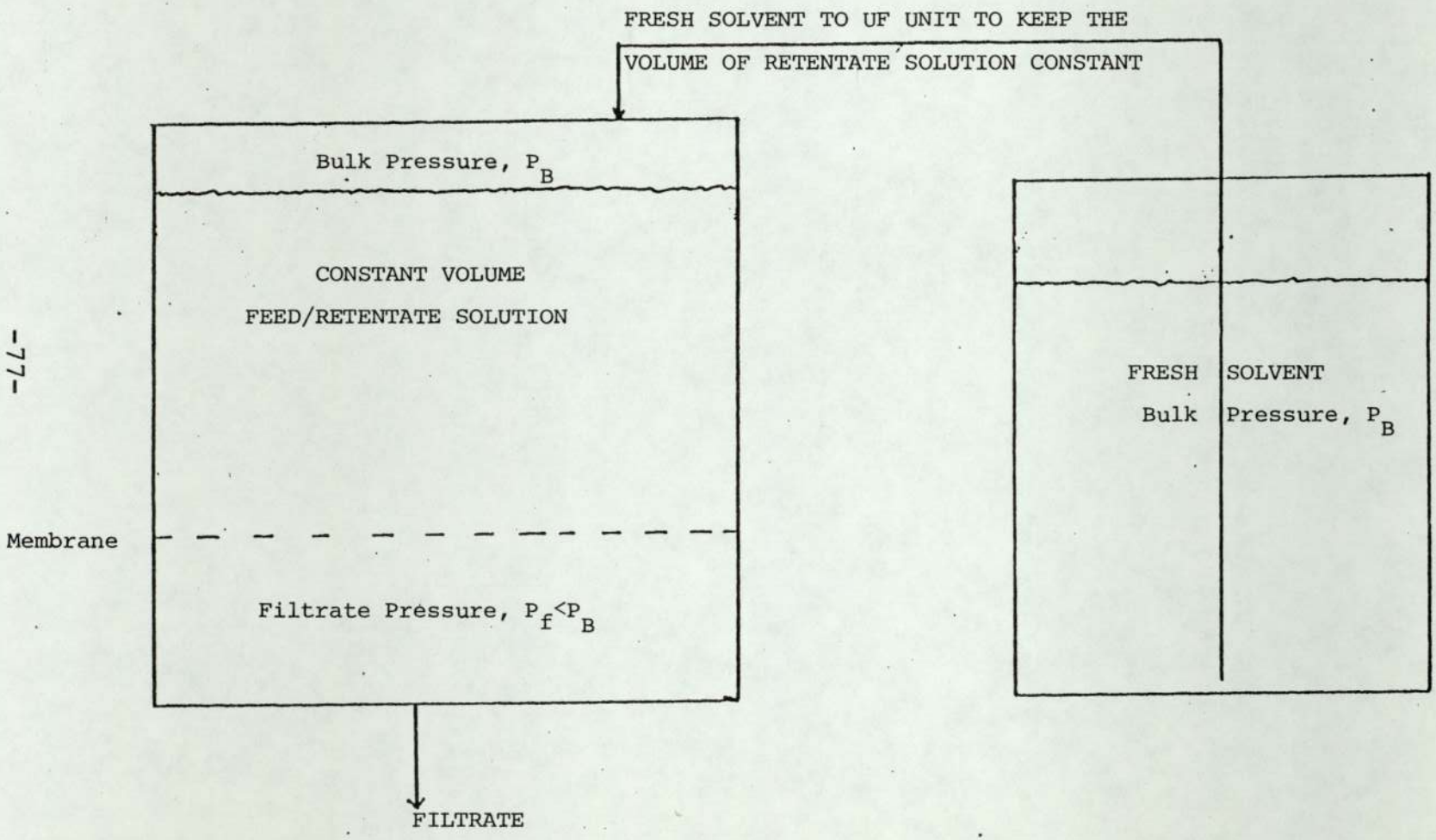
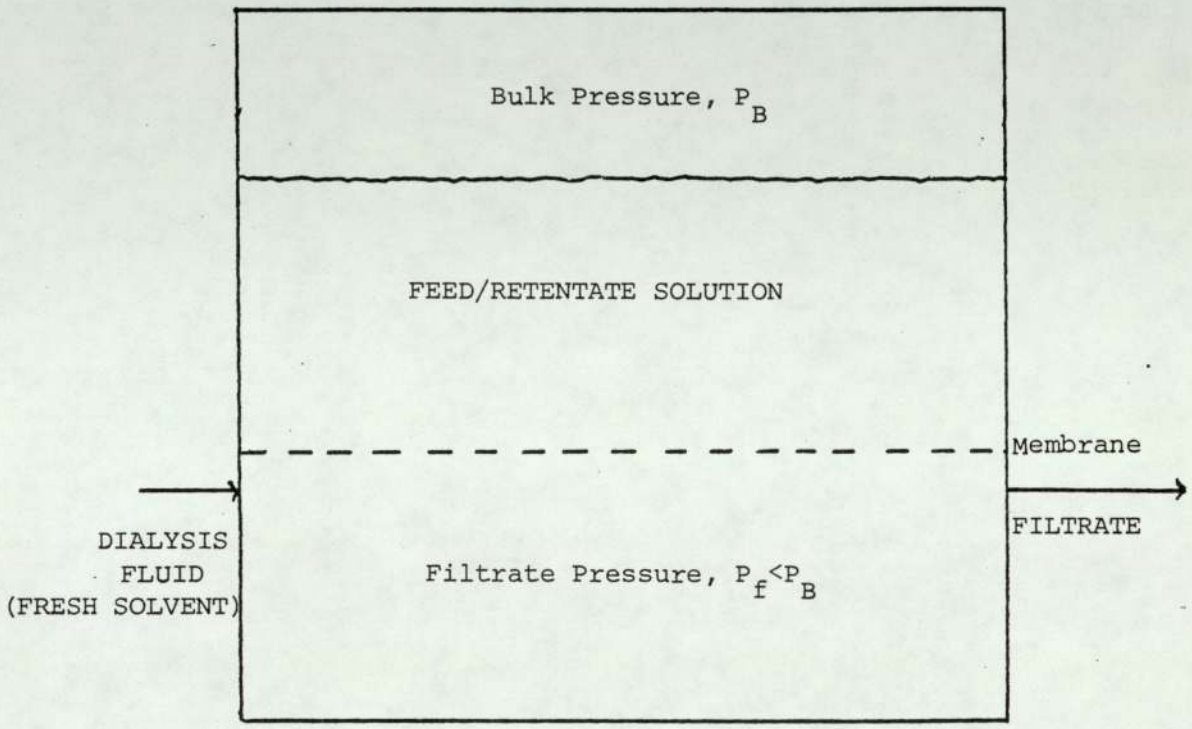


Fig. 2.20 UF Unit in Dialysis/Concentration Mode Operation



2.8.6 EQUIPMENT

The description of several laboratory and large scale equipments are available in the literature (39, 40,250-256). In this section only the general principles of the existing UF equipment are described.

Although laboratory scale equipment exist that do not try to reduce the concentration polarization effect, polarization control must be achieved to be able to evaluate the full potential of the process.

Polarization control can be achieved either by the laminar flow approach (243) or in membrane-coated porous tubes in turbulent regimes (257), or even by employing mechanical scrubbing to keep membranes clear (258). All have been employed in the design of UF equipment with varying degree of success.

Several laboratory equipment exist that use stirring to reduce the concentration polarization, but for commercial applications where the equipment needs to be compact and have high surface area of membrane, more sophisticated designs are used.

Initially plate and frame assemblies were the principal method for building up membrane area by using channels of restricted height along with pumps of sufficient output to maintain the shear requirements. A unique method, still keeping to thin channel concepts and allowing for more operational ease, was effected by casting the membrane on the inner surface of porous tubes (hollow fibres) with flow parallel to the membrane

along the channels of the tubes. A number of these tubes were incorporated into cartridges of fixed membrane area.

With the advent of fine hollow fibres, however, it would appear that a number of the criteria of large scale applications could be met by constructing systems utilizing such fibres. The ability to incorporate large amounts of membrane area into compact cartridge form, together with the shear induced by flow directed through the lumen should ensure high output sustained by good polarization control.

Thus hollow fibres systems are of more significant commercial interest than other UF systems.

2.8.7 APPLICATIONS

Ultrafiltration units are being used in three basic application modes: 1) to concentrate macromolecular components or to remove them from a solution, 2) to remove or exchange salts and other microsolutes, and 3) to resolve or fractionate complex mixtures.

The capability of UF devices for rapid concentration and purification by exclusion of macromolecules has led to such applications as the following:

1. Recovery of electro-deposition primer from rinse water in the motor industry.
2. Sewage treatment and recovery of process water for reuse.
3. Concentration and fractionation of cheese whey.

4. Concentration of egg albumin.
5. Recovery of meat proteins from animal blood and meat waste.
6. Albumin concentration and purification.
7. Harvesting of cells, removal of waste products and recovery of the products of fermentation.
8. Purification of iron dextran complex.
9. Preparation of ultrapure water.
10. UF utilization in the kidney machine.

Several of the UF applications are reviewed in detail in several papers and books (28-43,246,259).

3.0 ANALYTICAL GPC

3.0 ANALYTICAL GPC

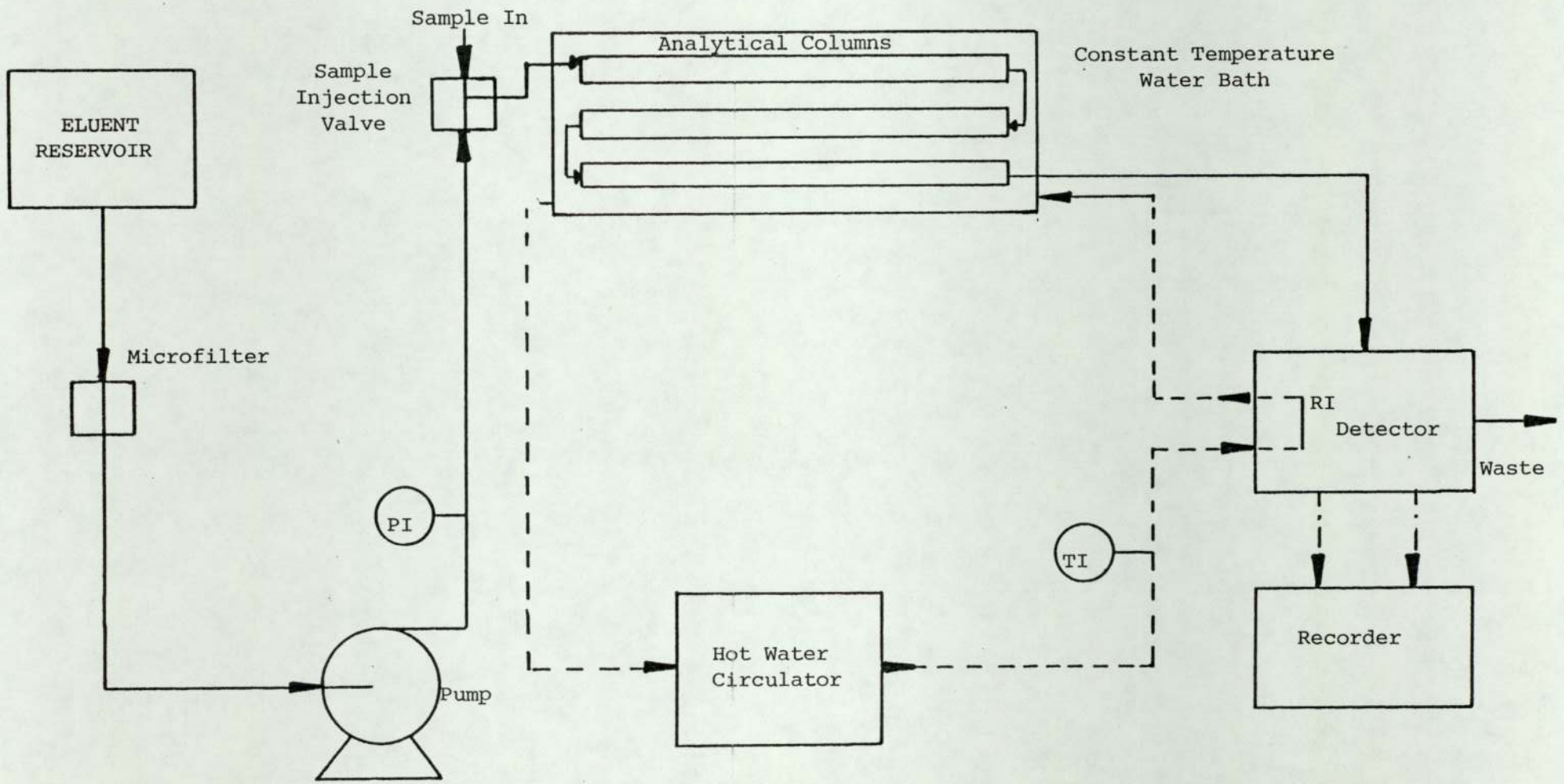
3.1 ANALYTICAL EQUIPMENT

A simple chromatographic system (Fig.3.1) was used for the analysis of the products, consisting of an eluent reservoir, microfilter, pump, a sample introduction valve, column(s), hot water circulator, water bath, detector and recorder.

The eluent used was a 0.02% w/v solution of sodium azide in distilled water. The sodium azide solution was used to prevent bacterial growth in the columns and to suppress the ionic exclusion peaks (260). The samples to be injected were made up to a concentration of 0.02% w/v sodium azide to prevent the presence of any negative (absence of azide) peaks on the chromatogram. The eluent was pumped with a positive displacement pump (Series II, Metering Pumps Ltd., London, U.K.). Samples were injected using a sample injection valve (type 30-501) supplied by Spectroscopic Accessory Co., and fitted with a constant volume (100 μm) sample loop. All the samples were filtered before being injected using a syringe filter (Millipore, London) that was fitted with a 0.46 μm disposable filter.

The fractionating column(s) used will be described in detail in Section 3.2. The column(s) were enclosed in a larger glass column (7.5 cm ID x 70 cm long, Corning Glass) filled with water and heated by passing it through a hot water circulator (C-400, Tecam, Cambridge),

Fig. 3.1 Schematic Diagram of the Analytical System



so that the operating temperature of the columns was kept constant. The water bath also allowed higher operating temperatures for the analytical system, creating better resolution and lower pressure drop across the column(s).

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 was registered on a flat-bed, two-pen, recorder (Venture Servoscribe, type 2, Smiths Ltd).

The resulting chromatograms were measured manually and then were treated to give the molecular weight distribution of the injected sample.

3.2 FRACTIONATING COLUMNS

Four types of fractionating columns were used during the research project.

DuPont Zorbax PSM Bimodal Columns

A pair of pre-packed DuPont Zorbax PSM columns, PSM60 and PSM1000 were arranged in series with the PSM60 column first. The initial characteristics of this pair of columns were very impressive, having an efficiency of 18400 plates and an analysis time of 12 minutes for glucose-dextran solutions. A flow-rate of 1 cm^3/min was maintained throughout the life of this pair of columns.

Unfortunately there was a marked drop in efficiency

with the application of a number of samples of Dextran 2000 (Pharmacia, Sweden), used to determine the void volume. But it was not the reduced efficiency which caused problems but the non-Gaussian peak shape. When the inlet ends of the columns were opened up it was found that a definite void of about 2 mm existed on the PSM60 column and a slight settlement of the PSM1000 also appeared to have taken place. The final efficiency of the columns was 5000 plates and the peak shape was very poor.

A second pair of Zorbax PSM Bimodal columns was brought into use, but in this case a precolumn packed with Lichroprep Si 60 was added to the system, before the injection valve, to reduce the silica dissolution from the DuPont columns. The initial efficiency of this pair of columns was 21,200 plates.

The fractionating range of both pairs of columns was ideal for the analysis of clinical dextran fractions with no noticeable lower limit and an exclusion limit of about 10^6 daltons.

Due to ionic exclusion peaks initially 0.02% w/v KH_2PO_4 solution was used as eluent, but after consulting a representative of DuPont, it was replaced by 0.02% w/v potassium hydrogen phthalate solution. After a sudden drop in efficiency the inlet ends of this pair of columns were opened up and it was found that there had been a considerable amount of settling with 2 mm and 1 mm voids on the PSM60 and PSM1000 columns respectively. The columns were "topped-up"

using the Zorbax BP-SIL material and the efficiency increased but the peak shape was poor, although there was no gap at the top of the columns. This was probably due to adsorption effects (87,88,260,261) of dextrans and glucose on the surface of the silica packing.

The column ends were gradually blocked after some days of use and also when the columns were left standing with water for a few days several shoulders appeared on the peak of the injected glucose sample. But it was found that if the columns were flushed with and stored in acetone after use, their lifetime was increased considerably.

The elution volumes of Dextran 2000 and glucose were recorded regularly to allow the use of the Wheaton-Bauman distribution coefficient (K_d), instead of elution volumes, for the system calibration. No change in the calibration of these columns was observed.

To prevent the drop in the efficiency of the columns and the poor shape of the peaks due to the packing dissolution, a pair of columns was designed and built using a moving piston at the inlet end to keep the packed bed always under compression as it dissolves.

Also a high pressure (480 atm) packing system was built to repack the damaged DuPont columns. However, neither the columns with the compression pistons nor the packing system were ever used, because most of the problems encountered with the DuPont columns were

eliminated later using an alternative packing.

Porasil C

Porasil is the trade name under which Spherosil was marketed by Waters Assoc., Milford, USA. The XOB-075 and the C grades are of equivalent porosity.

Because of the dissolution of the ultrafine silica particles in the DuPont columns larger size particles were to be tried for dextran analysis. Porasil, grade C, 35-75 µm, particles were packed in a 4 mm ID x 1000 mm long glass column. The eluent flowrate used was 0.25 cm^3/min and the analysis time was 42 min. The column efficiency was approximately 1000 plates.

The Porasil C column was in use for three weeks without any sign of deterioration. However, this column did not have sufficient resolution and it was observed that there was an extension of the dextran peak beyond the total liquid volume of the column.

TSK-Gel Toyopearl

TSK-gel Toyopearl is a new semi-rigid packing material for GPC. It is produced by the polymerisation of hydrophilic vinyl monomers and is marketed by Toyo Soda Manufacturing Co. Ltd., Tokyo, Japan.

Initially a 4mm ID x 1000 mm glass column was packed with Toyopearl HW 55s grade, 20-40 µm particle size. This column had a satisfactory efficiency of 5500 plates for glucose and a good resolution for the dextran used, but it had a low exclusion limit (262).

Then the same glass column was packed with Toyopearl HW 65s grade, 20-40 μm particle size. Although this column had a high enough exclusion limit, it had a low efficiency of 575 plates and poor resolution for dextran, i.e. it did not separate the chromatographic peaks of a dextran of $\bar{M}_w$ = 70000 daltons and glucose (180 daltons).

Since both grades of packing have advantages and disadvantages it was thought desirable to combine the high efficiency and good resolution of the HW55s packing with the high exclusion limit of the HW65s packing.

Several mixtures of the two packings were tried but only the mixture that contained 43.7% v/v of HW55s and 56.3% v/v of HW65s was found suitable for dextran analysis. This mixture had a suitable exclusion limit for dextrans; it also had good resolution and a satisfactory efficiency of 1500 plates. The only disadvantage with this packing was that the flowrates were very low (3-6 cm^3/hr) because of the working pressure of the packing (<7 kg/cm^2), and therefore the analysis time was long (2-3 hours).

TSK-Gel PW-Type

TSK-gel PW-type is a high performance polymeric gel marketed by Toyo Soda Manufacturing Co. Ltd., Tokyo, Japan.

The system used for dextran analysis consisted of two G5000 PW columns and one G3000 PW column. The columns were connected in series in sequence of

descending pore size. The operation of these columns was at 35°C.

The columns were in use for almost a year without any signs of deterioration. The flowrate through the columns was 1.1 cm^3/min, the analysis time for glucose-dextran solutions 50 minutes and the efficiency for ethylene glycol solution, 31500 plates, i.e. 17500 plates/metre.

Fisons Ltd., Pharmaceutical Division, Holmes Chapel, Cheshire (263) own another two sets of these columns and one of them has been used to control the quality of the clinical dextran for more than two years without any significant problems.

The set of columns used at Aston and the set used by Fisons for routine analysis were calibrated with the same standard fractions of dextran (Pharmacia T-fraction and Fisons standards) and the analysis of several samples in both systems were compared to produce a very good agreement between the sets of columns. A comparison of the molecular weight profiles of dextrans T40 and T70 produced on both sets of columns is given in Fig.3.2.

Conclusions

Silica packings suffer from dissolution and adsorption effects and most of the polymeric packings, because they are not rigid enough, require low flow-rates resulting in long analysis times.

Fig. 3.2 The Molecular Weight Profiles of Dextran T40 and T70 on Both Aston and Fisons Sets of PW-Type Columns

Integral Distribution % A	MW Profile of Dextran T40 at Aston B	MW Profile of Dextran T40 at Fisons C	MW Profile of Dextran T70 at Aston D	MW Profile of Dextran T70 at Fisons E	$\frac{B}{C}$x100 F	$\frac{D}{E}$x100 G
5.0	11425	11502	16034	17009	99	95
10.0	14689	14897	21252	22120	99	96
15.0	17250	17583	25403	26374	98	97
20.0	19577	19972	29191	30253	98	97
25.0	21740	22221	32820	34016	98	97
30.0	23923	24425	36475	37757	98	97
35.0	26126	26600	40217	41547	98	97
40.0	28441	28864	44131	45452	98	97
45.0	30839	31360	48375	49588	98	98
50.0	33469	33977	52814	54054	99	98
55.0	36299	36831	58012	58992	99	99
60.0	39505	39951	63587	64470	99	99
65.0	43019	43410	70108	70712	99	99
70.0	47273	47362	77957	78111	100	100
75.0	51966	52073	87287	87110	100	100
80.0	58195	58063	99068	98936	100	100
85.0	66325	66076	115182	114745	100	100
90.0	78007	77342	139498	138945	101	100
95.0	99841	99110	182075	186104	101	98

The TSK-gel PW-type is a polymeric packing rigid enough to be used with high flowrates and also it does not suffer from dissolution and adsorption.

Therefore the TSK-gel PW-type seems to be a satisfactory answer to all the problems that were encountered in this work for the characterisation of dextran polymer.

3.3 ANALYTICAL TECHNIQUES

The preparation of a sample to be injected on the analytical columns fell into one of three categories. These were:

(a) The preparation of solutions of known average molecular weights for the calibration of the columns. The above solutions were prepared by dissolving a known amount of dextran powder of known average molecular weights in a precise volume of 0.02% w/v sodium azide aqueous solution. Glucose and very high molecular weight dextran markers were added to those solutions to indicate the total inclusion and exclusion volumes respectively.

(b) The preparation of standard solution to calculate concentrations. A standard solution was prepared by dissolving a known amount of dextran powder in a precise volume of 0.02% w/v sodium azide aqueous solution. 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. Glucose was used as a marker.

(c) Feed and product solutions from the chromatographic unit or the UF systems did not usually need any preparation. Solutions too dilute for analysis were concentrated using a Buchi rotary evaporator and solutions too concentrated were diluted several times. Glucose was used for a marker. All solutions were made up to 0.02% w/v concentration of sodium azide.

The samples were filtered and injected into a six port injection valve with a constant volume sample loop. The chart recorder was adjusted to give a well defined peak so that it would be easy to measure the chromatogram manually for later data treatment. When the sample was injected on to the column the position was marked on the chart paper.

The eluent flowrates through the column were measured by weighing the eluate collected in a known period of time. The interstitial volume was taken to be at the peak maximum of the high molecular weight dextran (V_o) marker chromatogram. The total liquid volume for the column(s) was marked by peak maximum of the glucose peak.

3.4 DATA CONVERSION

3.4.1 CALIBRATION

To convert the chromatograms into a molecular weight distribution it was necessary to calibrate the column packing. The Nilsson and Nilsson (131) calibration approach was used to calibrate the TSK-gel PW-type columns.

A polynomial of the type

$$M = b_5+\exp\{b_4+b_1(K_d)+b_2(K_d)^2+b_3(K_d)^3\} \quad \ldots\ldots \quad (2.17)$$

was used. The K_d is related to elution volume, V_R according to the equation

$$V_R = V_o + V_i K_d \quad \text{or} \quad K_d = \frac{V_R - V_o}{V_i} \quad \ldots\ldots\ldots\ldots \quad (2.7)$$

The values of the constants b_1 to b_5 were obtained by the following procedure.

(1) A series of chromatograms were obtained for several Pharmacia T-fractions whose weight average molecular weights, $\bar{M}_w$'s had been measured previously by light scattering. The eluent flowrate for each chromatogram was measured.

(2) Each elution curve was divided into at least twenty vertical sections, the areas and elution volumes were measured and the elution volume (V_e) was converted to a K_d value.

(3) The areas, elution volumes and $\bar{M}_w$ (found by light scattering) of the T-fractions chromatograms together with guess values of b_1 to b_5 were entered

into a computer program (Appendix A1) that used Hartley's modification of the Gaussian-Newton method to give the new values of b_1 to b_5, which give the optimum agreement between the actual values of $\bar{M}_w$ measured by light scattering from each T-fraction and the calculated values obtained from the GPC elution profile.

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

3.4.2 DETERMINATION OF THE AVERAGE MOLECULAR WEIGHTS

To convert a chromatogram of a sample to average molecular weights it required that:

(1) The heights measured at regular intervals along the chromatogram, the elution volumes at these heights, the constants b_1 to b_5 found by the calibration program, and the pore and total liquid volumes for the fractionating column(s) were entered into a computer program called MWDC (Appendix A1).

(2) This computer program calculated the number ($\bar{M}_N$) and weight average ($\bar{M}_w$) molecular weights from the chromatogram using equations:

(a) $$K_d = \frac{V_R - V_o}{V_i} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.7)$$

(b) $$M_i = b_5 + \exp\{b_4 + b_1(K_{d_i}) + b_2(K_{d_i})^2 + b_3(K_{d_i})^3\} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2.17)$$

(c) $$\text{Normalised height, } h_i = \frac{\text{Chromatogram height, } h_i'}{\text{Total area of chromatogram, A}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.1)$$

(d) $$\bar{M}_w = \frac{\Sigma h_i M_i}{\Sigma h_i} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.20)$$

(e) $$\bar{M}_N = \frac{\Sigma h_i}{\Sigma h_i / M_i} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2.21)$$

3.4.3 DETERMINATION OF SAMPLE CONCENTRATION

The response of the refractometer was directly proportional to the concentration of the injected sample since a constant volume injection valve was used. Therefore concentrations were obtained by comparing the area of the chromatogram above the baseline of an injected sample against the area of a solution of known concentration. This was done by a subroutine in the MWDC program. The procedure was:

(1) The heights were measured at regular intervals along the chromatogram, the elution volumes and the pore and total liquid volumes for the fractionating column(s) were entered in the MWDC program.

(2) The elution volumes were converted into the Wheaton-Bauman distribution coefficients (K_d's).

(3) The area of the chromatogram was calculated by Simpson's rule.

(4a) If the chromatograph was 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.

(4b) 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.

3.5 COMMENTS ON THE ANALYTICAL SYSTEM

The introduction of the molecular weight interpretation technique allowed products from different runs to be compared directly by means of their average molecular weights and molecular weight distributions. The MWDC program provided a rapid and reproducible manner of quantitative and qualitative analysis of the products. 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.

4.0 SEMI-CONTINUOUS CHROMATOGRAPHY

4.0 SEMI-CONTINUOUS CHROMATOGRAPHY

4.1 PRINCIPLES OF OPERATION OF THE SCCR5

The SCCR5 machine is a semi-continuous counter-current, simulated moving bed, pilot plant scale chromatograph. The principles of operation of this machine are shown diagramatically in Fig. 4.1 and 4.2.

The equipment can be considered to be in two sections. The fractionating section is between E and P1 and consists of nine columns. The purge section is between PU and P2, it consists of one column and is isolated from the fractionating section by closing both the valves that connect this column to the other columns (transfer valves).

The feed enters a point F, the mid-point of the fractionating section. Pure eluent enters at point E and moves towards point F stripping out the large molecules that have preference for the mobile phase. At F there is a step change increase in the mobile phase flowrate and any material having a preference for the mobile phase is carried forward and finally 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, so that the simulated movement of the packing is in the opposite direction. Smaller molecules having greater

Fig. 4.1 Schematic Diagram of the SCCR5

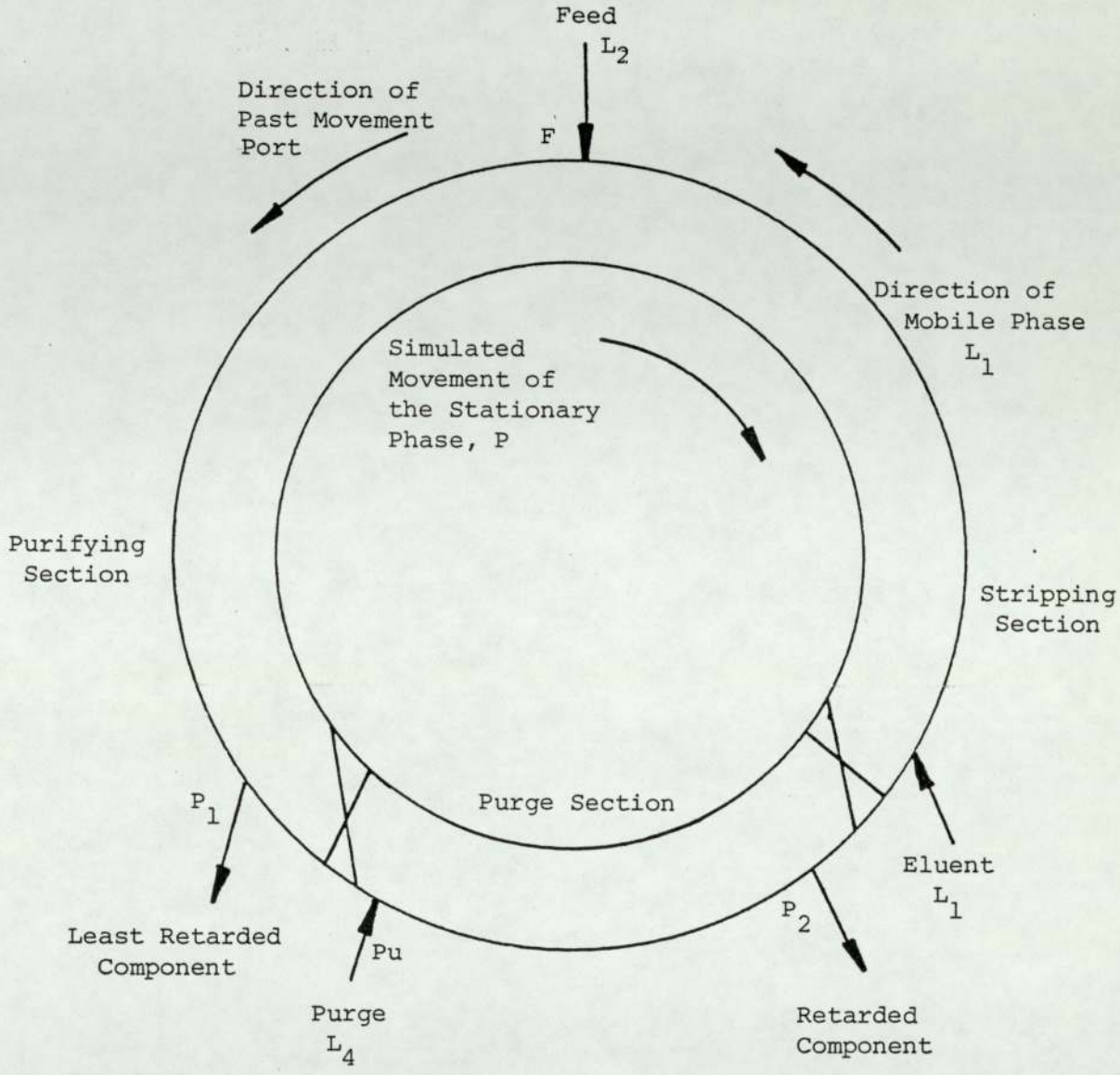
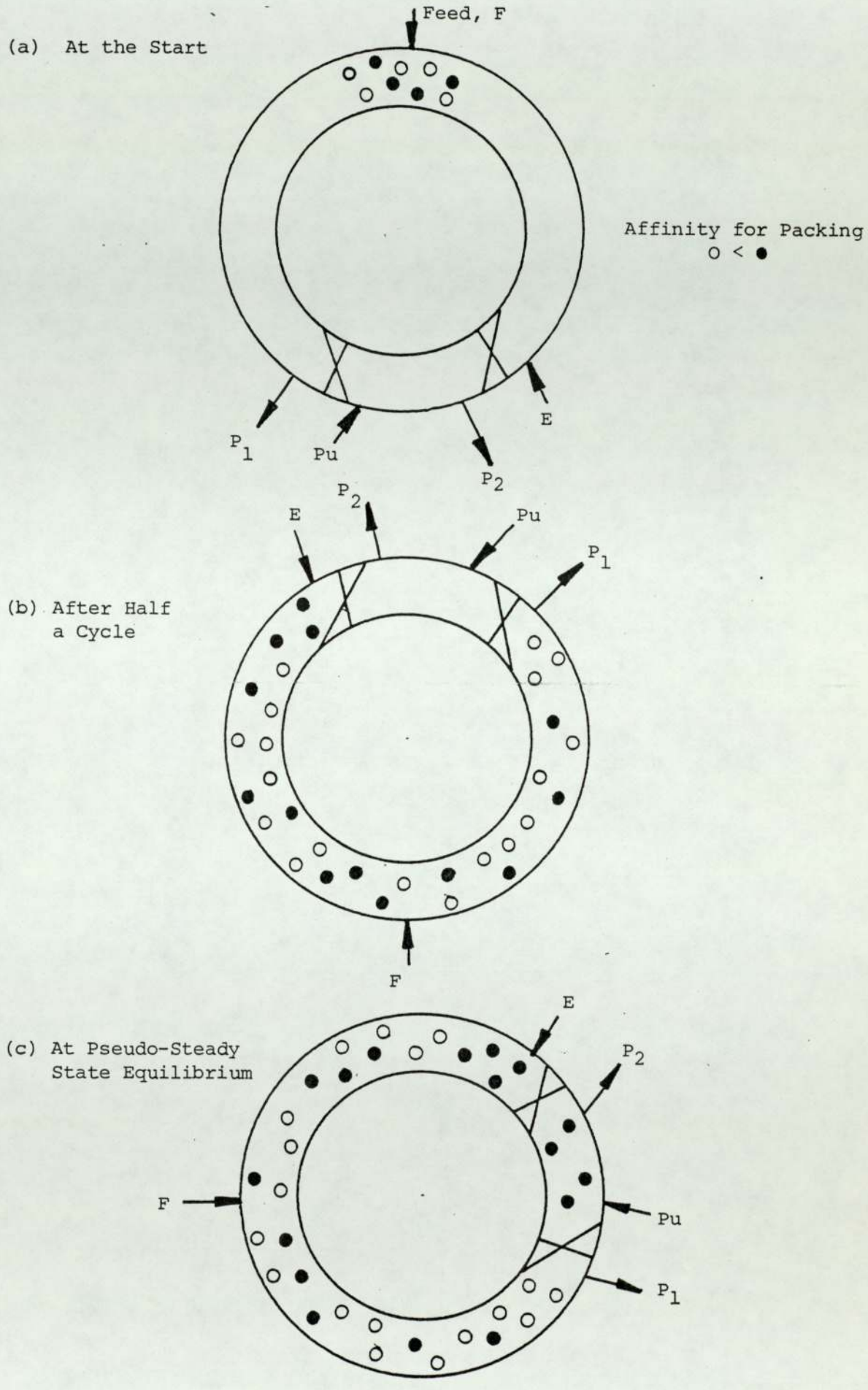


Fig. 4.2 Principal of Operation of the SCCR5



affinity for the stationary phase will still more in the direction of the mobile phase but with a lower velocity. The purge section advances towards the greater retained components although they are still moving with the mobile phase, so that the slowest moving (smaller) molecules are eventually enclosed in the purge section. These small molecules are removed from the purge section by flushing it through with excess quantities of mobile phase. When all columns in the chromatograph have operated as the purge section, the chromatograph is then said to have completed a cycle.

The volumetric throughput of the eluent, feed and 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 assigned to the 'apparent' movement of the stationary phase, P, when measured from the feed point. From the knowledge of the columns 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.

4.2 THE SELECTION OF EXPERIMENTAL OPERATING CONDITIONS

4.2.1 IDEALISED CASE. TWO COMPONENT FEED

The rate of movement of a component with the mobile phase, r'_{mi}, is proportional to the fraction of its molecules in the mobile phase at equilibrium.

Similarly the rate of movement of a component with the stationary phase, r'_{si}, is proportional to the fraction of its molecules in the stationary phase at equilibrium:

$$r'_{mi} = \bar{v}_m \cdot \frac{V'_o}{V'_o + K_{di} V'_i} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4.1)$$

and

$$r'_{si} = \bar{v}_s \cdot \frac{K_{di} \cdot V'_i}{V'_o + K_{di} V'_i} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4.2)$$

$\bar{v}_m$ = average velocity of mobile phase = L'_i/V'_o

L'_i = effective mobile phase flowrate

V'_o = average interstitial volume per unit length of column (i.e. $m^3.m^{-1}$)

V'_i = average pore volume per unit length of column

$\bar{v}_s$ = average velocity of stationary phase = P/V'_i

P = stationary phase flowrate

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:

$$L'_i = L_i - \frac{V'_o \ell}{s} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4.3)$$

where ℓ is the length per column and s the switch time.

A molecule will travel preferentially with the mobile phase if $r'_{mi} > r'_{si}$ or:

$$\frac{L'_i}{V'_o} \cdot \frac{V'_o}{V'_o + V'_i K_{di}} > \frac{P}{V'_i} \cdot \frac{V'_i K_{di}}{V'_o + V'_i K_{di}} \quad \ldots\ldots\ldots \quad (4.4)$$

or

$$\frac{L_i'}{P} > K_{di} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.5)$$

Similarly a molecule will travel preferentially with the stationary phase if:

$$\frac{L_i'}{P} < K_{di} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4.6)$$

For complete separation of two components 1 and 2:

$$K_{d_1} < \frac{L_i'}{P} < K_{d_2}$$

4.2.2 CUT POINTS FOR POLYMER FRACTIONATION

For a multi-component feed the differences in the distribution coefficients for molecules in ascending order in the polymer chain are very small. It is impractical to resolve completely 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.

4.2.3 NON-IDEALITIES OCCURRING IN A PRACTICAL SYSTEM

A number of factors influence the ability of the SCCR5 machine to separate a feed mixture. Factors which cause departures from the idealised case are:

(a) Chromatographic Zone Broadening

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

(b) The Sequential Nature of 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 increase as the number of columns was decreased.

(c) Finite Concentration Effects

Distribution coefficients in GPC (K_d) are affected by concentration (187). Ellison (47) operating a similar chromatograph showed how the product distributions can change when increasing the feed concentration from 10 to 200 g l^{-1}.

(d) Finite Feed Flowrate

The mobile phase flowrate has two values within the fractionating section of the SCCR5 machine, a pre-feed column value, L_1, and post-feed column value, $L_1+L_2=L_3$. If L_2 is significant, the unequality of equation (4.7) has to be modified to the form of:

$$K_{d_1} < \frac{L_1'}{P} < \frac{L_3'}{P} < K_{d_2} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (4.8)$$

Therefore as the feed throughput is increased the product purity is decreased.

4.3 DESCRIPTION OF THE SCCR5

The SCCR5 machine (Figs. 4.3a, 4.3b) consists of ten 5.1 cm I.D. x 70 cm long stainless steel columns mounted on a tubular frame construction.

The columns are packed with porous silica, Spherosil XOB 075 (Rhone-Poulenc, France) particles (200-400 μm). To each column are connected six pneumatically operated poppet valves (Fig. 4.4) (manufactured by Aston Technical Services Ltd.). The flow of liquid into each column was controlled by opening of the eluent, transfer, feed or purge valves. The control fluid out of the columns was to be either by the product valves opening or by opening of the transfer valve associated with the next column.

A pneumatic controller, constructed by Mr. M. Lea, the departmental electronics technician, that includes an accurate timing device was used to control the valve operation.

The liquids were fed into the columns by two pumps, a small reciprocating pump (MPL series 2, fitted with two No.4 polypropylene heads) for the feed and one of larger capacity (Twin k metering pump, MPL, London) for the eluent and purge.

For the operation of the SCCR5 machine at higher temperatures, an enclosure made of 50 mm thick fibre glass lagging surrounded the columns, which provided a constant temperature surrounding of up to 80°C. The system also included heaters for the air in the enclosure,

Fig. 4.3a Photograph of the SCCR5

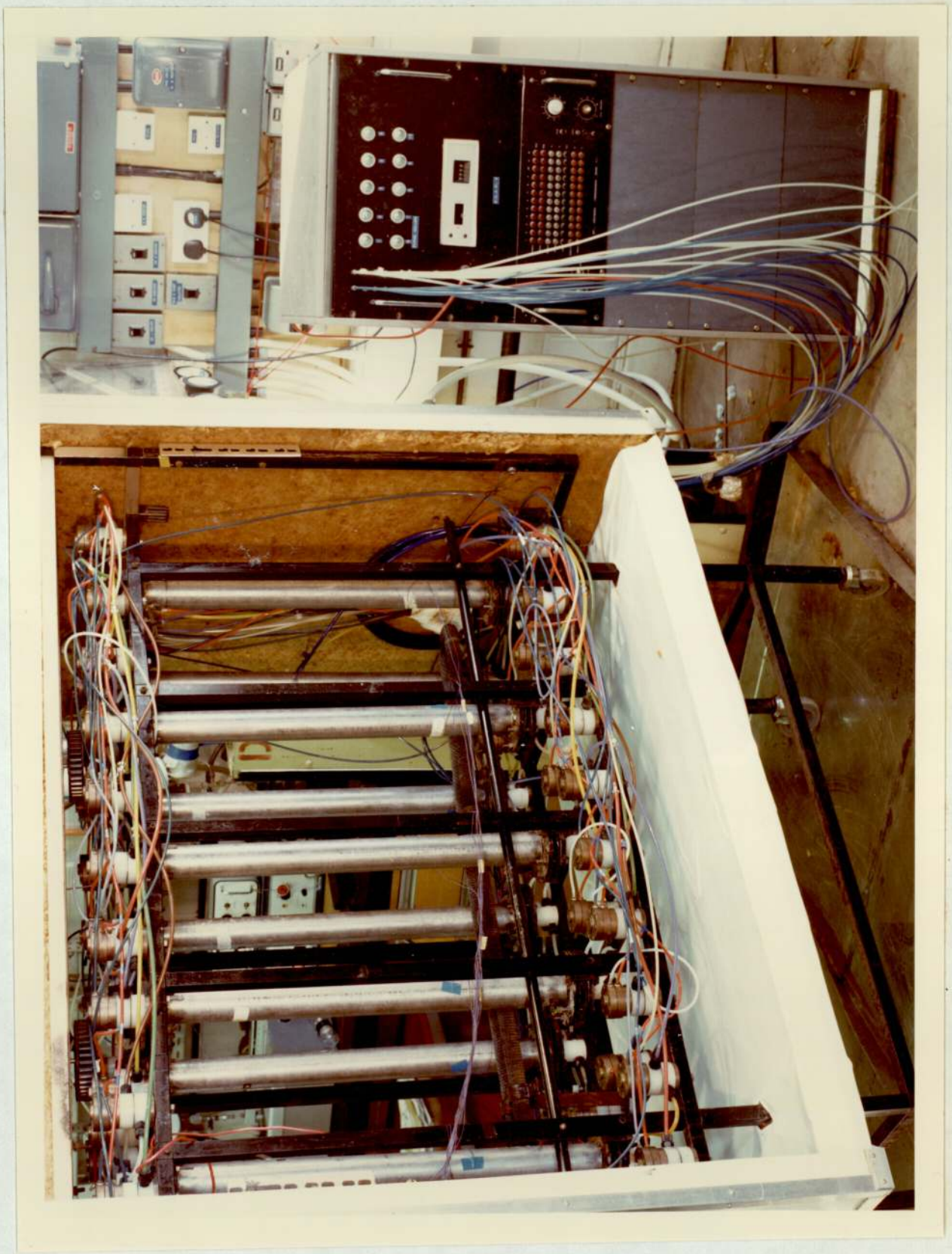


Fig. 4.3b A Schematic Diagram of the SCCR5

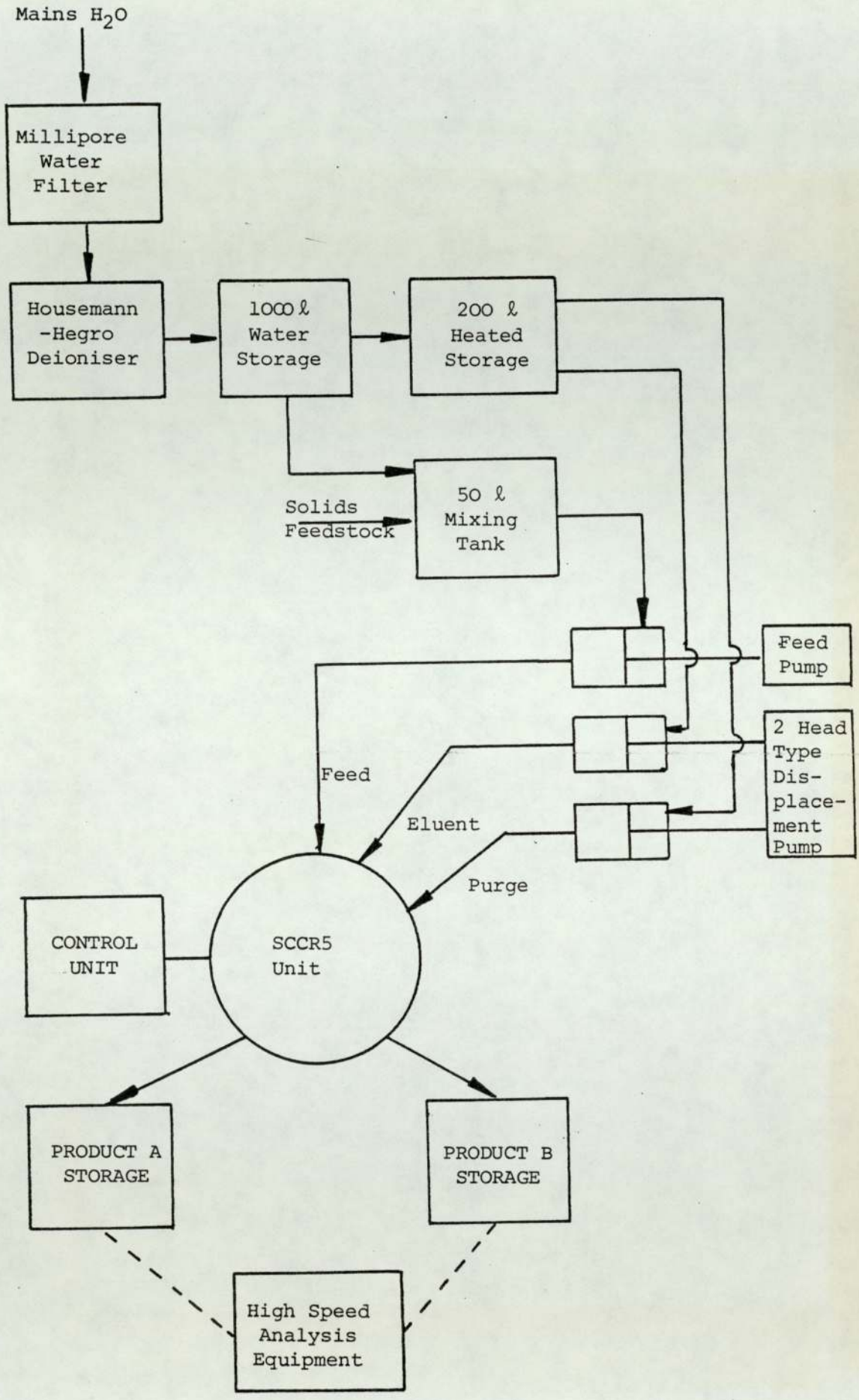


Fig. 4.4 Photograph of a Popped Valve used in the SCCR5

A = Assembled valve

B = Upper diaphragm chamber

C = Lower diaphragm chamber

D = Neoprene rubber diaphragm

E = Inlet chamber

F = Outlet chamber

G = Poppet and stem

H = Viton rubber gasket

I = Valve base

J = Diaphragm backing plates

K = Diaphragm assembly components

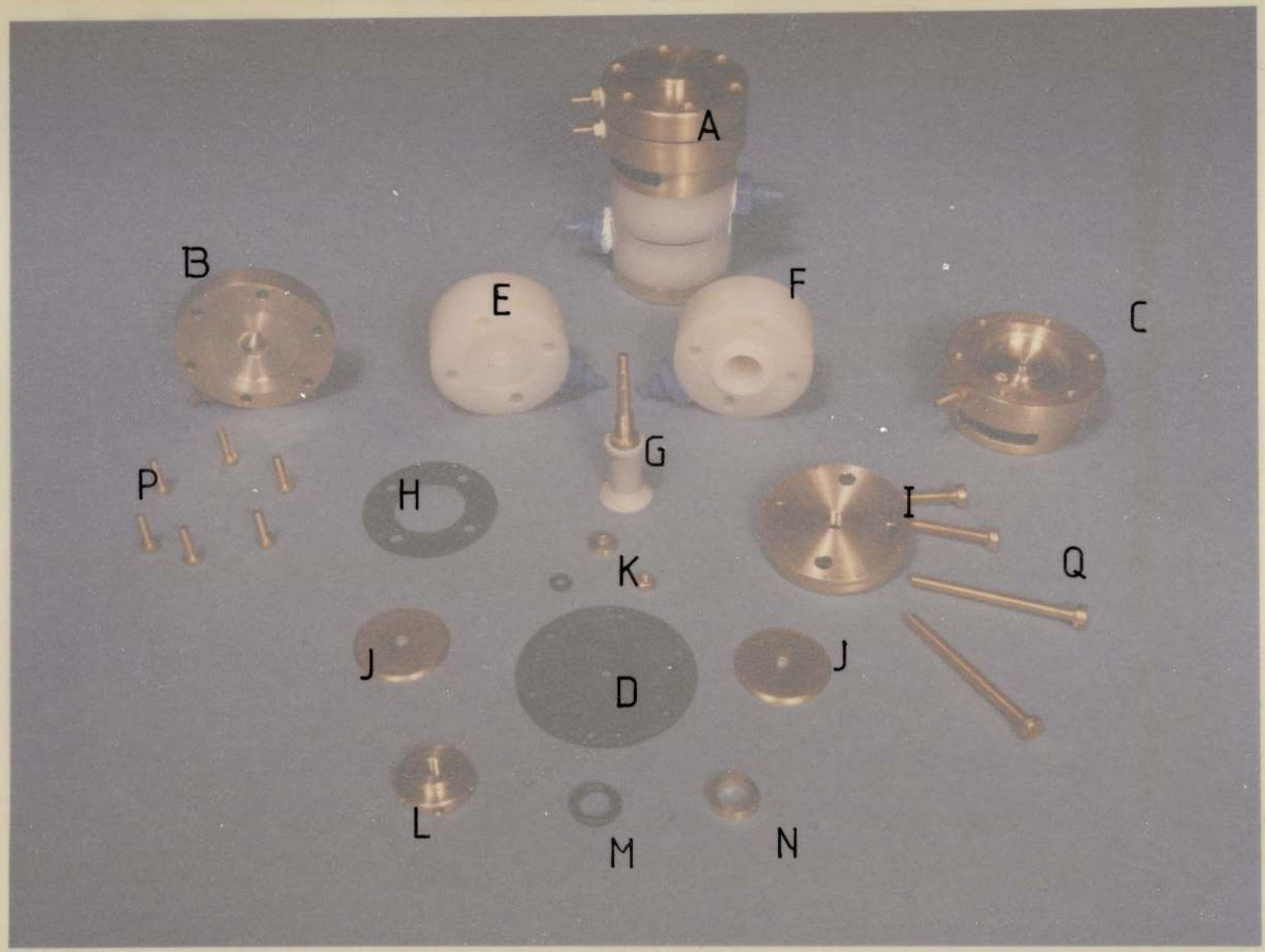
L = Adjustment nut

M = Viton 'O' ring

N = Thrust washer

P = 6 x 4BA cap screws

Q = 4 x 2BA cheese head body screws


A
B
E
F
C
G
P
H
I
Q
K
J
D
J
L
M
N

the dextran feed and the water (eluent, purge) entering the columns. The temperatures of the fluids entering the columns and of the air in the enclosure were regulated by temperature control systems supplied by Diamond H. Controls Ltd., Norwich.

A more detailed description of the SCCR5 was presented by K. England (46).

4.4 OPERATION OF THE SCCR5

The experimental runs lasted for up to 40 hours. Each set of runs was divided up into daily operations ranging from 12-14 hours. It had been established by this work and previous researchers (47,264) that no detectable change in the dextran distribution in the columns occurred during the period that the machine was shut down.

The operating procedure was as follows:

(a) When the experimental runs were performed at elevated temperatures, the temperatures of air, water and dextran feed were set on the temperature controllers and the appropriate heaters were switched on.

(b) The pneumatic supply to the chromatograph and the power supply to the control box were turned on. The switch time was selected on the control box.

(c) The purge, eluent and feed pumps were started and the flowrates were adjusted. This was the start of the run.

(d) During the run the flowrates, pressures and temperatures were checked and readjusted if it was necessary.

(e) The two products were collected in separate containers for each cycle or pair of cycles and at the end of the cycle(s) were weighed and analysed to check if the dextran output was equal to the input, i.e. if pseudo-steady state had been achieved. The SCCR5 was run for two to four cycles under pseudo-steady state conditions and then the experiment was stopped.

(f) At the end of the run the feed and eluent pumps were switched off and each column was purged individually. The purge product was weighed and analysed to obtain the on column concentration profile of dextran.

(g) Finally, all the heaters, pumps and the pneumatic and power supplies to the control box were switched off.

4.5 INITIAL GPC EXPERIMENTAL RUNS

4.5.1 EXPERIMENTAL CONDITIONS

The first experimental run, Run 1, on the SCCR5 was to remove the high molecular weight end of a

Fig. 4.5 The Overall Properties of the SCCR5 Columns before and after being Repacked

Property	Originally Packed Columns	Repacked Columns
$V_o + V_i$	1181	1190
V_o	574	546
V_i	607	644
V_o/V_i	1.061	1.179
Number of Plates	107	73

235 g l^{-1} dextran feed, operating at ambient temperature.

The purpose of this run was to be familiar with the operation of the SCCR5, to check its reproducibility and to reduce the purge rate so as to get more concentrated low molecular weight product.

The initial experimental conditions of the run were identical to those used by K. England (46) in Run B. But the initial flowrates of eluent, purge and feed were halved after the second cycle, because the pressure drop was (1675 kN.m^{-2}) higher than the maximum operating pressure of the system (1340 kN.m^{-2}). The switch time was then doubled to produce the same cut positions.

Because the pressure drop during Run 1 was higher than for the identical run by K. England (46) and because the columns were leaking, it was decided to dismantle the columns.

It was observed that the packing material in the columns had not shrunk at all during the four years of its use but a lot of dirt and bacterial debris was on the packing. Therefore the packing of the columns was emptied, washed with acid and water, and the columns were repacked. The overall properties of the packed columns before and after they were repacked have been measured using batch chromatography techniques, and presented in Fig.4.5. The difference in the number of plates is due to the different flowrates used to measure the properties of the columns, i.e. 50 $cm^3 min^{-1}$ before, 100 $cm^3 min^{-1}$ after.

Fig. 4.6 Theoretical Conditions of Run 1

Cycles	Switch Time (s)	Conc. of Feed ($g\ l^{-1}$)	Feedrate per		Eluent to Feed Ratio	Flowrates (theoretical) ($cm^3 min^{-1}$)			Theoretical Cut Positions		
			Cycle (g)	hr (g)		Eluent	Feed	Purge	Pre-Feed	Post-Feed	Purge
1-2	450	253.6	760.8	608.6	2.5	100	40	380	0.290	0.784	3.750
3-8	900	253.6	760.8	304.0	2.5	50	20	190	0.290	0.784	3.750
9-13	900	253.6	760.8	304.0	2.5	50	20	150	0.290	0.784	2.761
14-17	900	253.6	760.8	304.0	2.5	50	20	120	0.290	0.784	2.020

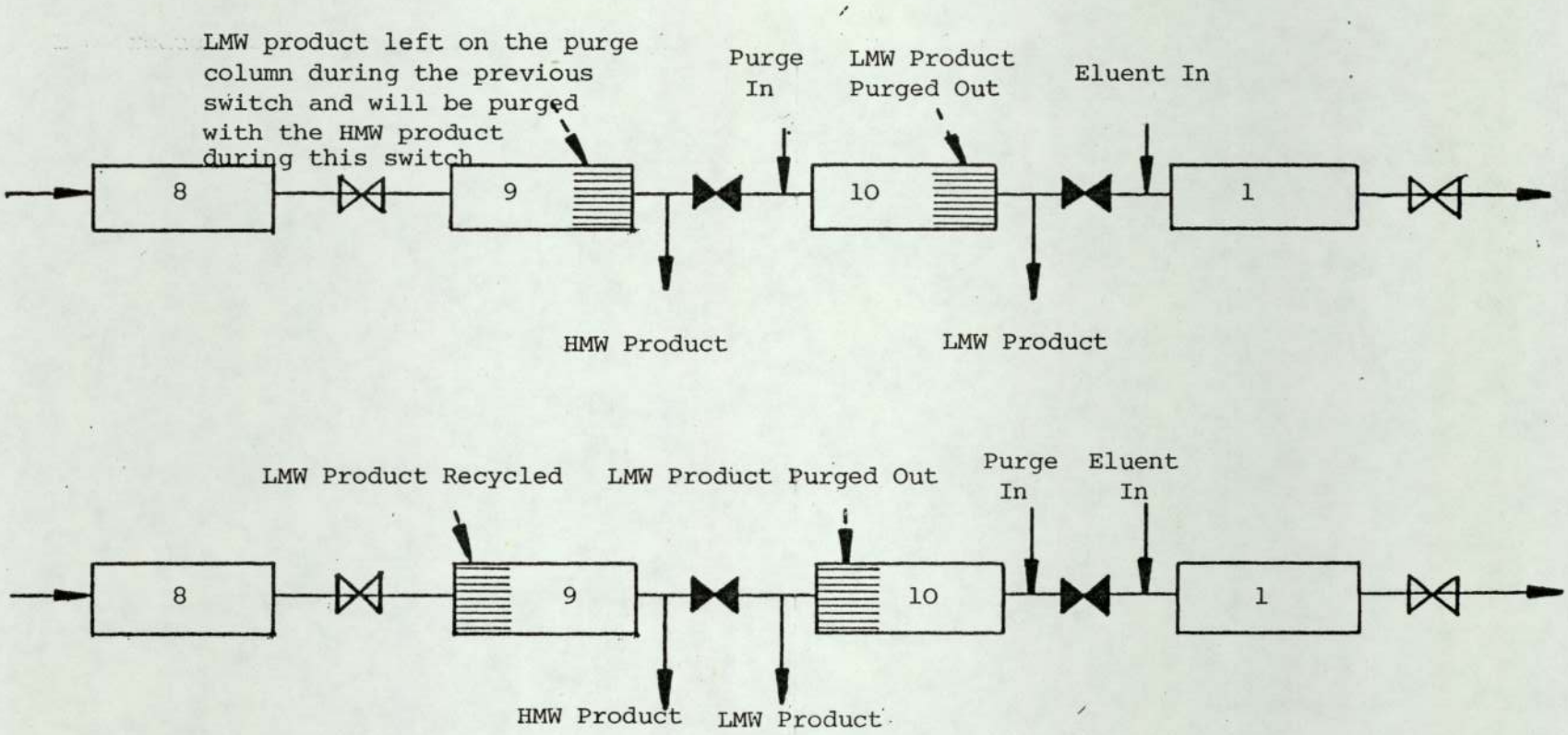
Feed (B161D)		
$\bar{M}_W$	$\bar{M}_N$	D
66000	23000	2.87

Fig. 4.7 Theoretical Conditions of Run 2

Cycles	Switch Time (s)	Conc. of Feed ($g\ l^{-1}$)	Feedrate per		Eluent to Feed Ratio	Flowrates (theoretical) ($cm^3 min^{-1}$)			Theoretical Cut Positions		
			Cycle (g)	hr (g)		Eluent	Feed	Purge	Pre-Feed	Post-Feed	Purge
1-3	450	241.3	723.8	578.0	2.5	100.0	40.0	380.0	0.316	0.786	3.578
4-15	900	241.3	723.8	289.5	2.5	50.0	20.0	190.0	0.316	0.786	3.578
16-19	900	241.3	723.8	289.5	2.5	50.0	20.0	100.0	0.316	0.786	1.481

Feed (B161D)		
$\bar{M}_w$	$\bar{M}_N$	$\bar{D}$
645000	20000	3.20

Fig. 4.8 Effect of Reversing the Purge



The flowrates and switch time of Run 2 were identical to those used for Run 1, but the pre-feed and post-feed cut position were higher since the interstitial and pore volumes of the repacked columns were found to be different from those values the columns originally had.

The direction of the liquids in and out of the purge column was reversed so that, when the purge rate was reduced and some low molecular weight material remained in the purge column after the switch, it would prevent its elution with the high molecular weight product during the next switch (see Fig. 4.8).

The experimental conditions of Run 1 and Run 2 are presented in Fig. 4.6 and Fig. 4.7 respectively.

4.5.2 RESULTS AND CONCLUSIONS

The results of Runs 1 and 2 are summarised in Fig. 4.9 to Fig. 4.12. The conclusions that can be drawn from these runs are:

(i) The cut positions and the feed input of Run 1 were identical to Run B done by K. England (46) three years previously. The results of the two runs agreed well which showed that there was no change in the fractionating ability of the system during the first three years of its operation.

Also the doubled switch time did not affect the separation.

(ii) When the purge rate was reduced to a value below 190 $cm^3 min^{-1}$ a considerable amount of dextran was

Fig. 4.9 Summary of the Results for Run 1

Cycles	Input Mass (g)	High Mol. Wt. Product						Low Mol. Wt. Product					
		Mass (g)	Mass (%)	Conc. (g l^{-1})	Mol. Wt. Distribution			Mass (g)	Mass (%)	Conc. (g l^{-1})	Mol. Wt. Distribution		
					$\bar{M}_W$	$\bar{M}_N$	$\bar{D}$				$\bar{M}_W$	$\bar{M}_N$	$\bar{D}$
7 and 8	772.0	134	17.0	12.36	176000	53000	3.28	655	83.0	22.69	50000	22000	2.28
12-13	764.0	185	24.2	16.00	167000	49000	3.37	579	75.8	24.38	46000	21000	2.10
14-17	765.0	215	28.1	21.0	142000	45000	3.25	550	71.9	33.00	44000	20000	2.31

Fig. 4.10 Concentration Profile of Run 1

(i.e. on Column Concentration of Dextran)

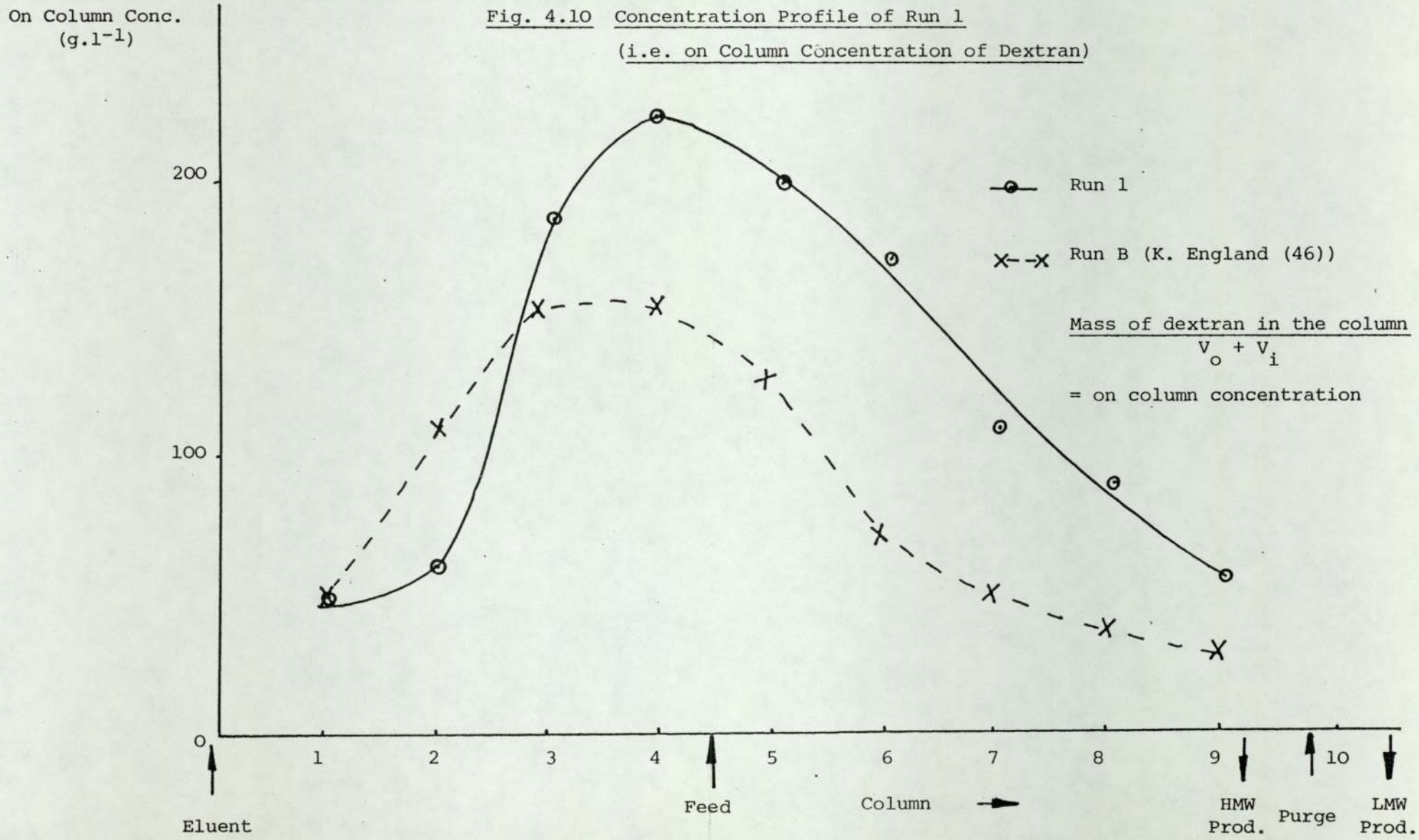


Fig. 4.11 Summary of the Results for Run 2

Cycles	Input Mass (g)	High Mol. Wt. Product						Low Mol. Wt. Product					
		Mass (g)	Mass (%)	Conc. (g l^{-1})	Mol. Wt. Distribution			Mass (g)	Mass (%)	Conc. (g l^{-1})	Mol. Wt. Distribution		
					$\bar{M}_w$	$\bar{M}_N$	$\bar{D}$				$\bar{M}_w$	$\bar{M}_D$	$\bar{D}$
13-15	728	182	25.3	15.7	120000	34000	3.52	538	74.7	19.0	44000	16500	2.71
18-19	740	268	37.8	23.4	74500	18500	4.00	442	62.2	29.7	47000	17000	2.76

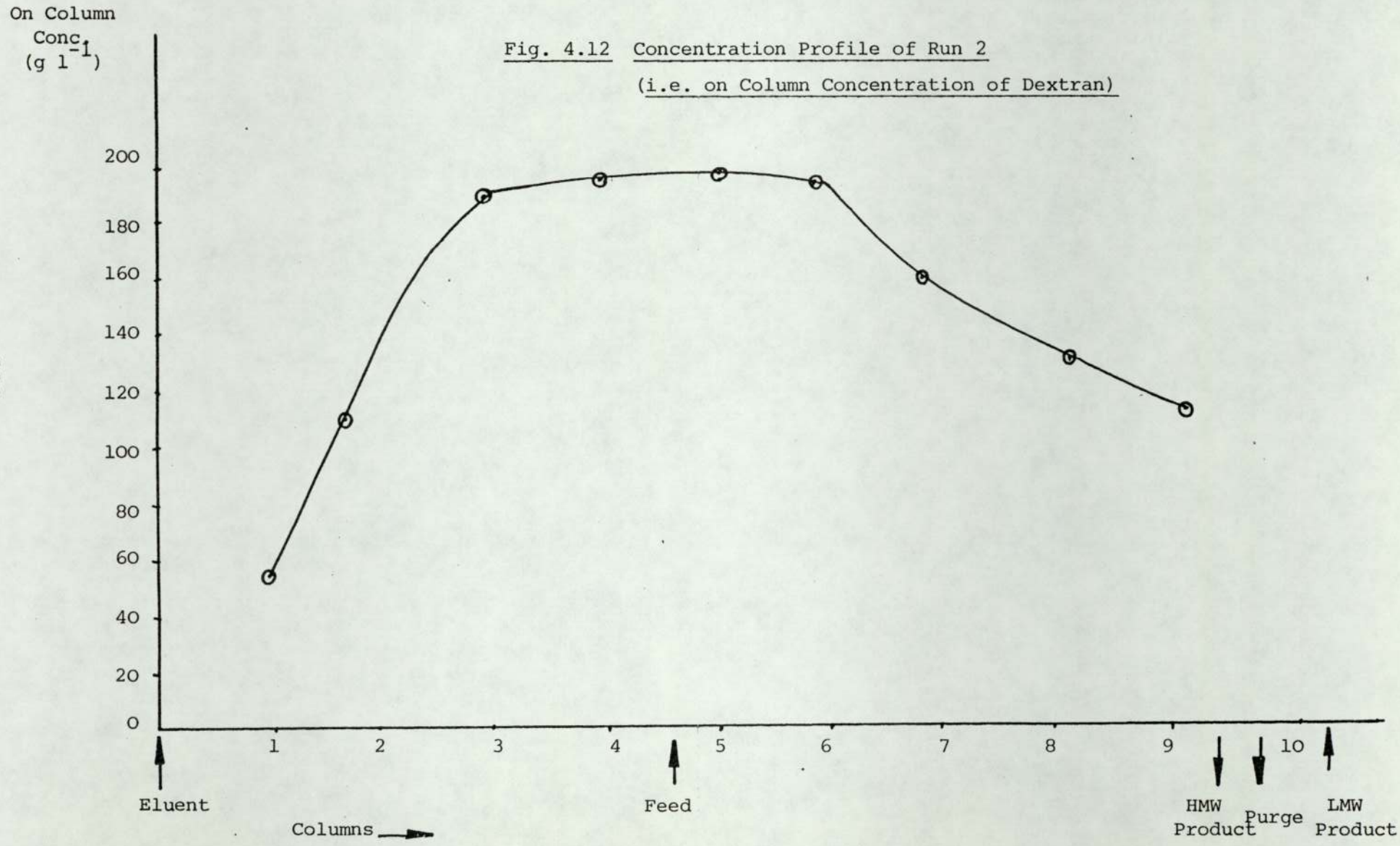
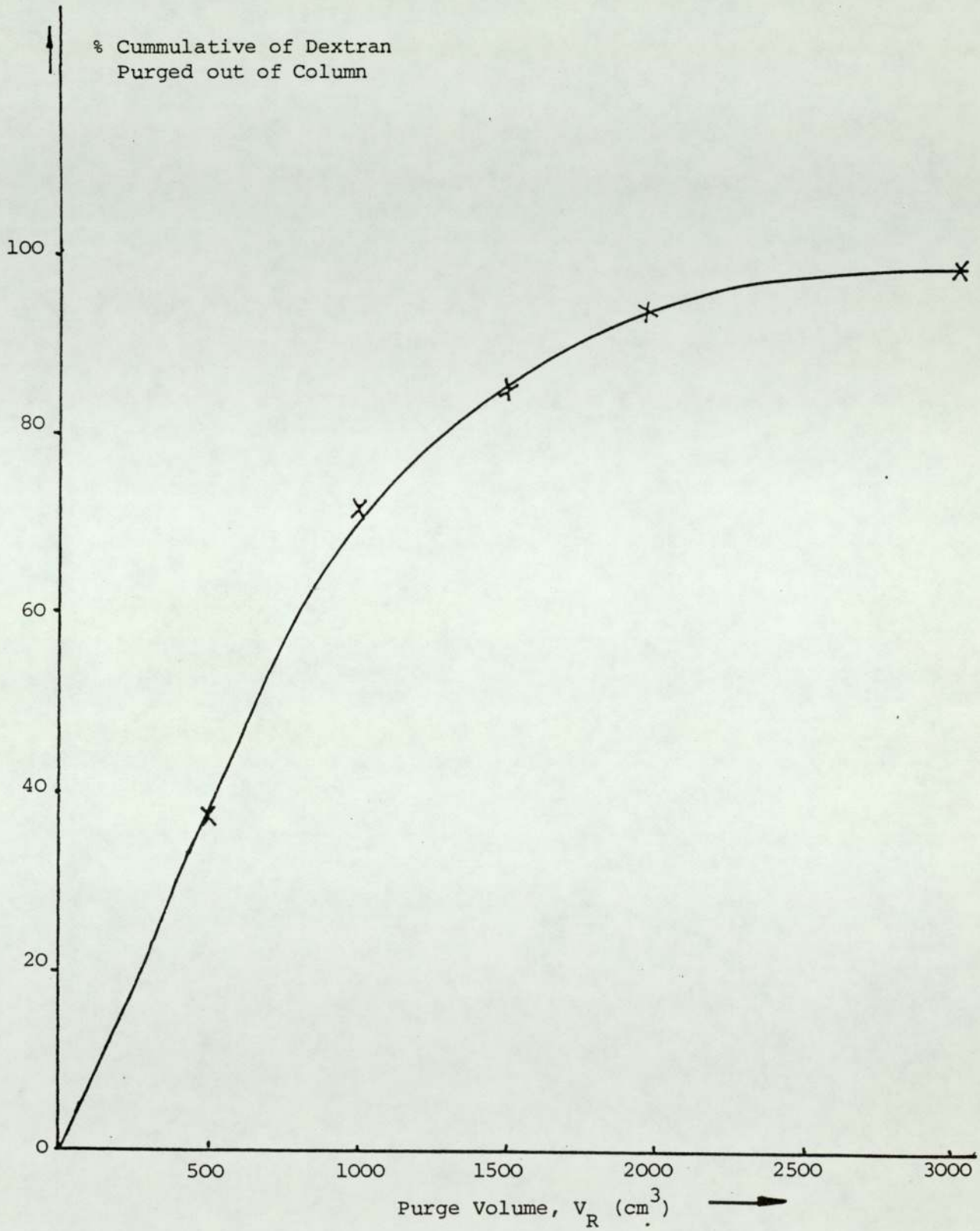


Fig. 4.12 Concentration Profile of Run 2

(i.e. on Column Concentration of Dextran)

Fig. 4.13 Concentration Profile of Purging Column 1



purged out of the columns in the last two minutes of a 15 minute switch. The amount of dextran purged in these two minutes is as much as 7% of the total amount of dextran purged during the switch.

This means that some of the low molecular weight dextran is not purged out of the columns by the end of the switch.

(iii) When the purge rate was reduced the percentage of dextran removed with the low molecular weight was also reduced. This is an effect of the purge rate not been enough to remove all the low molecular weight material of the column when in the purge position.

(iv) Comparing the results of the column concentration profiles of Run 1 with England's (46), Run B (Fig. 4.10) it can be seen that more material was retained by the columns due to the accumulation of unpurged low molecular weight material.

(v) Comparing Run 2 with Run 1 it is shown that by increasing the pre-feed and post-feed cut positions the percentage of dextran removed with the high molecular weight product is increased, and the average molecular weights of the products are reduced.

(vi) The change in the purge direction did not seem to have the expected effect on Run 2.

(vii) From the concentration profiles (Fig. 4.10, Fig. 4.12) it is observed that the higher on-column concentrations of dextran are near the feed column.

(viii) The concentration profile of purging dextran material out of column 1 was investigated (Fig. 4.13),

in a search for an optimum purge cut position. Column 1 was selected because it contains the lowest molecular weight material in the system. Purge cut positions 3.0 to 3.5 were found satisfactory.

5.0 SILICA DISSOLUTION

5.0 SILICA DISSOLUTION

5.1 INTRODUCTION

It was reported (46,265,266) and also found in this experimental work that the silica packing inside the SCCR5 columns dissolved slowly, and although the rate of dissolution was not sufficient to create any problems in the characteristics of the packed columns, the dextran products were contaminated with silica above the limit allowed by British Pharmacopeoia (1 ppm SiO_2 in 10% w/v dextran solution).

Hence ways for quantitative analysis of silica and methods to remove it from the dextran products were considered.

5.2 QUANTITATIVE ANALYSIS OF SILICA

Initially the quantitative analysis of silica in the dextran solutions was carried out using an Atomic Absorption Spectrophotometer (Model 151 aa/ae, Instrumentation Laboratory, Lexington, USA). But because the above analytical system relied on the Venturi effect for sucking the sample to the nebulizer and the dextran solutions were of different concentrations and therefore of different viscosity the amount of solution flowing to the nebulizer per unit time depended on the viscosity. To eliminate the viscosity effects the following ways were considered:

(a) To make all solutions to the same viscosity by diluting concentrated solutions.

(b) To add a viscous substance to raise the viscosity of all solutions to a constant value.

(c) To use periodic acid to oxidise the dextran in the solutions.

(d) To use a device to control flow in the nebulizer.

A Perkin-Elmer Model 603 Atomic Absorption Spectrophotometer equipped with an HGA-2200 Heated Graphite Atomizer was used in the Pilkington, Central Laboratory, St. Helens, for the analysis of silica present in the dextran solutions, but the silica levels that this equipment could detect were below the silica present in the dextran solutions and therefore further dilution of the solutions was necessary.

Finally the Molybdenum Blue Colorimetric Method (268) was used for the silica analysis, after Pilkington, Central Laboratory, St. Helens found that the viscosity and dextran background did not interfere with this analytical method. A Unicam SP1800 Ultraviolet Spectrophotometer, Pye Unicam, Cambridge was used to measure the optical densities of the solution at a wavelength of 700μm. The Molybdenum Blue Colorimetric Method is described in detail in Snenn-Ettre, Encyclopedia of Industrial Chemical Analysis (269).

5.3 SILICA REMOVAL (BY ION-EXCHANGE RESINS)

For the removal of silica, Ultrafiltration (UF) membranes and ion exchange resins were considered. The results of the Ultrafiltration runs are discussed in Sections 7.0, 8.0 and 9.0.

A 1 cm ID x 65 cm long glass column packed with Amberlite anion exchange resin IRA-900, particle size 0.5 mm (Rohm and Haas, Croydon, UK) was used to evaluate the applicability of this packing in the removal of silica from dextran aqueous solutions.

A 16 g l^{-1} dextran solution containing 100 ppm SiO_2 was passed through the column at a rate of 0.3 cm^3 min^{-1}. The SiO_2 content in the dextran solution after its passage through the packed column was 46 ppm. This resin was not that efficient to remove enough silica from the products so that they would be within the required limits.

Millipore, London, UK are marketing the Milli-Q systems for ultrapure water. These systems include a cartridge packed with a nuclear grade mixed-bed ion exchange resin. A 15 g l^{-1} dextran solution containing 7.6 ppm SiO_2 was passed through one of the above cartridges (Fig. 6.1) and the product out of the cartridge contained only 0.15 ppm SiO_2. The concentration of dextran in the solution did not change after its passage through the resin. Hence this resin appeared suitable for the removal of silica from the dextran products.

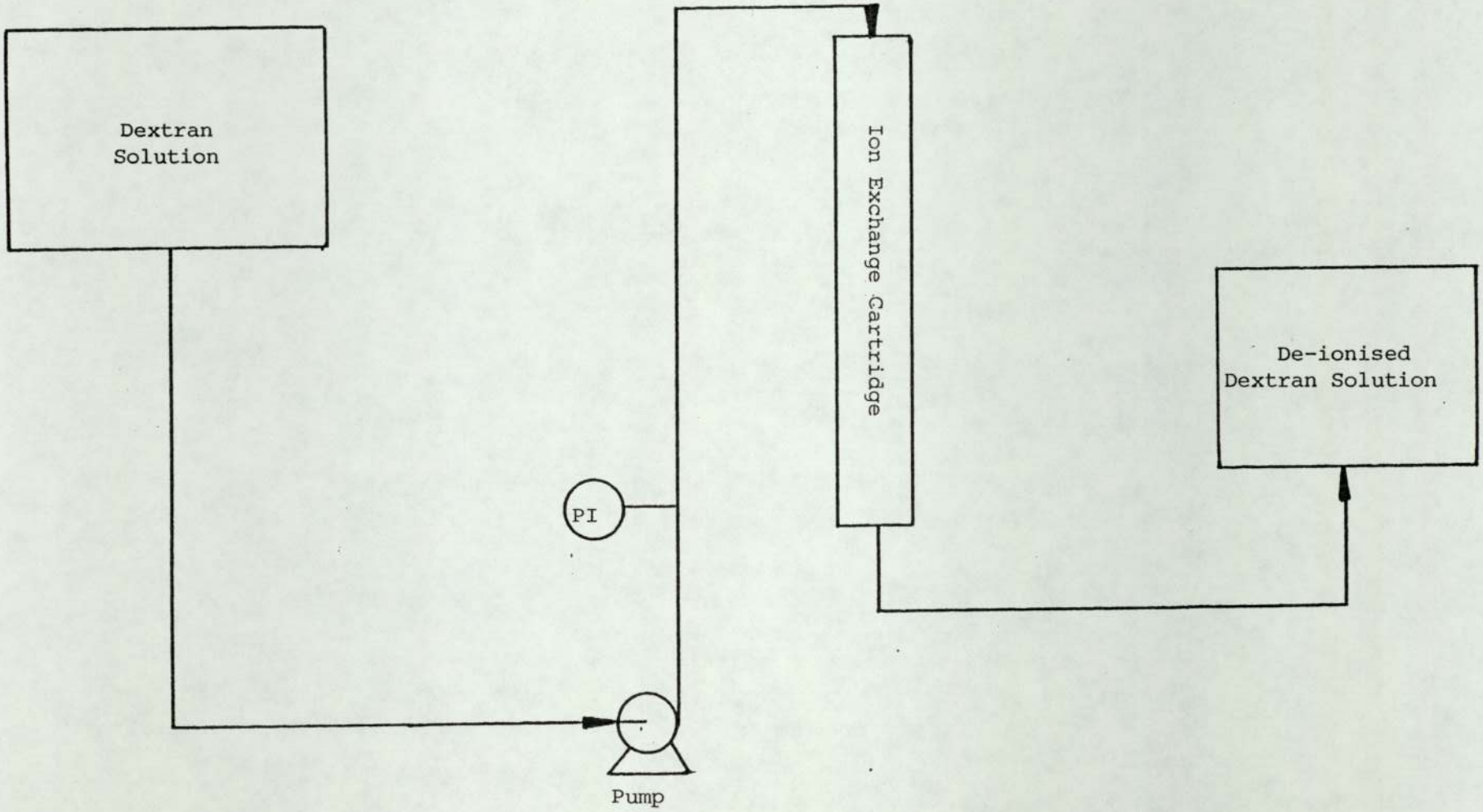
Dextran solutions of 16 g l^{-1} to 20 g l^{-1} were used because this was the concentration of dextran products out of the SCCR5.

5.4 CONCLUSIONS

The Molybdenum Blue Colorimetric Method seems to be the most suitable method for quantitative analysis of silica present in dextran solutions because it can detect all the forms of soluble silica and is not affected by the dextran presence in the solution.

Silica can be removed very efficiently from the dextran products using cartridges packed with nuclear grade mixed-bed ion exchange resin, and since these resins are of high purity will be suitable for pharmaceutical applications.

Fig. 5.1 Schematic Diagram of the De-ionization Process of Dextran Solutions



6.0 ULTRAFILTRATION OF DEXTRAN

6.0 ULTRAFILTRATION (UF) OF DEXTRAN

6.1 INTRODUCTION

Because the dextran solutions leaving the SCCR5 were contaminated with silica dissolved from the Spherosil packing, ultrafiltration was considered as a process for the removal of silica from these solutions. But ultrafiltration was also tried for fractionating and concentrating dextran solutions.

6.2 INITIAL ULTRAFILTRATION WORK

Initially a reverse osmosis (RO) cell (270) was used for the concentration of dilute dextran solutions with three types of membranes, a Sartorius type (270) and PM10 and YM5 Amicon (UK) (250) ultrafiltration membranes. The arrangement of the equipment used are shown in Fig. 6.1. The conditions and results of these runs are summarised in Figs. 6.2 and 6.3. From the results of these runs it was concluded that:

(1) Dextran solutions can be concentrated from 15 $g.l^{-1}$ to 70 $g.l^{-1}$ using UF (Fig. 6.2).

(2) Fractionation of dextran solutions can be achieved (Fig. 6.2).

(3) As the molecular weight cut off (i.e. the pores) of the membrane increased the rate of filtrate increased and the rejection of dextran by the membrane decreased (Fig. 6.2).

Fig. 6.1 Arrangement of the Equipment used for the Initial UF Runs

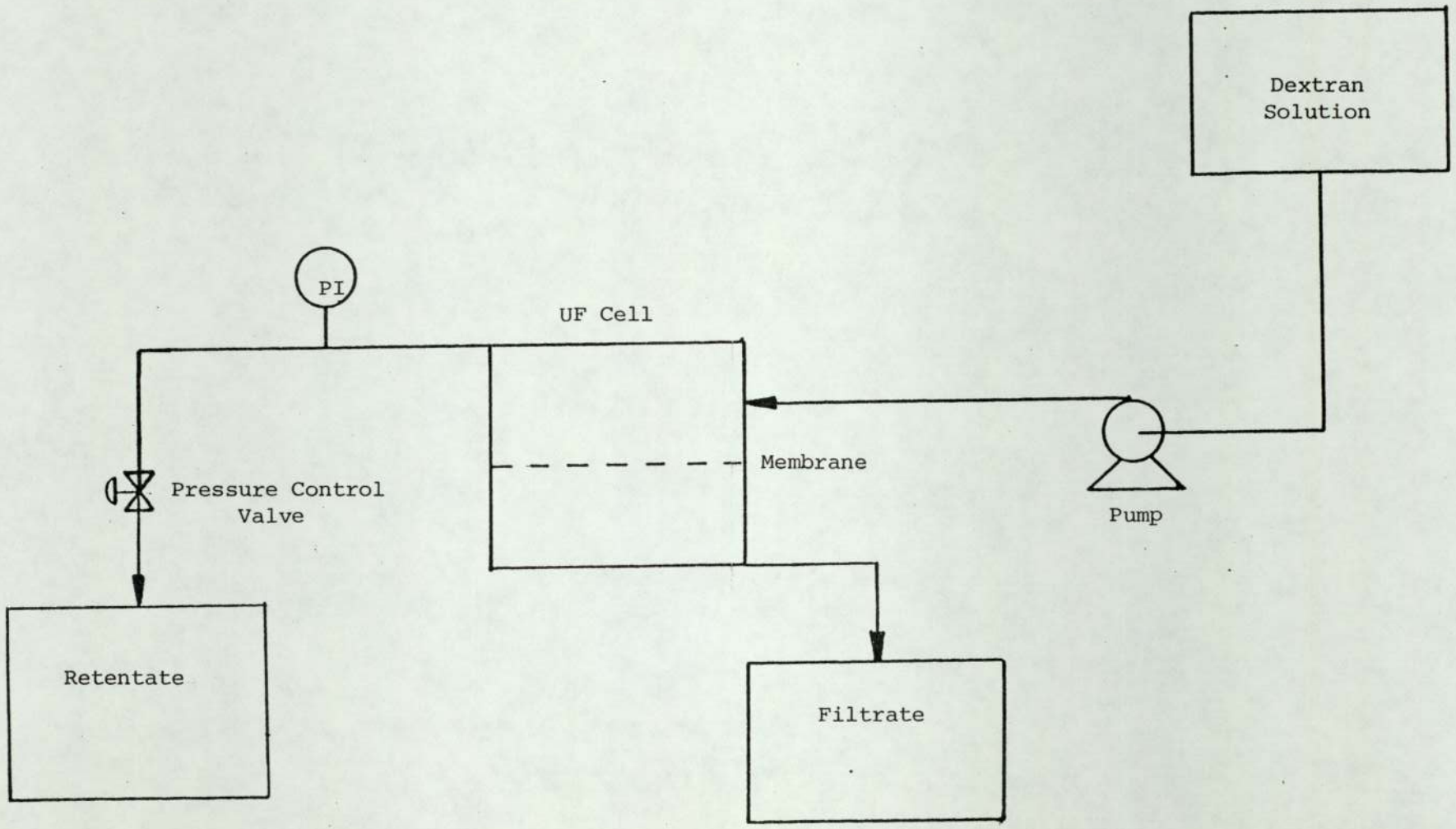


Fig. 6.2 Conditions and Results of the Initial UF Runs

Type of Membrane	Batch of Dextran Feed	Feed			Filtrate			Retentate			Rejection $\frac{\ln C_f/C_i}{\ln V_i/V_f}$
		Flowrate $cm^3 min^{-1}$	Conc. $g.l^{-1}$	$\bar{M}_w$	Flowrate $cm^3.min^{-1}$	Conc. $g.l^{-1}$	$\bar{M}_w$	Flowrate $cm^3.min^{-1}$	Conc. $g.l^{-1}$	$\bar{M}_w$	
Sartorius (Old Membrane)	B161 D40	0.01	16.6	70000	0.002	10.0	-	0.08 (at 145 psi)	17.6	-	26%
Amicon PM10*	B161 D40	0.095	13.5	70000	0.050	10.0	54000	0.045 (at 50 psi)	17.3	90000	33%
Amicon PM10	B192 B/M	0.45	15.6	35000	0.150	13.3	34000	0.300 (at 50 psi)	16.7	35500	17%
Amicon YM5**	B192 B/M	0.305	15.6	35000	0.230	1.54	-	0.075 (at 50 psi)	58.8	-	92%
Amicon YM5	B192 B/M	0.285	15.6	35000	0.230	2.50	18000	0.055 (at 50 psi)	69.1	37000	87%

* Number 10 stands for 10,000 MW cut off for globular molecules

** Number 5 stands for 5,000 MW cut off

Fig. 6.3 Silica Removal Using Ultrafiltration

Membrane	Feed Batch	Feed			Permeate			Retentate		
		Volume (cm^3)	Conc. ($g.l^{-1}$)	SiO_2 Content (ppm)	Volume (cm^3)	Conc. ($g.l^{-1}$)	SiO_2 Content (ppm)	Volume (cm^3)	Conc. ($g.l^{-1}$)	SiO_2 Content (ppm)
Amicon YM5	B192 B/M	67	15.6	44	54	2.5	42	13	69.1	49
	Dextran Removed with the: (%)				13.1			86.9		
	Silica Removed with the: (%)				78.0			22.0		

(4) The rejection of dextran by the membrane increased and the rate of filtrate decreased as the molecular weight of the dextran in the solution increased (Fig. 6.2).

(5) Silica was removed from dextran solution using ultrafiltration and it appeared that it was passing freely through the membranes (Fig. 6.3).

6.3 ULTRAFILTRATION RUNS ON AN AMICON 402 STIRRED CELL SYSTEM

An Amicon stirred cell UF system (Fig. 6.4) having a 400 cm^3 reservoir was used to determine the suitability of UF for the fractionation of dextran aqueous solutions and for the concentration of dilute dextran solutions.

Several Amicon membranes and two types of feed were used. The system was also run at ambient and 45^{o}C temperature. The conditions and the results of these runs are summarised in Fig. 6.4a to Fig. 6.9.

From these UF runs the following conclusions can be drawn:

(1) Concentration of dilute dextran solutions can be best achieved by using one of the YM5, UM10 or UM20 membranes. These membranes have high permeate flux, but they retain almost all the dextran (Figs. 6.4a, 6.4b, 6.4c).

(2) The removal of low molecular weight dextran from dextran aqueous solutions can be achieved. The YM10 and PM10 membranes are the most suitable for

Figure 6.4 The 402 Amicon UF System

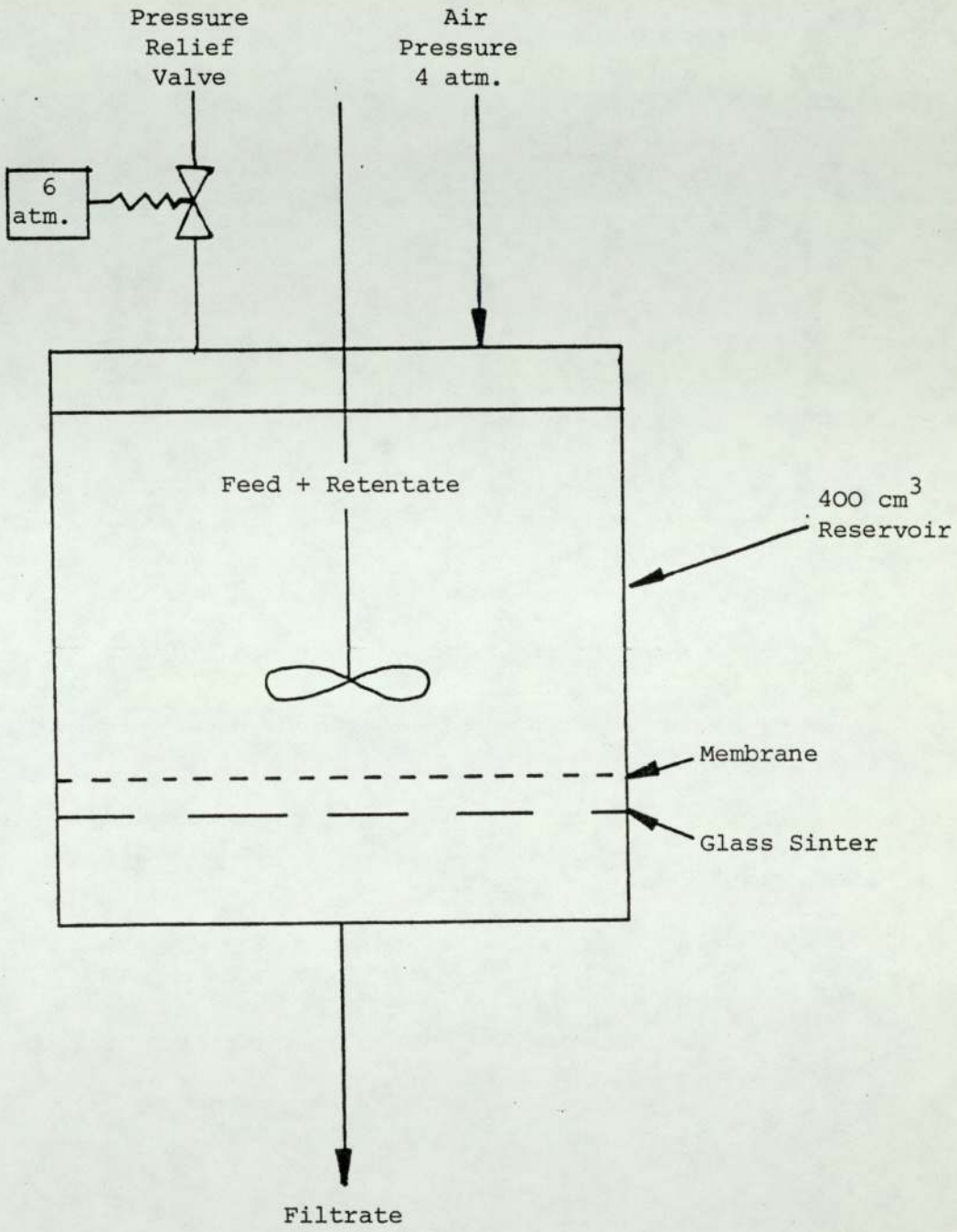


Fig. 6.4a UF Runs on an Amicon 402A Stirred Cell System

Run	1	2	3	4	5
Mode	Conc.	Conc.	Conc.	Conc.	Conc.
Membrane	UM05	UM2	UM10	UM10	UM20
Feed Batch	B192B/M	B192B/M	B192B/M	B161D40	B192B/M
Membrane Area (cm^2)	45	45	45	45	45
Pressure (atm.)	4	4	4	4	4
Initial Volume (cm^3)	400	400	400	400	400
Feed Conc. ($g.l^{-1}$)	17.14	17.14	17.14	17.14	17.14
$\bar{M}_w$ Feed	32000	32000	32000	32000	32000
$\bar{M}_N$ Feed	18000	18000	18000	24000	18000
Permeate Flow($cm^3.min^{-1}$)	0.35	1.0	1.0	1.3	1.2
Permeate Flow ($g.min^{-1}$)	<0.001	<0.001	<0.001	<0.001	0.00144
Permeate conc. ($g.l^{-1}$)	<0.1	<0.1	<0.1	<0.1	1.20
Dextran in Permeate(%)	<1.0	<1.0	<1.0	<1.0	6.7
$\bar{M}_w$ Permeate	-	-	-	-	-
$\bar{M}_N$ Permeate	-	-	-	-	-
Retentate Volume (cm^3)	45.0	40.0	42.5	35.0	32.5
Retentate Conc. ($g.l^{-1}$)	142.6	158.1	154.2	180.4	183.0
Dextran in Retentate(%)	98.0	96.6	94.7	97.9	90.8
Rejection (%)	99.1	98.5	97.6	99.1	96.2
$\bar{M}_w$ Retentate	31000	30000	31000	68000	30000
$\bar{M}_N$ Retentate	18500	18000	18000	23500	17500
$\bar{M}_w$ Residue	-	-	-	97500	-
$\bar{M}_N$ Residue	-	-	-	39000	-

Fig. 6.4b UF Runs on an Amicon 402A Stirred Cell System

Run	6	7	8	9	9A*
Mode	Conc.	Conc.	Conc.	Conc.	Conc.
Membrane	UM20	YM5	YM5	YM5	YM5
Feed Batch	B16D40	B192B/M	B161D40	B161D40	B161D40
Membrane Area (cm^2)	45	45	45	45	45
Pressure (atm)	4	4	4	4	4
Initial Volume (cm^3)	400	400	400	400	400
Feed Conc. ($g.l^{-1}$)	16.11	17.30	16.11	17.70	18.60
$\bar{M}_w$ Feed	71500	34000	71500	69000	69000
$\bar{M}_N$ Feed	24000	18000	24000	22500	22500
Permeate Flow ($cm^3.min^{-1}$)	1.35	1.0	1.43	1.2	1.2
Permeate Flow ($g.min^{-1}$)	0.00161	0.00050	0.00044	0.00055	0.00076
Permeate Conc. ($g.l^{-1}$)	1.19	0.50	0.31	0.46	0.64
Dextran in Permeate (%)	6.7	2.6	1.8	2.3	3.1
$\bar{M}_w$ Permeate	28500	-	14000	15500	14500
$\bar{M}_N$ Permeate	14000	-	9000	10500	10500
Retentate Volume (cm^3)	36.0	32.0	35.0	35.0	37.0
Retentate Conc.($g.l^{-1}$)	159.0	208.7	158.5	180.0	176.0
Dextran in Retentate(%)	89.5	96.5	86.7	89.0	87.5
Rejection (%)	95.4	98.6	94.1	95.2	94.4
$\bar{M}_w$ Retentate	68000	34000	67000	65000	64000
$\bar{M}_N$ Retentate	24000	18000	23500	22500	22000
$\bar{M}_w$ Residue	83500	-	88000	-	88000
$\bar{M}_N$ Residue	30500	-	29500	-	26000

Fig. 6.4c UF Runs on an Amicon 402A Stirred Cell System

Run	10	10A*	10B*	10C*	10D*
Mode	Conc.	Conc.	Conc.	Conc.	Conc.
Membrane	YM5	YM5	YM5	YM5	YM5
Feed Bach	B161D40	B161D40	B161D40	B161D40	B161D40
Membrane Area (cm^2)	45	45	45	45	45
Pressure (atm)	4	4	4	4	4
Initial Volume (cm^3)	400	400	400	400	400
Feed Conc. ($g.l^{-1}$)	19.0	19.0	19.0	19.0	19.0
$\bar{M}_w$ Feed	68500	68500	68500	68500	68500
$\bar{M}_N$ Feed	23000	23000	23000	23000	23000
Permeate Flow ($cm^3.min^{-1}$)	1.33	1.17	1.06	1.10	1.30
Permeate FLow ($g.min^{-1}$)	0.00031	0.00036	0.00033	0.00033	0.00048
Permeate Conc. ($g.l^{-1}$)	0.23	0.31	0.31	0.31	0.35
Dextran in Permeate (%)	1.3	1.5	1.5	1.5	1.5
$\bar{M}_w$ Permeate	14000	14500	15000	14500	14500
$\bar{M}_N$ Permeate	9000	9500	10000	9500	8500
Retentate Volume (cm^3)	40.0	37.5	40.0	37.5	47.5
Retentate Conc. ($g.l^{-1}$)	169.0	193.0	189.6	186.0	158.0
Dextran in Retentate(%)	88.9	95.2	99.7	92.1	89.0
Rejection (%)	95.0	97.9	99.8	96.4	99.4
$\bar{M}_w$ Retentate	64500	63500	63500	62500	63500
$\bar{M}_N$ Retentate	21500	21000	21500	20500	21000
$\bar{M}_w$ Residue	-	-	-	-	82000
$\bar{M}_N$ Residue	-	-	-	-	24000

Fig. 6.4d UF Runs on an Amicon 402A Stirred Cell System

Run	11	12	13	14	15
Mode	Conc..at 45°C	Conc.	Conc.	Conc.	Conc.
Membrane	YM5	YM10	YM10	PM10	PM10
Feed Batch	B161D40+	B192B/M	B161D40+	B161D40	B161D40
Membrane Area (cm^2)	45	45	45	45	45
Pressure (atm)	4	4	4	4	4
Initial Volume (cm^3)	400	400	400	400	400
Feed Conc. ($g.l^{-1}$)	21.50	16.40	18.00	16.11	16.11
$\bar{M}_w$ Feed	50000	35000	50000	71500	71500
$\bar{M}_N$ Feed	16000	20000	16000	24000	24000
Permeate Flow ($cm^3.min^{-1}$)	1.8	1.4	1.5	1.45	1.45
Permeate Flow ($g.min^{-1}$)	0.0047	0.0061	0.0084	0.0074	0.0067
Permeate Conc. ($g.l^{-1}$)	3.07	5.08	6.00	5.10	4.60
Dextran in Permeate (%)	14.0	29.1	30.6	27.7	25.9
$\bar{M}_w$ Permeate	16500	22000	17500	36500	36500
$\bar{M}_N$ Permeate	10000	11000	10500	16000	16000
Retentate Volume (cm^3)	20.0	22.5	27.0	37.0	37.0
Retentate Conc. ($g.l^{-1}$)	348.4	177.2	156.0	115.0	64.1
Dextran in Retentate (%)	81.4	60.8	58.6	66.0	64.1
Rejection (%)	93.0	82.8	81.1	82.6	81.0
$\bar{M}_w$ Retentate	56000	38000	62000	82000	77000
$\bar{M}_N$ Retentate	20000	22500	18000	28000	26500
$\bar{M}_w$ Residue	69000	-	90000	-	101000
$\bar{M}_N$ Residue	25500	-	31500	-	38000

Fig. 6.4e UF Runs on an Amicon 402A Stirred Cell System

Run	15	16	17	18	19
Mode	Conc.	Conc.	Conc.	Conc.	Conc.
Membrane	PM10	PM30	PM30	XM50	XM100
Feed Batch	B192B/M	B192B/M	B161D40	B192B/M	B161D40
Membrane Area (cm^2)	45	45	45	45	45
Pressure (atm)	4	4	4	4	4
Initial Volume (cm^3)	400	400	400	400	400
Feed Conc. ($g.l^{-1}$)	17.30	17.14	16.22	16.37	17.14
$\bar{M}_w$ Feed	34000	32000	69000	32000	69000
$\bar{M}_N$ Feed	19000	18000	23000	18000	23000
Permeate Flow ($cm^3.min^{-1}$)	1.3	1.5	2.0	1.5	1.5
Permeate Flow ($g.min^{-1}$)	0.0039	0.0085	0.014	0.0023	0.0064
Permeate Conc. ($g.l^{-1}$)	3.20	5.70	7.00	1.54	4.27
Dextran in Permeate(%)	15.8	32.0	39.8	8.6	22.6
$\bar{M}_w$ Permeate	24000	25000	47000	25500	30000
$\bar{M}_N$ Permeate	14000	14000	20000	11500	10500
Retentate Volume (cm^3)	45	32.5	32.0	32.5	37.5
Retentate Conc. ($g.l^{-1}$)	132.8	133.5	120.0	185.0	139.1
Dextran in Retentate(%)	85.2	66.3	59.2	91.8	76.1
Rejection (%)	93.0	82.0	79.0	96.0	88.0
$\bar{M}_w$ Retentate	35500	35000	83000	32000	71000
$\bar{M}_N$ Retentate	19500	20500	27000	19500	26000
$\bar{M}_w$ Residue	-	-	113000	-	-
$\bar{M}_N$ Residue	-	-	32000	-	-

Fig. 6.4f UF Runs on an Amicon 402A Stirred Cell System

Run	20
Mode	Conc.
Membrane	XM300A
Feed Batch	B161D40
Membrane Area (cm^2)	45
Pressure (atm)	4
Initial Volume (cm^3)	400
Feed Conc. ($g.l^{-1}$)	19.32
$\bar{M}_w$ Feed	70000
$\bar{M}_N$ Feed	24000
Permeate Flow ($cm^3.min^{-1}$)	1.5
Permeate Flow ($g.min^{-1}$)	0.0116
Permeate Conc. ($g.l^{-1}$)	7.73
Dextran in Permeate (%)	36.5
$\bar{M}_w$ Permeate	69000
$\bar{M}_N$ Permeate	22500
Retentate Volume (cm^3)	35.0
Retentate Conc. ($g.l^{-1}$)	138.2
Dextran in Retentate (%)	62.7
Rejection (%)	80.0
$\bar{M}_w$ Retentate	71000
$\bar{M}_N$ Retentate	24500
$\bar{M}_w$ Residue	-
$\bar{M}_N$ Residue	-

* In these runs the membrane was not cleaned after the previous run

\+ The same batch number feed but from another container. It had a different molecular weight from the other containers feed. Refer also to K. England's thesis (46)

Fig. 6.5 The Effect of Dextran Concentration on the Permeate Rates for a UM10 Membrane (Run 4)

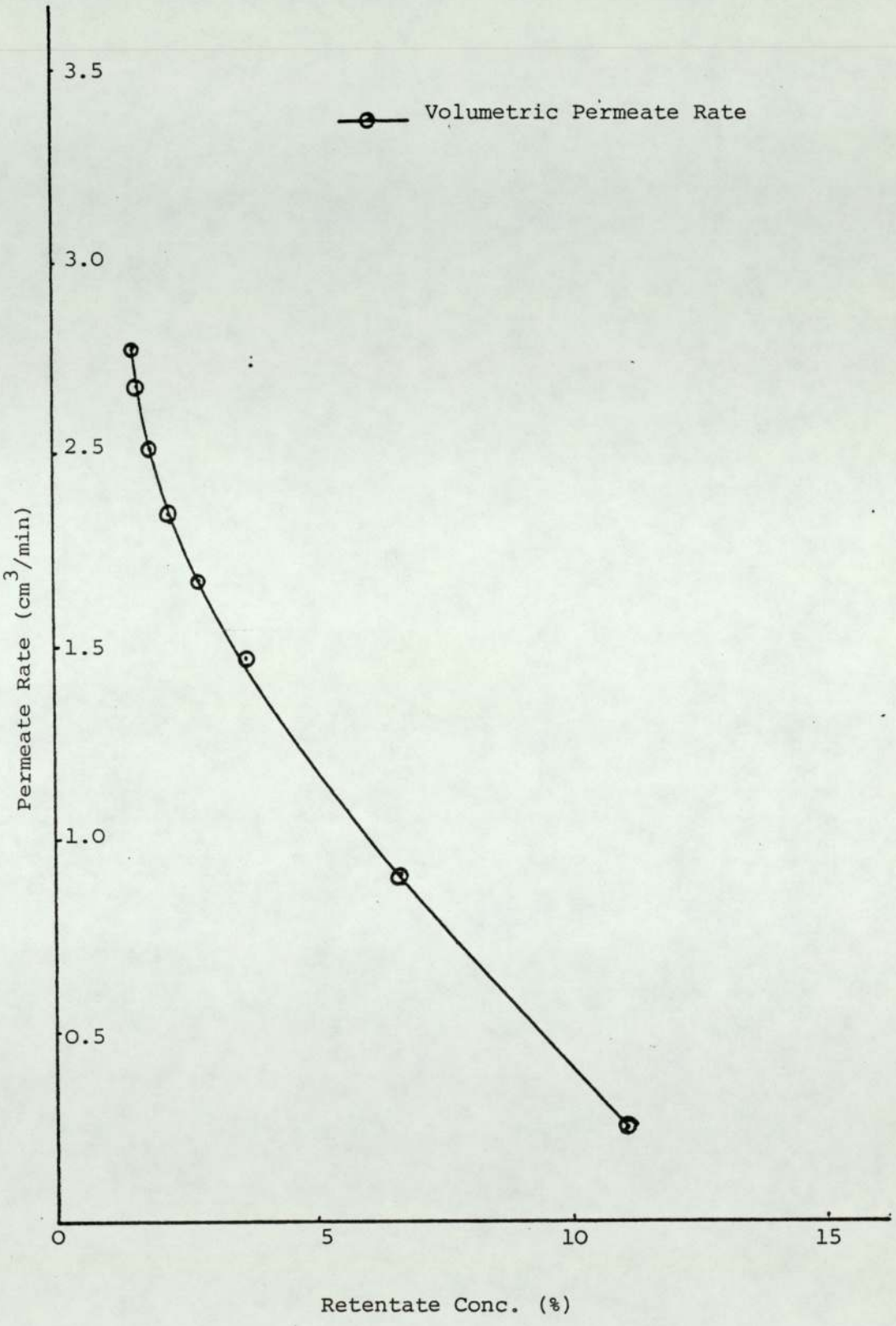


Fig. 6.6 The Effect of Dextran Concentration on the Permeate Rates for a UM20 Membrane (Run 6)

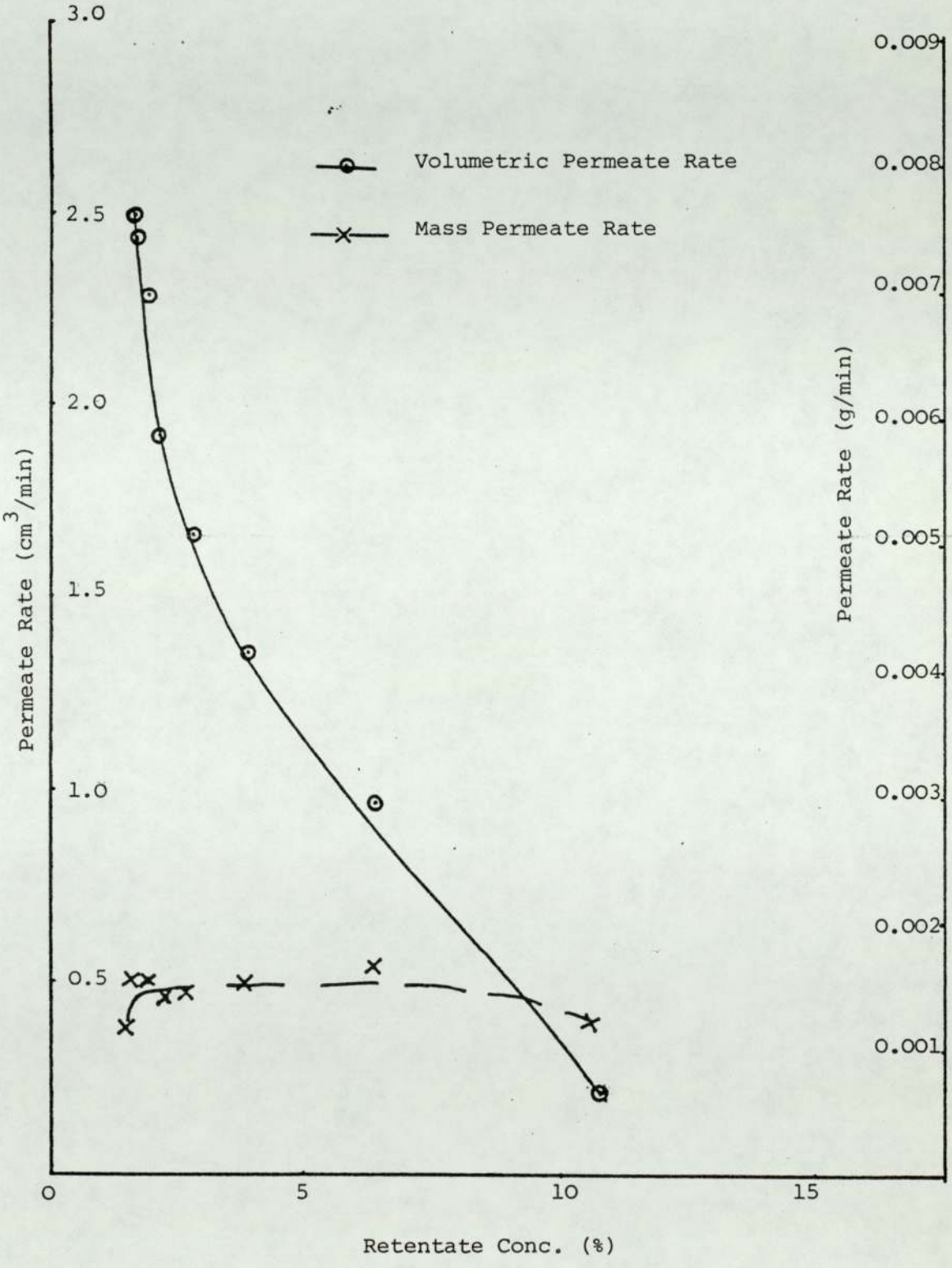


Fig. 6.7 The Effect of Dextran Concentration on the Permeate Rates for a YM5 Membrane at 20°C and 45°C (Run 8 and Run 11)

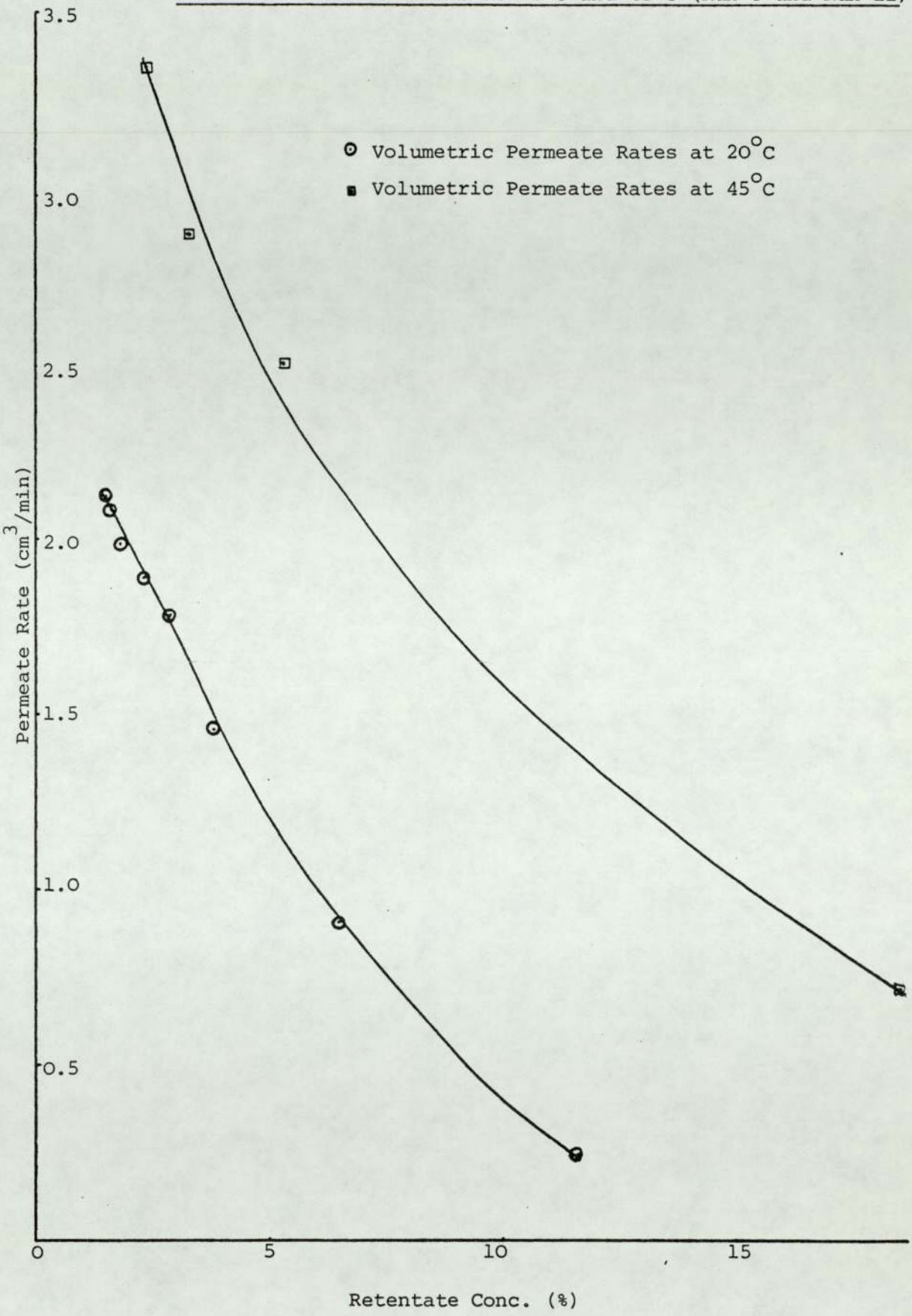


Fig. 6.8 The Effect of Dextran Concentration on the Permeate Rates for a YM10 Membrane (Run 13)

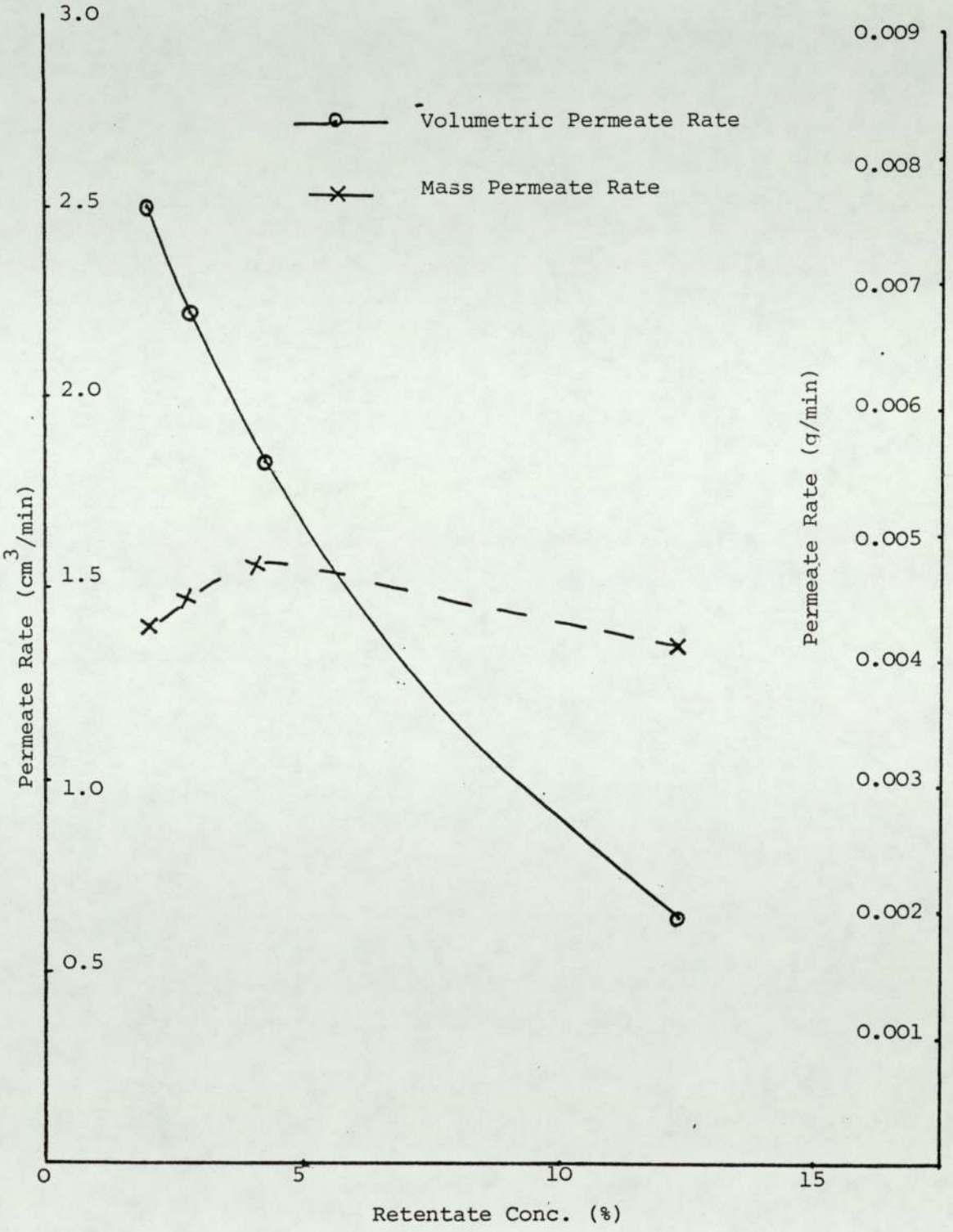
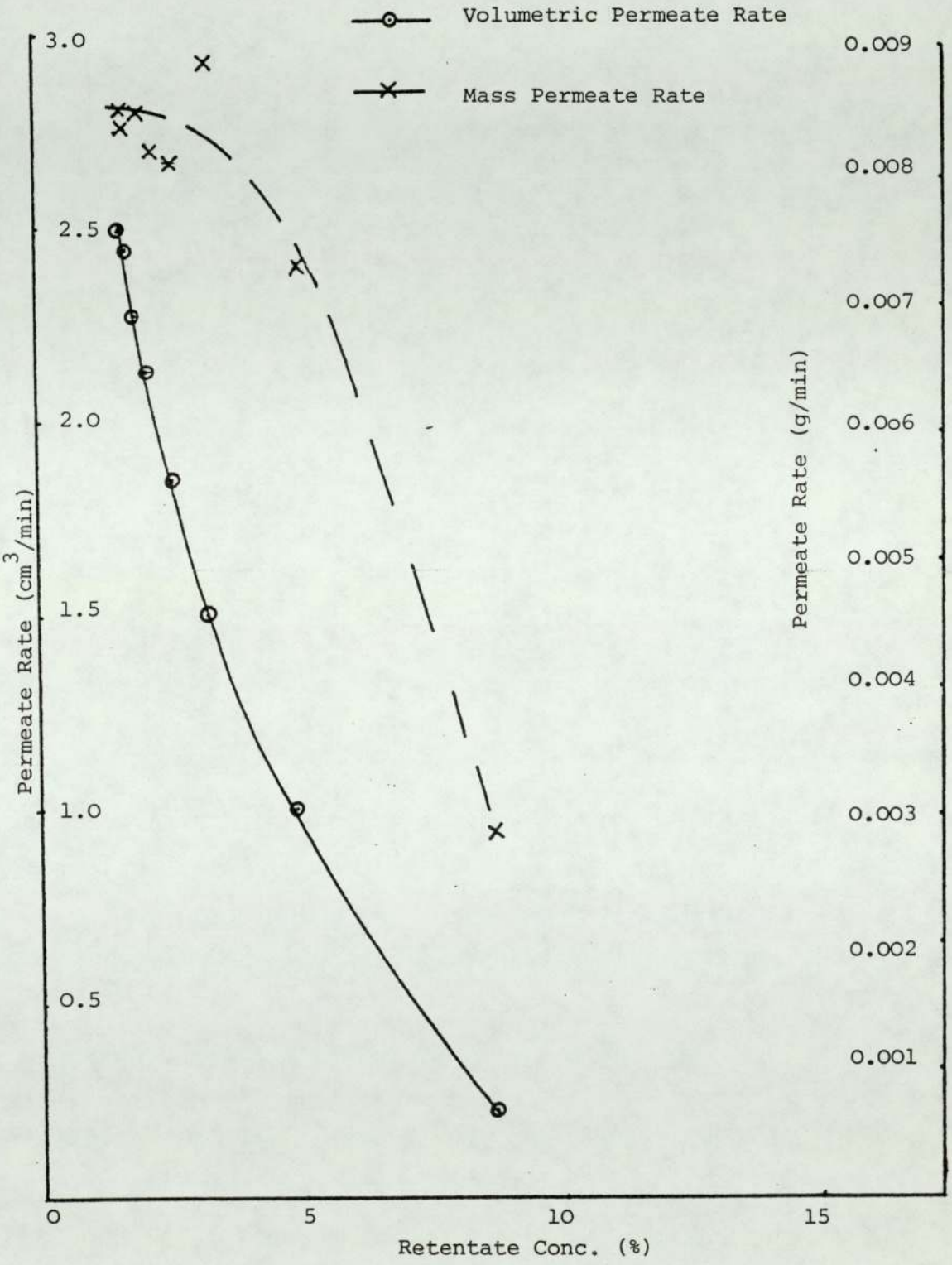


Fig. 6.9 The Effect of Dextran Concentration on the Permeate Rates for a PM10 Membrane (Run 14)



this operation (Fig. 6.4d). But removal of high molecular weight dextran from dextran solutions cannot be done efficiently (Figs. 6.4e, 6.4f).

(3) The very high molecular weight dextran seems to form a film on the surface of the membrane during the UF runs on this stirred cell system (Figs. 6.4a to 6.4e)

(4) Although the dextran feed batch B192B/M has lower average molecular weight ($\bar{M}_w$) than that of the B161D40 batch, the second contains more very low molecular weight material; hence using the same membrane and applying similar conditions, more dextran material is removed by passing through the membrane from the B161D40 feed, than the B192B/M feed (Figs. 6.4a to 6.4e).

(5) The permeate flow increases considerably as the operating temperature increases from ambient to 45^{o}C, but at the same time more and larger dextran molecules pass through the membrane (Figs. 6.4b to 6.4d, 6.7). Also higher concentrations can be achieved at higher temperatures (Fig. 6.4d). The above phenomena are due to the lower viscosity of the retentate at elevated temperatures and may be due to the thermal expansion of the pores of the membrane.

(6) The permeate volumetric rate decreases asymptotically as the concentration of retentate increases, but the mass rate of dextran through the membrane initially increases then reaches a maximum and

suddenly drops to very low values, probably because the low molecular weight material in the retentate is depleted (Figs. 6.5 to 6.9).

(7) The membranes performance is reproducible (Figs. 6.4b to 6.4d) and their life-time is long. More than ten UF runs were contacted on a YM5 membrane without any detrimental effects. Membranes can be cleaned effectively using 0.1M NaOH solution and can be stored in a 0.05% solutions of sodium azide.

6.4 ULTRAFILTRATION RUNS ON AN AMICON DC2A HOLLOW FIBRE CARTRIDGE SYSTEM

An Amicon hollow fibre cartridge UF system (Figs. 6.10 and 6.11) having a 2000 cm^3 reservoir was used to evaluate the suitability of such a system in the concentration and fractionation of dextran solutions and the removal of silica dissolved in these solutions.

As in the case of the Amicon 402 stirred cell several hollow fibre cartridges have been used with the DC2A system. Also two types of feed have been used.

The DC2A system was initially used in concentration mode (Fig. 6.10), but because at higher concentrations the membrane selectivity decreases (31) and because it is necessary to have high membrane selectivity for dextran fractionations, it was decided that the dextran fractionations should take place under dilute conditions. Hence the DC2A system was operated initially under diafiltration model (Fig. 6.11) (Section 2.8.5.2) until

Fig. 6.10 The Amicon DC2A UF System. Concentration Mode

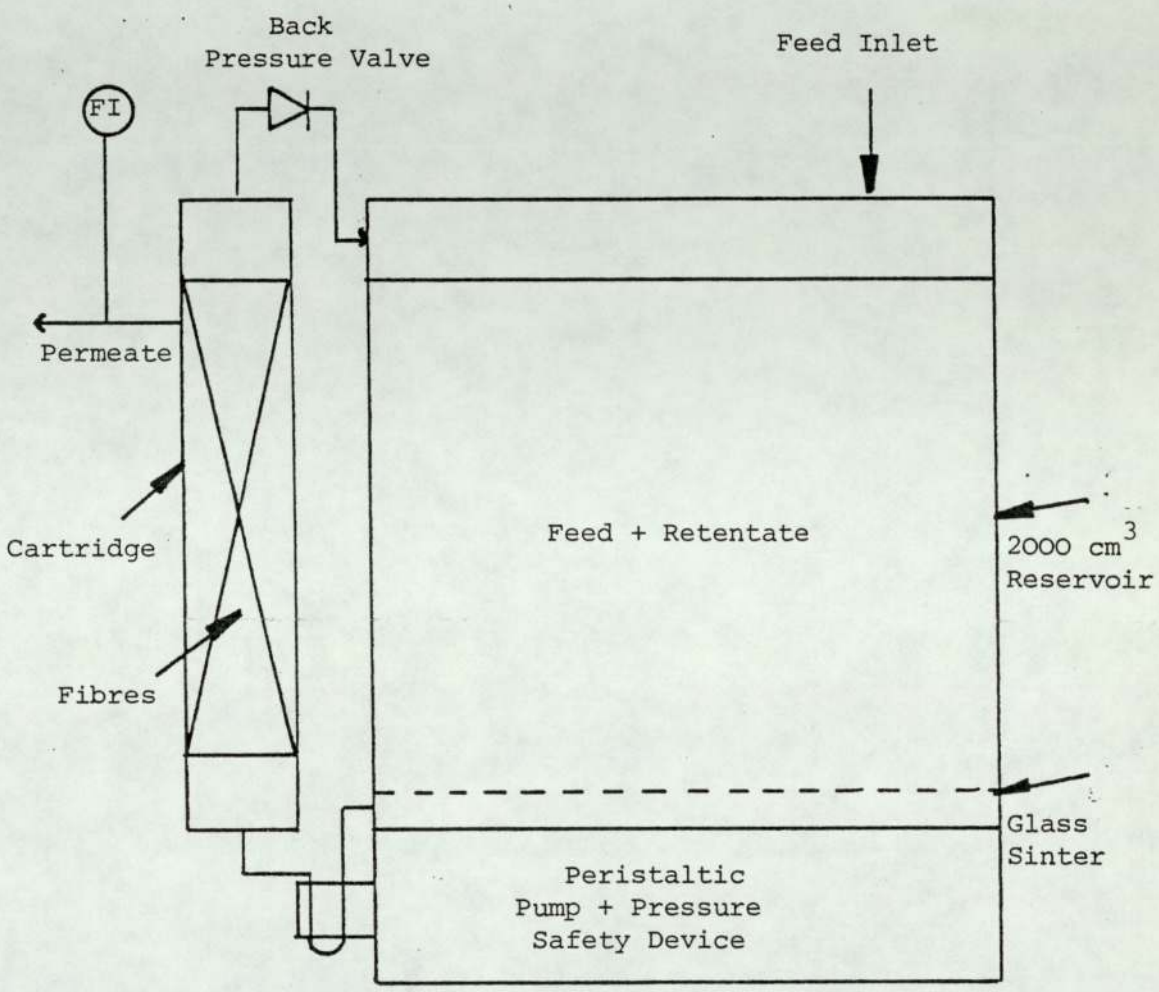
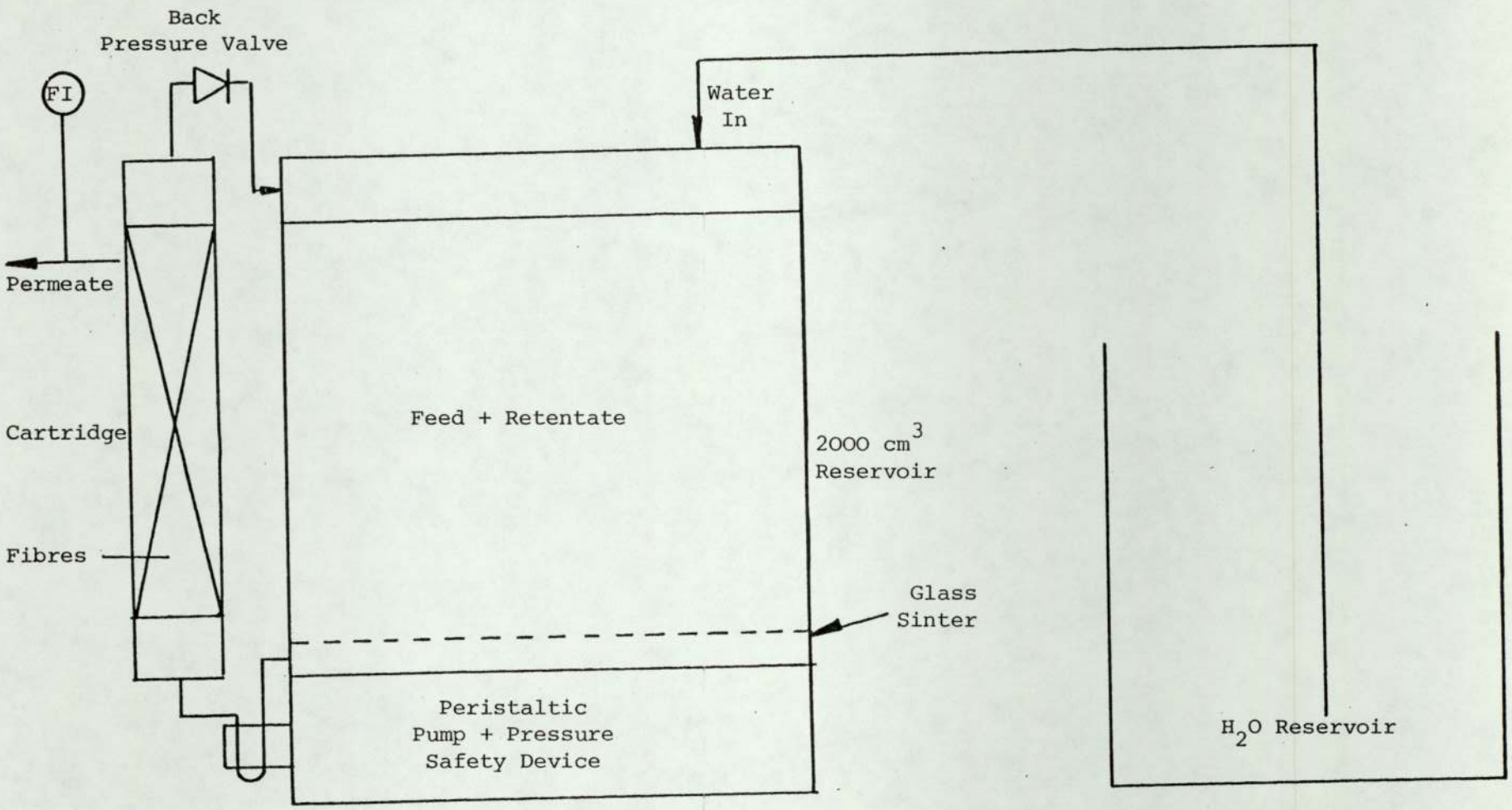


Fig. 6.11 The Amicon DC2A UF System. Diafiltration Mode



almost all of the material required to be removed has been removed and then it was operated under concentration mode conditions to concentrate the retentate.

The conditions and results of the UF runs on the DC2A system are summarised in Fig. 6.12 to Fig. 6.19.

The results of these runs have shown that concentration of dilute dextran solutions can be best achieved by using an H1P2 cartridge since most of the dextran molecules cannot pass through this fibre's pores (Fig. 6.12a).

The removal of low molecular weight dextran from dextran solutions can be carried out successfully using an H1P5 cartridge (Figs. 6.12b to 6.12e). Also removal of high molecular weight dextran can be achieved from dextran solutions using an H1P100 cartridge (Figs. 6.12e, 6.12f), but the results show that UF is less efficient than GPC (Fig. 4.9, Run 1) in removing high molecular weight dextran.

For the removal of low molecular weight dextran the diafiltration/concentration mode operation of the DC2A system is preferred to the concentration mode operation, since it removes much more low molecular weight material and hence enhances the separation (Figs. 6.12a, 6.12d, 6.12e).

As the concentration of retentate increases the permeate volumetric flow decreases assymptotically, but the mass rate of dextran through the membrane initially increases then reaches a maximum and suddenly drops to very low values, probably because the low molecular weight material in the retentate is depleted (Figs. 6.13, 6.14).

Fig. 6.12a UF Runs on a Amicon DC2A Hollow Fibre System

Run	1	2	3	4	5
Mode	Conc.	Conc.	Diafilt. & Conc.	Diafilt. & Conc.	Diafilt. & Conc.
Cartridge	H1P2	H1P2	H1P2	H1P2	H1P2
Feed Batch	B161D40	B161D40	B161D40	B161D40+	84% B161D40+ 16% D20
Membrane Area(cm^2)	300	300	300	300	300
Pressure (atm)	1	1	1	1	1
Initial Volume (cm^3) Water Volume (cm^3)	2000	2000	2000 6000	2000 12000	2000 6000
Feed Conc. ($g.l^{-1}$)	18.05	16.22	19.00	18.00	17.04
$\bar{M}_w$ Feed	70000	69000	68500	50000	46000
$\bar{M}_N$ Feed	24000	23500	23000	17000	16000
Permeate Flow ($cm^3.min^{-1}$)	5.0	5.1	6.8	7.1	6.4
Dextran in Filtrate(%)	4.1	5.0	12.4	22.5	15.1
$\bar{M}_w$ Permeate	11000	14000	21000	16000	15000
$\bar{M}_N$ Permeate	7500	9000	12000	10000	9500
Final Volume (cm^3)	230	195	230	200	200
Retentate Conc.($g.l^{-1}$)	149.7	148.0	134.0	140.6	138.0
Dextran in Retentate(%)	95.4	89.0	81.4	77.5	81.0
Rejection (%)	97.8	95.0	90.3	89.2	90.0
$\bar{M}_w$ Retentate	73000	75000	73000	57000	56000
$\bar{M}_N$ Retentate	25000	25500	26000	22000	21000

Fig. 6.12b UF Runs on an Amicon DC2A Hollow FIbre System

Run	6	7	8	9	10
Mode	Conc.	Conc.	Conc.	Conc.	Conc.
Cartridge	H1P5	H1P5	H1P5	H1P5	H1P5
Feed Batch	B192B/M	B192B/M	B161D40	B161D40	B161D40+
Membrane Area (cm^2)	600	600	600	600	600
Pressure (atm)	1	1	1	1	1
Initial Volume (cm^3) Water Volume (cm^3)	2000	2000	2000	2000	2000
Feed Conc. ($g.l^{-1}$)	17.30	16.37	16.22	19.00	67.31
$\bar{M}_w$ Feed	34000	35000	69000	69000	50000
$\bar{M}_N$ Feed	19500	20000	22000	22500	18000
Permeate Flow ($cm^3.min^{-1}$)	10	13	15	13	2.1
Dextran in Filtrate(%)	13.3	30.2	15.4	14.8	16.0
$\bar{M}_w$ Permeate	20000	26000	26000	28000	18000
$\bar{M}_N$ Permeate	12000	14500	13000	14000	9500
Final Volume (cm^3)	225	165	200	240	430
Retentate Conc.($g.l^{-1}$)	132.4	120.0	131.0	128.0	193.0
Dextran in Retentate(%)	86.1	64.3	80.8	80.8	62
Rejection (%)	93.1	80.0	91.0	90.0	68.5
$\bar{M}_w$ Retentate	35500	41000	80000	75000	59500
$\bar{M}_N$ Retentate	22000	24000	27000	27500	23000

Fig. 6.12c UF Runs on an Amicon DC2A Hollow Fibre System

Run	11	11A*	11B*	11C*	11D*
Mode	Conc.	Conc.	Conc.	Conc.	Conc.
Cartridge	H1P5	H1P5	H1P5	H1P5	H1P5
Feed Batch	B161D40+	B161D40+	B161D40+	B161D40+	B161D40+
Membrane Area (cm^2)	600	600	600	600	600
Pressure (atm.)	1	1	1	1	1
Initial Volume (cm^3) Water Volume (cm^3)	2000	2000	2000	2000	2000
Feed Conc. ($g.l^{-1}$)	19.51	19.51	19.51	19.51	19.51
$\bar{M}_w$ Feed	50000	50000	50000	50000	50000
$\bar{M}_N$ Feed	17000	17000	17000	17000	17000
Permeate Flow($cm^3.min^{-1}$)	14.8	12.7	12.0	13.3	13.2
Dextran in Filtrate (%)	15.4	16.7	12.3	13.1	12.6
$\bar{M}_w$ Permeate	20500	20000	20500	19000	18500
$\bar{M}_N$ Permeate	11000	11000	11500	11000	11000
Final Volume (cm^3)	220	200	210	215	215
Retentate Conc.($g.l^{-1}$)	131.0	146.3	141.3	159.8	140.5
Dextran in Retentate(%)	74.4	75.2	77.0	83.0	77.5
Rejection (%)	86.3	87.5	87.8	93.3	88.5
$\bar{M}_w$ Retentate	55500	56500	58000	58000	57000
$\bar{M}_N$ Retentate	21000	21000	22000	22000	21500

Fig. 6.12d UF Runs on an Amicon DC2A Hollow Fibre System

Run	12	12A*	12B*	13	14
Mode	Conc.	Conc.	Conc.	Conc.	Diafilt. & Conc.
Cartridge	H1P5	H1P5	H1P5	H1P5	H1P5
Feed Batch	B161D40	B161D40	B161D40	B161D40	B161D40
Membrane Area (cm^2)	600	600	600	600	600
Pressure (atm)	1	1	1	1	1
Initial Volume (cm^3) Water Volume (cm^3)	2000	2000	2000	800	2000 5000
Feed Conc. ($g.l^{-1}$)	17.7	18.0	22.0	128.0	17.7
$\bar{M}_w$ Feed	69000	69000	69000	69500	69500
$\bar{M}_N$ Feed	21500	21500	21500	21500	21500
Permeate Flow($cm^3.min^{-1}$)	14.0	12.0	10.0	0.78	23.5
Dextran in Filtrate(%)	25.9	24.3	20.0	13.7	32.5
$\bar{M}_w$ Permeate	28000	28000	26000	33000	32500
$\bar{M}_N$ Permeate	13500	13000	13500	17500	15000
Final Volume (cm^3)	200	195	220	460	200
Retentate Conc. ($g.l^{-1}$)	130.0	138.0	150.0	181.1	109.0
Dextran in Retentate(%)	73.5	76.0	75.0	81.3	61.6
Rejection (%)	86.6	88.0	87.0	63.0	79.0
$\bar{M}_w$ Retentate	78500	76000	75000	78000	95000
$\bar{M}_N$ Retentate	28000	27500	27000	29500	36000

Fig. 6.12e UF Runs on an Amicon DC2A Hollow Fibre System

Run	15	16	17	18	19
Mode	Diafilt. & Conc.	Diafilt. & Conc.	Diafilt. & Conc.	Conc.	Conc.
Cartridge	H1P5	H1P5	H1P5	H1X50	H1P100
Feed Batch	B161D40+	B161D40+	84% B161D40+ 16% D20	B161D40	B161D40
Membrane Area (cm^2)	600	600	600	600	600
Pressure (atm)	1	1	1	1	1
Initial Volume (cm^3) Water Volume (cm^3)	2000 18750	2000 28000	2000 5000	3000	2000
Feed Conc. ($g.l^{-1}$)	61.02	18.00	17.04	19.32	19.32
$\bar{M}_w$ Feed	51500	51500	46000	69000	70000
$\bar{M}_N$ Feed	17000	17000	16000	22000	23000
Permeate Flow($cm^3.min^{-1}$)	13.7	30.3	25.2	4.5	32.0
Dextran in Filtrate(%)	38.5	57.0	41.9	40.0	85.1
$\bar{M}_w$ permeate	22000	33500	22000	48000	60500
$\bar{M}_N$ Permeate	13000	19500	12500	20000	22500
Final Volume (cm^3)	760	160	195	175	100
Retentate Conc.($g.l^{-1}$)	99.8	97.1	101.3	196.5	55.0
Dextran in Retentate(%)	62.0	43.0	58.0	59.0	14.2
Rejection (%)	50.8	66.7	76.6	81.6	35.0
$\bar{M}_w$ Retentate	78000	91000	67000	98000	129500
$\bar{M}_N$ Retentate	29000	39000	23500	31000	28500

Fig. 6.12f UF Runs on an Amicon DC2A Hollow Fibre System

Run	20
Mode	Conc.
Cartridge	H1P100
Feed Batch	B161D40
Membrane Area (cm^2)	600
Pressure (atm)	1
Initial Volume (cm^3) Water Volume (cm^3)	2000
Feed Conc. ($g.l^{-1}$)	16.6
$\bar{M}_w$ Feed	70000
$\bar{M}_N$ Feed	21500
Permeate Flow($cm^3.min^{-1}$)	43.0
Dextran in Filtrate(%)	74.7
$\bar{M}_w$ Permeate	58000
$\bar{M}_N$ Permeate	18000
Final Volume (cm^3)	150
Retentate Conc. ($g.l^{-1}$)	41.4
Dextran in Retentate(%)	18.7
Rejection (%)	35.0
$\bar{M}_w$ Retentate	134000
$\bar{M}_N$ Retentate	30500

* In this runs the membrane was not cleaned from the previous run

\+ The same batch number feed but from another container. It had a different molecular weight from the other containers feed. Refer also to K. England (46)

Fig. 6.13 The Effect of Dextran Concentration on the Permeate Rates for H1P2 and H1P5 Hollow Fibre Cartridges (Runs 2 & 8)

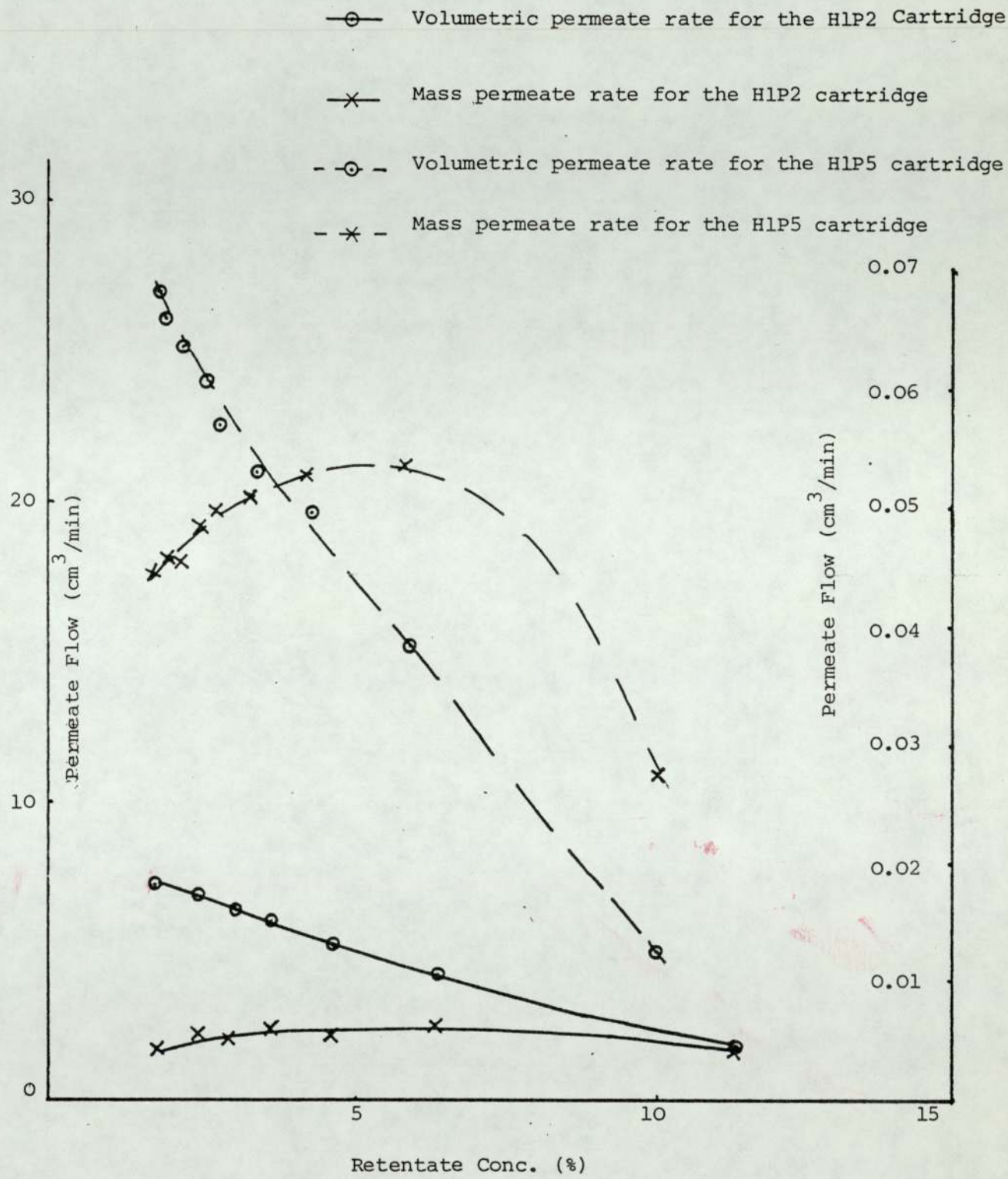


Fig. 6.14 The Effect of Dextran Concentration on the Permeate Rate for an H1P5 Hollow Fibre Cartridge

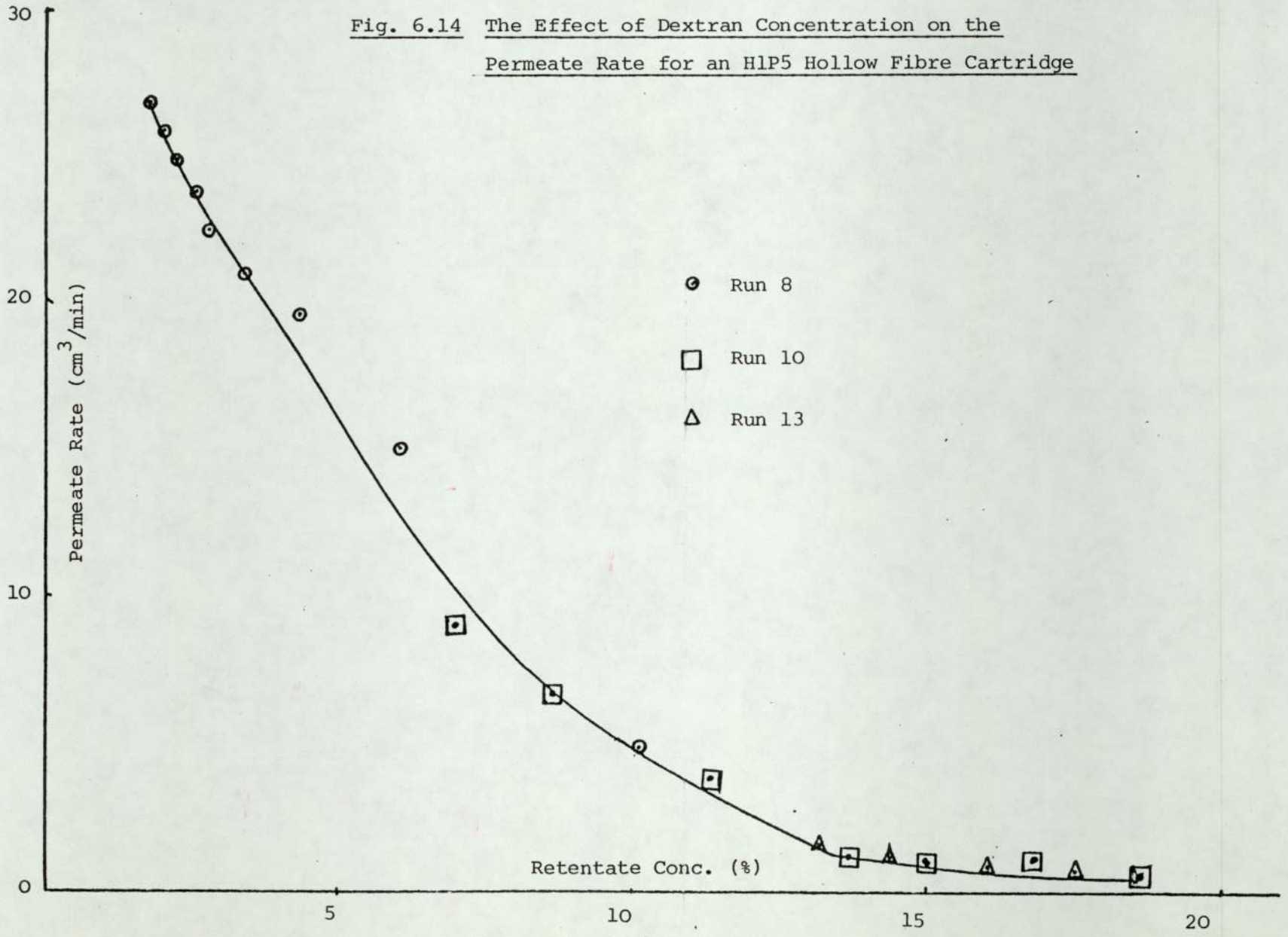


Fig. 6.15 The Diafiltration Stage of Run 4; Results; H1P2 Cartridge

Volume of Permeate / Volume of Feed	Retentate Rate (cm^3/min)	Conc. of Retentate (%)	$\bar{M}_w$ Retentate	$\bar{M}_N$ Retentate	Permeate Flow (cm^3/min)	Permeate Conc. (%)	Feed in the Permeate (%)	$\bar{M}_w$ Permeate	$\bar{M}_N$ Permeate
0	1950	1.800	50000	17000	-	-	-	-	-
1	1950	1.625	51500	18000	8.33	0.165	9.1	10000	7000
2	1950	1.548	53500	19000	7.85	0.093	14.3	13000	8000
3	1950	1.491	54000	195000	7.83	0.050	17.1	14000	8500
4	1950	1.460	54500	20000	7.94	0.028	18.6	17000	10000
5	1950	1.451	54500	20000	8.33	0.016	19.4	20000	12000
6	1950	1.448	55000	20500	7.97	0.010	20.0	30500	16000

Fig. 6.16 Dextran Passing through the H1P2 Cartridge During the Diafiltration Stage of Run 4

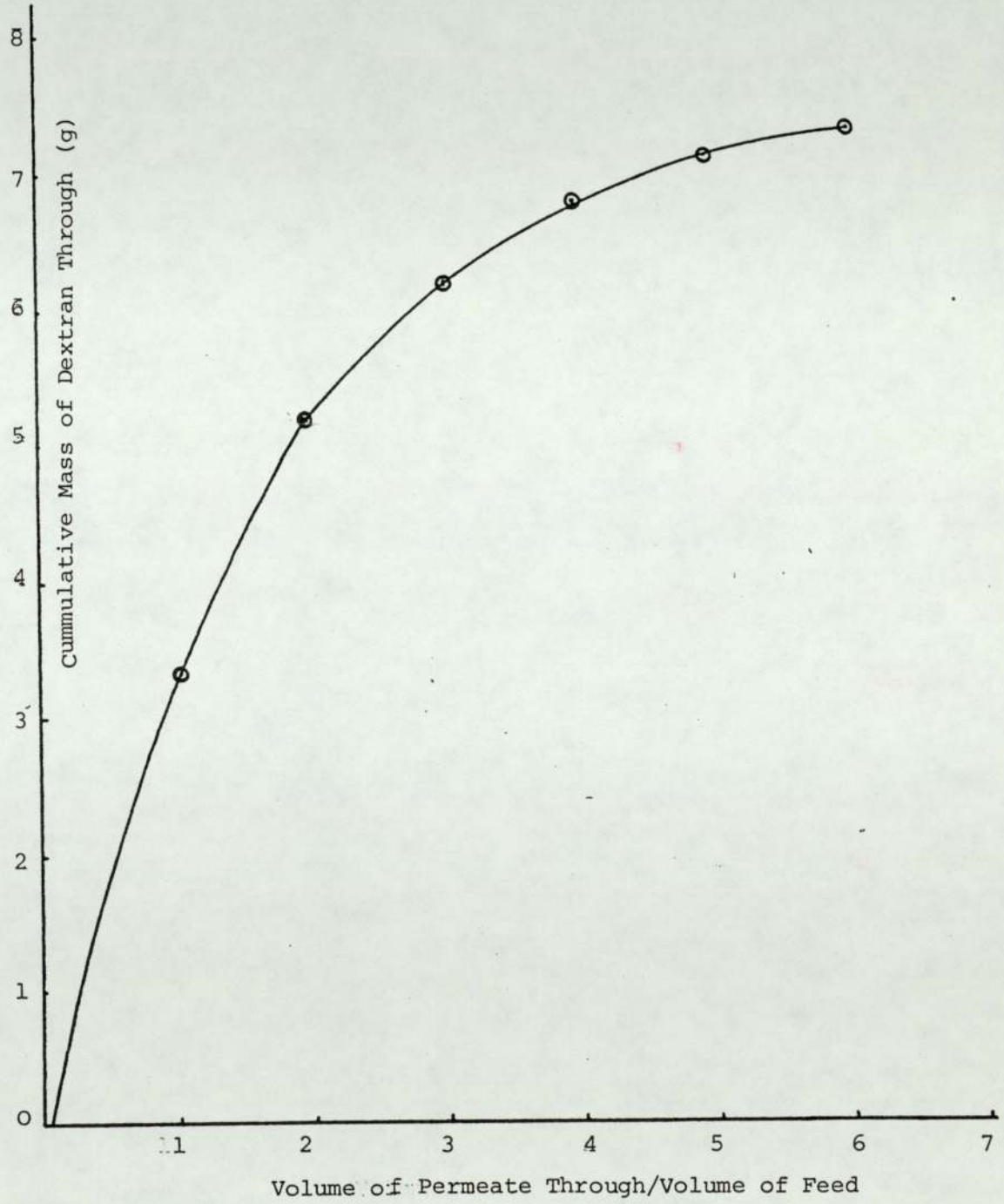


Fig. 6.17 The Diafiltration Stage of Run 16; Results; H1P5 Cartridge

Volume of Permeate / Volume of Feed	Retentate Rate (cm^3/min)	Conc. of Retentate (%)	$\bar{M}_W$ Retentate	$\bar{M}_N$ Retentate	Permeate Flow (cm^3/min)	Permeate Conc. (%)	Feed in Permeate (%)	$\bar{M}_W$ Permeate	$\bar{M}_N$ Permeate
0	1950	1.800	51500	17000	-	-	-	-	-
1	1950	1.558	58000	18000	30.3	0.242	13.4	24000	13000
2	1950	1.381	62000	19000	29.4	0.177	23.3	27000	15000
3	1950	1.231	64500	20000	29.9	0.130	30.5	27000	15500
4	1950	1.148	68500	21500	29.9	0.103	36.2	27000	15500
5	1950	1.076	71000	22500	30.8	0.072	40.2	27000	15500
6	1950	1.015	73500	23500	30.3	0.061	43.6	28000	16000
7	1950	0.961	75500	23500	30.3	0.054	46.6	28500	16000
8	1950	0.919	77500	24000	29.8	0.042	48.9	28000	17000
9	1950	0.890	80000	26000	30.8	0.035	50.9	33500	23000
10	1950	0.863	81500	27000	29.6	0.029	52.5	36500	25000
11	1950	0.834	82500	28000	29.9	0.023	53.8	35000	25000
12	1950	0.818	84000	28500	29.9	0.016	54.7	40000	25000
13	1950	0.808	85000	29500	29.4	0.010	55.2	42500	27000
14	1950	0.798	86000	30000	30.8	0.010	55.7	48000	30000

Fig. 6.18 Dextran Passing Through the H1P5 Cartridge During the Diafiltration Stage of Run 16

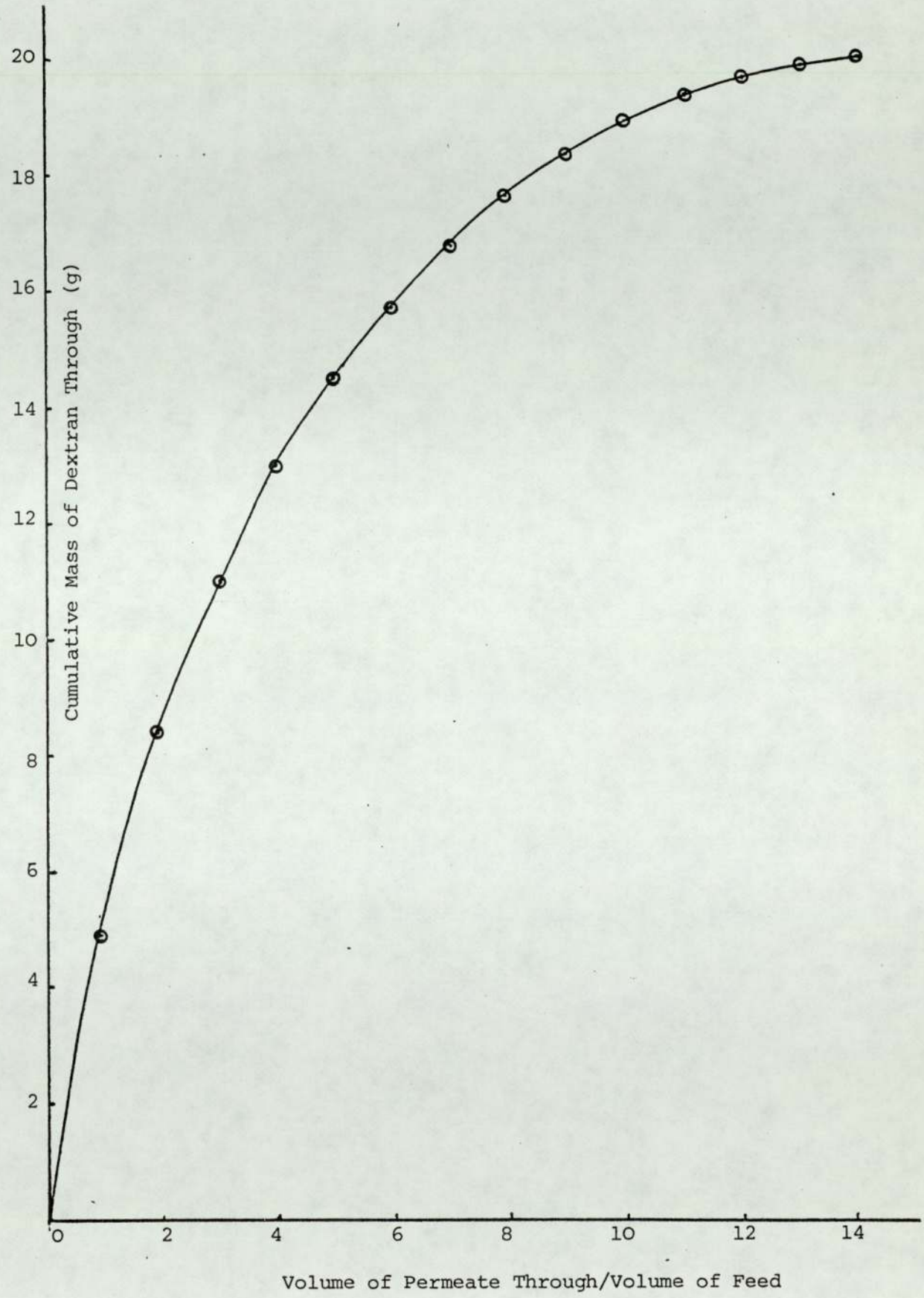


Fig. 6.19 Silica Removal Using the DC2A UF Hollow Fibre Cartridge System

Run	Cartridge	Feed B161D40			Permeate			Retentate		
		Volume (cm^3)	Conc. ($g.l^{-1}$)	SiO_2 Content (ppm)	Volume (cm^3)	Conc. ($g.l^{-1}$)	SiO_2 Content (ppm)	Volume (cm^3)	Conc. ($g.l^{-1}$)	SiO_2 Content
1	H1P2	2000	18.05	37	1770	0.84	33	230	149.7	45
		Dextran removed with: (%)			4.1			95.4		
		Silica removed with: (%)			85.0			15.0		

During the operation of the DC2A system in diafiltration mode it was observed that the mass rate of dextran passing through the membrane was reduced and the molecular weight of the permeate increased as the volume of permeate increased (Figs. 6.15 to 6.18). This was thought to be due to the depletion of very small molecules of dextran in the retentate.

Silica can be removed from dextran solutions using UF (Fig. 6.19), but this silica reduction will not be enough to produce a silica free product.

The cartridges performance is reproducible (Figs. 6.12a to 6.12e) and the life-time is long. More than thirty UF runs were carried out on an H1P5 cartridge and more than ten on an H1P2 cartridge without any detrimental effects. Cartridges can be cleaned using 0.1M NaOH solution and can be stored in a 0.05% solution of sodium azide.

6.5 DIAFILTRATION ON LARGE SCALE UF EQUIPMENT

A Patterson Candy International (PCI) reverse osmosis system (Fig. 6.20) was used with six BX6 ultrafiltration tubular membranes having a total membrane area 0.4 m^2.

Also an Amicon DC30 UF system (Fig. 6.21) having one H10P5 hollow fibre cartridge of 0.9 m^2 membrane area was tried. A dextran hydrolysate, HZ16K batch, was used as feed for the diafiltration runs on both the UF units.

Fig. 6.20 Flow Diagram of the Patterson-Candy International (PCI) RO System

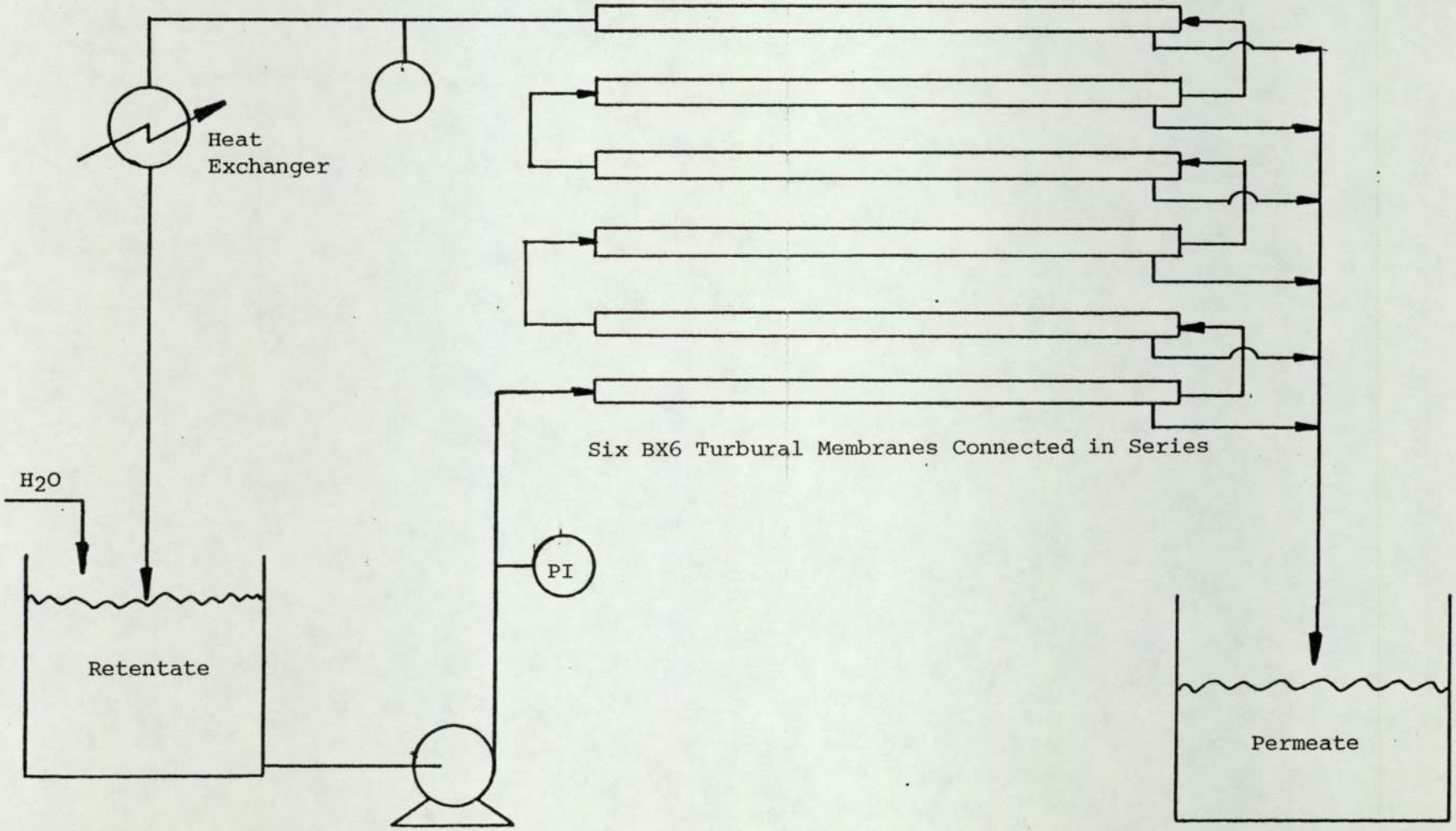
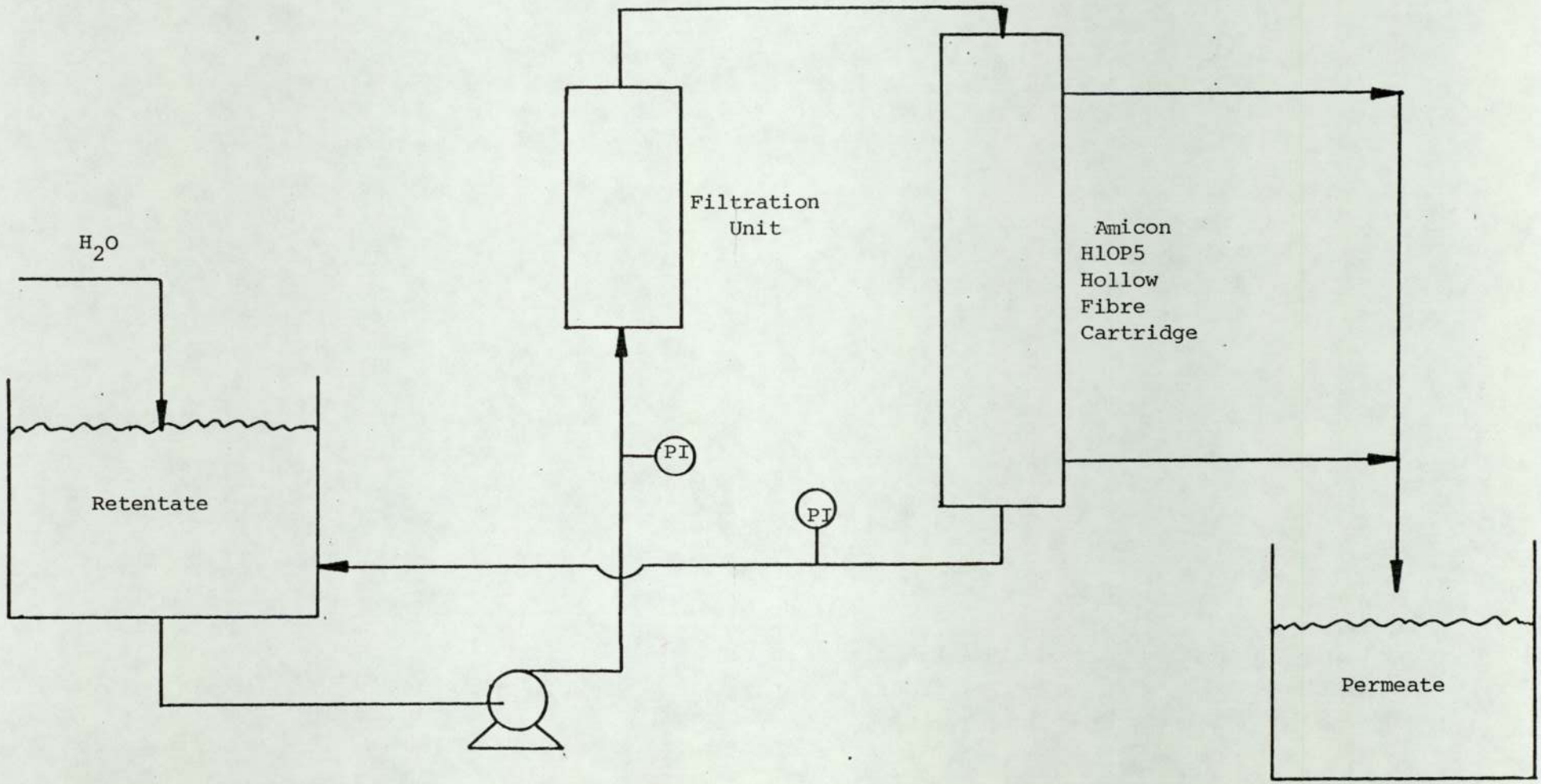


Fig. 6.21 Flow Diagram of the Amicon UF System



The condition and results of these runs are presented in Figs. 6.22 and 6.23.

From the results of these runs it can be seen that:

(1) The permeate rate is the same for both systems although the Amicon system has 0.9 m^2 membrane area compared to 0.4 m^2 for the PCI system, but the operating driving pressure of the PCI system is 2 atm instead of 1 atm for the Amicon system.

(2) The BX6 membrane appears more suitable for concentrating dextran solutions since dextran molecules pass with difficulty through the pores of the membrane. The H1OP5 is better for removing low molecular weight dextran.

(3) The molecular weight of the permeate passing through the H1OP5 cartridge increases due to the depletion of the low molecular weight material. This is justified by looking at the maximum and minimum molecular weights of the permeate. Although the minimum molecular weight increases, the maximum molecular weight is constant at around 70000 daltons. This means that the pores of the H1OP5 cartridge are of the size to remove molecules of molecular weight 70000 and below.

Fig. 6.22 Conditions and Results for the Diafiltration Run on the PCI RO System Using \ BX6 Membranes

Time hrs	RETENTATE						PERMEATE			
	Volume l	Conc. $g.l^{-1}$	Mass g	% of Dextran in Retentate	$\bar{M}_w$	$\bar{M}_N$	Volume l	Conc. $g.l^{-1}$	Mass g	% of Dextran in Permeate
0	53.000	24.0	1272	100	82400	9100	-	-	-	-
2	53.000	23.0	1219	95.9	82600	13000	52.000	1.0	52	4.1
4	53.000	22.1	1171	92.2	82400	15700	52.000	0.9	47	7.8
6	53.000	21.3	1129	88.9	81000	16500	53.000	0.8	42	11.1
8	53.000	20.7	1097	86.5	82500	17600	50.000	0.6	30	13.5
10	53.000	20.3	1076	84.9	81000	18000	53.000	0.4	21	15.1
12	53.000	20.0	1060	83.5	82800	19200	58.000	0.3	17	16.5

Fig. 6.23 Conditions and Results for the Diafiltration Run on the Amicon UF System Using H1OP5 Cartridge

Time hrs	RETENTATE						PERMEATE							
	Volume l	Conc. $g.l^{-1}$	Mass g	% of Dextran in Retentate	$\bar{M}_w$	$\bar{M}_N$	Volume l	Conc. $g.l^{-1}$	Mass g	% of Dextran in Permeate	$\bar{M}_w$	$\bar{M}_N$	Max. Mol. Wt.	Min. Mol. Wt.
0	53.000	23.0	1219	100	82000	8900	-	-	-	-	-	-	-	-
2	53.000	18.5	980	81.5	103000	18300	53.000	4.2	223	18.5	9000	3200	70500	182
4	53.000	16.0	848	71.1	113000	22000	58.000	2.1	122	28.9	9250	3700	70300	182
6	53.000	14.5	770	65.5	121000	29500	61.000	1.0	61	34.5	14850	8650	76000	1100
8	53.000	13.5	716	62.4	127000	32150	62.000	0.4	25	37.6	15800	10900	69000	2050
10	53.000	13.2	700	61.2	129000	33600	62.000	0.2	13	38.8	15950	10950	70500	2500

6.6 CONCLUSIONS

From all the runs carried on the small scale UF equipment it was concluded:

Dilute aqueous solution of dextran can be concentrated by using UF, and although the YM5, UM10 and UM20 flat membranes can be used successfully for concentrating dextran solutions, an H1P2 cartridge used with a hollow fibre UF system is preferred since these cartridges have a high membrane surface area per unit volume of equipment. Also operation of the system at elevated temperatures is desirable if it is required that the dextran solution reaches a concentration level suitable for spray drying (300 g/l), since a temperature increase will decrease the viscosity of the solution and will increase the permeate rate.

Low molecular weight dextran can be removed from dextran solutions using either flat membranes (YM10 and PM10) or hollow fibre cartridges (HIP5), but a hollow fibre system has the advantages of high membrane surface area per unit volume of equipment and can also be used in both concentration and diafiltration modes. The diafiltration mode operation is preferred in the case of dextran fractionations to the concentration mode operation since with the former more low molecular weight material can be removed.

High molecular weight material can be removed from dextran solutions, but in this case UF is less efficient than GPC as carried out on the SCCR5, hence

high molecular weight removal will not be considered any further.

Finally, a silica reduction can be achieved using UF, but a supplementary method of silica removal will be required if a silica free product is to be produced.

Because of the success of the DC2A system in removing low molecular weight dextran from dextran solutions two large scale equipments were tried. An Amicon DC30 system with an H1OP5 cartridge, made of the same material and having an equivalent molecular weight cut-off as the H1P5 cartridge, but 15 times larger membrane area, and a large scale PCI reverse osmosis system equipped with six, 183 cm long x 1.2 cm I.D. tubular membranes.

The H1OP5 cartridge was suitable for the removal of low molecular weight dextran, having the same success as the smaller H1P5 cartridge.

The BX6 membranes although they remove low molecular weight dextrans selectively, they do not allow much of the material to pass through, and are therefore more suitable for concentrating dextran solutions.

7.0 PRODUCTION OF CLINICAL DEXTRAN 40 FROM DEXTRAN HYDROLYSATE USING GPC, ULTRAFILTRATION (UF) AND ION EXCHANGE.

7.0 PRODUCTION OF CLINICAL DEXTRAN 40 FROM DEXTRAN HYDROLYSATE USING GPC, ULTRAFILTRATION (UF) AND ION EXCHANGE. I

7.1 INTRODUCTION

The production of clinical dextran 40 from dextran hydrolysate can be produced by removing some of the very large and very small dextran molecules.

The present industrial process involves fractional precipitation of the hydrolysate, from aqueous solutions, using ethanol but because ethanol is expensive to recover and is a fire hazard, an alternative method for fractionating dextran was considered. This method uses GPC for the removal of the high molecular weight dextran, and UF for the removal of the low molecular weight dextran and the concentration of the final solutions. The GPC operation is carried out on the SCCR5 machine (See Section 4.0). The UF operation is performed on a DC2A Amicon hollow fibre cartridge system (See Section 6.0). equipped with an H1P5 cartridge.

The final product must have an average molecular weight of around 40,000 daltons and 85% of the material must be between 12,000 and 98,000 daltons.

Silica dissolution of the GPC packing in the SCCR5 is reported by England (46), and since clinical dextran is required to be free from foreign compounds, silica removal is required. Although some of the silica will be removed during the UF operation another process

Fig. 7.1 The Properties of the SCCR5 Packed Columns

Column	Liquid Volume V_o+V_i (cm^3)	Void Volume V_o (cm^3)	Pore Volume V_i (cm^3)	$\frac{V_i}{V_o}$	Number of Plates N	HETP (cm)
1	1113	522	591	1.132	71	0.98
2	1152	527	625	1.185	83	0.84
3	1142	517	630	1.218	86	0.81
4	1139	515	624	1.212	87	0.80
5	1152	563	589	1.046	79	0.88
6	1176	551	625	1.134	88	0.79
7	1127	539	588	1.091	84	0.83
8	1164	534	630	1.180	92	0.76
9	1140	558	582	1.043	87	0.80
10	1116	546	570	1.044	79	0.88
Average	1142	537	605	1.127	84	0.83

for silica removal is required to produce a silica free product. A Millipore nuclear grade mixed bed ion exchange cartridge (see Section 5.0) is used for removing the remaining silica.

7.2 COLUMN PACKING FOR THE SCCR5

The SCCR5 machine is described in Section 4.0, and the reasons for the choice of the Spherosil XOB 075 packing used in the SCCR5 as well as its properties are presented by Ellison (47).

Before performing the following runs, the columns were emptied of packing and repaired for any leaks. Then they were slurry packed (47) again and then calibrated using batch chromatographic techniques (47). The properties of the repacked columns are presented in Fig. 7.1.

7.3 EXPERIMENTAL OPERATING CONDITIONS, PURPOSE OF THE RUNS

The purpose of these runs was to produce a clinical dextran 40 from dextran hydrolysate by removing the high molecular weight material using GPC and the low molecular weight material using UF. The average molecular weights for the hydrolysate feed (batch HZ16K) were:

$\bar{M}_W$ = 82000 $\bar{M}_N$ = 9250 D = 8.85

It was proposed that 15 to 20% of the feed would be removed by the GPC process and 25 to 40% by the UF

process, since the hydrolysate contains approximately 12% of material above 98000 daltons and 25.5% below 12000 daltons.

To evaluate the performance of mixed bed nuclear grade ion exchange cartridges for the removal of silica from dextran solutions, some of the main product from the GPC fractionation, contaminated with silica dissolved from the packing, was passed through such a cartridge.

A summary of the operating conditions for the GPC runs is presented in Fig. 7.2. The conditions of the GPC runs were primarily selected to remove 15 to 20% of the feed, but also to produce more optimum operating conditions. All the runs were carried out at 60°C in order to reduce the pressure drop in the system, except run 4.1 which was done at 40°C.

The aim of run 3.1 was to find a set of experimental conditions that will remove 15 to 20% of the dextran hydrolysate feed.

In run 3.2 the post-feed cut position was increased, keeping the same eluent rate as in run 3.1, in order to evaluate the effect of the change in the post-feed cut position and if possible to remove more dextran with the high molecular weight product.

Runs 4.2 and 4.3 had the same post-feed cut positions as run 3.1 and 3.2 respectively, but the eluent rate was increased and therefore the eluent to feed ratio was increased. The purpose of this run was to predict the effect of changing the pre-feed cut position.

Run 4.1 was identical to run 4.2 but it was done at 40°C instead of 60°C in order to evaluate temperature effects.

In run 5.1 the feed input and post-feed cut position were the same as in run 4.2, but lower eluent rate and feed concentrations were used. In this run the effect of varying the pre-feed cut position was to be evaluated when the feed input was kept constant.

In run 5.2 the post-feed cut position was increased, but the eluent rate was kept constant as in run 5.1, in an attempt to remove more high molecular weight product than in run 5.1.

Runs 5.1 and 5.2 also had the same eluent and feed rates, i.e. the same pre-feed and post-feed cut positions, as runs 3.1 and 3.2 respectively, but they had a lower feed concentration and hence a lower feed input in order to predict the concentration effects.

Runs 6.1 and 6.2 were identical to runs 5.1 and and 5.2 respectively, but the feed was introduced into the SCCR5 at column 7 instead of column 5, so that the effect of the change in the feed position can be found.

Run 7.2 was a repeat of runs 5.2 and 6.2, but in this case the feed was introduced into the SCCR5 at column 3. The effect of the change in the feed position was also the purpose of this run.

Finally, in run 7.1 the feed rate and feed concentration were kept the same as in run 7.2, but both the pre-feed and post-feed cut position were increased in order to remove more dextran with the high molecular

weight product than run 7.2, in case run 7.2 did not remove enough high molecular weight dextran.

For the removal of the low molecular weight material by UF the diafiltration/concentration mode operation (see Section 6.4) was used, and the concentration and volume of the permeate were measured regularly during the runs to check if enough material has been removed. When the material removed was approximately the amount required the process was stopped.

Finally, the dextran product from some GPC runs was pumped through an ion exchange cartridge at a rate of 200 $cm^3 . min^{-1}$. The product was collected and analysed for silica (see Chapter 5.0).

7.4 RESULTS AND DISCUSSION

The experimental operating conditions and the results of the runs are presented in Figs. 7.2 to 7.43.

For the GPC runs several experimental variables have been changed in order to achieve a more detailed knowledge of the process. From these GPC runs several conclusions were drawn.

(1) An increase in the post-feed cut position results in an increase of the material removed as high molecular weight product (Runs 3.1 and 3.2, 4.2 and 4.3, 5.1 and 5.2, 6.1 and 6.2). This is due to higher mobile phase flowrate in the post-feed fractionating section, and especially in the feed column, that forces more of the dextran molecules

Fig. 7.2 Theoretical Conditions for the GPC Runs

Run	Feed Column	Temperature (°C)	Switch Time (s)	Conc. of Feed ($g.l^{-1}$)	Feedrate per		Eluent to Feed Ratio	Flowrates ($cm^3.min^{-1}$)			Theoretical Cut Position		
					Cycle (g)	Hour (g)		Eluent	Feed	Purge	Pre-Feed	Post-Feed	Purge
3.1	5	60	450	234	702	561	2.375	95	40	300	0.290	0.786	2.831
3.2	5	60	450	234	1035	828	1.580	95	60	300	0.290	1.034	2.831
4.1	5	40	450	215	403	323	4.400	110	25	300	0.476	0.786	2.831
4.2	5	60	450	215	403	323	4.400	110	25	300	0.476	0.786	2.831
4.3	5	60	450	215	726	581	2.440	110	45	300	0.476	1.034	2.831
5.1	5	60	450	134	402	322	2.375	95	40	300	0.290	0.786	2.831
5.2	5	60	450	134	603	483	1.580	95	60	300	0.290	1.034	2.831
6.1	7	60	450	134	402	322	2.375	95	40	300	0.290	0.786	2.831
6.2	7	60	450	134	603	483	1.580	95	60	300	0.290	1.034	2.831
7.1	3	60	450	134	603	483	1.830	110	60	300	0.476	1.220	2.831
7.2	3	60	450	134	603	483	1.580	95	60	300	0.290	1.034	2.831

Fig. 7.3 Operating Conditions for GPC Run 3

Run	Cycle	Pressures ($kN.m^{-2}$)			Temperature (^{o}C)			Input Flowrates $cm^3.min^{-1}$			Product Flows $cm^3.min^{-1}$		Cut-Positions		
		Eluent	Feed	Purge	Purge	Eluent	In-line	L_1	L_2	L_3	HMW	LMW	Pre-Feed	Post-Feed	Purge
3.1	5&6	380	241	70	60	60	60	90	37	127	133	280	0.260	0.685	2.585
3.1	7&8	380	241	70	60	60	60	90	38	128	133	290	0.260	0.700	2.710
3.1	9	380	241	70	60	60	60	90	40	130	134	300	0.260	0.725	2.830
3.1	10	380	241	70	60	60	60	90	40	130	134	300	0.260	0.725	2.830
3.2	15	1000	725	70	60	60	60	95	58	153	155	315	0.290	1.010	3.030
3.2	16	1000	725	70	60	60	60	95	58	153	156	345	0.290	1.010	3.390
3.2	17	1000	725	70	60	60	60	95	58	153	155	345	0.290	1.010	3.390
3.2	18	1000	725	70	60	60	60	95	58	153	157	330	0.290	1.010	3.200

Fig 7.4 Products from GPC Run 3

Run	Cycle	Dextran Input per cycle (g)	High Mol. Wt. Product			Low Mol. Wt. Product			HMWP Output	LMWP Output	Mass Balance
			Conc. g/l	Vol..l	Mass g	Conc. g/l	Vol. l	Mass g			
3.1	9	702	7.2	9.75	70	26.9	22.2	597	0.105	0.895	0.950
3.1	10	702	8.2	9.75	80	28.0	22.2	622	0.114	0.886	1.000
3.2	17	1018	18.1	11.51	210	29.3	25.9	760	0.216	0.784	0.960
3.2	18	1018	18.8	11.51	217	32.4	24.8	802	0.213	0.787	1.000

		High Mol. Wt. Product			Low Mol. Wt. Product		
		$\bar{M}_W$	$\bar{M}_N$	D	$\bar{M}_W$	$\bar{M}_N$	D
3.1	9	468000	53000	8.83	35400	8600	4.11
3.1	10	465000	54000	8.61	35200	9000	3.92
3.2	17	284000	41000	6.92	36700	8900	4.12
3.2	18	287000	40600	7.07	35300	8900	3.97

Fig. 7.5 Purge Products from GPC Run 3.2

Column	Purged Volume l	Purge Conc. l	Dextran Mass l	Column Conc. $g.l^{-1}$	Col. Conc. / Feed Conc.	$\bar{M}_w$	$\bar{M}_N$	D
1	3.53	21.3	75.2	66.0	0.282	37300	8300	4.49
2	3.70	54.0	199.8	175.0	0.748	43400	9400	4.61
3	3.70	59.3	219.3	192.0	0.820	45200	9300	4.86
4	3.70	59.3	219.3	192.0	0.820	47000	9700	4.84
5	3.60	58.4	210.2	184.1	0.787	49700	10100	4.92
6	3.89	43.7	170.1	149.0	0.637	63000	12500	5.04
7	3.75	37.8	141.6	124.0	0.530	73000	14500	5.03
8	3.70	27.7	102.5	89.8	0.384	84500	17500	4.83
9	3.72	22.3	83.2	72.9	0.311	112000	22300	5.03

Fig. 7.5a On Column Concentration Profile for Run 3.2

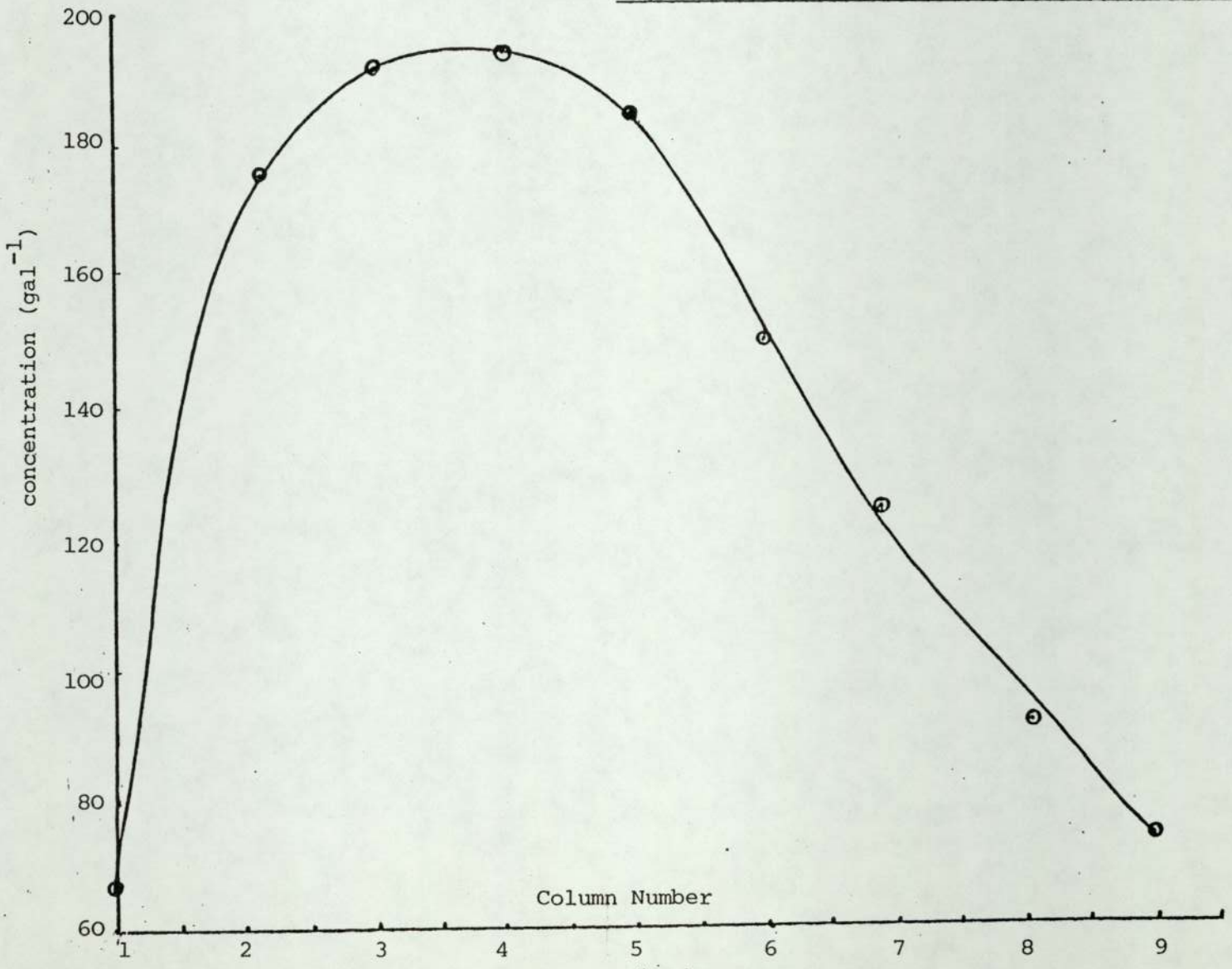


Fig. 7.6 Conditions and Results for UF Run 3

Run	3.1	3.2
Feed Volume (l)	2.000	2.000
Feed Conc. (g/l)	28.0	33.0
Feed Mass (g)	56	66
Feed, $\bar{M}_w$	35200	35300
Feed, $\bar{M}_N$	9000	8900
Diafiltration water (l)	3.000	1.500
Permeate Vol.(l)	4.770	3.180
Permeate Conc. (g/l)	4.8	6.8
Permeate Mass (g)	22.9	21.6
Permeate Output (%)	41.5	32.0
Permeate, $\bar{M}_w$	17000	15250
Permeate, $\bar{M}_N$	4500	4100
Retentate Vol. (l)	0.230	0.320
Retentate Conc. (g/l)	140.0	143.6
Retentate Mass (g)	32.2	46.0
Retentate Output (%)	58.5	68.0
Retentate, $\bar{M}_w$	47100	43250
Retentate, $\bar{M}_N$	23400	19800

Fig. 7.7 GPC and UF Fractionation of Dextran for Run 3.1

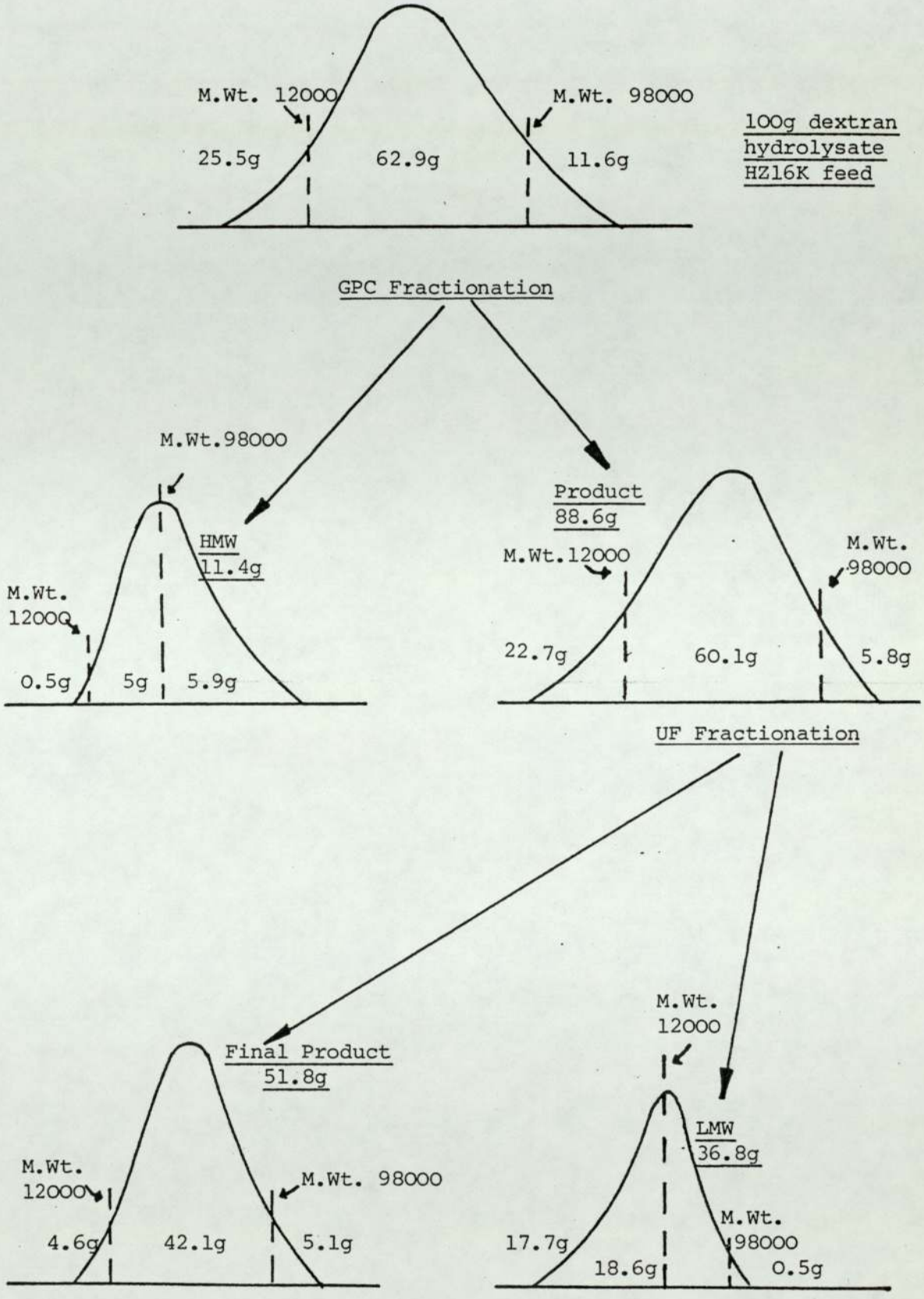


Fig. 7.8 GPC and UF Fractionation of Dextran for Run 3.2

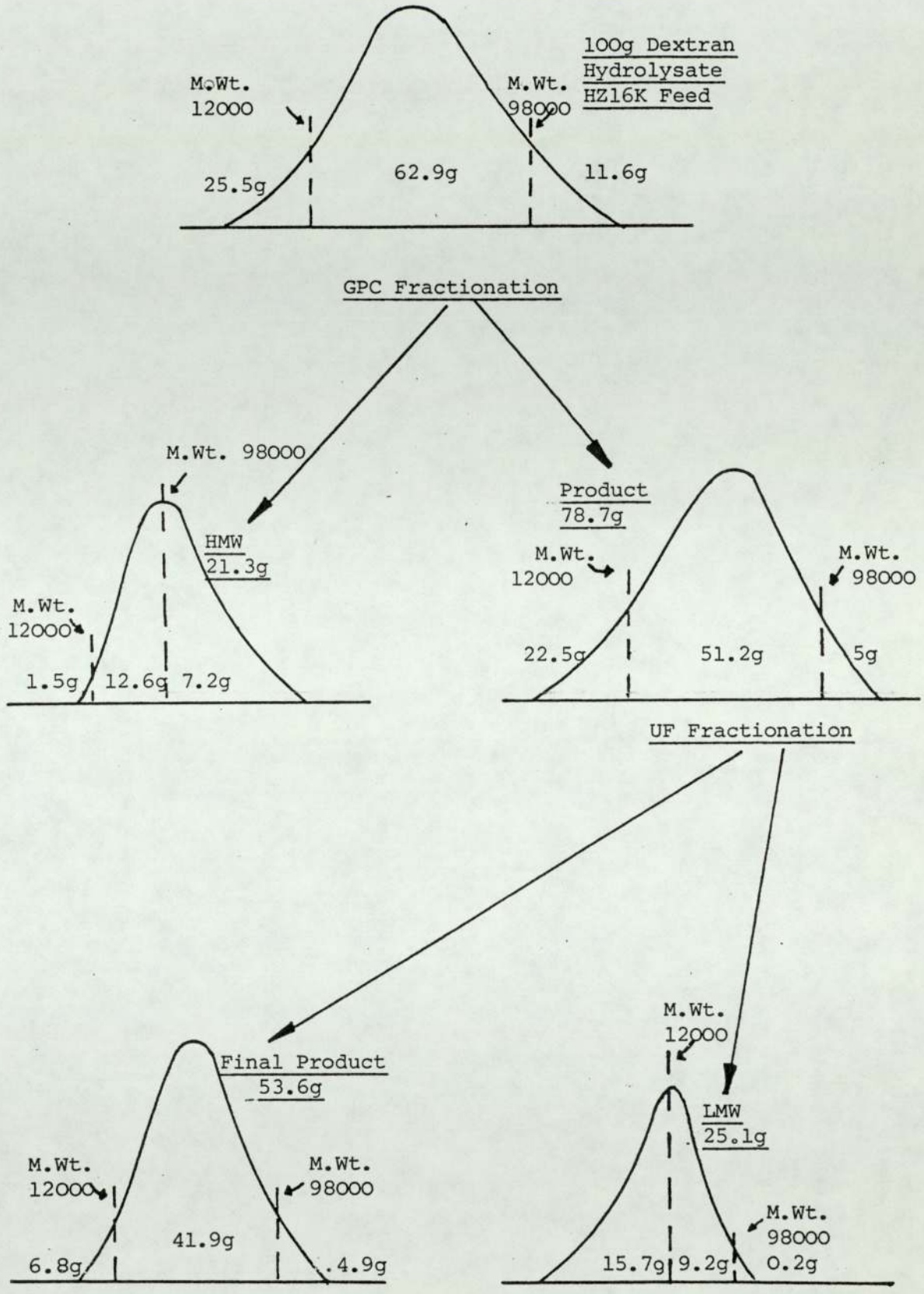


Fig. 7.9 Silica Mass Balance during the GPC and UF Run 3.1

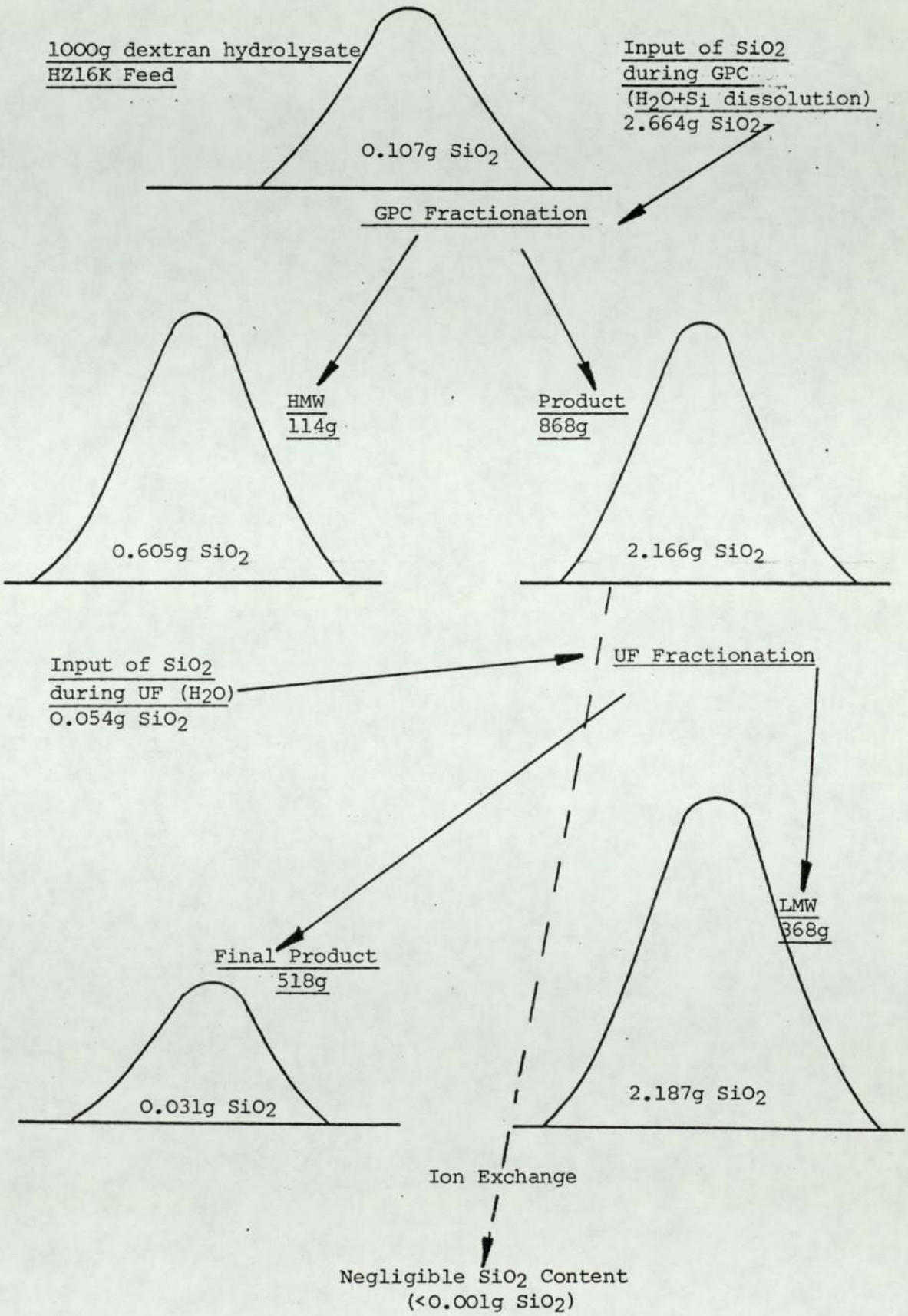


Fig. 7.10 Silica Mass Balance during the GPC and UF Run 3.2

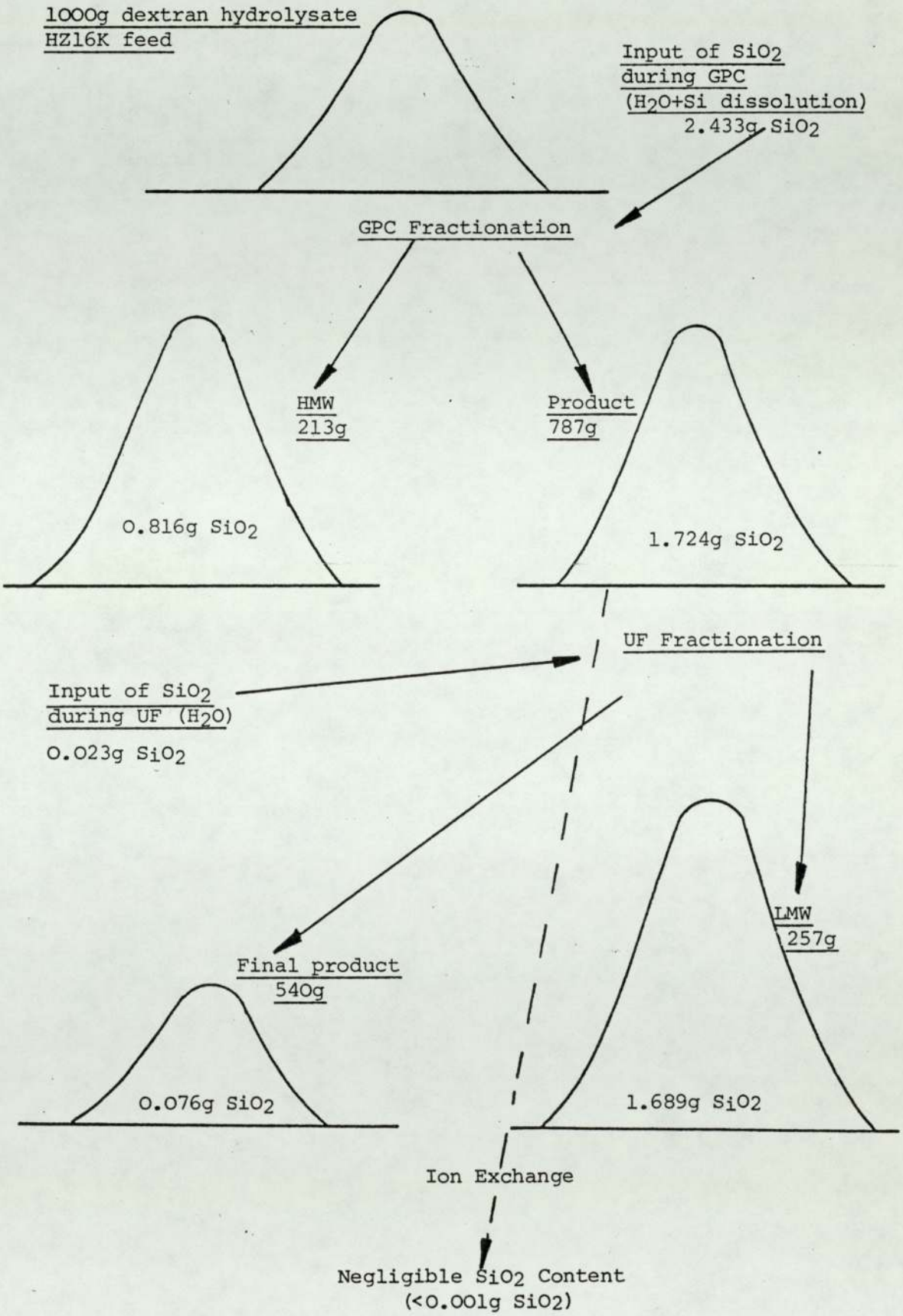


Fig. 7.11 Operating Conditions for GPC Run 4

Run	Cycle	Pressures ($kN.m^{-2}$)			Temperatures (^{o}C)			Input Flowrates $cm^3.min^{-1}$			Product Flows $cm^3.min^{-1}$		Cut-Positions		
		Eluent	Feed	Purge	Purge	Eluent	In-Line	L_1	L_2	L_3	HMW	LMW	Pre-Feed	Post-Feed	Purge
4.1	5&6	414	276	97	40	40	40	110	25	135	135	297	0.476	0.786	2.794
4.1	7&8	414	276	97	40	40	40	110	25	135	134	297	0.476	0.774	2.794
4.1	9&10	414	276	97	40	40	40	110	25	135	135	297	0.476	0.786	2.794
4.1	11	414	276	97	40	40	40	111	23	134	134	297	0.488	0.774	2.794
4.1	12	414	276	97	40	40	40	111	24	135	135	297	0.488	0.786	2.794
4.2	13	345	276	70	60	60	60	111	24	135	135	300	0.488	0.786	2.831
4.2	14	345	276	70	60	60	60	111	24	135	136	301	0.488	0.786	2.844
4.2	15	345	276	70	60	60	60	110	24	134	134	302	0.476	0.774	2.856
4.2	16	345	276	70	60	60	60	110	24	134	134	301	0.476	0.774	2.844
4.3	17	794	690	70	60	60	60	110	44	155	155	312	0.488	1.034	2.980
4.3	18	794	690	70	60	60	60	110	45	155	155	315	0.476	1.034	3.017
4.3	19	794	690	70	60	60	60	111	44	155	155	322	0.488	1.034	3.104
4.4	20	794	690	70	60	60	60	111	44	155	155	321	0.488	1.034	3.091

Fig. 7.12 Products from GPC Run 4

Run	Cycle	Dextran Input per Cycle (g)	High Mol. Wt. Product			Low Mol. Wt. Product			HMWP Output	LMWP Output	Mass Balance
			Conc.g/l	Vol.l	Mass g	Conc.g/l	Vol. l	Mass g			
4.1	11	371	6.3	10.0	63	13.8	22.3	308	0.170	0.830	1.000
4.1	12	387	6.6	10.1	67	13.4	22.3	299	0.182	0.818	0.950
4.2	15	387	7.5	10.0	75	13.8	22.7	313	0.193	0.807	1.000
4.2	16	387	7.7	10.0	77	13.7	22.7	311	0.198	0.802	1.000
4.3	19	709	25.3	11.7	295	16.3	24.6	401	0.424	0.576	0.980
4.3	20	709	24.1	11.9	297	16.2	24.2	392	0.432	0.568	0.970

		High Mol. Wt. Product			Low Mol. Wt. Product		
		$\bar{M}_W$	$\bar{M}_N$	D	$\bar{M}_W$	$\bar{M}_N$	D
4.1	11	378000	56000	6.75	30400	7100	4.27
4.1	12	373000	56500	6.61	32900	7400	4.42
4.2	15	339000	53500	6.33	30400	7200	4.25
4.2	16	336000	54000	6.22	28900	7200	4.03
4.3	19	220000	27500	8.00	30000	6000	5.00
4.3	20	220000	27200	8.09	27500	6500	4.25

Fig. 7.13 Purge Products from GPC Run 4.3

Column	Purged Volume l	Purge Conc. $g.l^{-1}$	Dextran Mass g	Column Conc. $g.l^{-1}$	Col. Conc. / Feed Conc.	$\bar{M}_w$	$\bar{M}_N$	D
1	3.47	11.3	39.2	34.3	0.160	31200	8600	3.62
2	3.65	42.3	144.4	126.4	0.589	32500	9200	3.53
3	3.60	51.5	185.5	162.4	0.755	34800	9300	3.74
4	3.55	53.9	191.3	167.5	0.779	37700	9450	3.99
5	3.60	51.8	186.3	163.1	0.758	42000	9800	4.28
6	3.70	43.9	162.4	142.2	0.661	58000	11150	5.20
7	3.65	36.6	133.7	117.1	0.554	62800	14200	4.42
8	3.70	30.8	114.0	89.8	0.464	73000	18100	4.03
9	3.70	21.3	78.9	69.1	0.321	83000	21450	3.91

Fig. 7.13a On Column Concentration Profile for Run 4.3

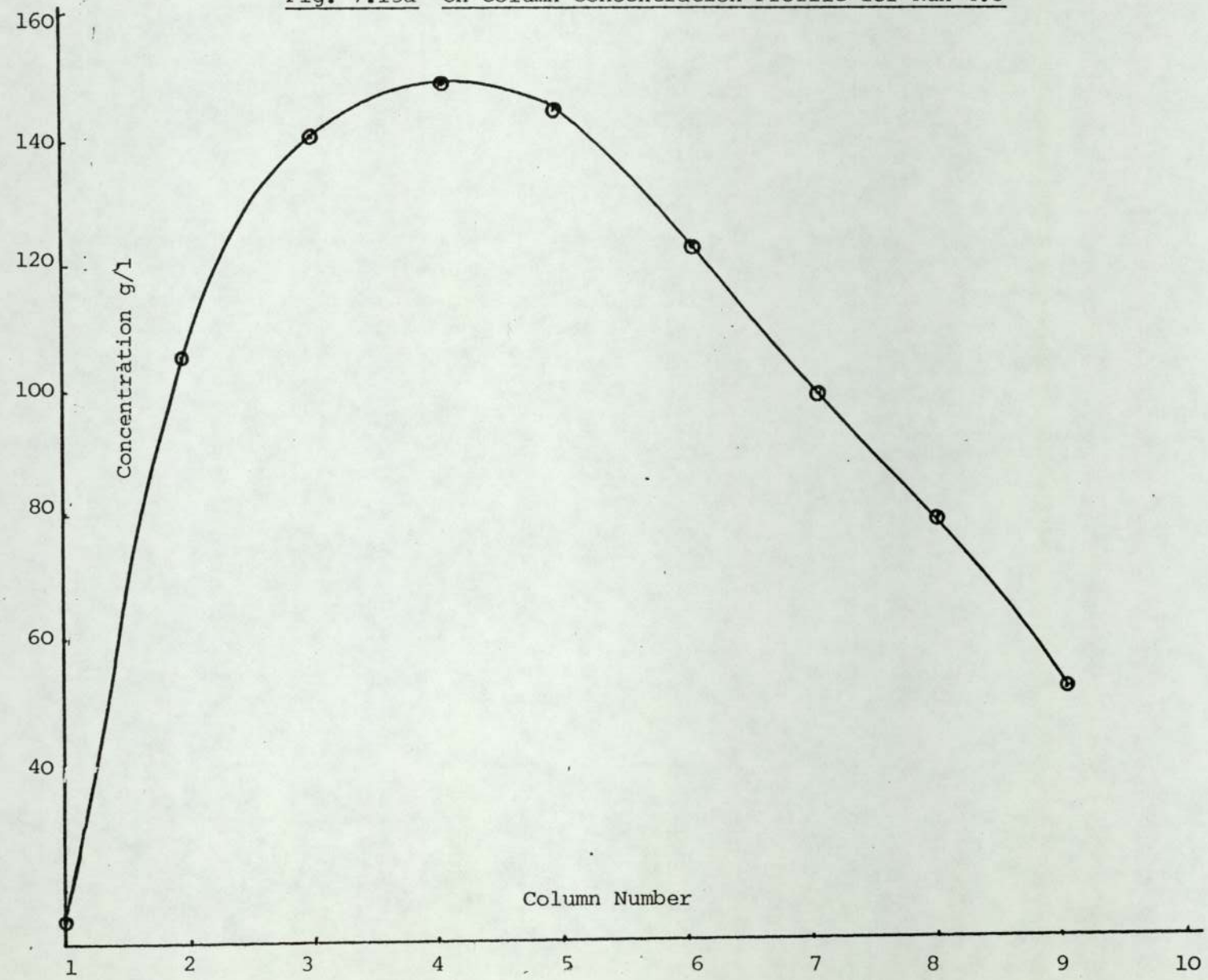


Fig. 7.14 Conditions and Results for UF Run 4

Run	4.1	4.2	4.3
Feed Vol. (l)	2.000	2.000	2.000
Feed Conc. (g/l)	13.4	13.7	16.2
Feed Mass (g)	26.8	27.4	32.4
Feed, $\bar{M}_w$	32900	28900	27500
Feed, $\bar{M}_N$	7400	7200	6500
Diafiltration Water (l)	6.000	6.000	4.000
Permeate Vol. (l)	7.810	7.810	5.825
Permeate Conc. (g/l)	1.3	1.2	2.3
Permeate Mass (g)	10.2	9.3	13.4
Permeate Output (%)	38.2	34.4	41.9
Permeate, $\bar{M}_w$	18100	17220	14280
Permeate, $\bar{M}_N$	5050	4410	3990
Retentate Vol. (l)	0.190	0.190	0.175
Retentate Conc. (g/l)	86.5	93.1	106.5
Retentate Mass (g)	16.5	17.7	18.6
Retentate Output (%)	61.8	65.6	58.1
Retentate, $\bar{M}_w$	41830	35280	36930
Retentate, $\bar{M}_N$	23950	19540	20690

Fig. 7.15 GPC and UF Fractionation of Dextran for Run 4.1

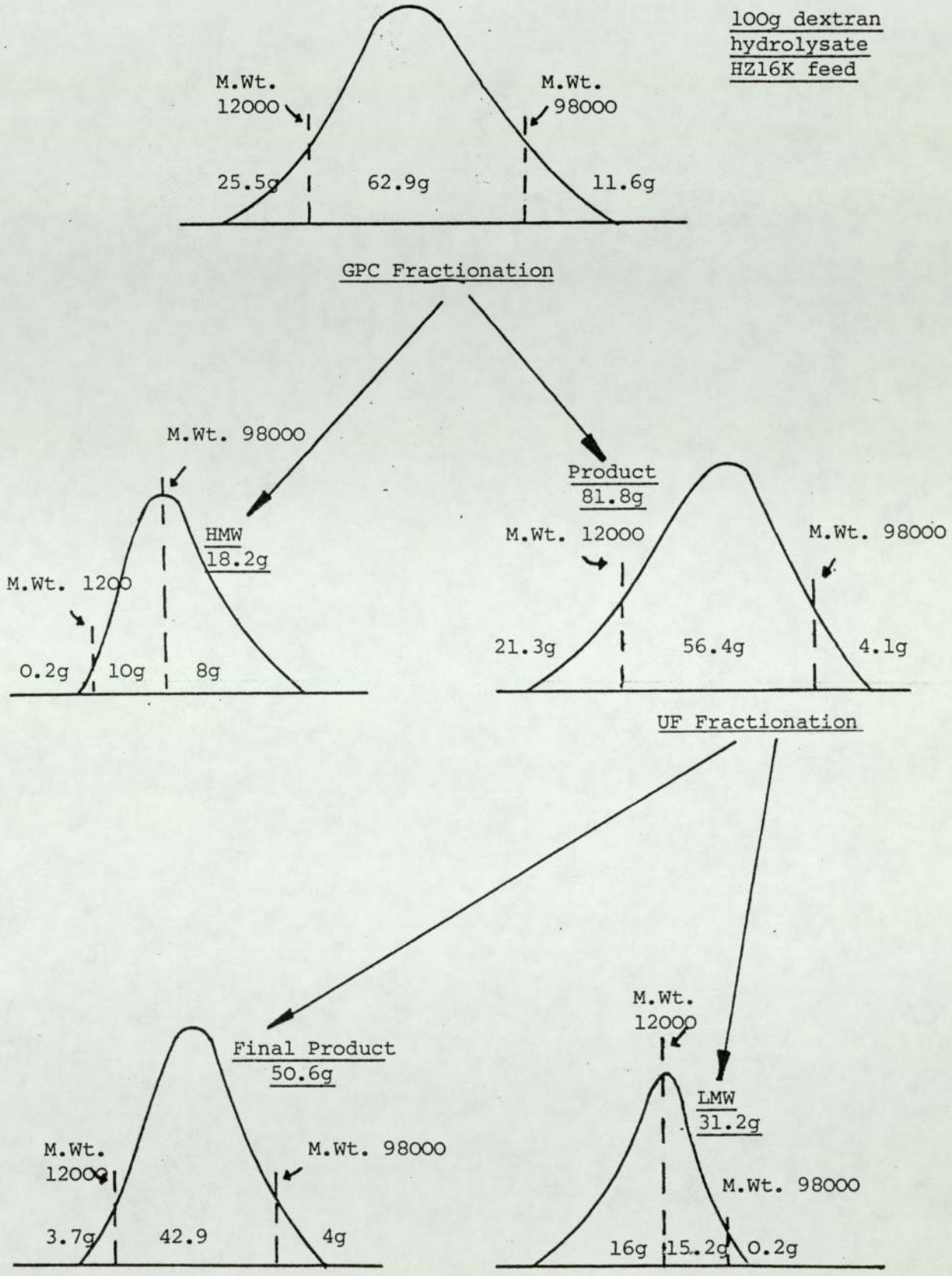


Fig. 7.16 GPC and UF Fractionation of Dextran for Run 4.2

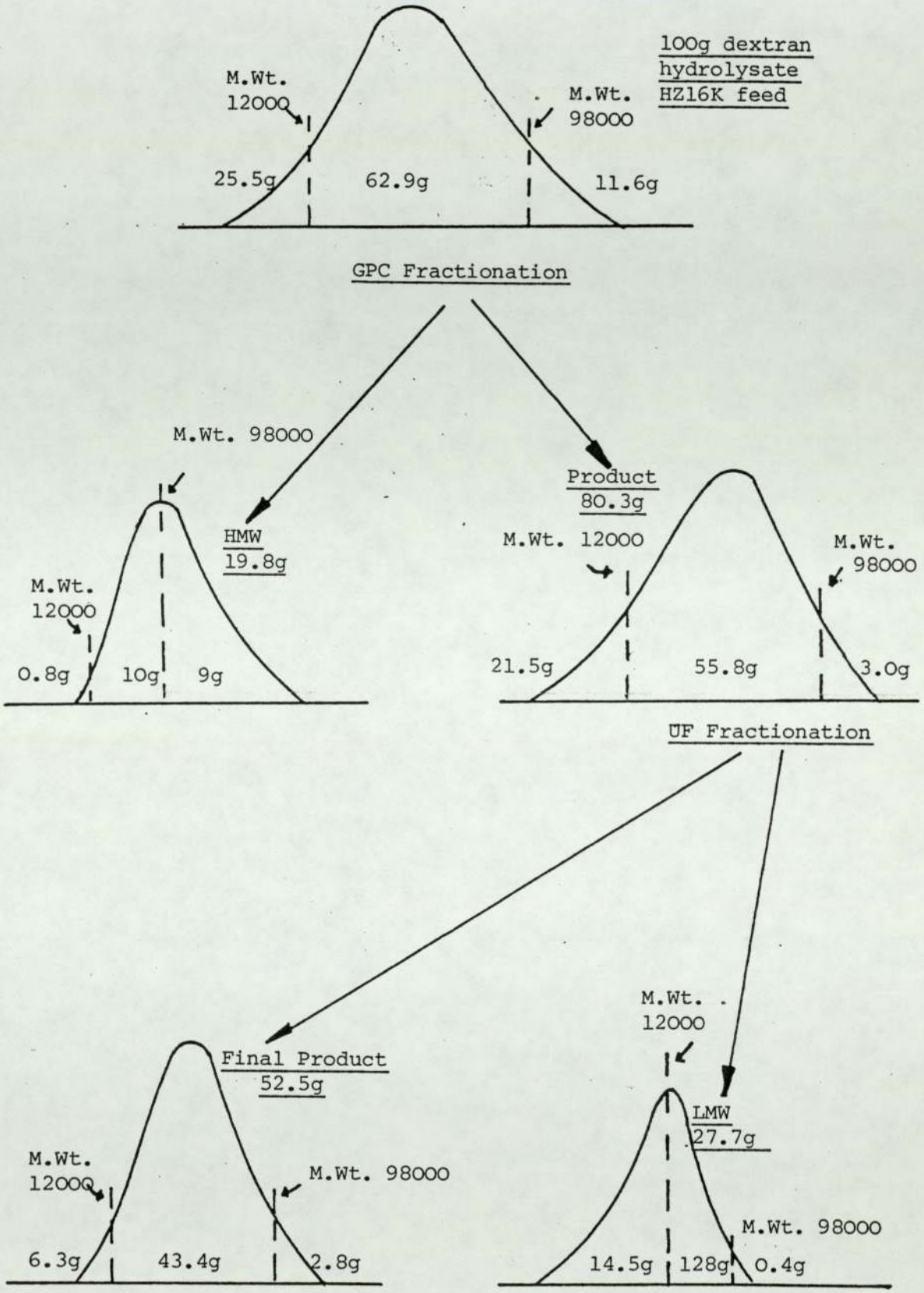


Fig. 7.17 GPC and UF Fractionation of Dextran for Run 4.3

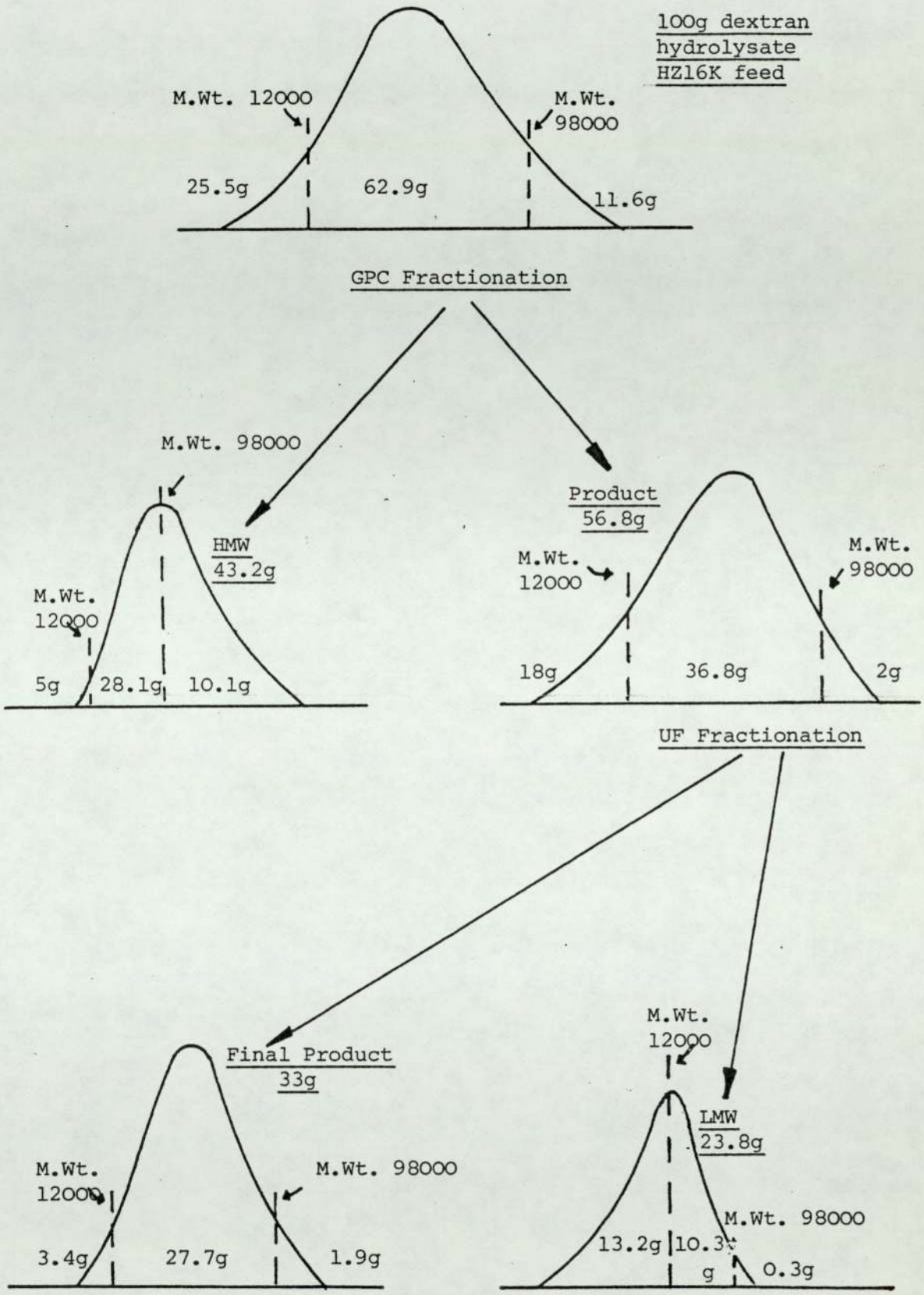


Fig. 7.18 Silica Mass Balance during the GPC and UF Run 4.1

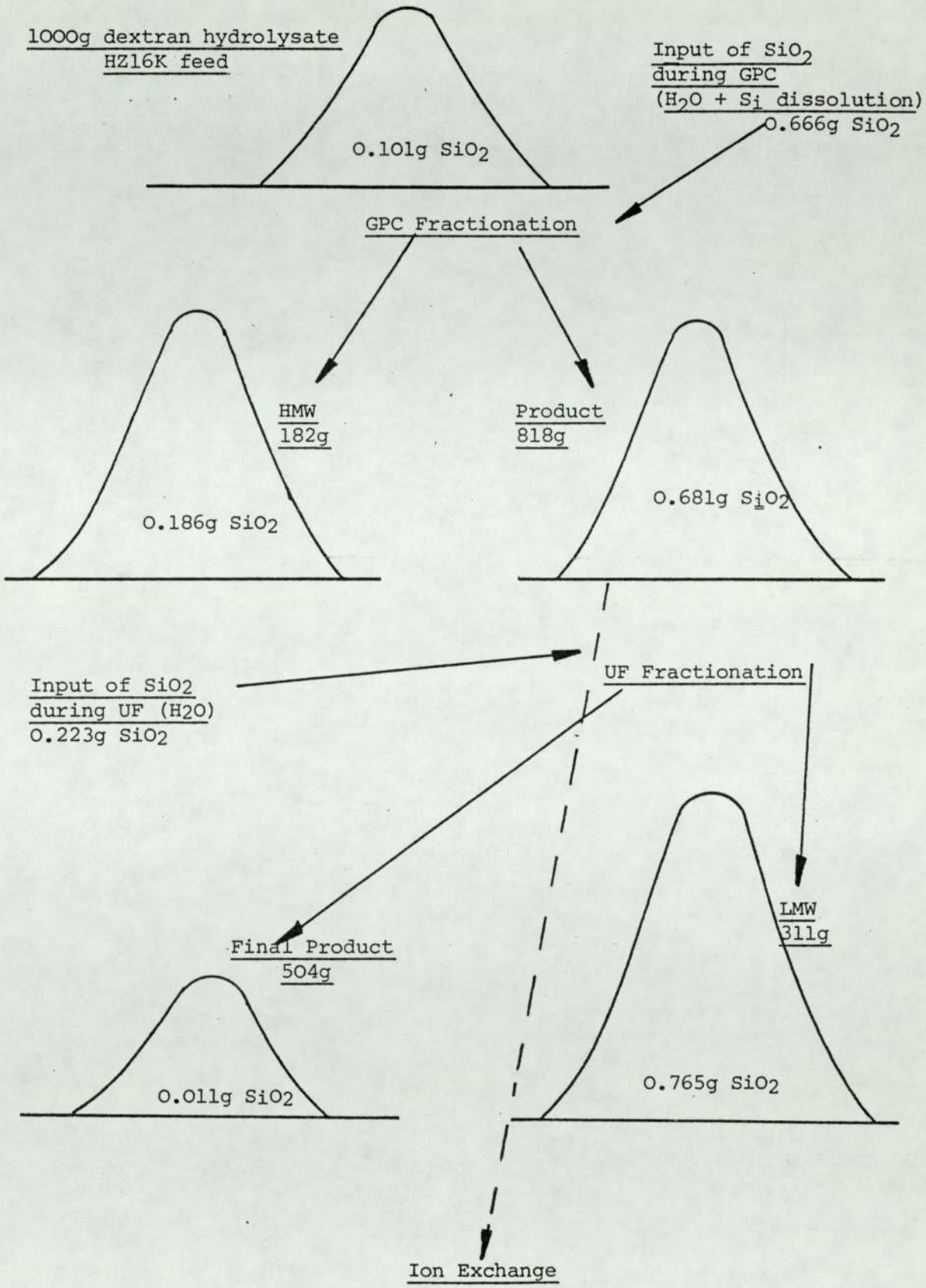


Fig. 7.19 Silica Mass Balance during the GPC and UF Run 4.2

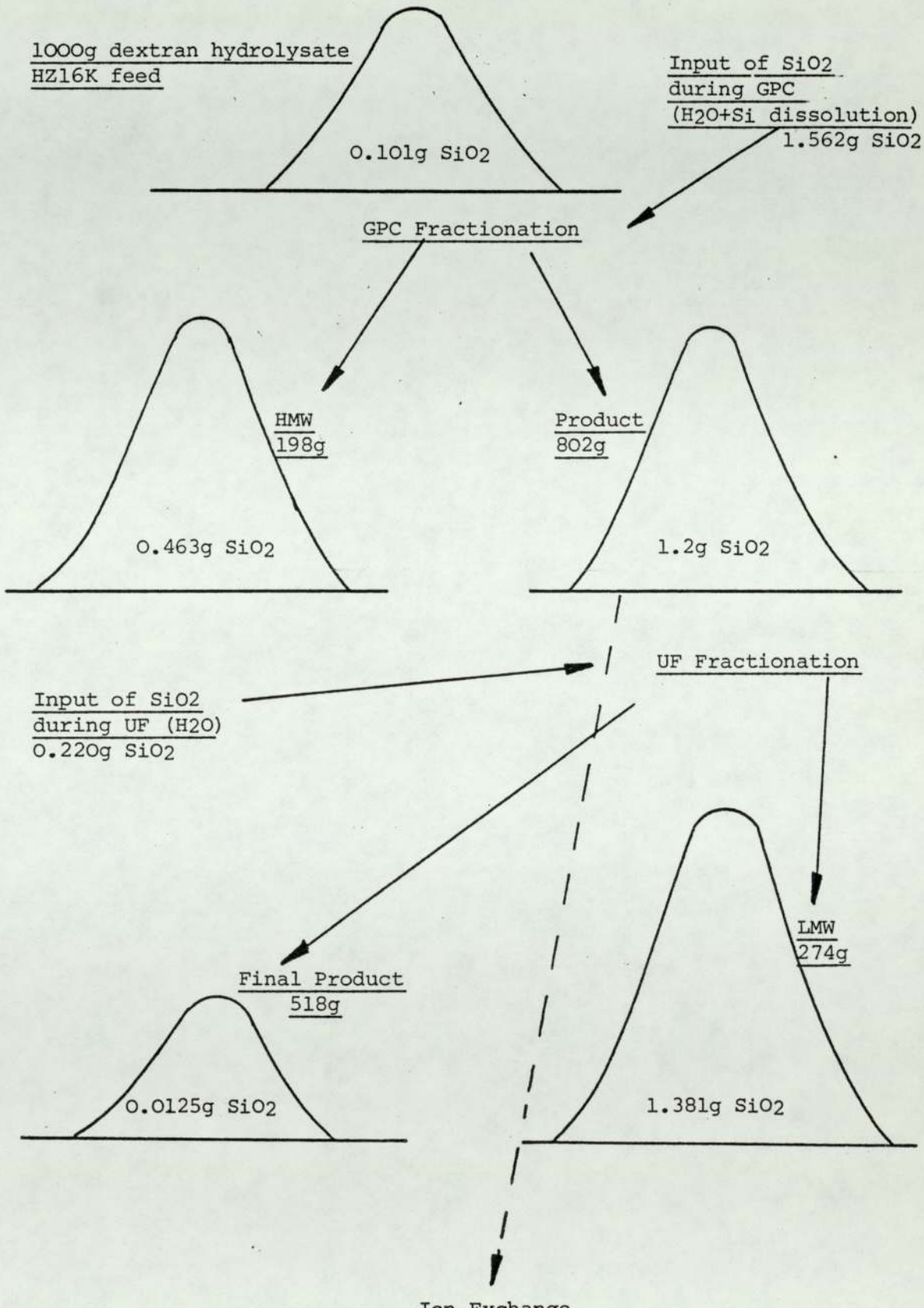


Fig. 7.20 Silica Mass Balance during the GPC and UF Run 4.3

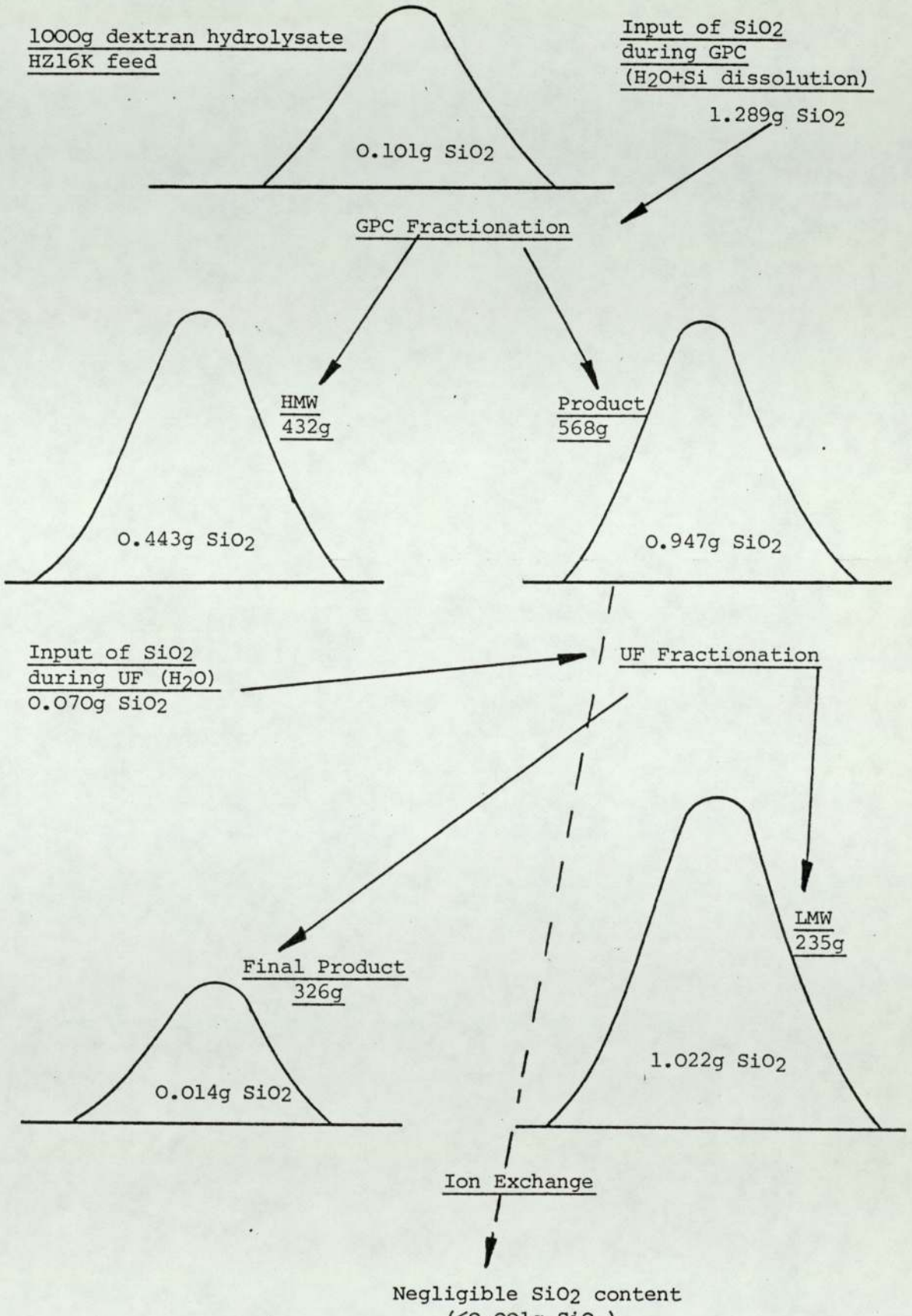


Fig. 7.21 Operating Conditions for GPC Run 5

Run	Cycle	Pressures ($kN.m^{-2}$)			Temperatures (^{o}C)			Input Flowrates $cm^3.min^{-1}$			Product Flows $cm^3.min^{-1}$		Cut-Positions		
		Eluent	Feed	Purge	Purge	Eluent	In-line	L_1	L_2	L_3	HMW	LMW	Pre-Feed	Post-Feed	Purge
5.1	5&6	256	206	70	60	60	60	95	41	136	136	286	0.290	0.798	2.658
5.1	7	256	206	70	60	60	60	95	41	136	134	287	0.290	0.798	2.670
5.1	8	256	206	70	60	60	60	95	41	136	134	287	0.290	0.798	2.670
5.2	12	376	246	70	60	60	60	95	59	154	155	294	0.290	1.022	2.757
5.2	13	376	246	70	60	60	60	96	58	153	155	300	0.290	1.010	2.831
5.2	14	376	70	60	60	60	60	95	59	154	155	303	0.290	1.022	2.856

Fig. 7.22 Products from GPC Run 5

Run	Cycle	Dextran Input per Cycle (g)	High Mol. Wt. Product Conc. g/l	Vol. l	Mass g	Low Mol. Wt. Product Conc. g/l	Vol. l	Mass g	HMPW Output	LMWP Output	Mass Balance
5.1	7	412	5.0	10.0	50	17.0	21.5	365	0.120	0.880	1.000
5.1	8	412	5.0	10.0	50	17.0	21.5	365	0.120	0.880	1.000
5.2	12	583	11.2	11.6	130	19.0	22.5	430	0.232	0.768	0.960
5.2	13	583	11.8	11.6	137	19.5	22.7	445	0.235	0.765	0.980

		High Mol. Wt. Product			Low Mol. Wt. Product		
		$\bar{M}_W$	$\bar{M}_N$	D	$\bar{M}_W$	$\bar{M}_N$	D
5.1	7	468500	56150	8.34	33200	9000	3.69
5.1	8	446000	52000	8.55	32200	9100	3.54
5.2	12	269000	40000	6.72	32000	8700	3.68
5.2	13	279000	42000	6.62	29000	7500	3.86

Fig. 7.23 Purge Products from GPC Run 5.2

Column	Purged Volume l	Purge Conc. $g.l^{-1}$	Dextran Mass g	Column Conc. $g.l^{-1}$	Col. Conc. / Feed Conc.	$\bar{M}_W$	$\bar{M}_N$	D
1	4.75	9.12	43.3	37.9	0.283	29500	8500	3.47
2	3.58	30.69	10.0	96.3	0.719	33400	9200	3.63
3	4.26	29.44	25.5	109.5	0.817	35600	9300	3.82
4	3.95	33.72	133.1	116.5	0.869	39550	9600	4.11
5	4.63	26.76	123.8	108.4	0.809	48000	10800	4.44
6	4.58	24.67	113.0	98.9	0.738	62500	12400	5.04
7	4.49	20.18	90.6	79.3	0.592	68000	15000	4.53
8	4.17	16.93	70.6	61.8	0.461	76300	16700	4.56
9	4.17	13.11	54.7	47.9	0.357	93500	22000	4.25

Fig. 7.23a On Column Concentration Profile for Run 5.2

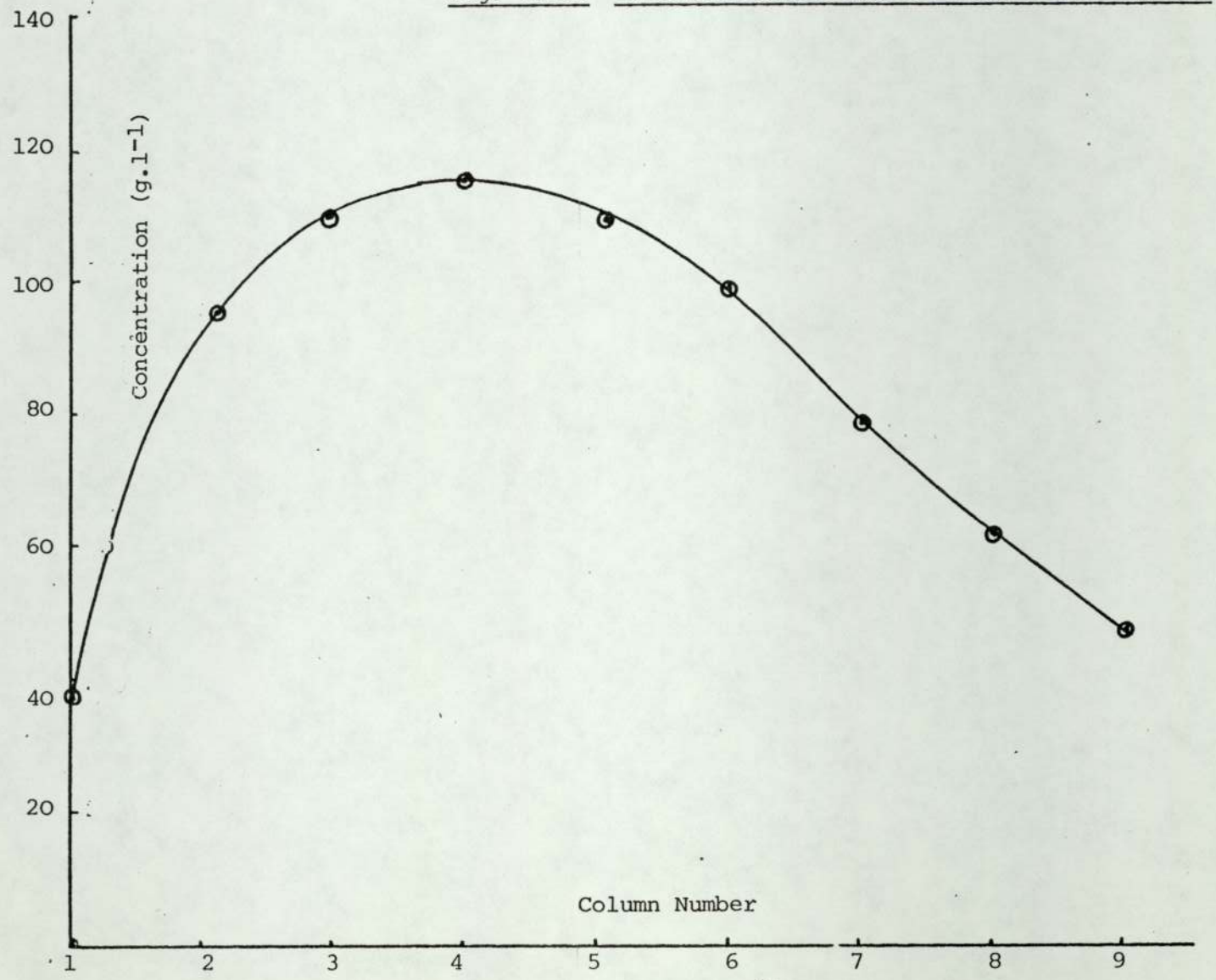


Fig. 7.24 Conditions and Results for UF Run 5

Run	5.1	5.2
Feed Vol. (l)	2.000	2.000
Feed Conc. (g/l)	17.0	19.5
Feed Mass (g)	34.0	39.0
Feed, $\bar{M}_w$	32200	29000
Feed, $\bar{M}_N$	9100	7500
Diafiltration Water (l)	10.000	8.000
Permeate Vol. (l)	11.830	9.845
Permeate Conc. (g/l)	1.23	1.65
Permeate Mass (g)	14.7	16.3
Permeate Output (%)	43.2	41.8
Permeate, $\bar{M}_w$	15260	13810
Permeate, $\bar{M}_N$	4640	4440
Retentate Vol. (l)	0.170	0.155
Retentate Conc. (g/l)	113.5	146.0
Retentate Mass (g)	19.3	22.7
Retentate Output (%)	56.8	58.2
Retentate, $\bar{M}_w$	44650	40950
Retentate, $\bar{M}_N$	27150	24300

Fig. 7.25 GPC and UF Fractionation of Dextran for Run 5.1

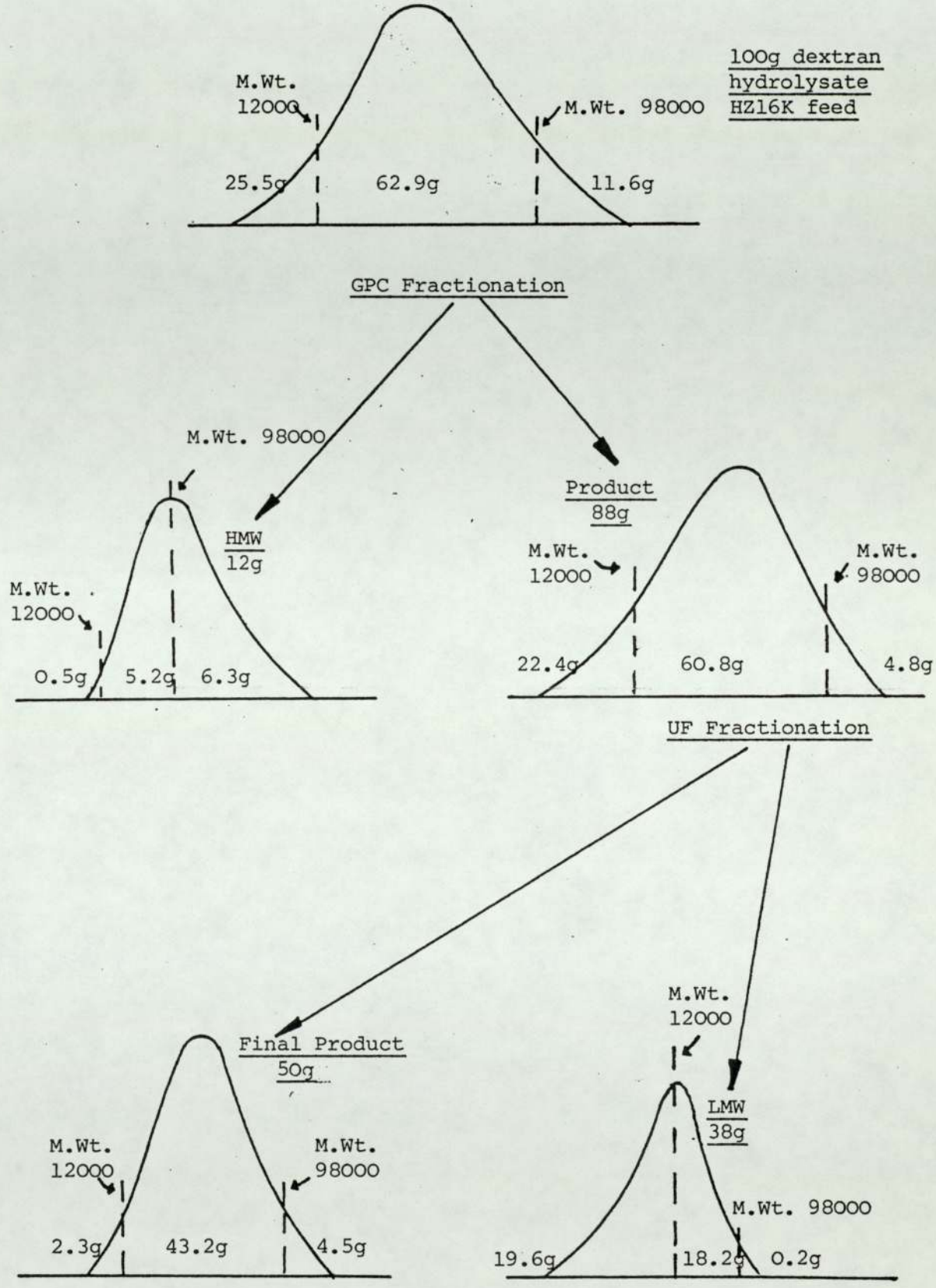


Fig. 7.26 GPC and UF Fractionation of Dextran for Run 5.2

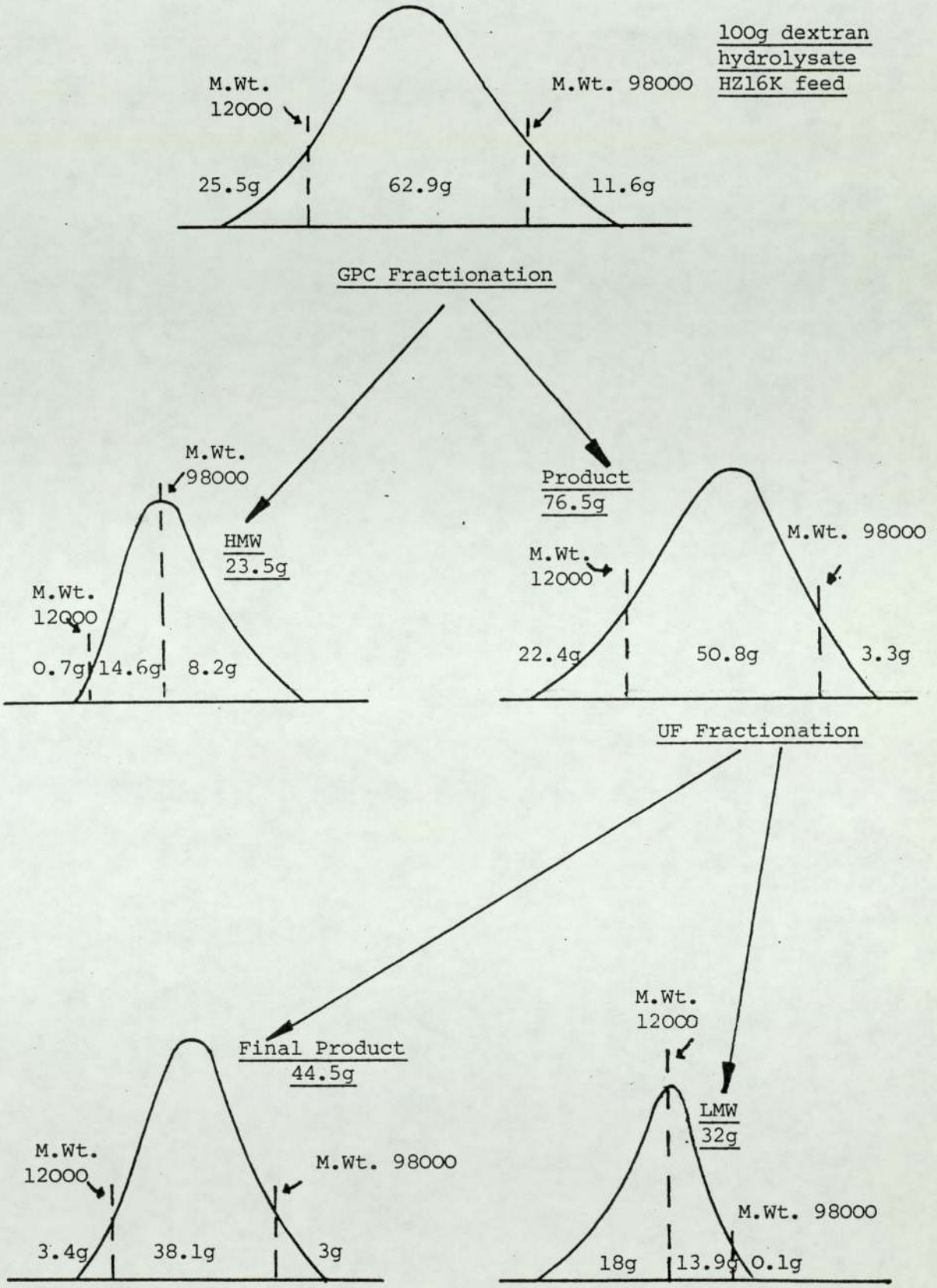


Fig. 7.27 Operating Conditions for GPC Run 6

Run	Cycle	Pressures ($kN.m^{-2}$)			Temperatures (^{o}C)			Input Flowrates $cm^3.min^{-1}$			Product Flowrates $cm^3.min^{-1}$		Cut-Positions		
		Eluent	Feed	Purge	Purge	Eluent	In-Line	L_1	L_2	L_3	HMW	LMW	Pre-Feed	Post-Feed	Purge
6.1	5&6	228	186	70	60	60	60	95	40	135	135	300	0.290	0.786	2.831
6.1	7	228	186	70	60	60	60	95	40	135	135	302	0.290	0.786	2.856
6.1	8	228	186	70	60	60	60	95	40	135	135	291	0.290	0.786	2.730
6.2	12	352	214	70	60	60	60	95	59.5	154.5	155	296	0.290	1.028	2.782
6.2	13	352	214	70	60	60	60	95	59.5	154.5	155	297	0.290	1.028	2.794
6.2	14	352	214	70	60	60	60	95	59.5	154.5	155	300	0.290	1.028	2.831

Fig. 7.28 Products from GPC Run 6

Run	Cycle	Dextran Input	High Mol. Wt. Product			Low Mol. Wt. Product			HMWP Output	LMWP Output	Mass Balance
		per cycle (g)	Conc.g/l	Vol. l	Mass g	Conc. g/l	Vol. l	Mass g			
6.1	7	402	6.5	10.2	65	14.8	22.7	336	0.162	0.838	1.000
6.1	8	402	6.3	10.2	64	15.0	21.8	327	0.164	0.836	0.975
6.2	13	598	14.0	11.6	163	19.3	22.3	430	0.275	0.725	0.998
6.2	14	598	14.2	11.6	165	19.2	22.5	432	0.276	0.724	1.000

		High Mol. Wt. Product			Low Mol. Wt. Product		
		$\bar{M}_w$	$\bar{M}_N$	D	$\bar{M}_w$	$\bar{M}_N$	D
6.1	7	400000	61000	6.65	31600	8300	3.80
6.1	8	353000	60000	5.84	29900	8100	3.71
6.2	13	224000	30000	7.58	30000	8500	3.55
6.2	14	242000	34000	7.05	31000	8500	3.71

Fig. 7.29 Purge Products from GPC Run 6.2

Column	Purged Volume l	Purge Conc. $g.l^{-1}$	Dextran Mass g	Column Conc. $g.l^{-1}$	Col. Conc. / Feed Conc.	$\bar{M}_w$	$\bar{M}_N$	D
1	3.49	11.5	40.1	35.1	0.262	31250	8100	3.85
2	3.81	31.7	120.8	105.8	0.790	33300	9150	3.64
3	4.08	33.0	134.6	117.9	0.880	35400	9450	3.75
4	3.72	37.3	138.8	121.5	0.907	35600	9200	3.87
5	3.90	35.0	136.5	119.5	0.892	36250	9450	3.84
6	3.95	33.7	133.2	116.6	0.870	37700	9350	4.03
7	3.95	33.0	130.4	114.2	0.852	42800	9200	4.65
8	3.45	36.2	124.9	109.4	0.816	56600	9700	5.83
9	4.08	21.2	86.5	75.7	0.565	80650	14500	5.56

Fig. 7.29a On Column Concentration Profile for Run 6.2

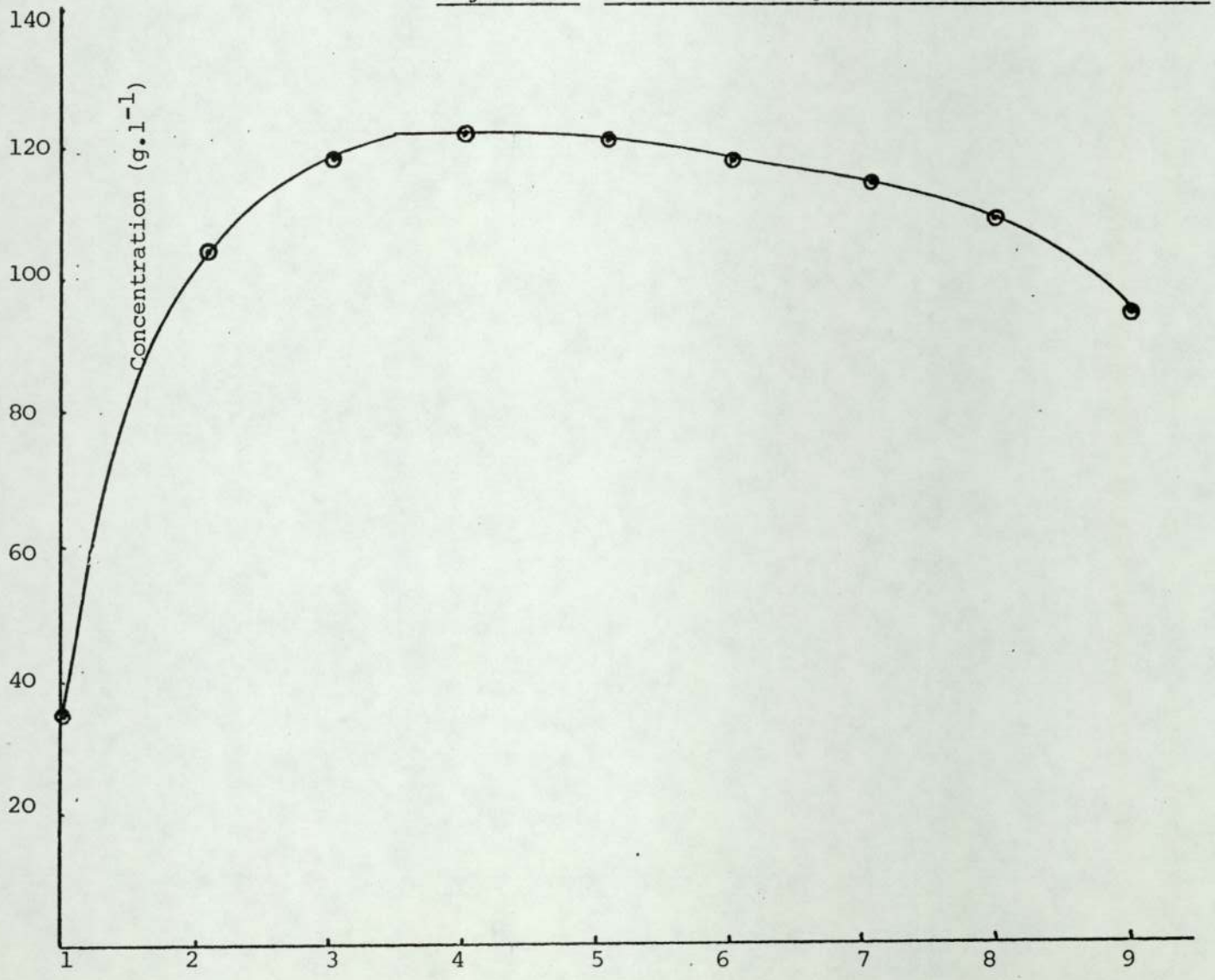


Fig. 7.30 Conditions and Results for UF Run 6

Run	6.1	6.2
Feed Vol. (l)	2.000	2.000
Feed Conc. (g/l)	15.0	19.2
Feed Mass (g)	30.0	38.4
Feed, $\bar{M}_w$	29900	31000
Feed, $\bar{M}_N$	8100	8500
Diafiltration Water (l)	6.500	6.000
Permeate Vol. (l)	8.350	7.840
Permeate Conc. (g/l)	1.72	2.37
Permeate Mass (g)	14.36	18.6
Permeate Output (%)	48.4	49.5
Permeate, $\bar{M}_w$	17400	17500
Permeate, $\bar{M}_N$	5400	4700
Retentate Vol. (l)	0.150	0.160
Retentate Conc. (g/l)	102.2	118.8
Retentate Mass (g)	15.33	19.0
Retentate Output (%)	51.6	50.5
Retentate, $\bar{M}_w$	42900	42250
Retentate, $\bar{M}_N$	25800	24650

Fig. 7.31. GPC and UF Fractionation of Dextran for Run 6.1

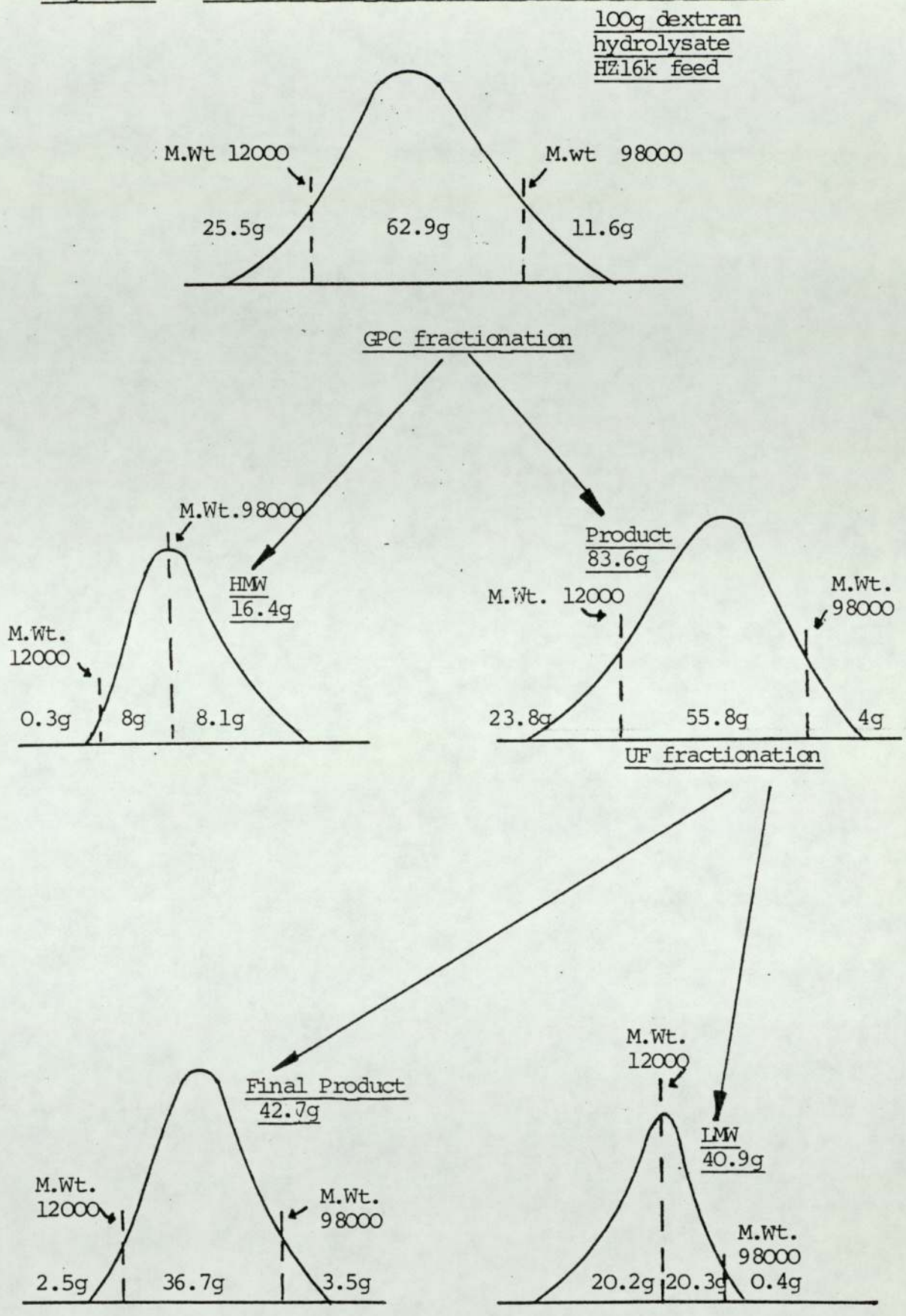


Fig. 7.32 GPC and UF Fractionation of Dextran for Run 6.2

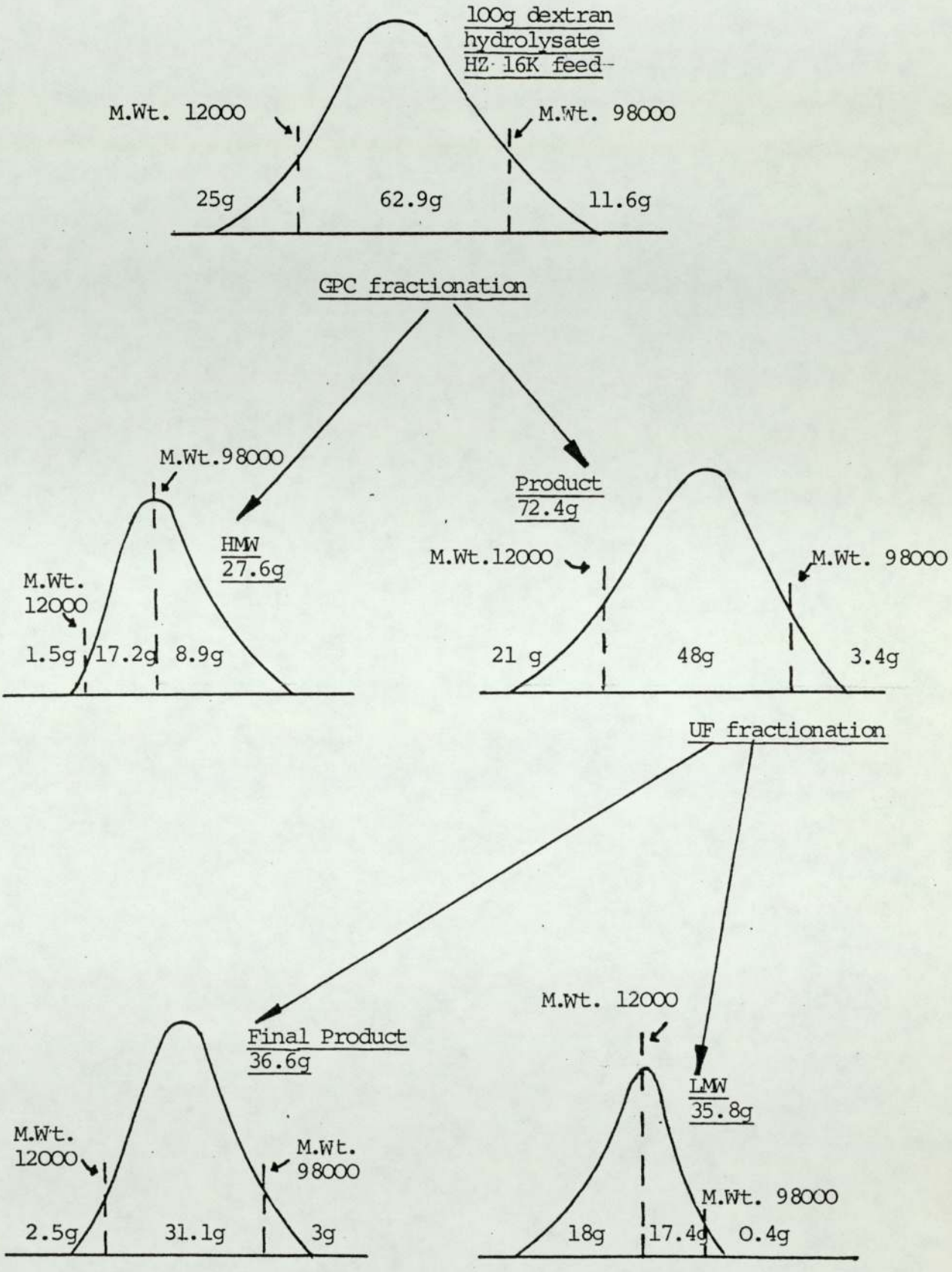


Fig. 7.33 Operating Conditions for GPC Run 7

Run	Cycle	Pressures ($kN.m^{-2}$)			Temperatures (^{o}C)			Input Flowrates $cm^3.min^{-1}$			Product Flows $cm^3.min^{-1}$		Cut-positions		
		Eluent	Feed	Purge	Purge	Eluent	In-line	L_1	L_2	L_3	HMW	LMW	Pre-feed	Post-feed	Purge
7.1	6	552	552	70	60	60	60	116	60	170	170	291	0.476	1.220	2.720
7.1	7	552	552	70	60	60	60	110	60	170	170	296	0.476	1.220	2.782
7.1	8	552	552	70	60	60	60	110	59	169	169	296	0.476	1.207	2.782
7.2	12	483	482	70	60	60	60	94	60	154	154	291	0.278	1.021	2.720
7.2	13	483	483	70	60	60	60	95	59	154	154	312	0.290	1.021	2.980
7.2	14	483	483	70	60	60	60	94	60.5	154.5	154	293	0.278	1.021	2.745

Fig. 7.34 Products from GPC Run 7

Run	Cycle	Dextran input	High Mol. wt. Product			Low Mol. wt. Product			HMWP Output	LMWP Output	Mass Balance
		per cycle (g)	Conc. g/ℓ	Vol. ℓ	Mass g	Conc. g/ℓ	Vol.	Mass g			
7.1	7	603	22.0	12.75	283	14.0	22.23	311	0.476	0.533	0.990
7.1	8	592	22.5	12.70	285	13.0	22.23	290	0.495	0.505	0.970
7.2	13	592	12.0	11.55	138	18.0	23.45	422	0.245	0.755	0.950
7.2	14	608	12.5	11.55	144	22.4	22.0	493	0.226	0.774	1.050

		High Mol. Wt. Product			Low Mol. Wt. Product		
		$\bar{M}_W$	$\bar{M}_N$	D	$\bar{M}_W$	$\bar{M}_N$	D
7.1	7	158000	18000	8.84	27450	7650	3.59
7.1	8	134000	17000	7.97	27200	7350	3.68
7.2	13	250000	30000	8.30	33000	8000	4.12
7.2	14	249000	28500	8.79	30100	7700	3.90

Fig. 7.35 Purge Products from GPC Run 7.2

Column	Purged Volume ℓ	Purge Conc. $g.\ell^{-1}$	Dextran mass g	Column Conc. $g.\ell^{-1}$	Col. Conc. / Feed Conc.	$\bar{M}_W$	$\bar{M}_N$	D
1	4.36	9.9	43.2	37.8	0.282	29000	7900	3.67
2	4.05	31.6	128.0	112.1	0.836	33150	8500	3.90
3	4.00	34.8	139.2	121.9	0.909	44600	8650	5.16
4	3.68	35.5	130.6	114.4	0.853	49650	8950	5.55
5	3.71	32.8	121.7	106.6	0.796	53050	10200	5.71
6	3.84	28.6	109.8	96.1	0.717	57000	10200	5.59
7	3.68	23.7	87.2	76.4	0.570	58000	10200	5.65
8	3.58	19.6	70.2	61.5	0.459	63800	10450	6.12
9	3.73	13.8	51.5	45.1	0.337	83000	12400	6.58

Fig. 7.35a On Column Concentration Profile for Run 7.2

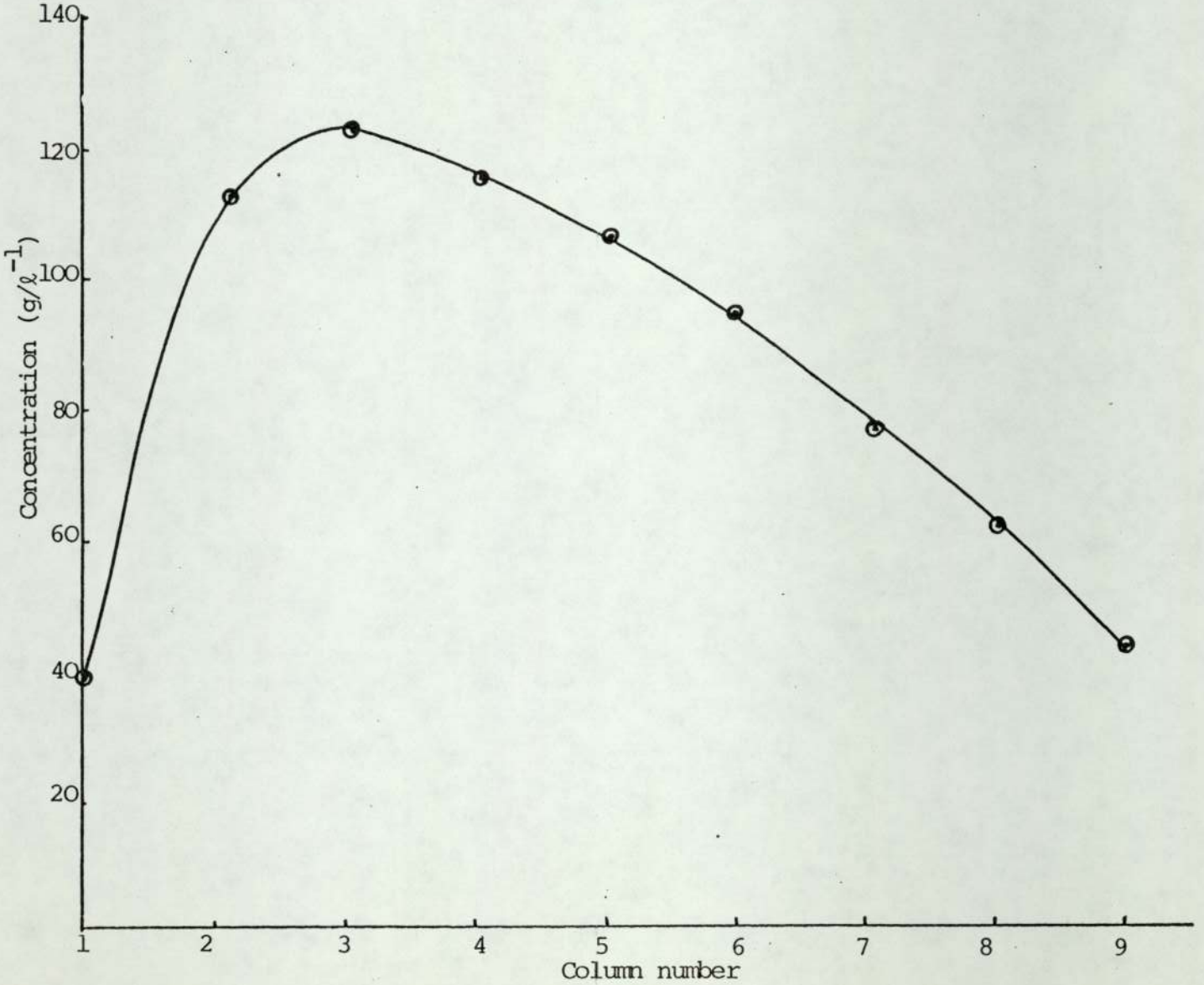


Fig. 7.36 Conditions and Results for UF Run 7

Run	7.1	7.2
Feed Vol. (ℓ)	2.000	2.000
Feed Conc. (g/ℓ)	13.0	22.4
Feed Mass (g)	26.0	44.8
Feed, $\overline{M}_W$	27200	30100
Feed, $\overline{M}_N$	7350	7700
Diafiltration water (ℓ)	2.000	3.000
Permeate vol. (ℓ)	3.840	4.785
Permeate conc. (g/ℓ)	3.0	4.2
Permeate Mass (g)	11.5	20.1
Permeate Output (%)	44.9	43.9
Permeate, $\overline{M}_W$	14450	15570
Permeate, $\overline{M}_N$	4150	4350
Retentate Vol. (ℓ)	0.160	0.215
Retentate conc. (g/ℓ)	88.0	119.5
Retentate Mass (g)	14.1	25.7
Retentate Output (%)	55.1	56.1
Retentate, $\overline{M}_W$	33750	40750
Retentate, $\overline{M}_N$	20300	21050

Fig. 7.37 GPC and UF Fractionation of Dextran for Run 7.1

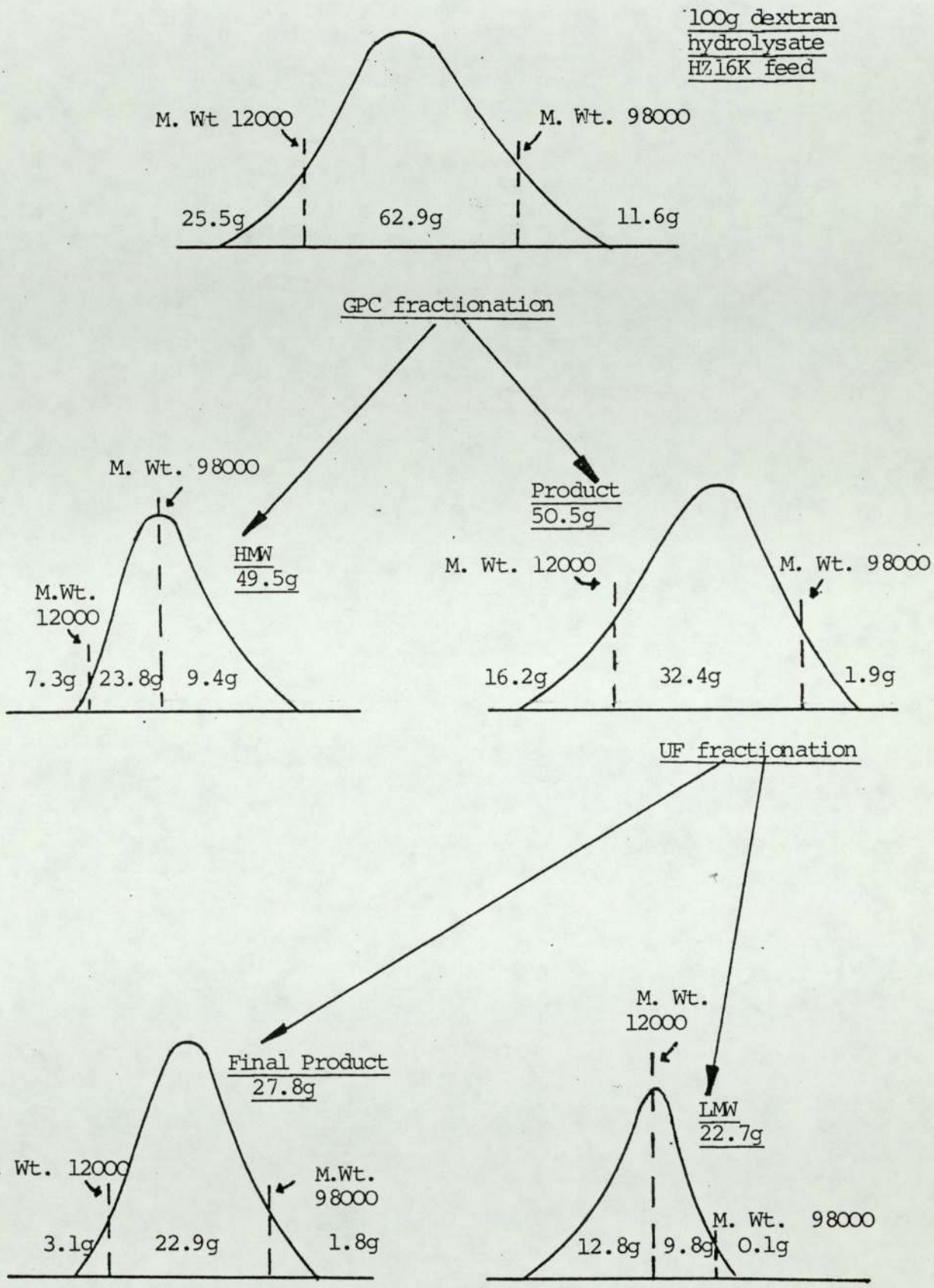


Fig. 7.38 GPC and UF Fractionation of Dextran for Run 7.2

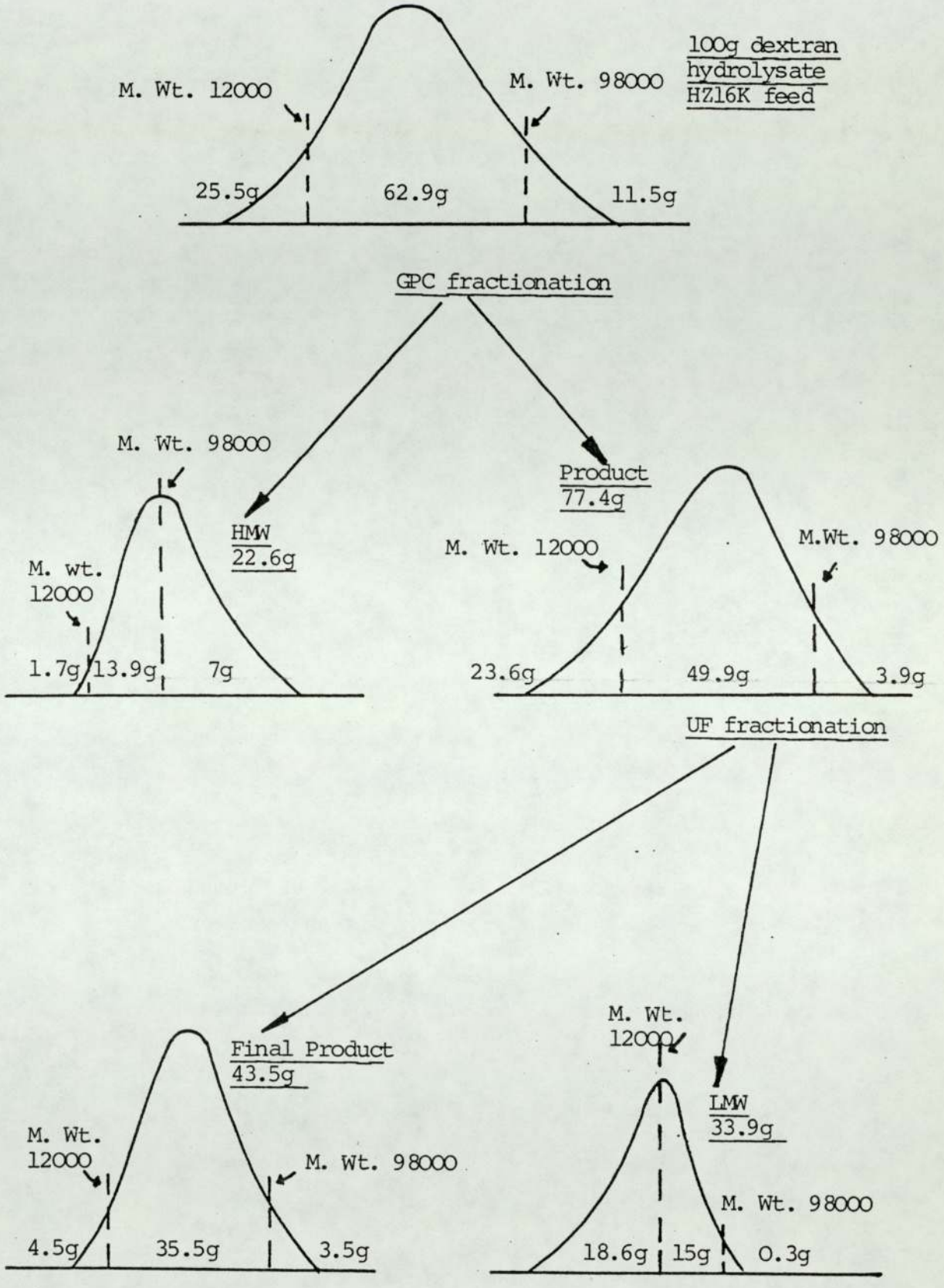


Fig. 7.39 Purge Profile on Column 1, i.e. the Column with the Lowest Mol. Wt. Dextran

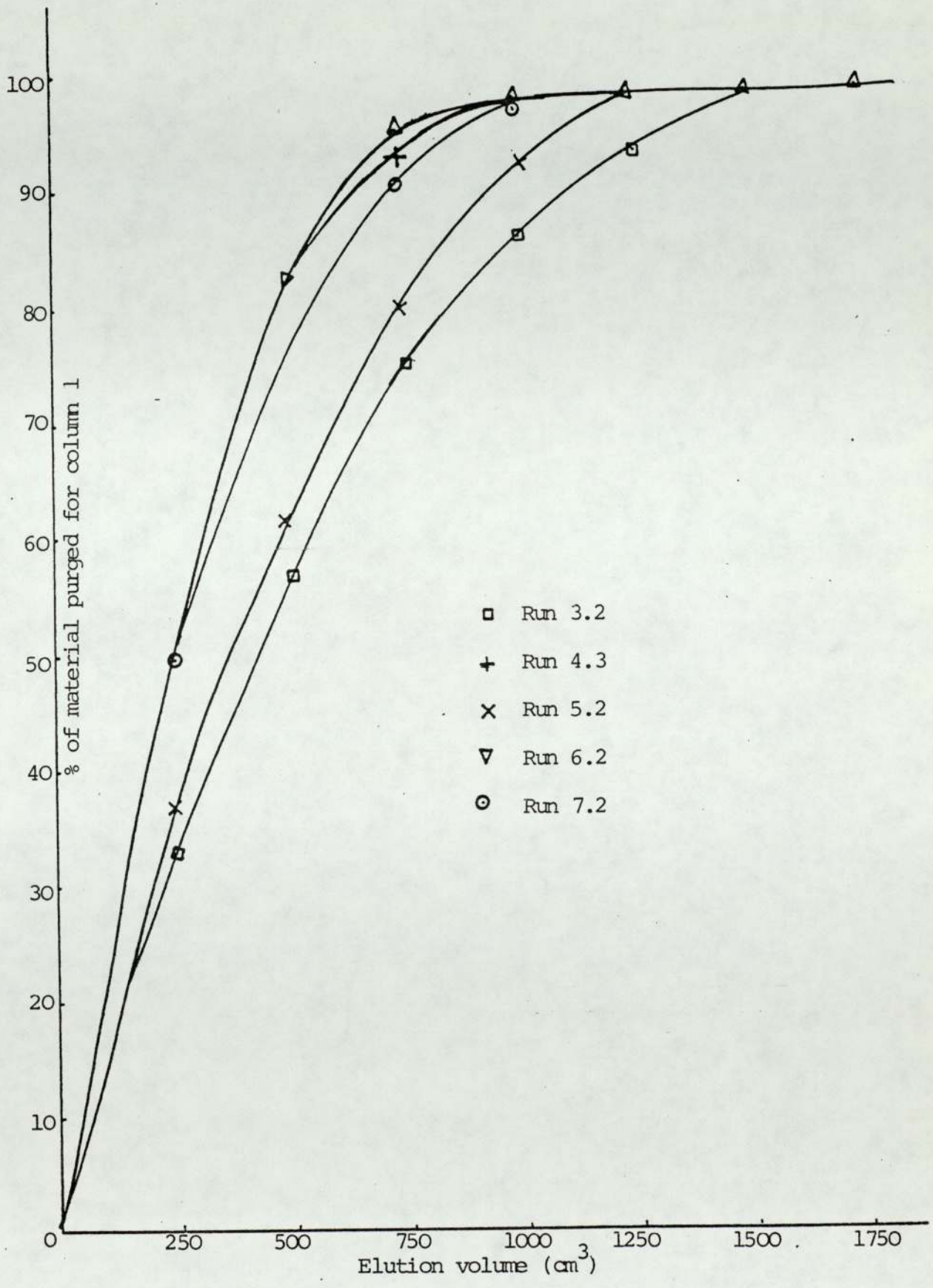


Fig. 7.40 Summary of the Results for the GPC Runs

Run	Input Mass (g)	High Mol. Wt. Product					Low Mol. Wt. Product				
		Mass (g)	Conc. ($g.\ell^{-1}$)	Mol. Wt. Distribution			Mass (g)	Conc. ($g.\ell^{-1}$)	Mol. Wt. Distribution		
				$\bar{M}_W$	$\bar{M}_N$	D			$\bar{M}_W$	$\bar{M}_N$	D
3.1	702	80	8.2	465000	54000	8.61	622	28.0	35200	9000	3.92
3.2	1018	217	18.8	287000	40600	7.07	802	32.4	35300	8900	3.97
4.1	387	87	6.6	373000	56500	6.61	299	22.3	32900	7400	4.42
4.2	387	77	7.7	336000	54000	6.22	311	22.7	28900	7200	4.03
4.3	709	297	24.1	220000	27200	8.09	392	24.2	27500	6500	4.25
5.1	412	50	5.0	446000	52000	8.55	365	17.0	32200	9100	3.54
5.2	593	137	11.8	279000	42000	6.62	445	19.5	29000	7500	3.86
6.1	402	64	6.3	353000	60000	5.84	327	15.0	29900	8100	3.71
6.2	598	165	14.2	242000	34000	7.05	432	19.2	31000	8500	3.71
7.1	593	285	22.5	134000	17000	7.97	290	13.0	27200	7350	3.68
7.2	608	144	12.5	249000	28500	8.73	493	22.4	30100	7700	3.90

Fig. 7.41 Summary of the Results for the GPC and UF Runs

Run	Input Mass g	GPC Runs HMW Dextran removed (%)	UF Runs LMW dextran removed (%)	Final Products Final Product (%)	$\overline{MW}$	$\overline{MN}$	Material < 12000 (%)	Material > 98000 (%)	Material off specification (%)
3.1	702	11.4	36.8	51.8	47000	23400	8.2	10.6	18.8
3.2	1018	21.3	25.1	53.6	43250	19800	11.9	9.3	21.2
4.1	387	18.2	31.2	50.6	35300	19550	7.3	7.9	15.2
4.2	387	19.8	27.7	52.5	35300	19550	12.2	5.4	17.6
4.3	709	43.2	23.8	33.0	36900	20700	10.5	5.8	16.3
5.1	412	12.0	38.0	50.0	44650	27150	4.6	8.9	13.5
5.2	593	23.5	32.0	44.5	40950	24300	6.9	7.4	14.3
6.1	402	16.4	40.9	42.7	42900	25800	5.7	8.2	13.9
6.2	598	27.6	35.8	36.6	42250	24650	6.8	8.2	15.0
7.1	593	49.5	22.7	27.8	38350	20300	10.9	6.5	17.4
7.2	608	22.6	33.9	43.5	40750	21050	10.2	8.0	18.2

Fig. 7.42 The Efficiencies of the GPC Runs

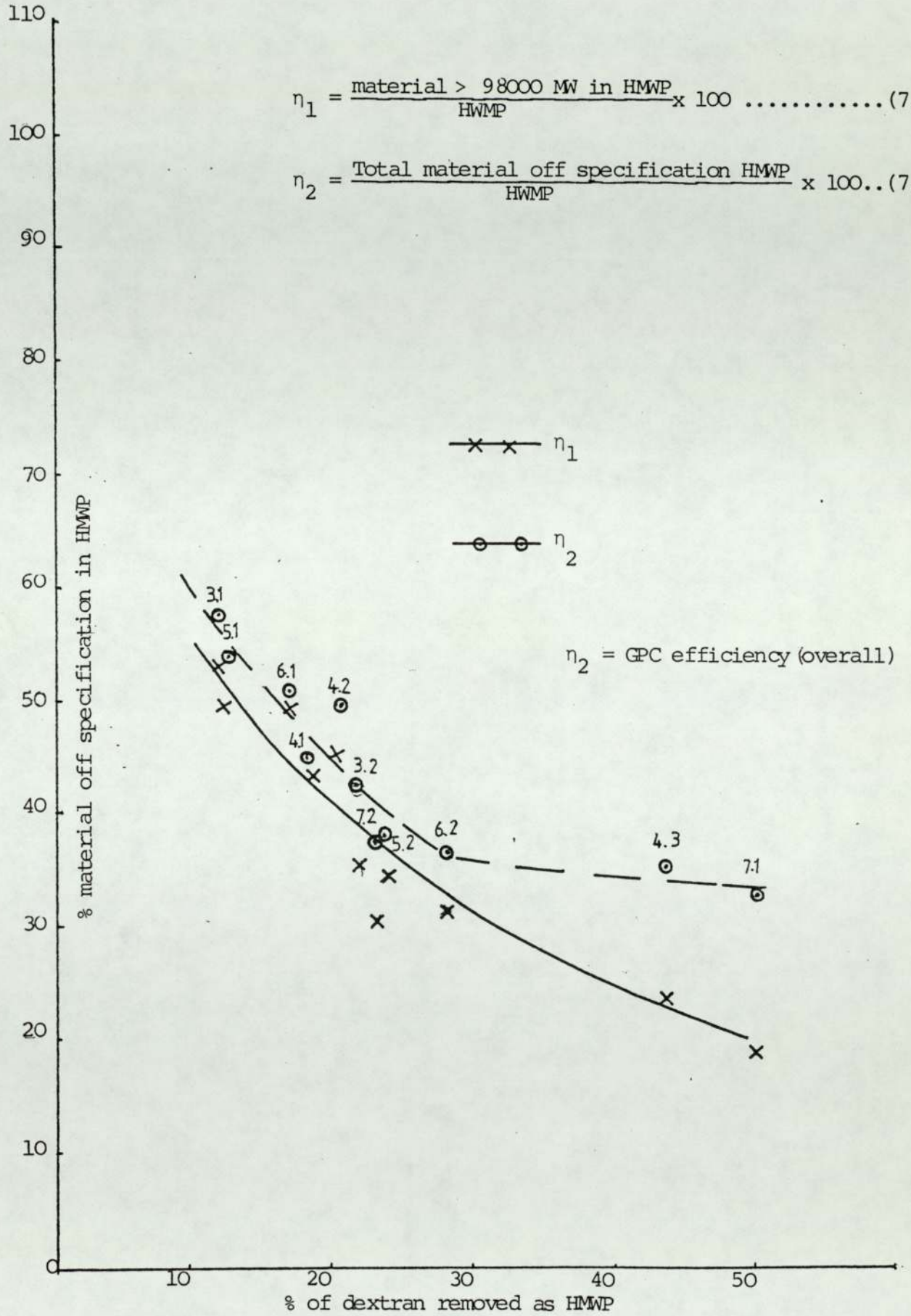
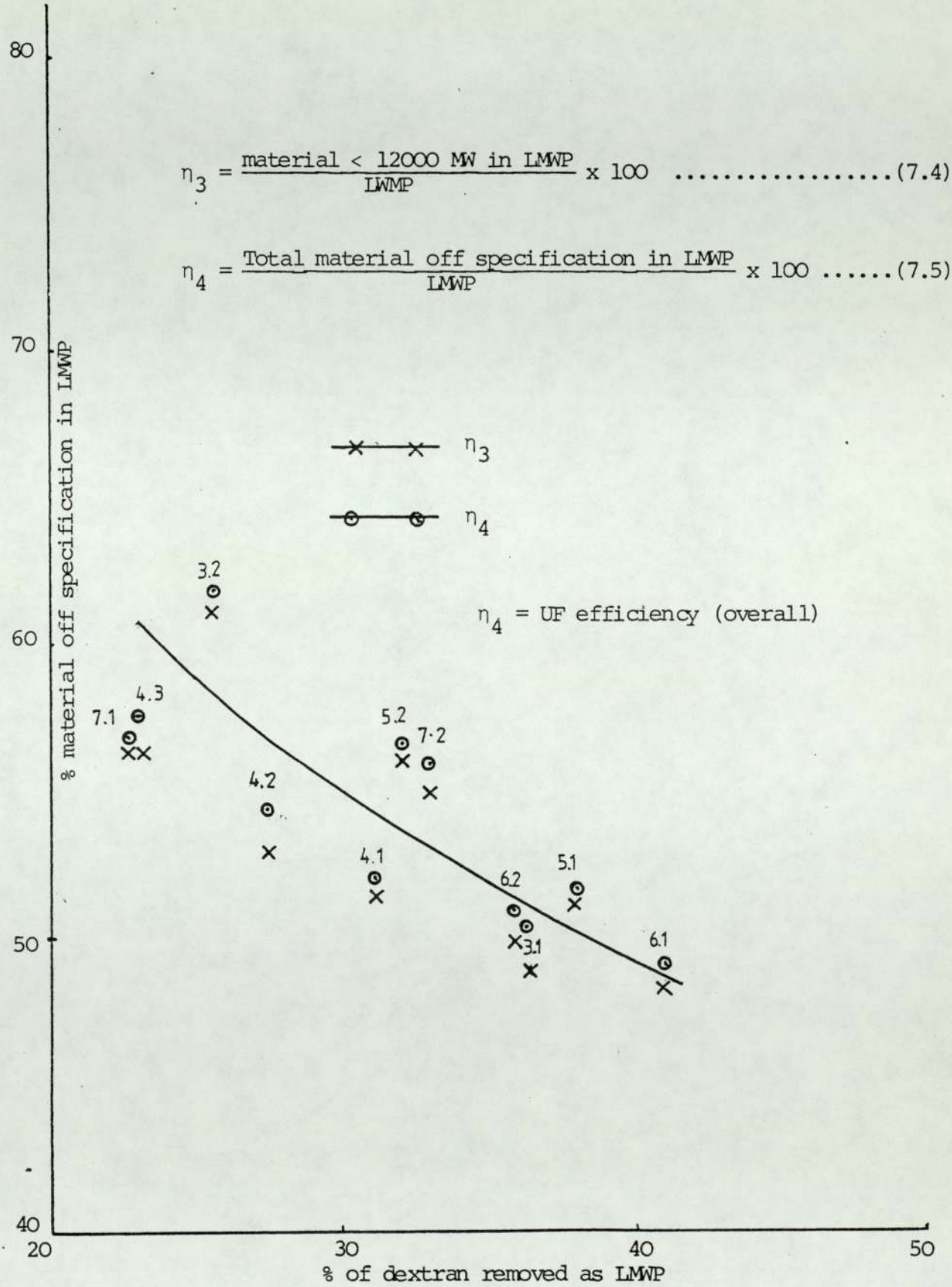


Fig. 7.43 The Efficiencies of the UF Runs

$$\eta_3 = \frac{\text{material} < 12000 \text{ MW in LMWP}}{\text{LWMP}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (7.4)$$

$$\eta_4 = \frac{\text{Total material off specification in LMWP}}{\text{LMWP}} \times 100 \quad \ldots\ldots (7.5)$$



to travel with the mobile phase according to equation (4.8).

(2) Also as the pre-feed cut position increases the amount of dextran in the high molecular weight product increases (Runs 3.1 and 4.2, 5.1 and 4.2, 7.2 and 7.1). This is due to the effect of higher mobile flowrate in the fractionating section that washes more molecules towards the high molecular weight product outlet port in agreement with equation (4.8).

(3) As the feed concentration increases, the dextran removed with the low molecular weight product also increases (Runs 3.1 and 5.1, 3.2 and 5.2). The same observation was reported by England (46) and Ellison (47). This is because the elution volumes of dextran molecules are higher in concentrated solutions than in dilute solutions (Section 2.6.4).

(4) A temperature increase will cause less dextran molecules to be purged out with the low molecular weight product (Runs 4.1 and 4.2), because the elution volumes of dextran molecules decreases as the temperature increases (187), probably due to thermal expansion of the dextran molecules.

(5) The position at which the feed is introduced effects the cut of a GPC run on the SCCR5. The closer the feed position is to high molecular weight product outlet port, more dextran molecules are removed with this product (Runs 5.1 and 6.1,

5.2, 6.2 and 7.2), since the purge section takes longer to reach the feed column and hence more eluent will be able to pass through this column so that more dextran molecules will be eluted (Fig. 4.2). Also the efficiency of the GPC fractionations increases slightly as the feed inlet position moves away from the eluent inlet port (Fig. 4.1 and Fig. 7.42, Runs 5,6 and 7).

(6) To purge a SCCR5 column, packed with Spherosil XOB 075, completely from its dextran molecules, during a run, it is necessary to pass at least 2 l of water through it (Fig. 7.39).

(7) The efficiency of the GPC fractionations decreases as the dextran removed as high molecular product is increased (Fig. 7.42). This is probably due to the depletion of the very high molecular weight dextran.

(8) The efficiency also increases as the eluent to feed ratio (Runs 3.2 and 4.2) and temperature (Runs 4.1 and 4.2) increase (Fig. 7.42).

(9) The on column concentration of dextran increases as the feed concentration increases (Figs. 7.5, 7.13 and 7.23).

(10) The dissolution of silica packing in the SCCR5 increases significantly as the temperature increases (Figs. 7.18, 7.19). This is due to higher silica dissolution at elevated temperatures.

For the UF fractionations it was observed that as the dextran removed with the permeate increased, the efficiency of the UF runs decreased (Fig. 7.43), probably due to the depletion of the very small dextran molecules.

Also most of the silica in the GPC main product can be removed by using UF (Figs. 7.9, 7.10, 7.18 to 7.20) and it appears that the higher the permeate volume is, the more silica is removed, because silica is passing freely with the water molecules through the pores of the membrane. But completely silica free product can be only produced by passing the dextran products through a mixed bed, nuclear grade, ion exchange cartridge (Figs. 7.9, 7.10, 7.18 to 7.20)

7.5 CONCLUSIONS

From these runs it was found that clinical dextran 40 can be produced from dextran hydrolysate combining GPC, UF and ion exchange (Fig. 7.41).

It is necessary to concentrate the final dextran product solution at least up to 250 g l^{-1}, before it can be spray dried. To achieve such a high solution concentration with the UF system used, at ambient temperatures, will take very long time, therefore the system needs to be modified so that it will be able to work at elevated temperatures in order to achieve the required concentration within acceptable time limits (Fig. 6.4d, Run 11).

Also the GPC runs carried out on the SCCR5 had an efficiency between 35 and 60% depending on the percentage of dextran hydrolysate removed as high molecular weight product. This means that for every 100 g of material removed as high molecular weight product, 40 to 65 g were useful material (on specification). Therefore the efficiency of GPC process must be improved so as not to remove so much of useful material. The change in the feed position and the higher eluent to feed ratio although they looked promising, initially did not have any significant effect. Probably the efficiency of the SCCR5 can be increased by using a higher porosity packing, a smaller particle size packing, or more columns.

The efficiency of the UF runs for the removal of the low molecular weight material from the dextran hydrolysate feed, on the DC2A Amicon system using an H1P5 cartridge lies between 50 and 62%, and probably will be increased by using an H1P2 cartridge, but it will take considerably longer to achieve the same fractionation.

Finally nuclear grade, mixed bed, ion exchange cartridges, that are used with Milli Q ultrapure water system, marketed by Millipore (UK), can be used to produce a silica free dextran.

8.0 PRODUCTION OF CLINICAL DEXTRAN 40 FROM DEXTRAN HYDROLYSATE USING GPC, ULTRAFILTRATION (UF) AND ION EXCHANGE. II

8.0 PRODUCTION OF CLINICAL DEXTRAN 40 FROM DEXTRAN HYDROLYSATE USING GPC, ULTRAFILTRATION (UF) AND ION EXCHANGE. II

8.1 INTRODUCTION

During the previous dextran fractionations on the SCCR5 (Chapter 7.0) it was found that for every 100 g of dextran removed as high molecular weight product, 40-65 g were useful material. Hence a way of improving the efficiency of the GPC fractionations on the SCCR5 was required.

The Spherosil XOB 075 packing used in the SCCR5 was to be replaced with a GPC packing having larger pores in a trial to increase the efficiency of the GPC fractionations.

8.2 SPHEROSIL XOB 030. THE CHOICE OF PACKING, ITS PROPERTIES AND PROPERTIES OF THE PACKED COLUMNS

To reduce the amount of useful material removed as high molecular weight product, during the dextran hydrolysate GPC fractionations, a packing having larger pores than the existing Spherosil XOB 075 was to be tried.

It was decided to use a larger pore size packing, because this packing will be able to fractionate dextrans of higher molecular weight than the Spherosil XOB 075.

Fig. 8.1 Properties of the SCCR5 Columns Packed with Spherosil XOB 030

Column	Liquid Volume $V_o + V_i$ (cm^3)	Void Volume V_o (cm^3)	Pore Volume V_i (cm^3)	$\frac{V_i}{V_o}$	Number of Plates N	HETP (cm)
1	1129	578	551	1.05	78	0.90
2	1144	550	594	0.93	79	0.89
3	1104	550	554	0.99	73	0.96
4	1144	525	619	0.85	92	0.76
5	1148	572	576	0.99	64	1.09
6	1181	575	606	0.95	67	1.04
7	1140	569	571	1.00	72	0.97
8	1125	515	610	0.84	72	0.97
9	1136	555	581	0.96	72	0.97
10	1123	535	588	0.91	73	0.96
Average	1138	552	586	0.94	74	0.94

* These properties were measured at a flowrate of 100 $cm^3.min^{-1}$

Fig. 8.2 Calibration of the Spherosil XOB 075 and XOB 030 packings using Pharmacia T-fractions of dextran

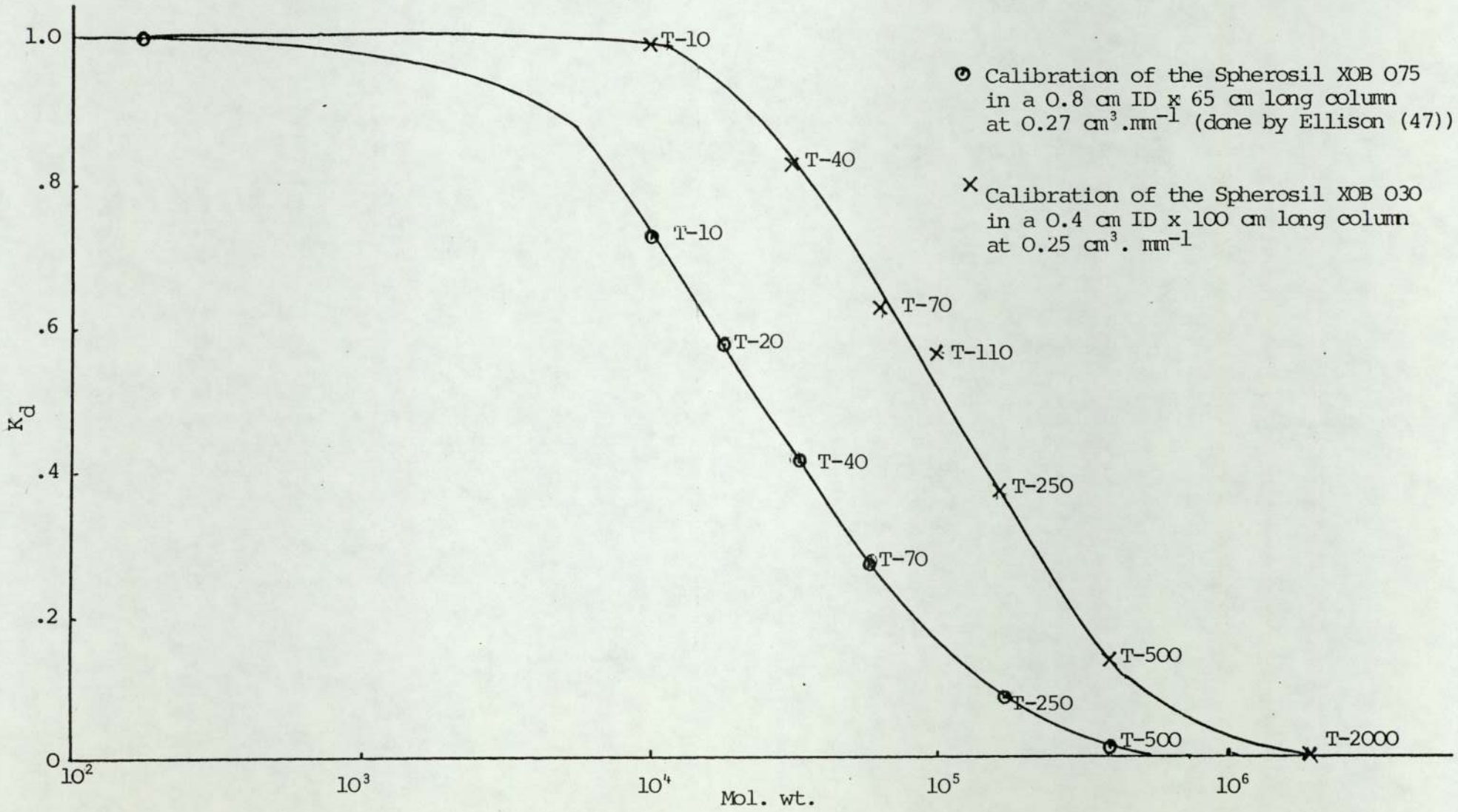


Fig. 8.3 Comparison between Spherosil XOB 075 and XOB 030 packings and packed columns

Properties	Spherosil XOB 075	Spherosil XOB 030
+Average Pore size (Å)	350	700
+Pore volume ($cm^3.g^{-1}$)	1.2	0.94
+Specific surface area ($m^2.g^{-1}$)	0.9	0.45
Average particle diameter (µm)	285	340
Material between 200 - 400 µm (%)	88	98
Fractionation range (daltons)	$1x10^4$- 1.5 x 10^5	7.5 x 10^4-$5x10^5$
$V_o + V_i$ for the SCCR5 packed colums (cm^3)	1142	1138
V_o for the SCCR5 packed columns (cm^3)	537	552
V_i for the SCCR5 packed columns (cm^3)	605	586
Number of plates per SCCRS column	84	74

+These packing properties were measured by the manufacturer.

Finally, Spherosil XOB 030, of the same particle size as the Spherosil XOB 075, was selected as a replacement, due to the similarities in the properties of the two Spherosils and because of their comparatively low price. Ellison (47) in his thesis presents all the reasons that led to the selection of the Spherosil XOB 075 as the GPC packing.

The columns of the SCCR5 were emptied from the Spherosil XOB 075 packing and were slurry packed (47) with the Spherosil XOB 030 packing. The repacked columns were calibrated using batch chromatographic techniques (47). The properties of the packed columns are shown in Fig. 8.1. A comparison of the properties of the two Spherosil packings as well as of the packed columns in the SCCR5, before and after they have been repacked, is made in Figs. 8.2 and 8.3.

8.3 EXPERIMENTAL OPERATING CONDITIONS. PURPOSE OF THE RUNS

In the attempts to achieve more optimum conditions for the production of clinical dextran 40 from dextran hydrolysate, the Spherosil XOB 075 packing in the SCCR5 was replaced by the Spherosil XOB 030 packing. A new H1P5 hollow fibre cartridge replaced the old one in the DC2A UF system and the H1P2 cartridge was to be tried in the DC2A system in order to compare its fractionation performance with that of the H1P5 cartridges.

Also a large scale Patterson Candy International (PCI) reverse osmosis system equipped with six, 183 cm long x 1.2 cm I.D. tubular membranes was used for the removal of low molecular weight dextran.

The experimental conditions of the GPC runs are presented in Figs. 8.4, 8.5 and 8.16.

Run 8.2 used identical operating conditions to run 5.2, done on the Spherosil XOB 075 packing, in an attempt to evaluate the effects of different porosity packings in the fractionation of dextran.

In run 8.1 the eluent and feed flowrates were adjusted so that approximately 10 to 25% of the dextran hydrolysate would be removed as high molecular weight product (see Section 7.3).

Runs 9.1, 9.2, 9.3 and 9.4 used more dilute conditions, than any of the previous runs, in order to be sure that the GPC columns were not overloaded.

In run 9.1 the pre-feed and post-feed cut positions were kept the same as in run 8.1, but in order to reduce the feed input the feed concentration was reduced.

From the GPC runs that were performed on the Spherosil XOB 075, the runs having the higher eluent to feed ratio looked promising in improving the efficiency of the GPC fractionations, hence in run 9.2 the eluent to feed ratio was increased, compared with run 9.1, by decreasing the feed rate, and keeping the same eluent rate and feed concentration.

In run 9.3 the eluent to feed ratio was increased, compared with run 9.2, by increasing the eluent rate,

Fig. 8.4 Theoretical conditions for the GPC runs

Run	Feed Column	Temperature (^{o}C)	Switch time (S)	Conc. of feed ($g.\ell^{-1}$)	Feedrate (g)		Eluent to feed ratio	Flowrates ($cm^3.min^{-1}$)			Theoretical cut positions		
					Cycle	Hour		Eluent	Feed	Purge	Pre-feed	Post-feed	Purge
8.1	5	60	450	131	344	275	3.285	115	35	300	0.530	0.978	2.831
8.2	5	60	450	131	590	472	1.600	96	60	300	0.290	1.042	2.831
9.1	5	60	450	71	186	149	3.285	115	35	325	0.530	0.978	3.217
9.2	5	60	450	71	134	108	5.000	125	25	325	0.530	0.850	3.217
9.3	5	60	450	71	134	108	5.000	125	25	325	0.658	0.978	3.217
9.4	5	60	450	118	134	108	8.300	125	15	325	0.658	0.850	3.217

Fig. 8.5 Operating conditions for GPC run 8

Run	Cycle	Pressures (kN^{-2})			Temperatures (^{o}C)			Input Flowrates $cm^3 \cdot min^{-1}$			Product Flows $cm^3 \cdot min^{-1}$		Cut-positions		
		Eluent	Feed	Purge	Purge	Eluent	In-line	L_1	L_2	L_3	HMW	LMW	Pre-feed	Pre-feed	Purge
8.1	8	275	206	70	60	60	60	115	34	150	150	300	0.530	0.978	2.831
8.1	9	275	206	70	60	60	60	115	34	150	150	318	0.530	0.978	3.128
8.1	10	275	206	70	60	60	60	115	34	150	150	301	0.530	0.978	2.910
8.2	13	344	310	70	60	60	60	96	59	155	155	287	0.290	1.042	2.731
8.2	14	344	310	70	60	60	60	96	59	155	155	304	0.290	1.042	2.949
8.2	15	344	310	70	60	60	60	96	59	155	155	306	0.290	1.042	2.974

Fig. 8.6 Products from GPC run 8

Run	Cycle	Dextran Input per cycle (g)	High Mol. Wt. Product Conc. g/ℓ	Vol. ℓ	Mass g	Low Mol. Wt. Product Conc. g/ℓ	Vol. ℓ	Mass g	HMWP Output	LMWP Output	Mass Balance
8.1	8	334	4.0	11.34	46	12.0	22.50	270	0.145	0.855	0.950
8.1	9	334	3.7	11.34	42	11.0	23.86	263	0.138	0.862	0.920
8.1	10	334	4.1	11.34	46	12.2	22.60	276	0.142	0.858	0.960
8.2	13	579	6.4	11.57	75	20.4	21.55	440	0.146	0.854	0.890
8.2	14	579	7.0	11.57	80	20.1	22.80	460	0.149	0.851	0.940
8.2	15	579	7.0	11.57	80	20.0	23.00	460	0.149	0.851	0.940

		High Mol. Wt. Product			Low Mol. Wt. Product		
		$\bar{M}_W$	$\bar{M}_N$	D	$\bar{M}_W$	$\bar{M}_N$	D
8.1	8	432000	52000	8.33	36000	10500	3.43
8.1	9	428000	54000	7.93	36300	10400	3.49
8.1	10	422000	52500	8.03	35750	10100	3.53
8.2	13	405000	39000	10.38	43000	11000	3.90
8.2	14	402000	39500	10.18	41500	9800	4.23
8.2	15	382000	38000	10.08	42400	10100	4.20

Fig. 8.7 Purge Products from GPC run 8.2

Column	Purged Volume ℓ	Dextran Conc. $g.\ell^{-1}$	Dextran Mass g	Column Conc. $g.\ell^{-1}$	Col. Conc. / Feed Conc.	$\bar{M}_W$	$\bar{M}_N$	D
1	3.26	14.5	47.2	41.5	0.316	38950	9250	4.21
2	3.06	40.1	122.9	108.0	0.824	39500	9250	4.26
3	3.04	45.7	138.8	122.0	0.931	42000	10250	4.09
4	3.23	44.8	144.5	127.0	0.969	49000	9800	5.01
5	2.94	48.8	143.4	126.0	0.961	55000	10500	5.24
6	3.18	38.3	121.8	107.0	0.816	61500	13000	4.71
7	2.28	39.4	89.9	79.9	0.603	70000	14000	5.00
8	2.07	32.4	67.1	59.0	0.450	94000	16100	5.82
9	3.01	13.6	41.0	36.0	0.274	166000	18000	9.23

Fig. 8.8 Dextran profile in the mobile phase in the SCCR5 for run 8

Outlet of Column	Run 8.1				Run 8.2			
	Conc. $g.\ell^{-1}$	$\bar{M}_W$	$\bar{M}_N$	D	Conc. $g.\ell^{-1}$	$\bar{M}_W$	$\bar{M}_N$	D
1	71	37000	9150	4.04	77	38000	8850	4.29
2	114	39800	9250	4.30	112	40500	9150	4.43
3	117	51000	10100	5.04	115	50500	10600	4.76
4	115	69000	12150	5.67	113	65000	13200	4.92
5	70	87000	12750	6.82	106	83000	13400	6.19
6	36	126000	18500	6.81	82	155000	22300	6.95
7	28	186000	25400	7.32	61	204000	27400	7.44
8	16	375000	41000	9.14	51	353000	31300	11.28
9	12	505000	49500	10.20	20	453000	35600	12.72

Fig. 8.9 Mobile phase concentration profiles for run 8.1. Profiles for three consecutive cycles

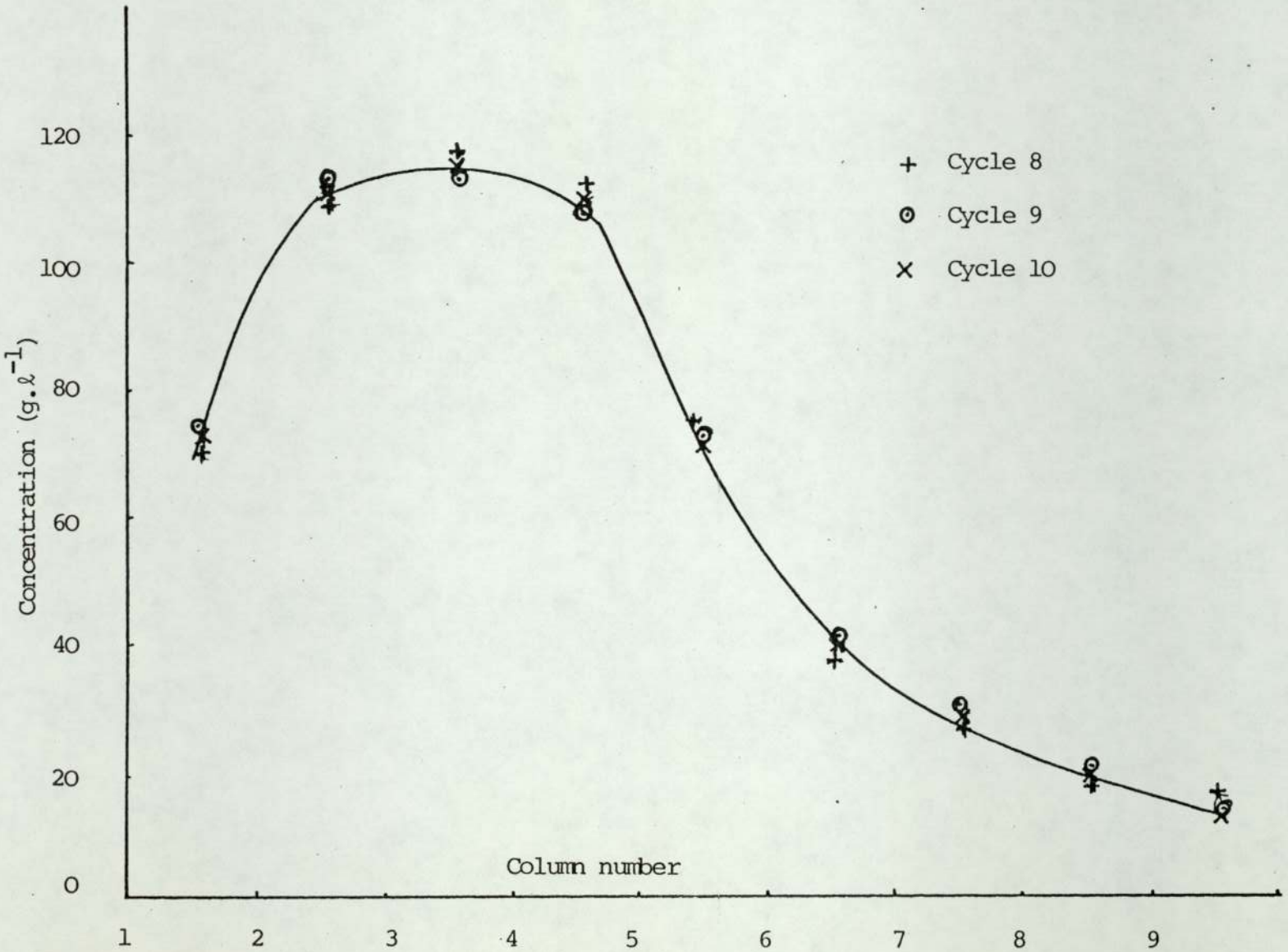


Fig. 8.10 Concentration profiles for run 8.2

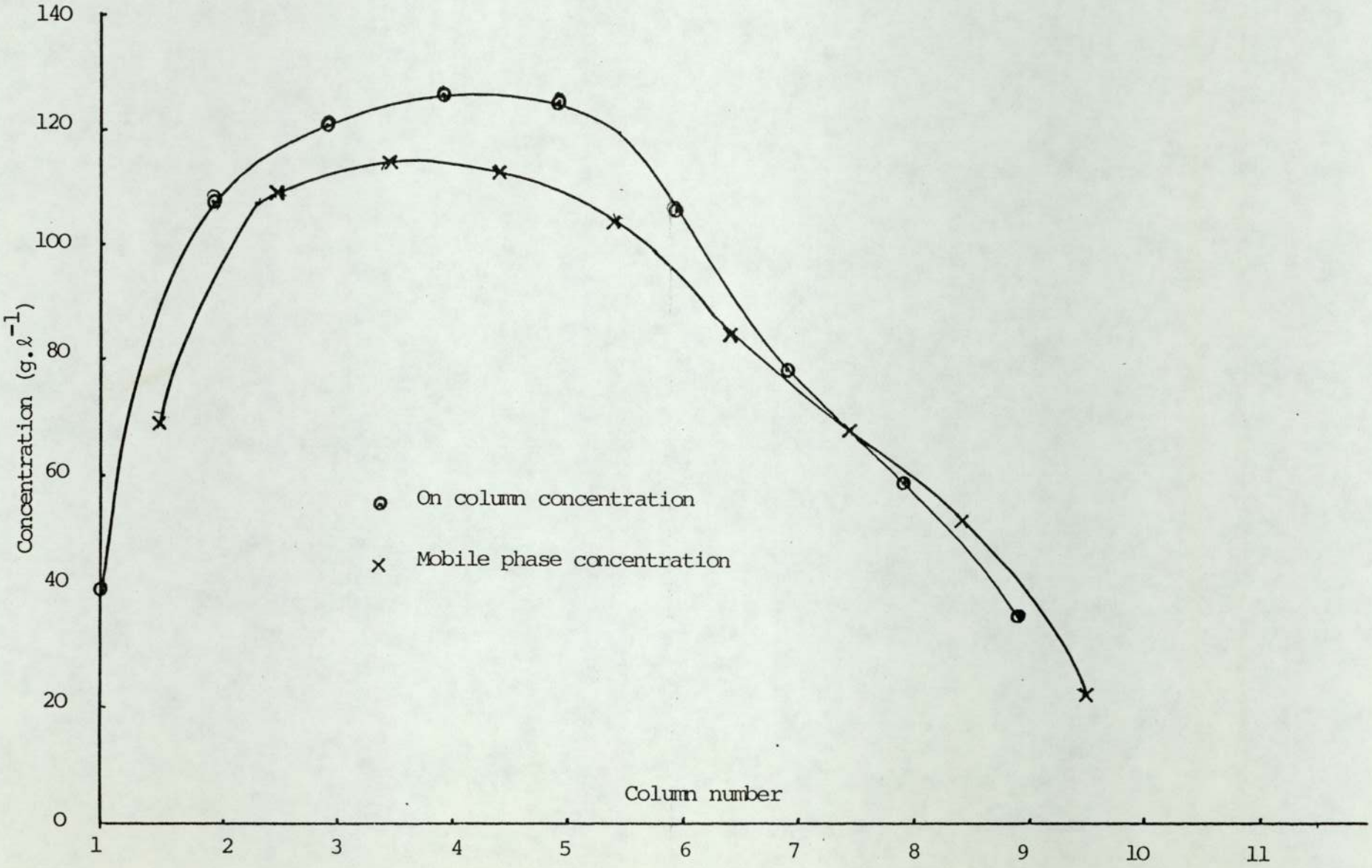


Fig. 8.11 Purge profile of column 1, Run 8 i.e. the column with the lowest molecular weight dextran

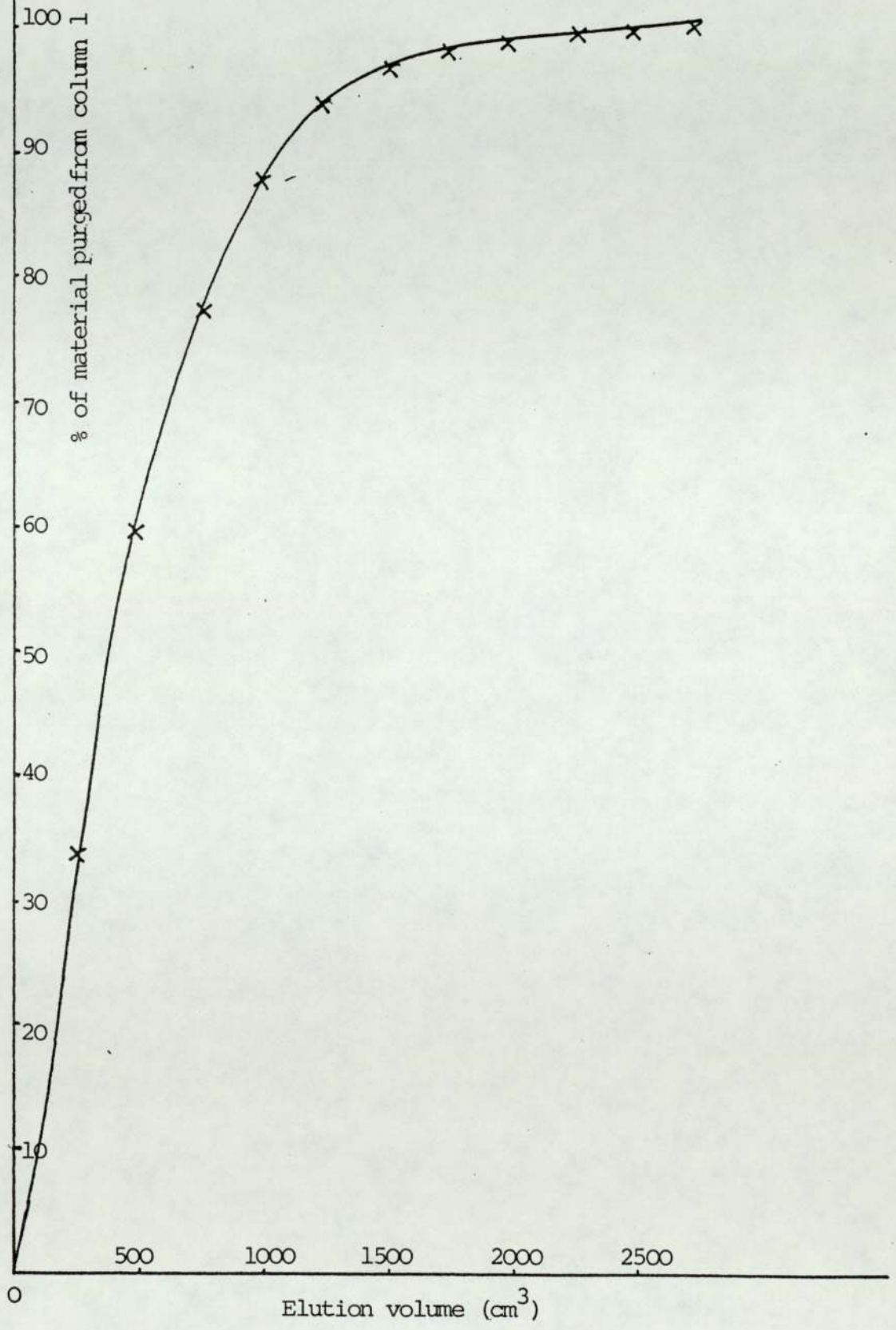


Fig. 8.12 Conditions and results for UF run 8

Run	8.1a	8.1b	8.2
Cartridge	HIP2	HIP5	HIP5
Feed Vol. (ℓ)	2.000	2.000	2.000
Feed Conc. (g/ℓ)	12.2	12.2	20.0
Feed Mass (g)	24.4	24.4	40.0
Feed, $\bar{M}_w$	35750	35750	42400
Feed, $\bar{M}_N$	10100	10100	10100
Diafiltration Water (ℓ)	25.250	2.835	5.260
Permeate Vol. (ℓ)	27.000	4.000	7.000
Permeate Conc. (g/ℓ)	0.26	2.0	2.25
Permeate Mass (g)	7.1	8.0	15.7
Permeate Output (%)	28.7	31.9	37.8
Permeate, $\bar{M}_w$	13750	13800	14800
Permeate, $\bar{M}_N$	3300	3100	3200
Retentate Vol. (ℓ)	0.250	0.835	0.260
Retentate Conc. (g/ℓ)	70.0	20.5	100.0
Retentate Mass (g)	17.7	17.1	25.9
Retentate Output (%)	71.3	68.1	62.2
Retentate, $\bar{M}_W$	45300	502500	53150
Retentate, $\bar{M}_N$	25300	25700	28700

Fig. 8.13 GPC and UF fractionation of dextran for run 8.1a

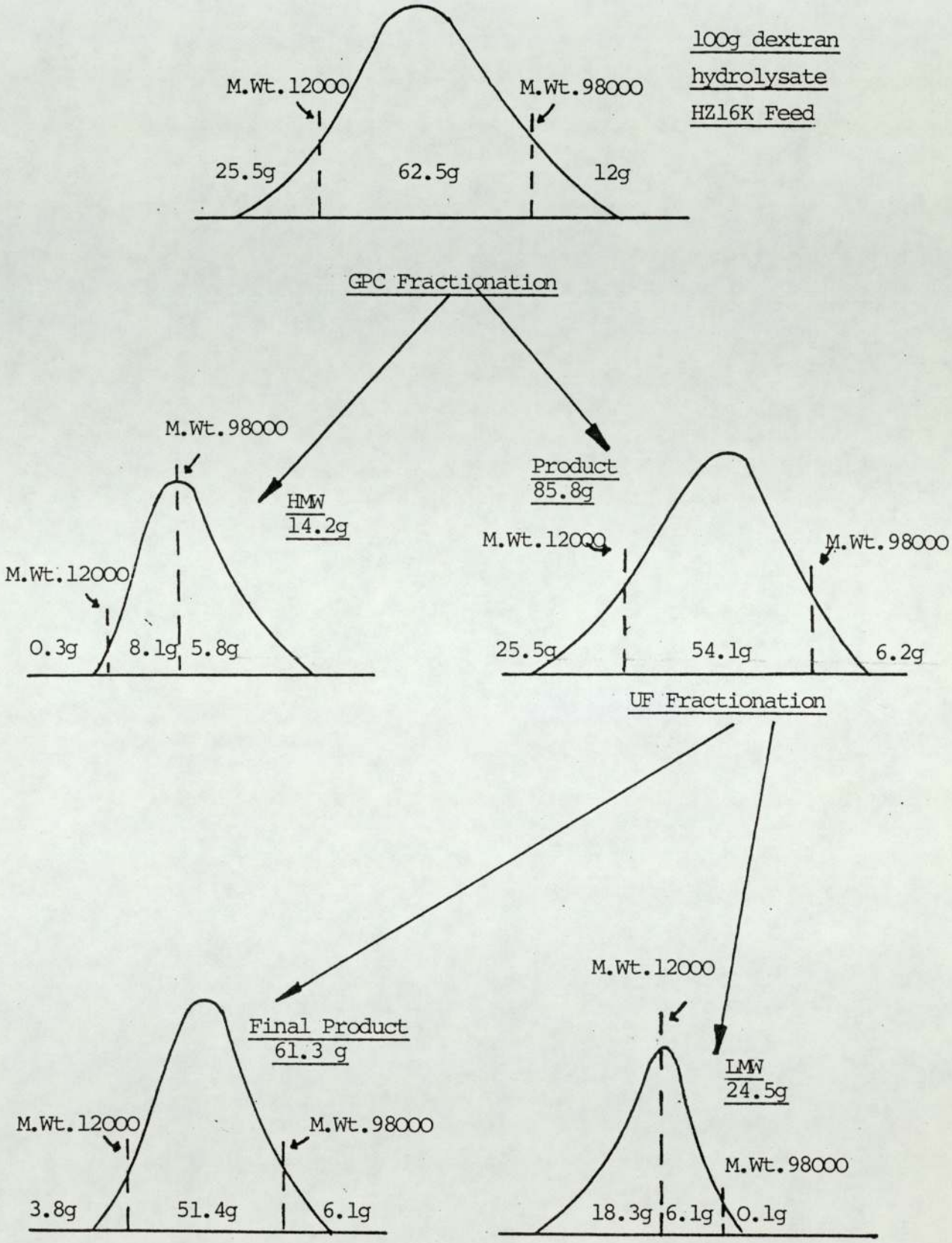


Fig. 8.14 GPC and UF fractionation of dextran for run 8.1b

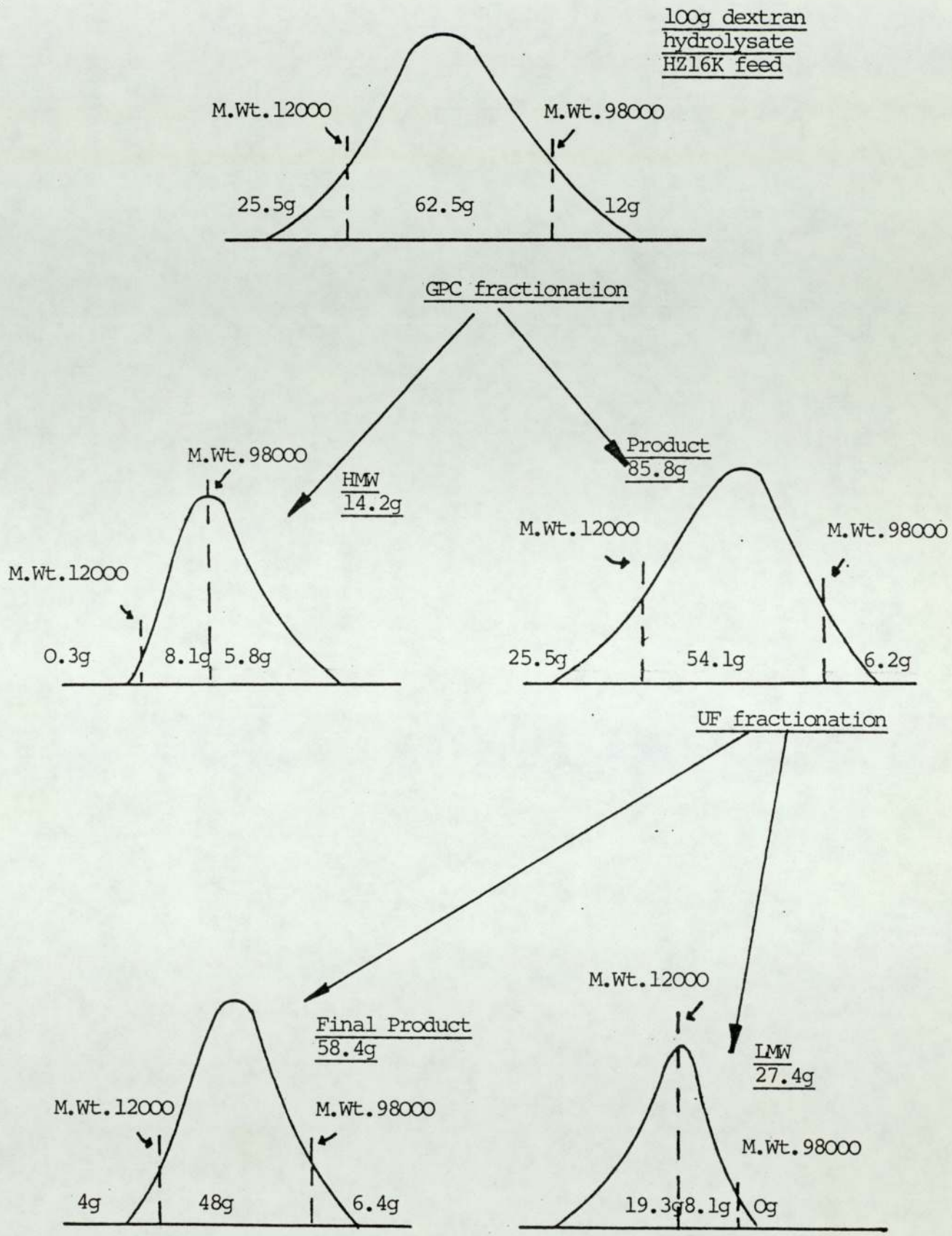


Fig. 8.15 GPC and UF fractionation of dextran for run 8.2

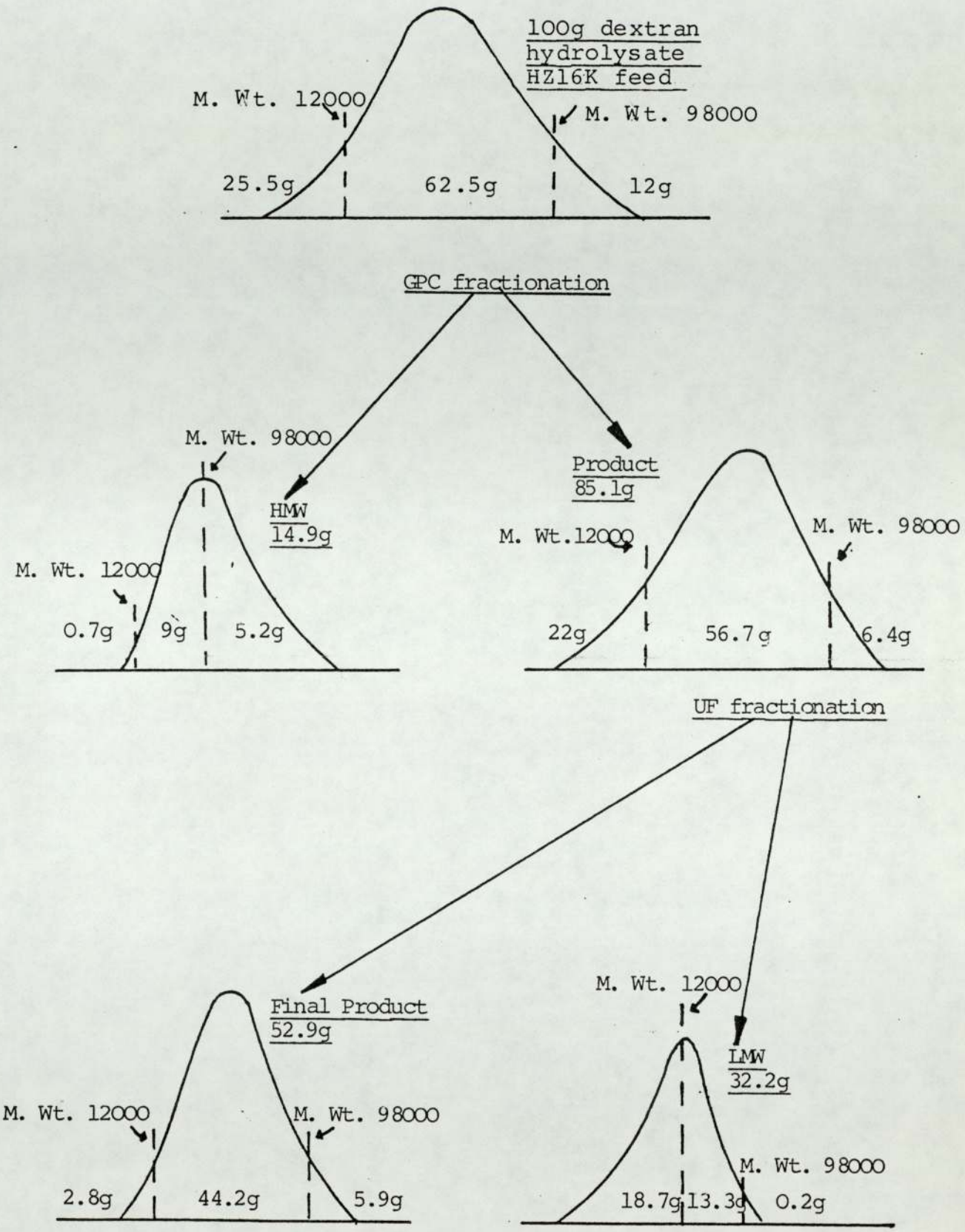


Fig. 8.16 Operating conditions for GPC run 9

Run	Cycle	Pressures ($kN.m^{-2}$)			Temperatures (^{o}C)			Input Flowrates			Product Flows		Cut-positions		
								$cm^3.min^{-1}$			$cm^3.min^{-1}$		Pre-feed	Post-feed	Purge
		Eluent	Feed	Purge	Purge	Eluent	In-line	L_1	L_2	L_3	HMW	LMW			
9.1	6	207	207	83	60	60	60	115	35	150	151	314	0.530	0.991	3.078
9.1	7	207	207	83	60	60	60	115	35	150	151	326	0.530	0.991	3.280
9.1	8	207	207	83	60	60	60	115	35	150	149	333	0.530	0.965	3.320
9.2	11	207	207	83	60	60	60	115	25	140	140	325	0.530	0.850	3.218
9.2	12	207	207	83	60	60	60	115	25	140	140	325	0.530	0.850	3.218
9.2	13	207	207	83	60	60	60	115	25	140	140	325	0.530	0.850	3.218
9.3	16	207	207	83	60	60	60	125	25	150	151	326	0.658	0.991	3.230
9.3	17	207	207	83	60	60	60	125	25	150	151	326	0.658	0.991	3.230
9.3	18	207	207	83	60	60	60	125	25	150	151	326	0.658	0.991	3.230
9.4	23	207	207	83	60	60	60	125	15	140	140	323	0.658	0.850	3.192
9.4	24	207	207	83	60	60	60	125	15	140	140	300	0.658	0.837	2.898
9.4	25	207	207	83	60	60	60	125	15	140	140	325	0.658	0.850	3.230

Fig. 8.17 Products from GPC run 9

Run	Cycle	Dextran input	High Mol. Wt. Product			Low Mol. Wt. Product			HMWP	LMWP	Mass
		per cycle (g)	Conc. g/ℓ	Vol. ℓ	Mass g	Conc. g/ℓ	Vol.ℓ	Mass g	Output	Output	Balance
9.1	7	186.4	2.76	11.34	31.5	6.60	24.45	162.5	0.162	0.838	1.030
9.1	8	186.4	2.70	11.17	30.0	6.00	25.00	150.0	0.167	0.833	0.970
9.2	12	134.0	1.75	10.45	18.0	5.65	24.35	137.0	0.116	0.884	1.150
9.2	13	134.0	1.62	10.45	17.0	5.45	24.35	133.0	0.114	0.885	1.120
9.3	17	134.0	2.85	11.34	32.0	3.90	24.43	96.0	0.250	0.750	0.960
9.3	18	134.0	2.90	11.34	33.0	3.95	24.49	97.0	0.253	0.747	0.970
9.4	24	134.0	1.90	10.48	20.0	5.00	24.27	121.5	0.141	0.859	1.050
9.4	25	134.0	1.99	10.48	20.5	4.86	24.38	118.5	0.147	0.853	1.040

		High Mol. Wt. Product			Low Mol. Wt. Product		
		$\overline{M}_W$	$\overline{M}_N$	D	$\overline{M}_W$	$\overline{M}_N$	D
9.1	7	406000	36500	11.1	30000	6450	4.66
9.1	8	382000	35000	10.9	30700	6400	4.80
9.2	12	408000	43800	9.3	32250	7450	4.34
9.2	13	400000	44000	9.0	31000	7700	4.01
9.3	17	241000	33000	7.3	27000	6900	3.90
9.3	18	265000	37000	7.2	27800	6500	4.30
9.4	24	392000	44000	8.9	30250	7500	4.10
9.4	25	385000	41000	9.4	29500	7250	4.06

Fig 8.18 Purge Products from GPC run 9.4

Column	Purged Volume ℓ	Purge Conc. $g.\ell^{-1}$	Dextran Mass g	Column Conc. $g.\ell^{-1}$	Col. Conc. / Feed Conc.	$\overline{M}_W$	$\overline{M}_N$	D
1	5.44	1.6	8.7	7.6	0.107	28500	6500	4.38
2	3.85	16.6	64.0	56.2	0.792	32000	7100	4.51
3	4.49	17.2	77.2	67.8	0.955	39500	10200	3.91
4	3.99	20.1	80.2	70.5	0.993	43500	10500	4.14
5	4.31	17.9	77.5	68.1	0.959	54000	10900	4.95
6	5.13	8.3	42.5	37.3	0.525	60500	12850	4.71
7	3.31	7.4	24.5	21.5	0.303	69000	13800	5.00
8	4.17	3.7	15.4	13.5	0.190	96000	16800	5.71
9	4.13	2.5	10.3	9.1	0.128	178000	18500	9.62

Fig 8.19 Dextran profile in the mobile phase in the SCCR5 for run 9.4

Outlet of Column	conc. $g.\ell^{-1}$	$\bar{M}_w$	$\bar{M}_N$	D
1	30.5	29000	6600	4.39
2	77.5	34000	7300	4.65
3	90.0	47800	10000	4.78
4	86.5	67500	12000	5.62
5	66.5	85000	12400	6.85
6	35.0	124000	16450	7.53
7	24.0	198000	23800	8.31
8	13.5	385000	37800	10.18
9	7.0	487000	45000	10.83

Fig 8.20 Concentration profiles for run 24

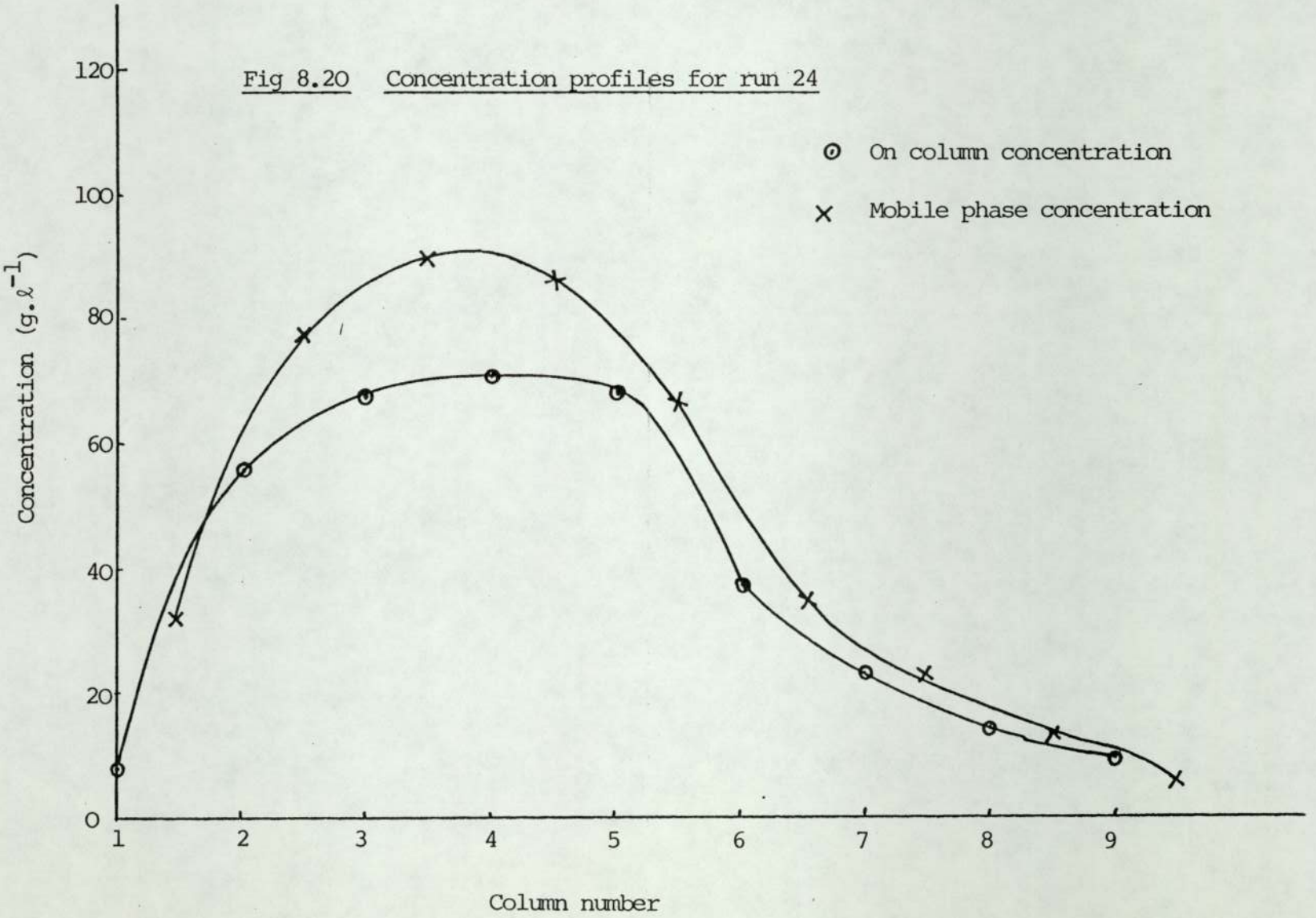


Fig 8.21 GPC fractionation of dextran for run 9.1

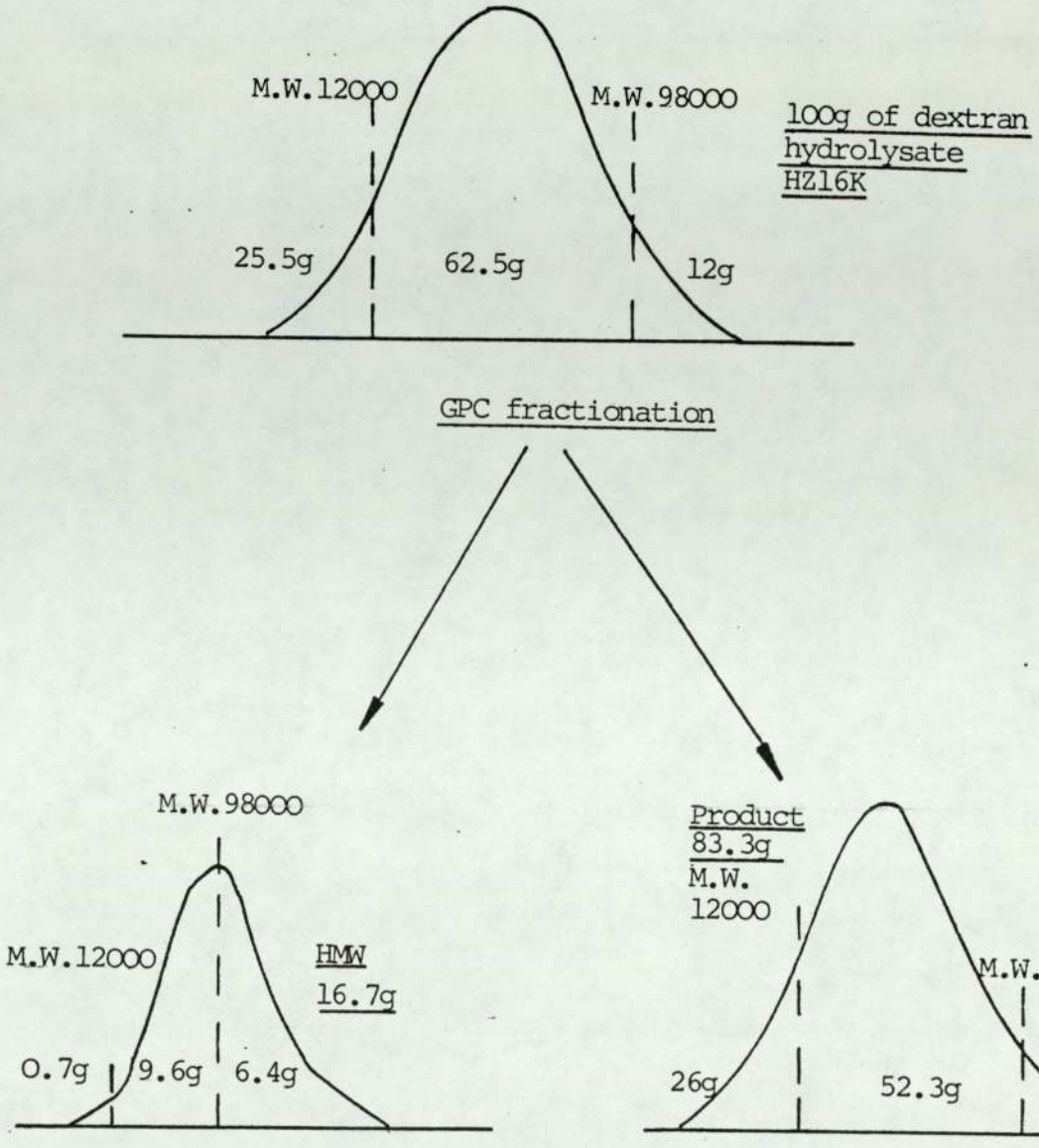


Fig 8.22 GPC fractionation of dextran for run 9.2

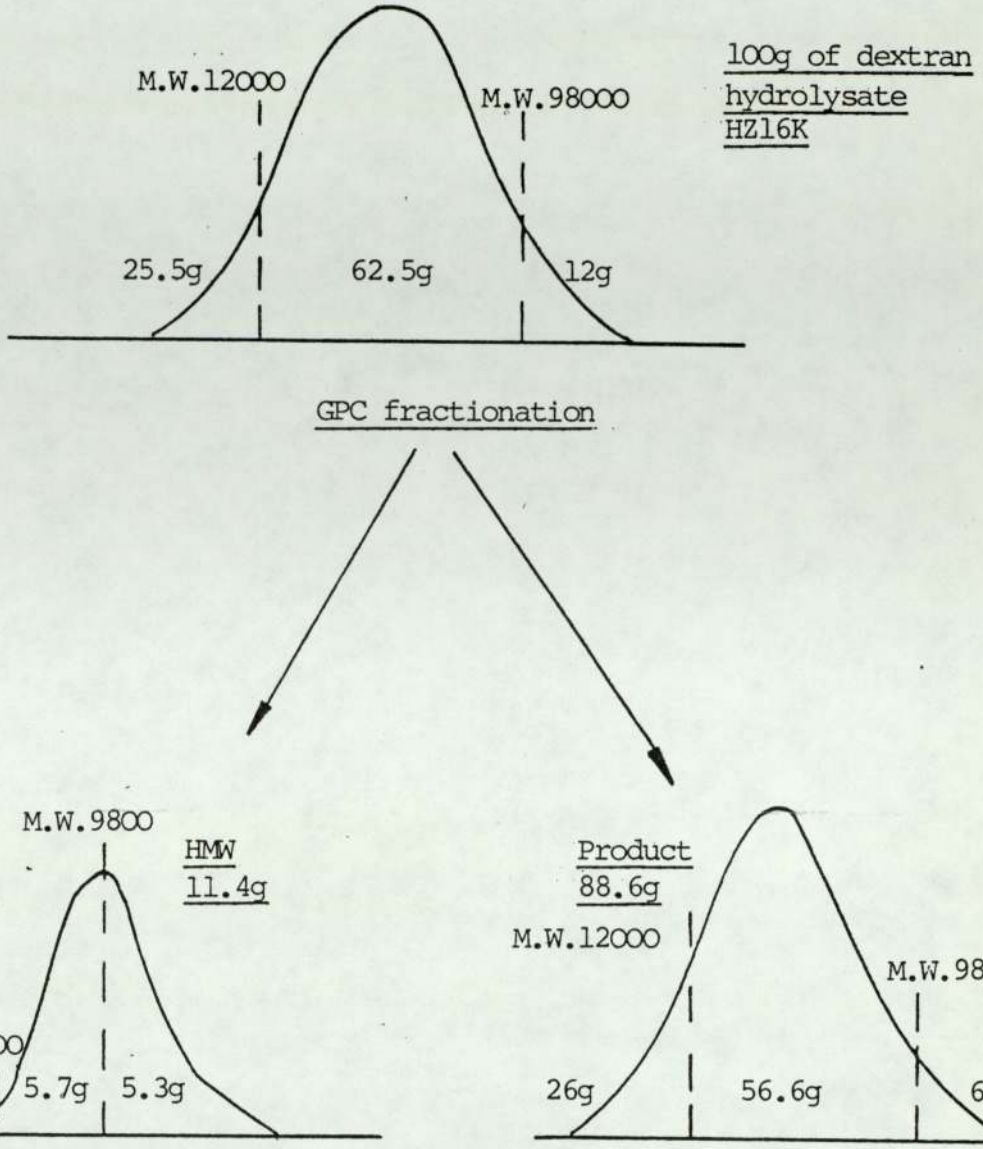


Fig 8.23 GPC fractionation of dextran for run 9.3

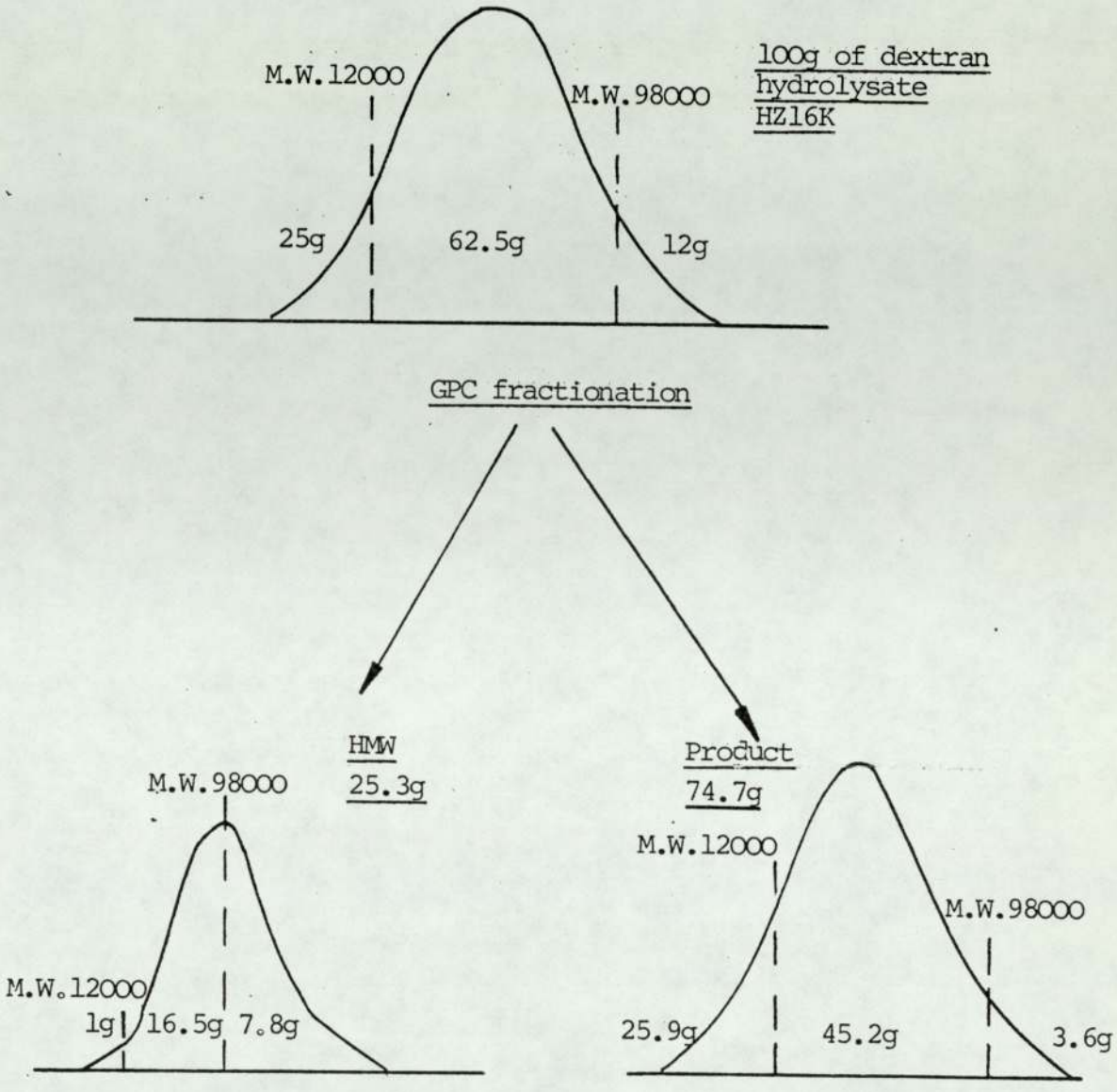


Fig 8.24 Conditions and results for UF run 9.4

Run	9.4
System	PCI reverse osmosis
Membrane	BX6
Feed Vol (ℓ)	96.0
Feed conc. (g/ℓ)	5.0
Feed Mass (g)	480.0
Feed, $\overline{M}_W$	32000
Feed, $\overline{M}_N$	8200
Diafitration water (ℓ)	248.0
Permeate vol (ℓ)	332.0
Permeate conc. (g/ℓ)	0.24
Permeate mass (g)	78.0
Permeate output (%)	18.2
Permeate, $\overline{M}_W$	8000
Permeate, $\overline{M}_W$	2500
Retentate vol (ℓ)	12.0
Retentate conc. (g/ℓ)	29.0
Retentate mass (g)	348.0
Retentate output (%)	81.8
Retentate, $\overline{M}_W$	33000
Retentate, $\overline{M}$	17500

Fig 8.25 GPC and UF fractionation of dextran for run 9.4

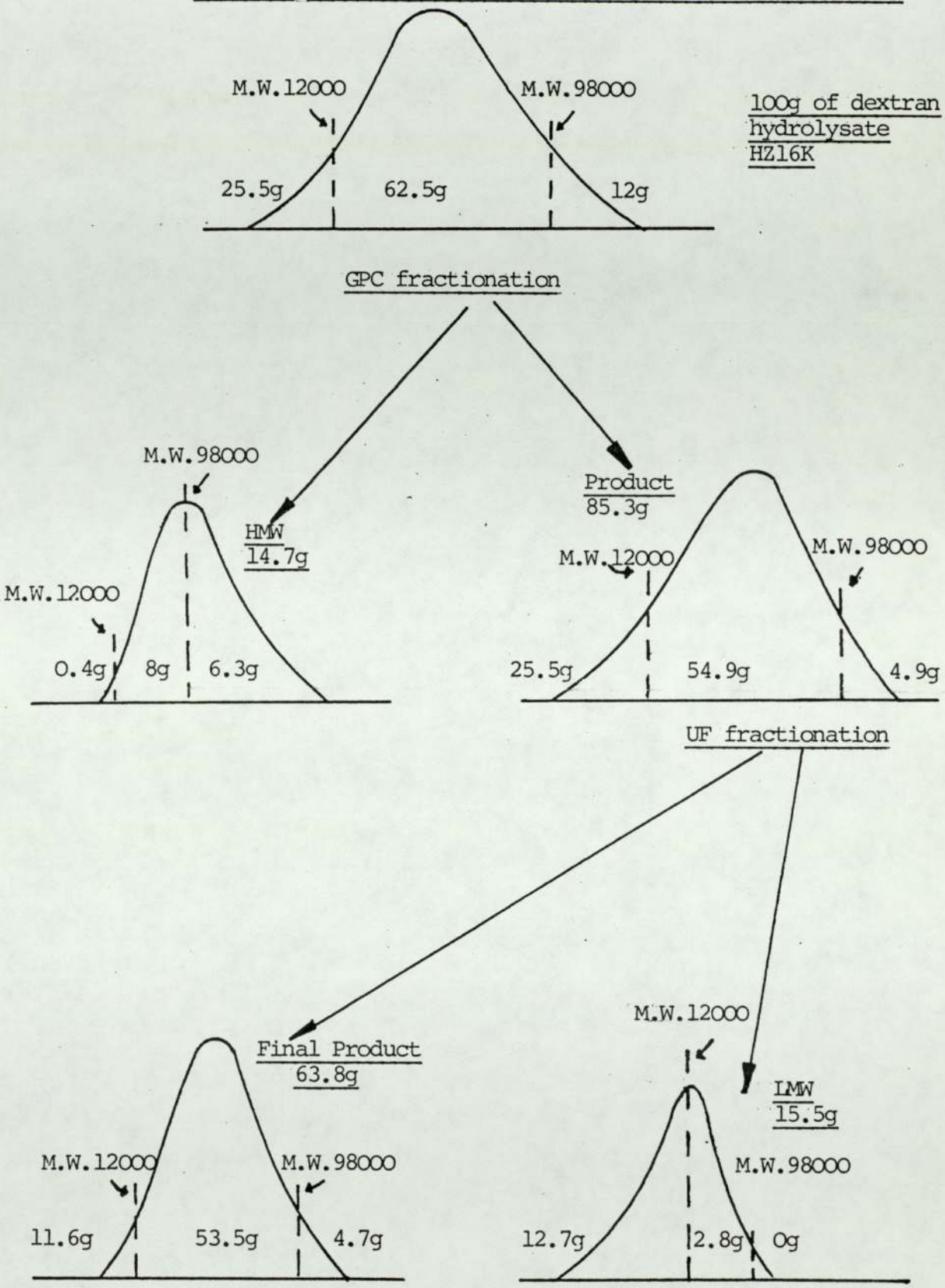


Fig. 8.26 Summary of the results for the GPC runs

Run	Input Mass (g)	High Mol. Wt. Product					Low Mol. Wt. Product				
		Mass (g)	Conc. ($g.\ell^{-1}$)	Mol. Wt. Distribution			Mass (g)	Conc. ($g.\ell^{-1}$)	Mol. Wt. Distribution		
				$\bar{M}_w$	$\bar{M}_N$	D			$\bar{M}_w$	$\bar{M}_N$	D
8.1	334	46.0	4.LO	422000	52000	8.33	276.0	12.2	35750	10100	3.53
8.2	579	80.0	7.00	382000	38000	10.05	460.0	20.0	42400	10100	4.20
9.1	187	30.0	2.70	382000	35000	10.90	140.0	6.00	30700	6400	4.80
9.2	134	17.0	1.62	400000	44000	9.02	133.0	5.45	31000	7700	4.01
9.3	134	33.0	2.90	265000	37000	7.2	97.0	3.95	27800	6500	4.30
9.4	134	20.5	1.99	385000	41000	9.4	118.5	4.86	29500	7250	4.06

Fig. 8.27 Summary of the results for the GPC and UF runs

Run	Input Mass (g)	GPC RUNS: HMW dextran removed (%)	UF RUNS: LMW dextran removed (%)	FINAL PRODUCTS: Final product (%)	$\overline{M}_w$	$\overline{M}_N$	Material <12000 (%)	Material >98000 (%)	Material off Specification (%)
8.1a	334	14.2	24.5	61.3	45300	25300	6.15	10.05	16.2
8.1b	334	14.2	27.4	58.4	50250	25700	6.80	11.00	17.8
8.2	579	14.9	32.2	52.9	53150	28700	5.40	11.40	16.8
9.1	187	16.2	-	83.3	30700	6400	31.20	6.00	37.8
9.2	134	11.4	-	88.6	31000	7700	29.30	6.80	36.1
9.3	134	25.3	-	74.7	27800	6500	34.60	4.80	39.4
9.4	134	14.7	15.5	69.8	33000	17500	16.60	6.70	23.3

Fig. 8.28 The efficiencies of the GPC runs

$$\eta_1 = \frac{\text{material >98000 M.W. in HMWP}}{\text{HMWP}} \times 100 \quad \cdots\cdots\cdots \quad (7.1)$$

$$\eta_2 = \frac{\text{total material off specification in HMWP}}{\text{HMWP}} \times 100 \quad (7.2)$$

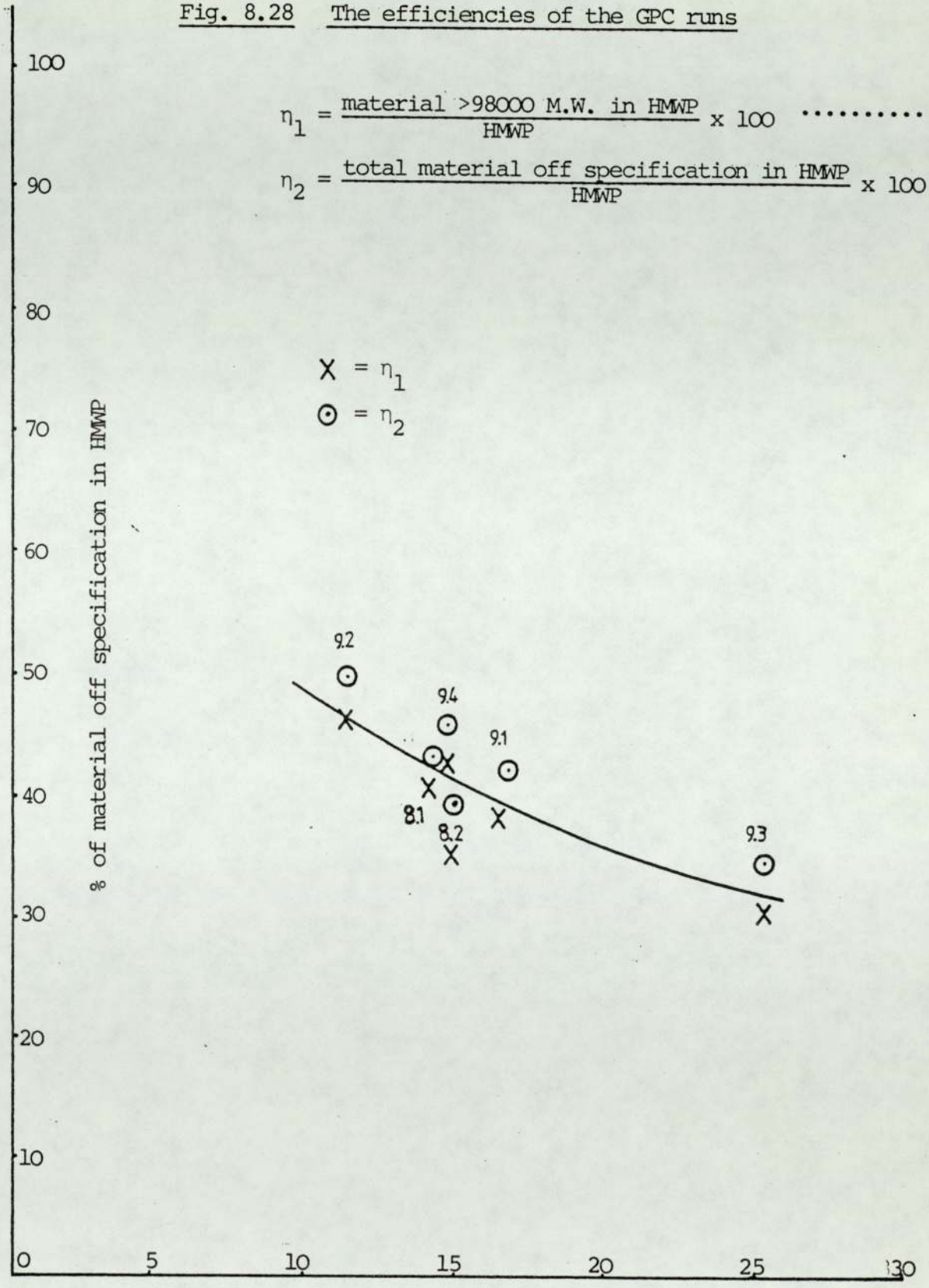


Fig. 8.29 The efficiencies of the UF runs

$$\eta_3 = \frac{\text{material <12000 M.W. in LMWP}}{\text{LWMP}} \times 100 \quad \text{.....(7.4)}$$

$$\eta_4 = \frac{\text{total material off specification in LMWP}}{\text{LMWP}} \times 100 \quad \text{.....(7.5)}$$

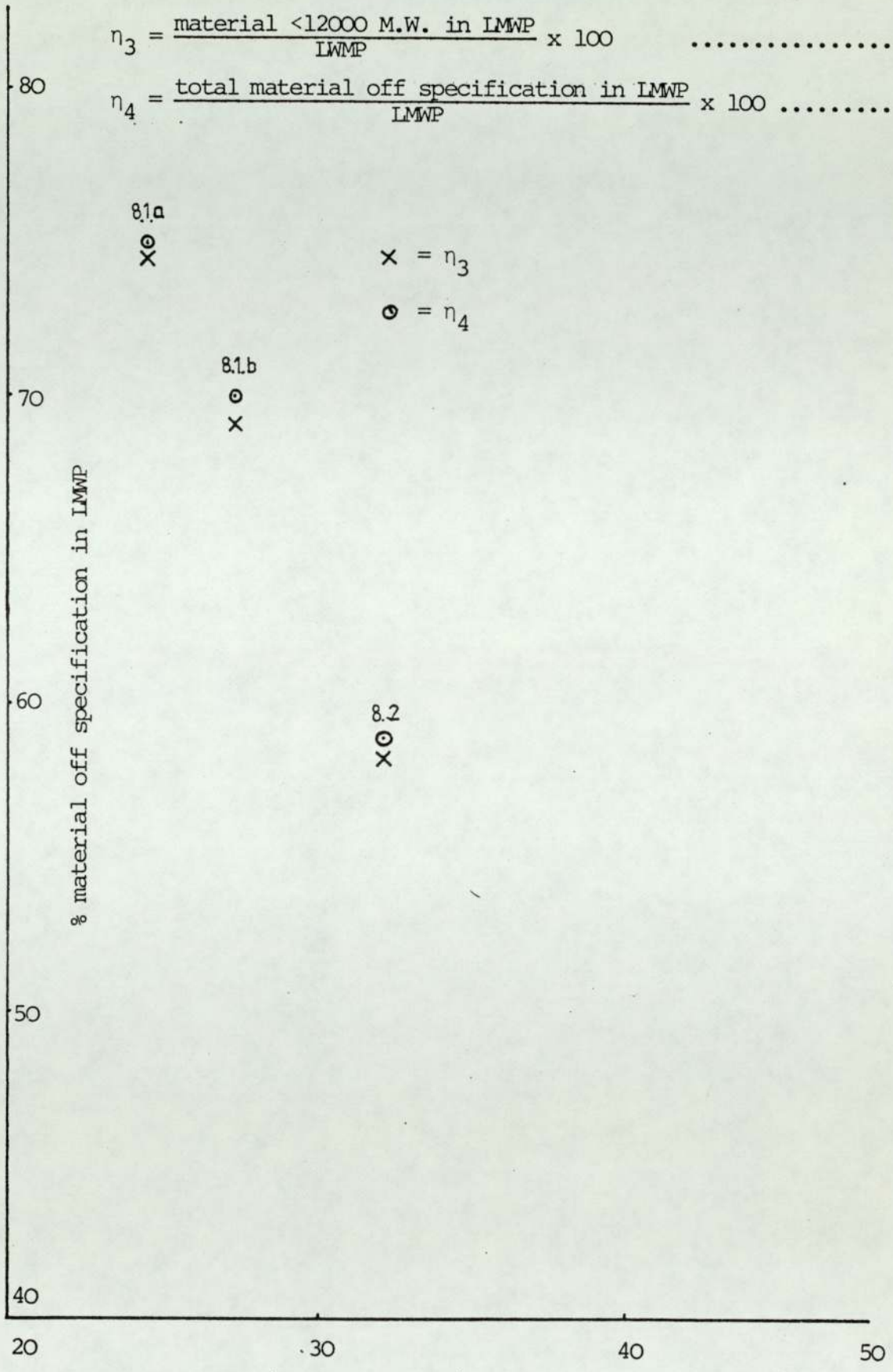


Fig. 8.30 Profile of the K_d in the SCCR5

Run	8.2			9.4		
Column	On column concentration α ($g.\ell^{-1}$)	Mobile phase concentration β ($g.\ell^{-1}$)	K_d at the middle of the column	On column concentration α ($g.\ell^{-1}$)	Mobile phase concentration β ($g.\ell^{-1}$)	K_d at the middle of the column
1	42.0	—	--	7.5	--	--
2	109.0	100.0	1.174	56.5	61.6	0.842
3	122.0	116.5	1.092	67.5	86.0	0.582
4	127.0	116.5	1.175	70.5	89.5	0.587
5	126.0	112.0	1.242	68.2	81.0	0.693
6	107.0	97.0	1.200	45.0	37.5	0.676
7	79.0	75.0	1.103	26.5	21.5	0.634
8	59.0	60.0	0.968	17.0	13.5	0.600
9	36.0	41.5	0.742	9.0	11.5	0.578

and keeping the same feed rate and feed concentration.

Run 9.4 had the same feed input, and eluent rate as run 9.3, but higher feed concentration and higher eluent to feed ratio because of the lower feed rate.

The conditions of the UF runs are presented in Figs. 8.12 and 8.24. The feed for runs 8.1a and 8.1b was the product of GPC run 8.1, but UF runs 8.1b and 8.2 were performed on the new H1P5 cartridge and run 8.1a on an H1P2 cartridge.

In run 9.4 the PCI reverse osmosis system, equipped with six tubular membranes was used for the removal of the low molecular weight dextran.

Finally, it was not necessary to pass the products through the ion exchange cartridge, since it is known from runs 3 and 4 (Figs. 7.9, 7.10 and 7.18 to 7.20) that silica free dextran solutions can be achieved by passing them through this type of ion exchange cartridge.

8.4 RESULTS AND DISCUSSION

The conditions and results of the runs are presented in Figs. 8.4 to 8.30.

GPC run 8.2 was performed under identical conditions to run 5.2, but the SCCR5 was packed with Spherosil XOB 030 instead of XOB 075. In run 5.2, 23.5% of the dextran hydrolysate was removed as high molecular weight product, but only 14.9% was removed as high molecular weight product in run 8.2. This is

because the Spherosil XOB 030 packing has larger pores than the XOB 075 packing and hence more molecules have higher affinity for the packing, than the mobile phase, so that more of them will move towards the low molecular weight product port (Fig. 4.2).

Then from runs 5.2 and 8.2 it is concluded that if the same percentage of dextran hydrolysate is to be removed as high molecular weight product during a GPC fractionation on the SCCR5 using either of the packings, the fractionation on the Spherosil XOB 030 packing will require a higher eluent rate than the fractionation on the XOB 075 packing for the same feed rate and feed input. Therefore, the runs on the Spherosil XOB 030 will give more dilute high molecular weight products than the runs on the XOB 075 packing.

The efficiencies of the two packings, for the GPC fractionations, were very similar (Figs. 7.42 and 8.28). This means that the change in the pore size of the packing does not have any significant effect on the fractionating efficiency, probably due to the similar shapes of the calibration curves of the two packings (Fig. 8.2). Perhaps, if the calibration curves were steeper, i.e. if the packings had a narrower pore size distribution, the GPC efficiency would be better.

Also more water is required to purge a column, packed with Spherosil XOB 030, completely from its dextran molecules than a column packed with the Spherosil XOB 075 (Figs. 7.39 and 8.11), hence more dilute products will be produced from the SCCR5 when

it is packed with the XOB 030 packing, than with the XOB 075 packing. The need for more water to purge the dextran completely from larger pore size packing is probably due to the fact that more molecules are inside the pores of the packing, since more of them can penetrate the pores.

The eluent to feed ratio seems to have an effect on the efficiency of the GPC fractionations. The higher the eluent to feed ratio the higher is the GPC fractionation efficiency (Fig. 8.28, Run 9.4 and 8.2), but less dextran hydrolysate is processed by the SCCR5.

At the end of each run samples were taken from the outlet of each column. The concentrations of these samples were measured and were plotted against the distance of the sample point from the eluent inlet column. These are the mobile phase concentration profiles (Figs. 8.9, 8.10, 8.20).

Also at the end of each set of runs the columns were purged out, and the mass of dextran in each column was found; knowing the dextran mass and the liquid volume of the column, the on column concentrations were calculated and plotted against the distance of the column from the eluent column. These are the on column concentration profiles (Figs. 8.10, 8.20).

The mobile phase concentration in a column and the average on column concentration at the end of a run are known from the concentration profiles. Assuming first that the columns are divided in N well mixed theoretical plates and second that the plate in the

middle of a column has a dextran concentration equal to the average on column concentration, hence the distribution coefficient, $K_d = \frac{c}{x}$, for this plate can be found from equation (8.6).

e.g.

mobile phase concentration, $x=\beta$ ..... (8.1)

On column concentration, $\alpha=\frac{V_o.x + V_i.c}{V_o + V_i}$ .... (8.2)

where c is the stationary phase concentration

Distribution coefficient, $K_d = \frac{c}{x}$ ..... (8.3)

From equations (8.2) and (8.3)

$$\alpha = \frac{V_o.x + V_i.K_d.x}{V_o + V_i} \quad \text{..... (8.4)}$$

Combine equations (8.1) and (8.4)

$$\alpha = \frac{V_o.\beta + V_i.K_d.\beta}{V_o + V_i} \quad \text{..... (8.5)}$$

Rearranging equation (8.5)

$$K_d = \{\frac{\alpha}{\beta}\ (V_o+V_i)-V_o\}/V_i \quad \text{..... (8.6)}$$

From Fig. 8.30 it can be seen that the K_d decreases from column 1 to column 9, as to be expected, since the molecular weight of the dextran increases and therefore the stationary phase concentration decreases, except for columns 3, 4 and 5 where K_d increases due to concentration effects described by England (46), because these are the columns with the highest concentrations (Figs. 8.10, 8.20 and 8.30).

The mobile phase concentration profiles were also used to check if steady-state has been reached, i.e. if the concentration profiles for two or three cycles

agreed well it was assumed that the system had reached steady-state (Fig. 8.9).

From the UF runs performed on the H1P2 and H1P5 cartridges, the H1P2 cartridge was more efficient (Fig. 8.20), but its permeate rate was much slower than that of the H1P5 cartridge (approximately ten times). Therefore the H1P5 cartridge was to be preferred. Also the new H1P5 cartridge was slightly more efficient than the old one.

The BX6 tubular membranes although they removed low molecular weight dextran selectively (Fig. 8.24), the quantity of dextran allowed to pass through them was insufficient. The BX6 membranes appear more suitable for concentrating dextran solutions, but in this case (Run 9.4) high concentrations were not achieved because of the low concentration of UF feed used, and the high dead volume of the PCI system (12ℓ).

8.5 CONCLUSIONS

From these runs it was found that the efficiencies of the Spherosil XOB 075 and XOB 030 packings were similar, but when the XOB 030 packing is used the products are more dilute, hence the Spherosil XOB 075 packing is preferable.

The efficiency of the SCCR5 probably can be increased by adding more columns to the system, or replacing the packing for another one of smaller particle size or of narrower pore size range.

Although the H1P2 cartridge is more efficient, for the removal of the low molecular weight material using UF than the H1P5 cartridge, the second is preferred since it can process the dextran solutions at a much faster rate. Finally the BX6 membranes were not able to remove sufficient low molecular weight material to provide a product within the British Pharmacopoeia specifications.

9.0 MATHEMATICAL MODELLING OF THE SCCR5

9.0 MATHEMATICAL MODELLING OF THE SCCR5

9.1 INTRODUCTION

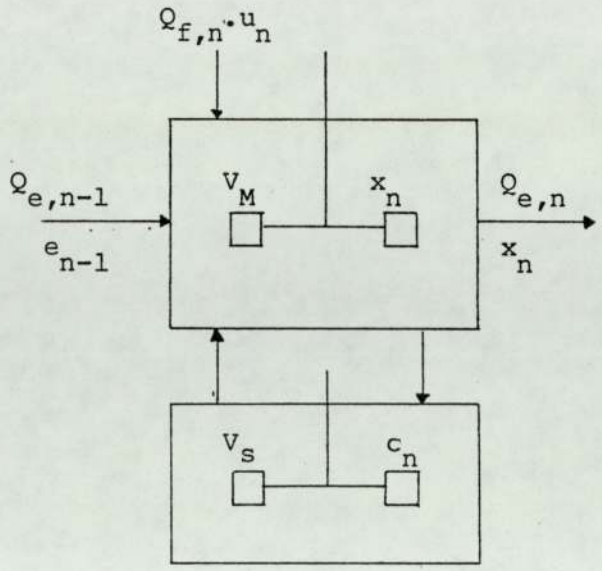
The models for the GPC separation mechanisms are reviewed in section 2.5. The more recent models of the GPC processes are based on the non-equilibrium descriptions, but the complexity of these models together with the insufficient knowledge of the thermodynamic properties of the dextran system prevented the use of such a model. A simple linear exclusion controlled model has been used in this thesis, but this model was modified to take into account the concentration and temperature effects on the performance of the SCCR5.

9.2 LINEAR EXCLUSION CONTROLLED MODEL

The "dispersion model" is perhaps the most widely used model for the description of chemical processes. A digital computer program based on this model has been used by England (46) to simulate the operation of the SCCR5 liquid-liquid chromatograph.

A chromatographic column was considered to consist of a series of idealised mixing stages where the mobile phase leaving a stage was in equilibrium with the stationary phase in the column. For the operating conditions used in the SCCR5, correlations (271) suggest that the axial dispersion coefficient was small and so a plug flow model could be used.

A mass balance over a general stage, n, for the solute gives:



$$Q_{e,n-1} \cdot x_{n-1} + Q_{f,n} \cdot u_n = Q_{e,n} \cdot x_n + V_M \frac{dx_n}{dt} + V_S \frac{dc_n}{dt} \quad \ldots \quad (9.1)$$

where

Q = volumetric flowrate

x = mobile phase concentration

u = feed concentration

c = stationary phase concentration

V = volume of phase

Subscripts

e = eluent

M = mobile

S = stationary phase

f = feed

Assuming equilibrium between the mobile and stationary phase:

$$C_n = K_d x_n \quad \ldots \quad (9.2)$$

equation (9.1) becomes:

$$Q_{f,n} \cdot u_n + Q_{e,n-1} \cdot x_{n-1} - Q_{e,n} \cdot x_n = (V_M + K_d V_S) \frac{dx_n}{dt}$$

..... (9.3)

Rearranging equation (9.3) to get:

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

where

$$a_{n-1} = \frac{Q_{e,n-1}}{V_M + K_d V_S}$$

$$b_n = \frac{Q_{e,n}}{V_M + K_d V_S}$$

$$c_n = \frac{Q_{f,n}}{V_M + K_d V_s}$$

Assuming that a chromatographic column was divided into N stages a set of equations was produced according to equation (9.4):

$$\dot{x}_1 = a_o x_o - b_1 x_1 + c_1 u_1$$

$$\dot{x}_2 = a_1 x_1 - b_2 x_2 + c_2 u_2$$

$$\dot{x}_n = a_{n-1} x_{n-1} - b_n x_n + c_n u_n$$

$$\dot{x}_N = a_{N-1} x_{N-1} - b_N x_N + c_N u_N$$

or

$$\underline{\dot{x}}(t) = \underline{A}\ \underline{x}(t) + \underline{B}\ \underline{u}(t) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (9.5)$$

Then the general equation (9.5) is solved and the solution is:

$$\underline{x}(t) = e^{\underline{A}t} \cdot \underline{x_o} + \int_o^t e^{\underline{A}(t-\tau)} \cdot B \cdot u(\tau) \cdot d\tau \quad \ldots\ldots\ldots \quad (9.6)$$

If the feed input is not time dependent equation (9.6) can be solved further to:

$$\underline{x}(t) = e^{\underline{A}t}.\underline{x}_o + \underline{A}^{-1}\{e^{\underline{A}t}-\underline{I}\}.\underline{B}.\underline{u} \quad \ldots\ldots\ldots\ldots \quad (9.7)$$

or

$$\underline{x}(t) = \underline{\phi}(t).\underline{x}_o + \underline{\Delta}(t).\underline{B}.\underline{u} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (9.8)$$

where

$$\underline{\phi}(t) = e^{\underline{A}t}$$

$$\underline{\Delta}(t) = \underline{A}^{-1}\{e^{\underline{A}t} - \underline{I}\}.\underline{B}.\underline{u} = \underline{A}^{-1}\{\underline{\phi}(t)-\underline{I}\}$$

The detailed solution of equation (9.5) is given by England (46).

9.3 CONCENTRATION AND TEMPERATURE EFFECTS

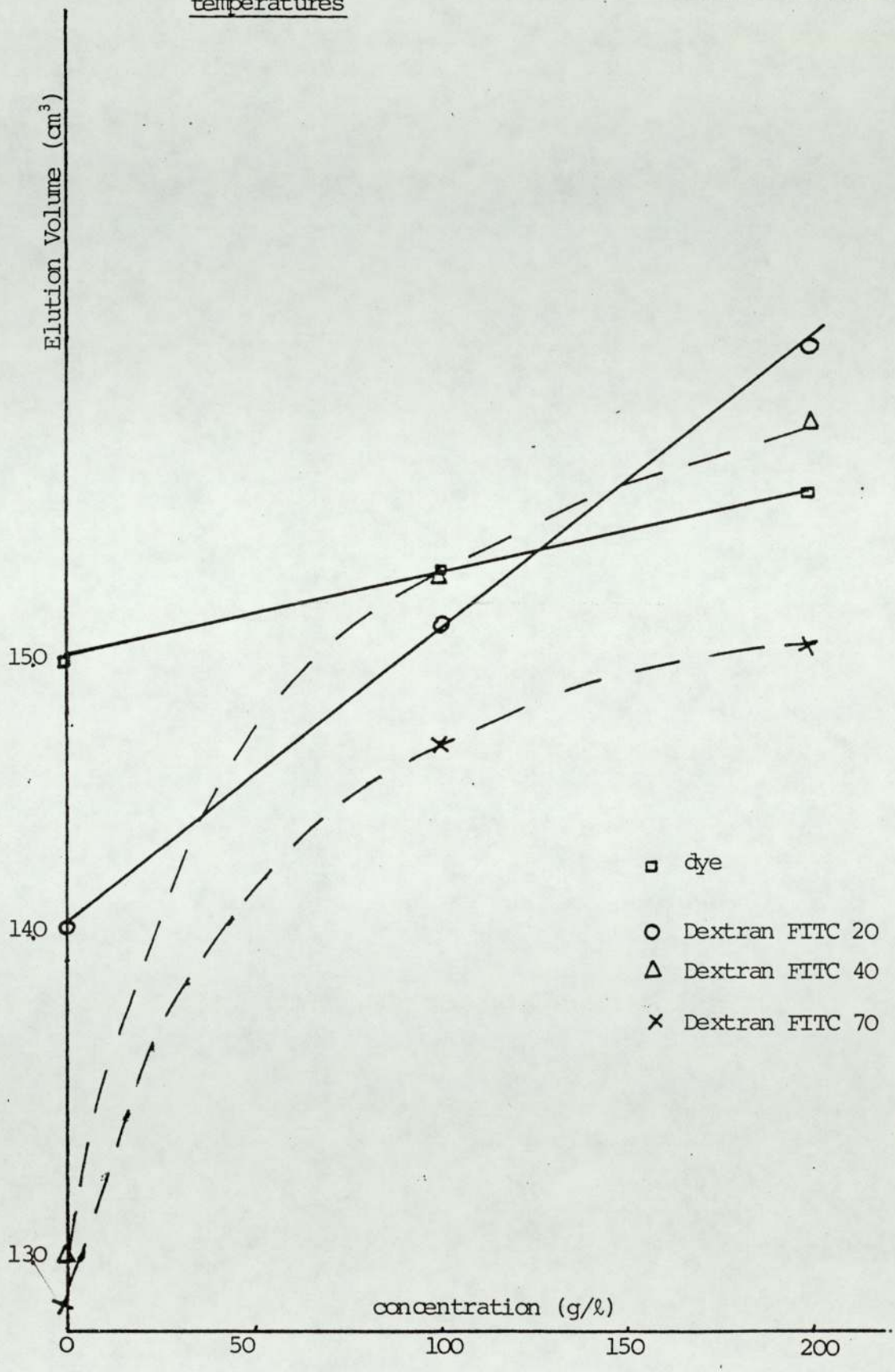
Because the fractionating performance of the SCCR5 changes significantly as the dextran concentration in the system increases, the computer program used to simulate the dextran fractionation on the SCCR5, based only on the linear exclusion controlled model was insufficient to predict the GPC fractionations on the SCCR5 (46). Hence this program had to be modified in order to take into account the concentration effects.

The data used in order to modify the existing simulation program was provided by the experimental work carried out in this laboratory by Holding (187). He used FITC dextrans (i.e. labelled dextrans of narrow molecular weight distribution, marketed by Pharmacia Fine Chemicals Co., Upsala, Sweden), at different dextran

Fig. 9.1 Results of the concentration and temperature work carried out by Holding (187)

Dextran	FITC 70			FITC 40			FITC 20			Dye (marker)		
Temperature ^{o}C	Ambient	40	60	Ambient	40	60	Ambient	40	60	Ambient	40	60
Concentration background ($g.\ell^{-1}$)	Elution Volume (cm^3)	Elution Volume (cm^3)	Elution Volume (cm^3)	Elution Volume (cm^3)	Elution Volume (cm^3)	Elution Volume (cm^3)	Elution Volume (cm^3)	Elution Volume (cm^3)	Elution Volume (cm^3)	Elution Volume (cm^3)	Elution Volume (cm^3)	Elution Volume (cm^3)
0	12.90	12.36	12.07	13.04	12.74	12.76	14.10	13.82	13.72	14.97	14.89	14.74
100	14.69	14.06	13.88	15.23	14.87	14.62	15.09	15.21	15.27	15.24	15.25	15.29
200	15.03	14.86	14.54	15.76	15.58	15.52	15.99	15.87	15.59	15.51	15.53	15.46

Fig. 9.2 The effect of concentration on the elution volumes of of dextrans of different molecular weights at ambient temperatures



concentration backgrounds and at different operating temperatures in a batch chromatographic system in an attempt to evaluate the concentration and temperature effects on different molecular weight dextrans.

Results of his work are presented in Fig. 9.1 and some of these results are plotted in Fig. 9.2. From these results can be seen that the elution volume of dextran increases as the on columns concentration increases and the elution volume decreases as the operating temperature increases. The change of the elution volume is a linear function of concentration for FITC dextran 20 (i.e. 20000 molecular weight), but logarithmic functions for higher molecular weight dextrans such as FITC dextran 40 and 70.

Because the molecular weight, at the peak of the dextran feeds used and main products, is around 40,000 similar to the FITC dextran 40, the results for this dextran were used in order to evaluate the concentration effects on the SCCR5.

The elution volume and hence K_d (equation (9.3)) were taken to be logarithmic functions of concentration (Fig. 9.2, FITC 40), e.g.

$$V_R = \alpha \log(x+c) + \beta \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (9.9)$$

where α and β are constants and can be calculated from the change in the elution volume for a given change in the column concentration, for FITC dextran 40 (Figs. 9.1 and 9.2).

From Fig. 9.1 it was calculated that the average decrease in the elution volume for an increase in temperature from ambient to 40°C was 1.5% and from ambient to 60°C was 2.5%. Therefore the change in elution volume, V_R, was defined as:

$$V_R^{40^\circ C} = V_R^{20^\circ C}(1-0.015) \text{ at } 40^\circ C$$

and $$V_R^{60^\circ C} = V_R^{20^\circ C}(1-0.025) \text{ at } 60^\circ C$$

Finally the existing simulation program was modified and the effects of concentration and temperature were introduced into it. A flowsheet and a listing of the program together with a list of variables is contained in Appendix A2.

9.4 SIMULATION RUNS

The accuracy of the numerical method for the simulation of the SCCR5 has been investigated by England (46). For constant operating conditions, the number of stages per column, the number of terms included in the calculation of the matrices $\phi(\underline{t})$ and $\Delta(\underline{t})$, the step-time increment and number of components were decided in order to achieve a high accuracy in the shortest time possible (Fig. 9.3).

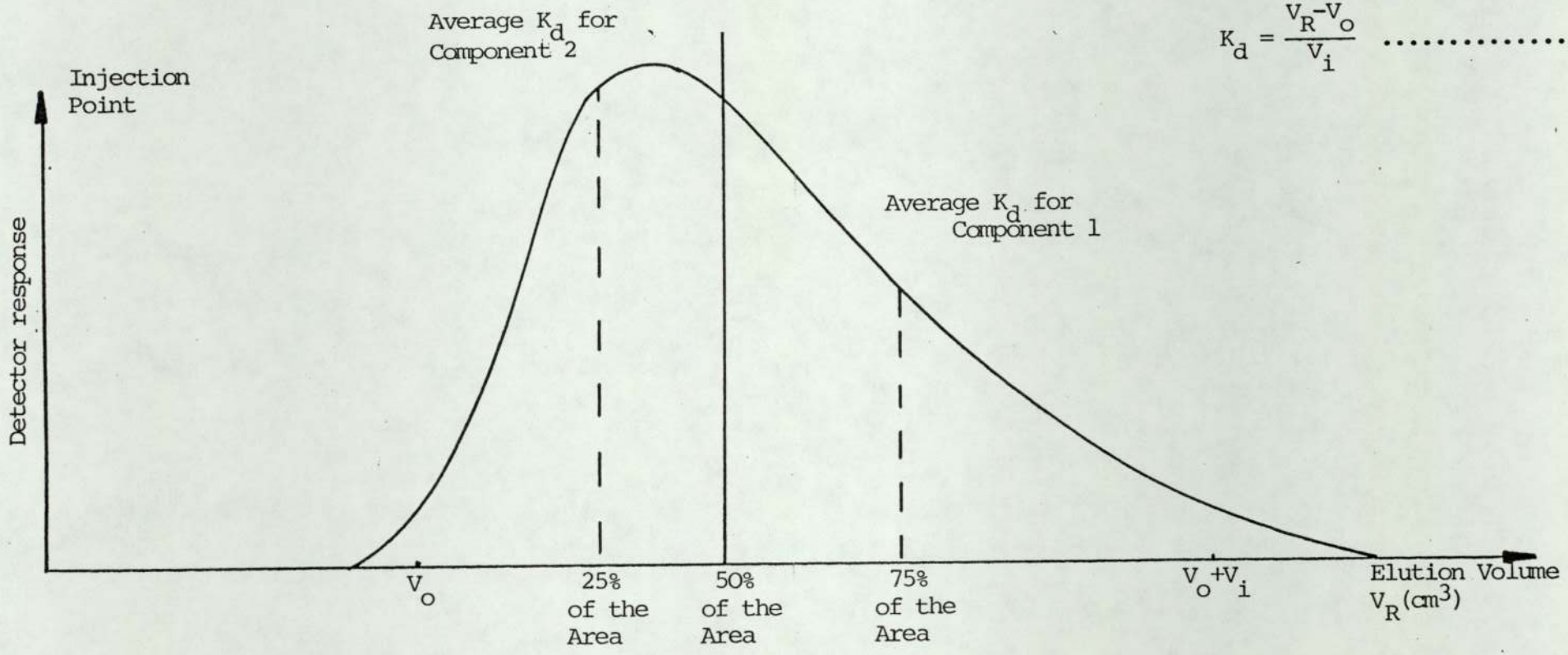
Each column was divided into five theoretical stages, and five terms were used in the calculation of the matrices $\underline{\phi}(t)$ and $\underline{\Delta}(t)$ as decided by England (46), in order to achieve high accuracy in a short time. However during my earlier simulation runs checking the accuracy

Fig. 9.3 Preliminary simulation runs carried out by England (46) to fix the simulation terms. Conditions and results.

Run	Switch time s	Eluent rate cm^3/s	Feed rate cm^3/s	Mass input g	Terms in $\underline{\Delta}$ matrix	Step-increment s	Stages per column	No. of components	Mass in LMWP g	Mass in HMWP g	Computer time taken (Manchester) s
1.2.0	450	1.667	0.333	30	3	10	10	2	10.2	1.3	> 1270
1.2.1	450	1.667	0.333	30	5	10	10	2	16.7	13.3	295
1.2.2	450	1.667	0.333	30	7	10	10	2	16.7	13.4	385
1.2.3	450	1.667	0.333	30	10	10	10	2	16.7	13.4	385
1.2.4*	450	1.667	0.333	30	5	90	5	2	12.8	8.9	> 1270
1.2.5*	450	1.667	0.333	30	5	50	5	2	16.5	13.2	60
2.2.1	450	1.667	0.333	30	7	10	7	2	16.5	13.2	230
2.2.2	450	1.667	0.333	30	7	2	7	2	16.6	13.2	1250
1.2.1	450	1.667	0.333	30	5	10	10	2	16.7	13.3	295
2.2.1	450	1.667	0.333	30	7	10	7	2	16.5	13.2	230
3.2.1	450	1.667	0.333	30	5	10	5	2	16.5	13.1	165
3.2.2	450	1.667	0.333	30	5	10	2	2	18.8	11.1	125
4.2.1	450	1.667	0.666	60	5	10	5	2	28.6	30.9	205
1.5.2	450	1.667	0.667	60	5	10	5	5	28.7	30.9	435

* Runs carried out by the author of this thesis.

Fig. 9.4 An example of how to obtain the relative concentrations and average K_d values for the components of a binary feed mixture



$$K_d = \frac{V_R - V_o}{V_i} \quad \text{.....(2.7)}$$

Fig. 9.5 Summary of the non-experimental parameters for the simulation runs

Packing Used	XOB 075	XOB 030
Number of Stages per column	5	5
Number of Stages	(10-1) x 5 = 45	(10-1) x 5 = 45
Components	2	2
Terms in the matrices	5	5
Time increment (s)	50	50
V_{o_1} (cm^3)	537	552
V_i (cm^3)	605	586
K_{d_1}*	1.020	1.112
Constant, α_1*	51.0	52.0
Constant, β_1 (cm^3)*	1258	1302
K_{d_2}*	0.500	0.640
Constant, α_2*	36.5	40.0
Constant, β_2 (cm^3)*	905	997

*subscripts 1 and 2 refer to components 1 and 2

of the program showed that time-step increments of up to 50 seconds can be used, instead of 10 seconds that England (46) recommended, without any significant loss in accuracy or effect in stability (Fig. 9.3).

Since dextran is a polymer and contains many hundreds of different solute species it was necessary to choose a few components to represent the system. England (46) in his simulation runs considered dextran both as a binary and a five component system (Fig. 9.3). In this project dextran feed was described as a binary mixture for two reasons: (1) because when the simulation program was modified, so that it will take into account concentration and temperature effects, the computer time to run a program for a binary component feed was very close to the maximum time allowed (1270s) for the Manchester (largest computer available to me) computer jobs. Hence by trying to describe dextran as a multi-component mixture, the simulation runs will fail due to the time limit, and because (2) from Fig. 2.3, runs 4.2.1 and 1.5.2, it can be seen that the dextran outputs in the low and high molecular weight products are not affected significantly by the number of components used. The relative concentrations and average K_d of each component were obtained from an elution chromatogram of dextran HZ16K on Spherosil XOB 075 or Spherosil XOB 030 as shown in Fig. 9.4.

The non-experimental parameters for the simulation runs are summarised in Fig. 9.5.

The simulation of GPC runs was carried out and the conditions and results of these runs together with those of the experimental runs are presented in Figs. 9.5 to 9.15. From these runs it can be seen that simulation and experimental results are not very different, although further improvement in the simulation can be achieved. It was also observed from the concentration profiles (Figs. 9.8 to 9.15) that the simulation runs predict lower concentrations than the experimental values, particularly towards the high molecular weight product port.

Simulation runs 8.1 and 8.1.1 were identical, except for the constants α and β, for component 2. The values of α and β, in run 8.1.1 were 48 and 1018 respectively, instead of 40 and 997 that were in run 8.1. The results of these runs (Figs. 9.16 and 9.17) showed that the change in the constants of equation (9.9) did not have any effect on the actual shape of the peak, but affected the amount of material to be removed with the high molecular weight product.

The deficiencies of the simulation model are probably due to the simple model used to describe such a complicated process, due to limited amount of data available for the concentration effects and because the concentration and temperature effects were evaluated under batch mode conditions instead of continuous operation.

Finally, simulation runs 8.1 and 8.1.2 were identical except to the fact that the system is assumed

Fig. 9.6a Results and conditions of the experimental and simulation runs

Run	3.1	3.2	4.1	4.2	4.3
Packing	XOB 075	XOB 075	XOB 075	XOB 075	XOB 075
Switch time (min)	7.5	7.5	7.5	7.5	7.5
Temperature (^{o}C)	60	60	40	60	60
Feed column no.	5	5	5	5	5
Feed rate (cm^3/min)	40	60	25	25	45
Feed Conc. (g/ℓ)	234	234	215	215	215
Feed input (g/cycle)	702	035	403	403	726
Eluent rate (cm^3/min)	95	95	110	110	110
Dextran in HMWP (%) (predicted)	12.0	24.7	17.3	19.3	34.2
Dextran in LMWP (%) (predicted)	88.0	75.3	82.7	80.7	65.8
Dextran in HMWP (%) (Experimental)	11.4	21.3	18.2	19.8	43.2
Dextran in LMWP (%) (Experimental)	88.6	78.7	81.8	80.2	56.8

Fig. 9.6b Results and conditions of the experimental and simulation runs

Run	5.1	5.2	6.1	6.2	7.1	7.2
Packing	XOB 075	XOB 075	XOB 075	XOB 075	XOB 075	XOB 075
Switch time (min)	7.5	7.5	7.5	7.5	7.5	7.5
Temperature (^{o}C)	60	60	60	60	60	60
Feed column number	5	5	7	7	3	3
Feed rate (cm^3/min)	40	60	40	60	60	60
Feed conc. (g/ℓ)	134	134	134	134	134	134
Feed inputs (g/cycle)	402	603	402	603	603	603
Fluent rate (cm^3/min)	95	95	95	95	110	95
Dextran in HMWP (%) (predicted)	13.3	25.5	16.0	27.8	36.7	23.8
Dextran in LMWP (%) (predicted)	86.7	74.5	84.0	72.2	63.3	76.2
Dextran in HMWP (%) (predicted)	12.0	23.5	16.4	27.6	49.5	22.6
Dextran in LMWP (%) (experimental)	88.0	76.5	83.6	72.4	50.5	77.4

Fig. 9.6c Results and conditions of the experimental and simulation runs

Run	8.1	8.2	9.1	9.2	9.3	9.4
Packing	XOB 030	XOB 030	XOB 030	XOB 030	XOB 030	XOB 030
Switch time (min)	7.5	7.5	7.5	7.5	7.5	7.5
Temperature (^{o}C)	60	60	60	60	60	60
Feed column no.	5	5	5	5	5	5
Feed rate (cm^3/min)	35	60	35	25	25	15
Feed conc. (g/ℓ)	131	131	71	71	71	118
Feed input (g/cycle)	344	590	186	134	134	134
Eluent rate (cm^3/min)	115	96	115	115	125	125
Dextran in HMWP (%) (predicted)	17.7	15.3	19.4	10.3	26.5	14.3
Dextran in LMWP (%) (predicted)	82.3	84.7	80.6	89.7	73.5	85.7
Dextran in HMWP (%) (experimental)	14.2	14.9	16.2	11.4	25.3	14.7
Dextran in LMWP (%) (experimental)	85.8	85.1	83.8	88.6	74.7	85.3

Fig 9.7 Results of the simulation runs

Run	3.1		3.2		4.1		4.2		4.3	
Component	1	2	1	2	1	2	1	2	1	2
Dextran in LMWP (g)	33.2	27.1	49.8	28.1	18.6	13.5	18.6	12.7	33.6	13.8
Dextran in HMWP (g)	2.6×10^{-4}	8.3	5.3×10^{-2}	25.6	1.3×10^{-4}	6.7	1.9×10^{-4}	7.5	4.6×10^{-2}	23.4

Run	5.1		5.2		6.1		6.2		7.1		7.2	
Component	1	2	1	2	1	2	1	2	1	2	1	2
Dextran in LMWP (g)	19.8	15.0	28.5	15.6	19.0	14.4	28.1	14.7	27.5	9.0	28.5	15.7
Dextran in HMWP (g)	2.1×10^{-4}	5.2	4.1×10^{-2}	15.1	2.9×10^{-2}	6.4	5×10^{-1}	16.6	3.7×10^{-1}	19.4	3.6×10^{-3}	13.7

Run	8.1		8.2		9.1		9.2		9.3		9.4	
Component	1	2	1	2	1	2	1	2	1	2	1	2
Dextran in LMWP (g)	16.0	11.5	28.0	21.1	8.6	5.9	6.2	5.3	6.0	3.3	6.0	4.8
Dextran in HMWP (g)	2×10^{-3}	5.9	1×10^{-2}	8.9	1.5×10^{-3}	3.5	1.1×10^{-4}	1.3	1.3×10^{-3}	3.4	1×10^{-4}	1.7

Fig. 9.8 Experimental and simulation of on column concentration profiles for run 3.2

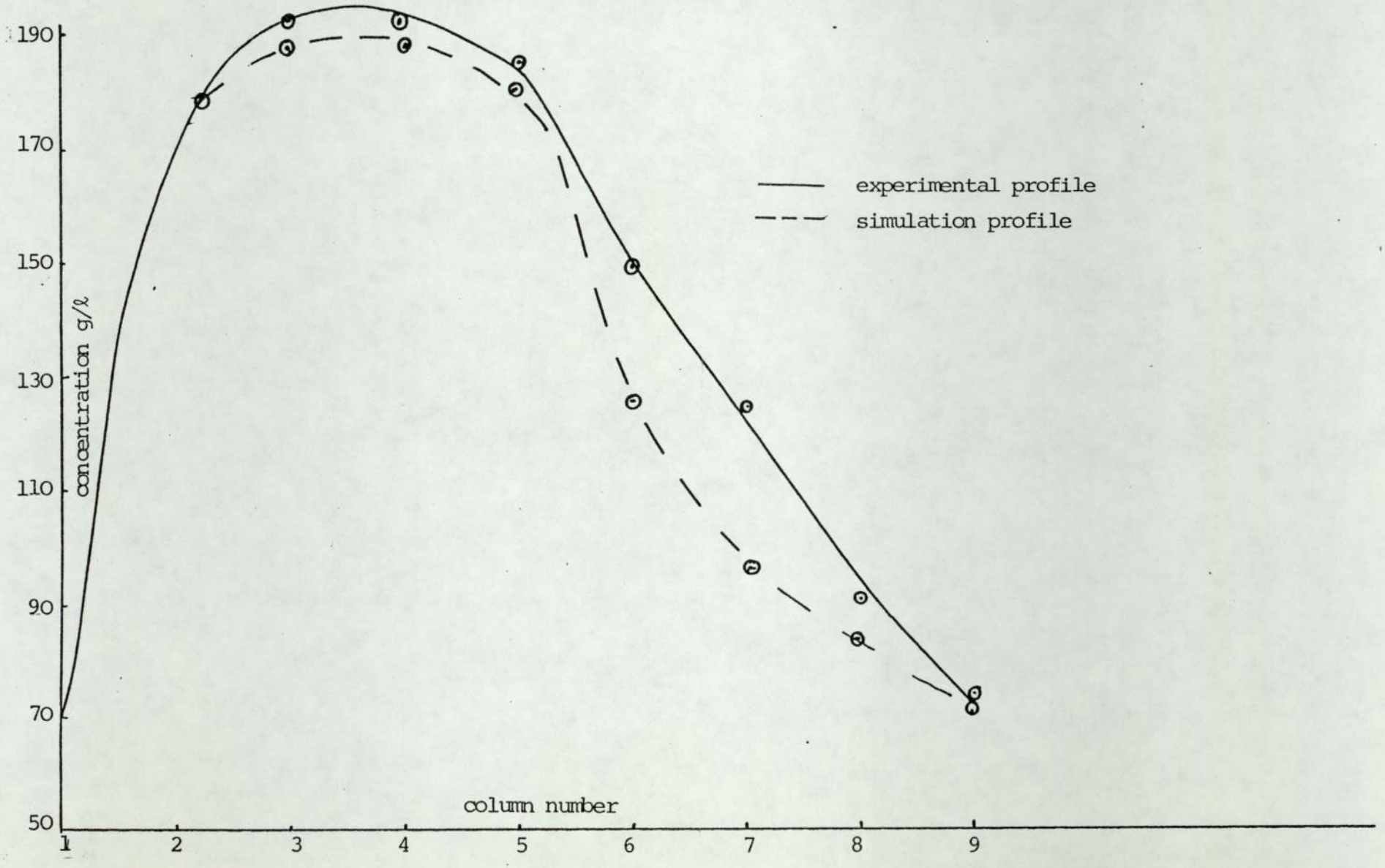


Fig. 9.9 Experimental and simulation of on column concentration profiles for run 4.3

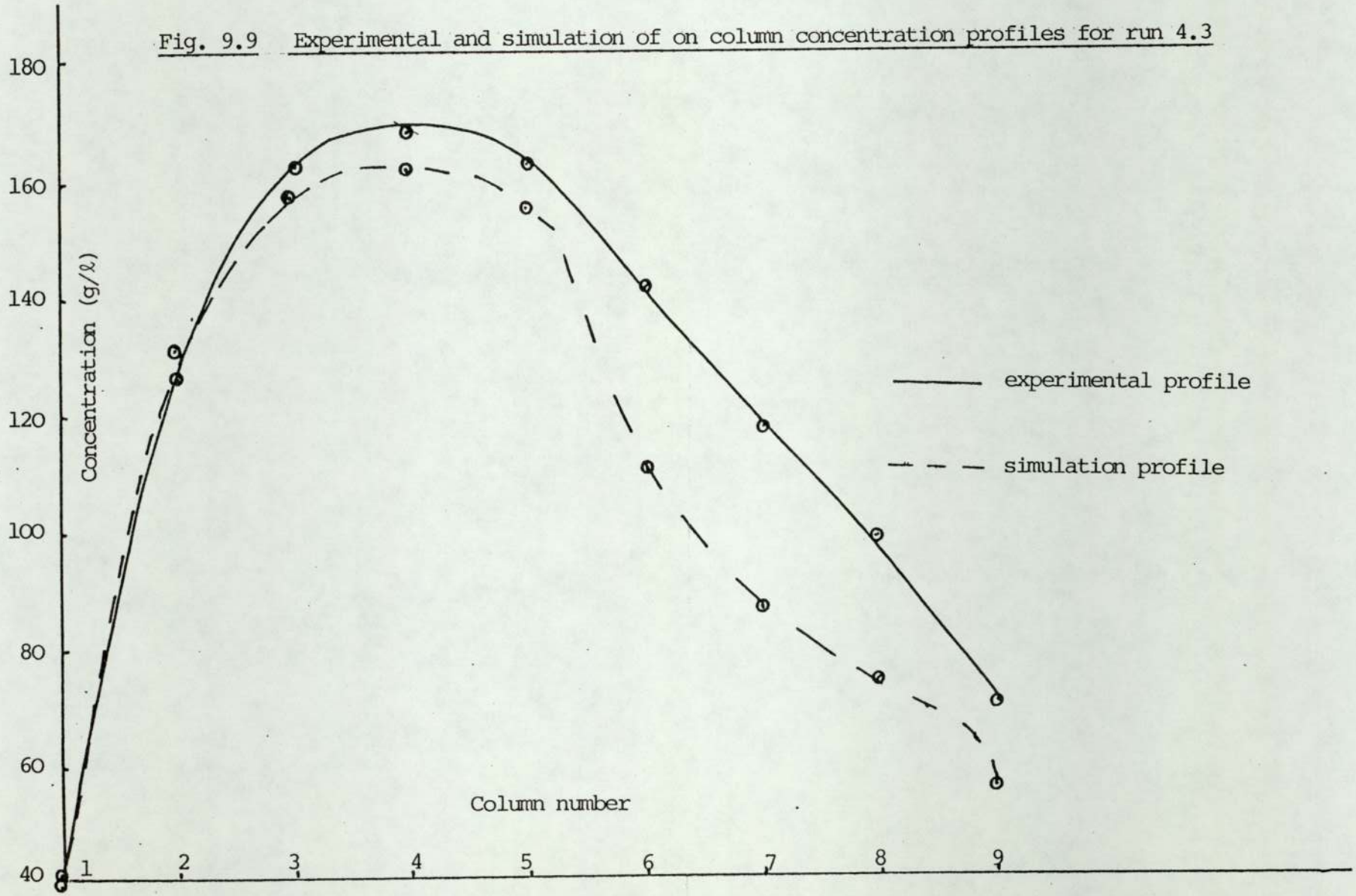


Fig. 9.10 Experimental and simulation of on column concentration profiles for run 5.2

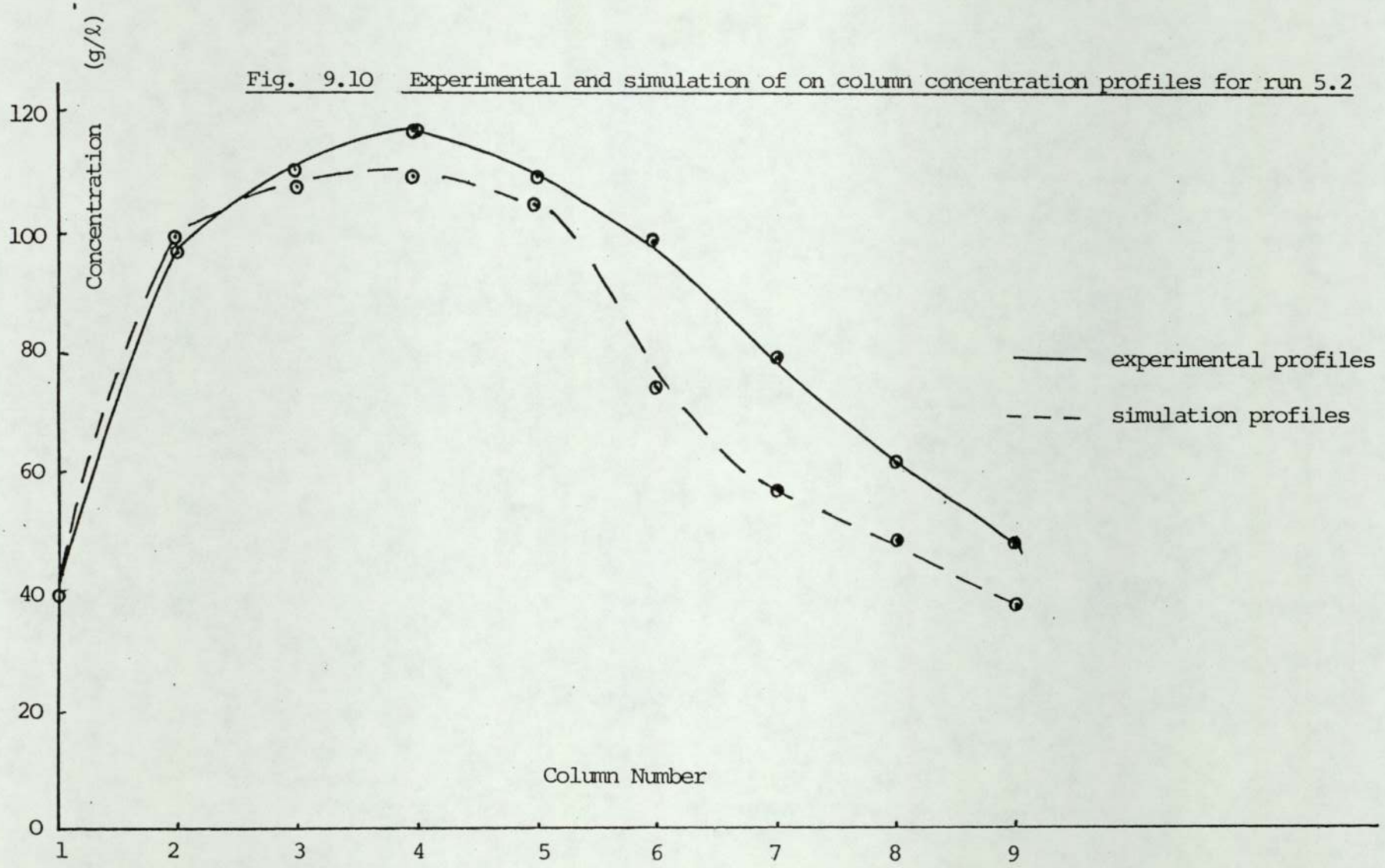
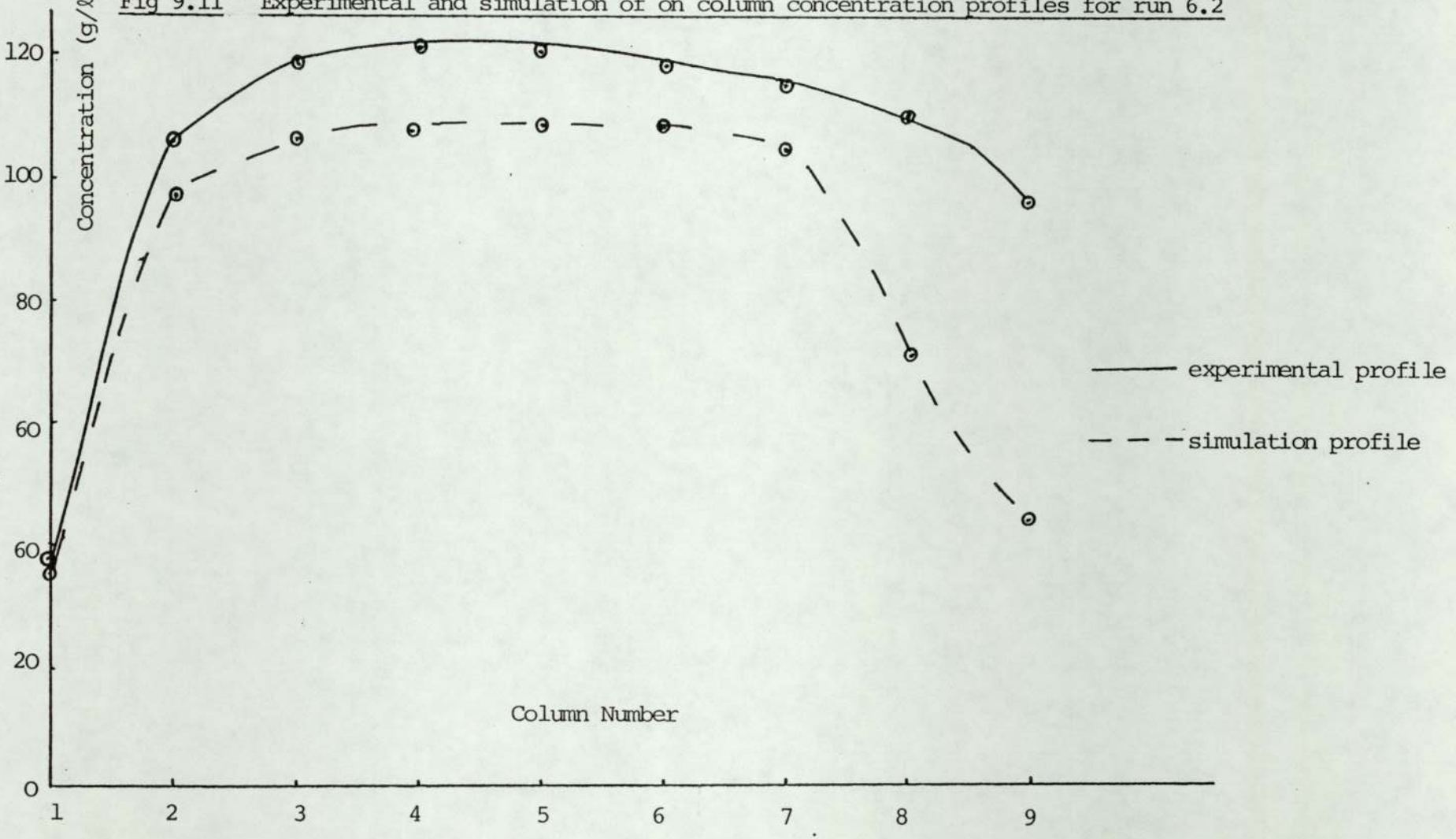


Fig 9.11 Experimental and simulation of on column concentration profiles for run 6.2



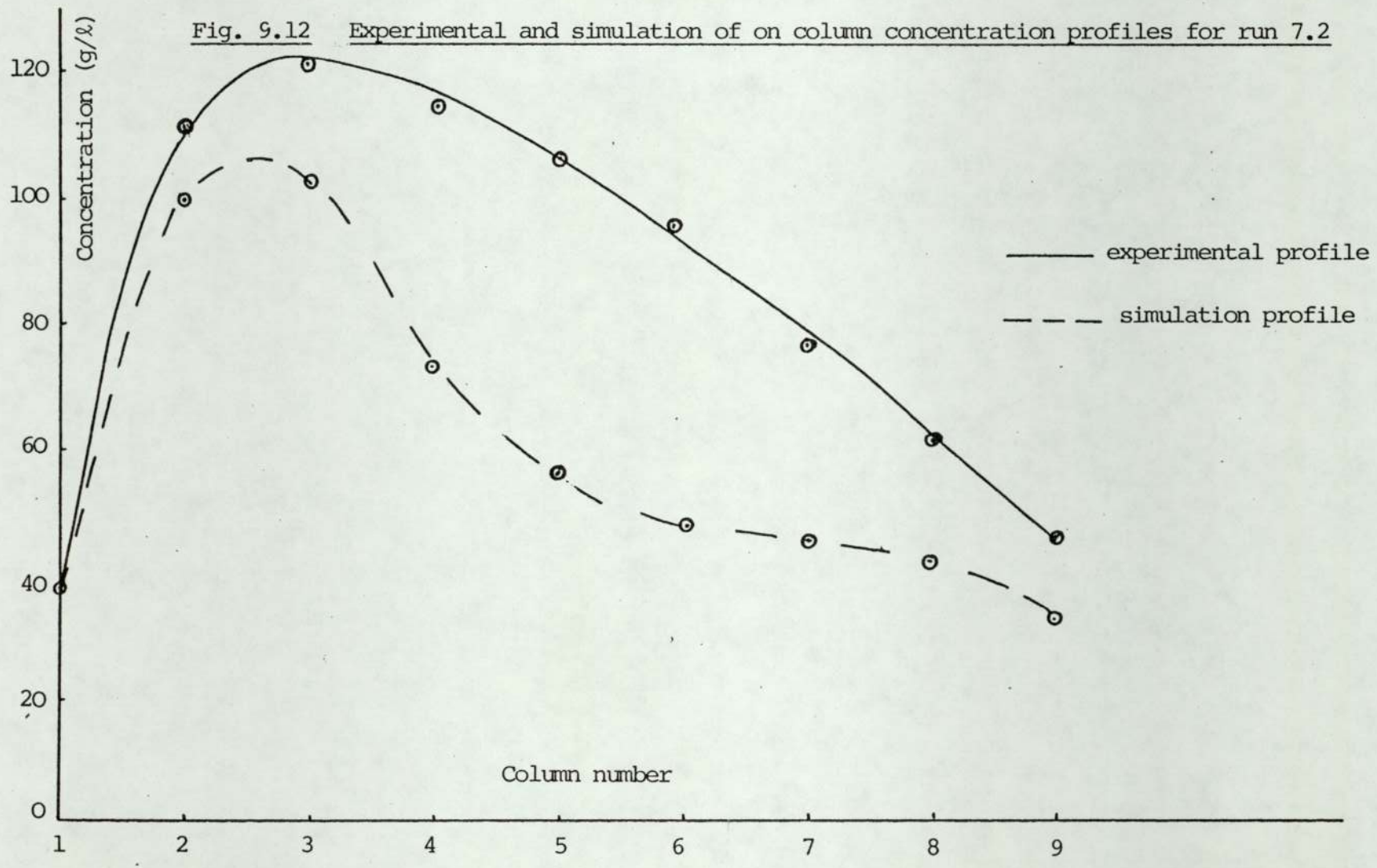


Fig. 9.12 Experimental and simulation of on column concentration profiles for run 7.2

Fig. 9.13 Experimental and simulation of mobile phase concentration profiles for run 8.1

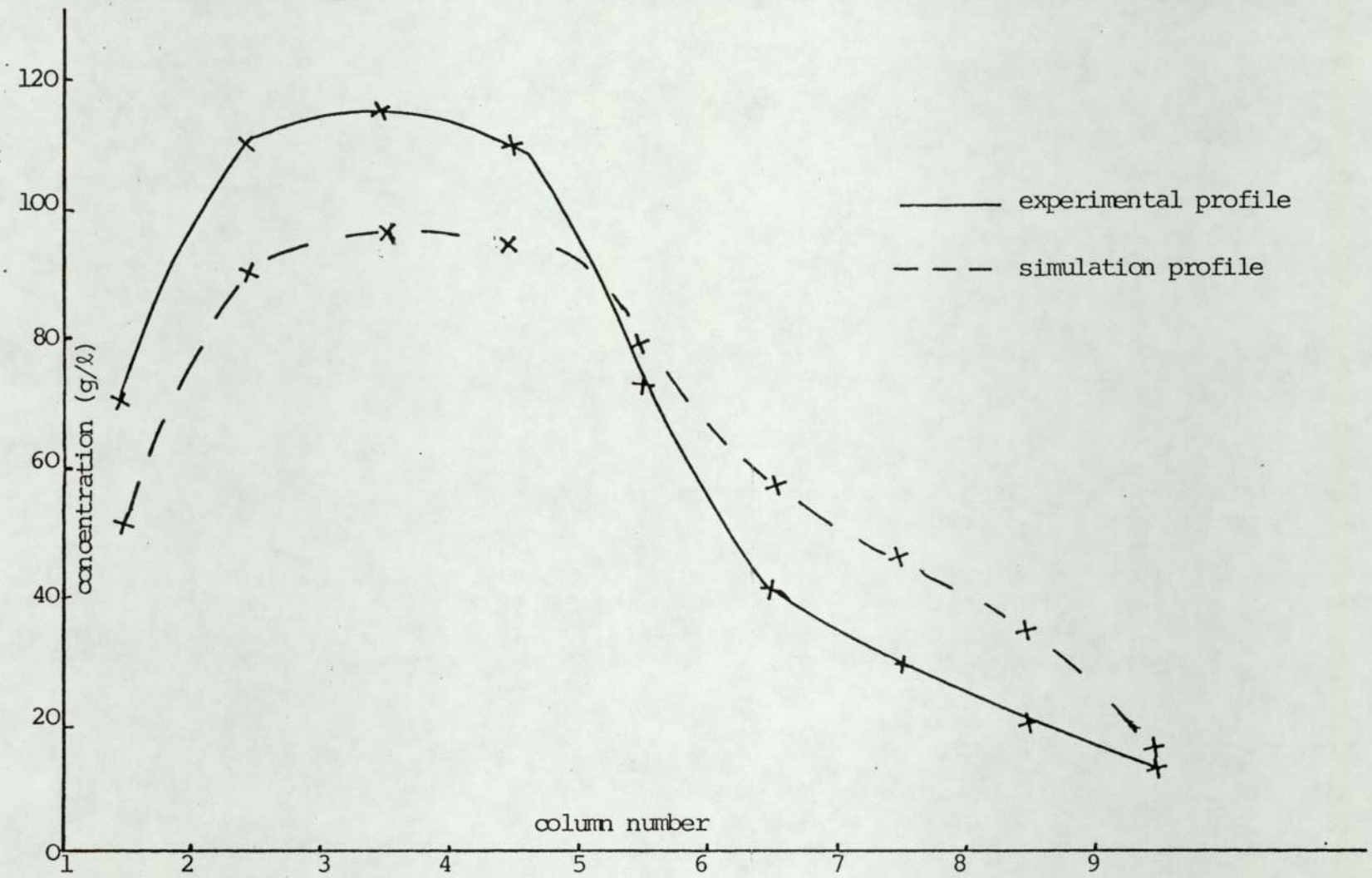


Fig. 9.14 Experimental and simulation of on column concentration and mobile phase concentration profiles for run 8.2

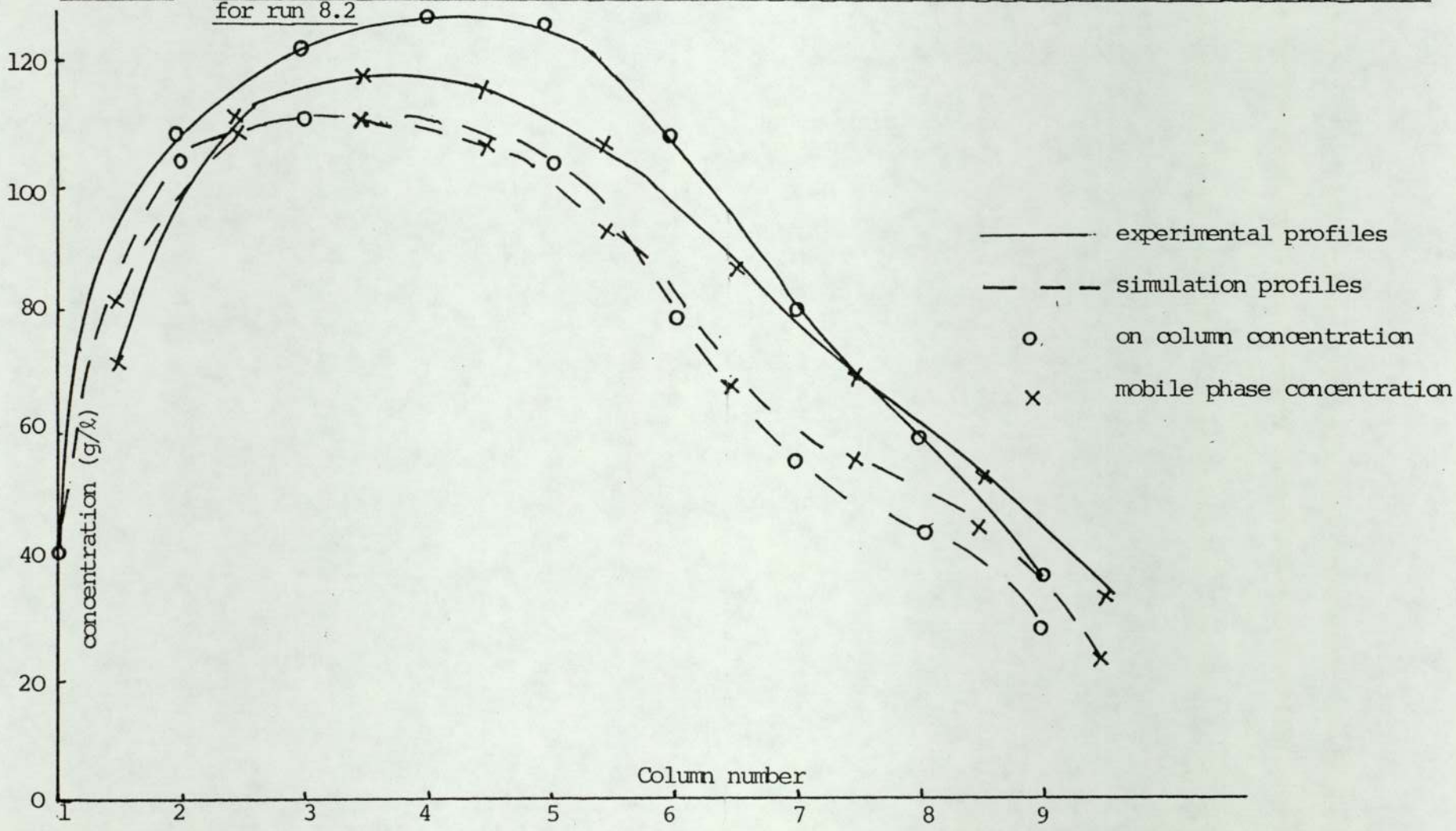


Fig. 9.15 Experimental and simulation of on column concentration and mobile phase concentration profiles for run 9.4

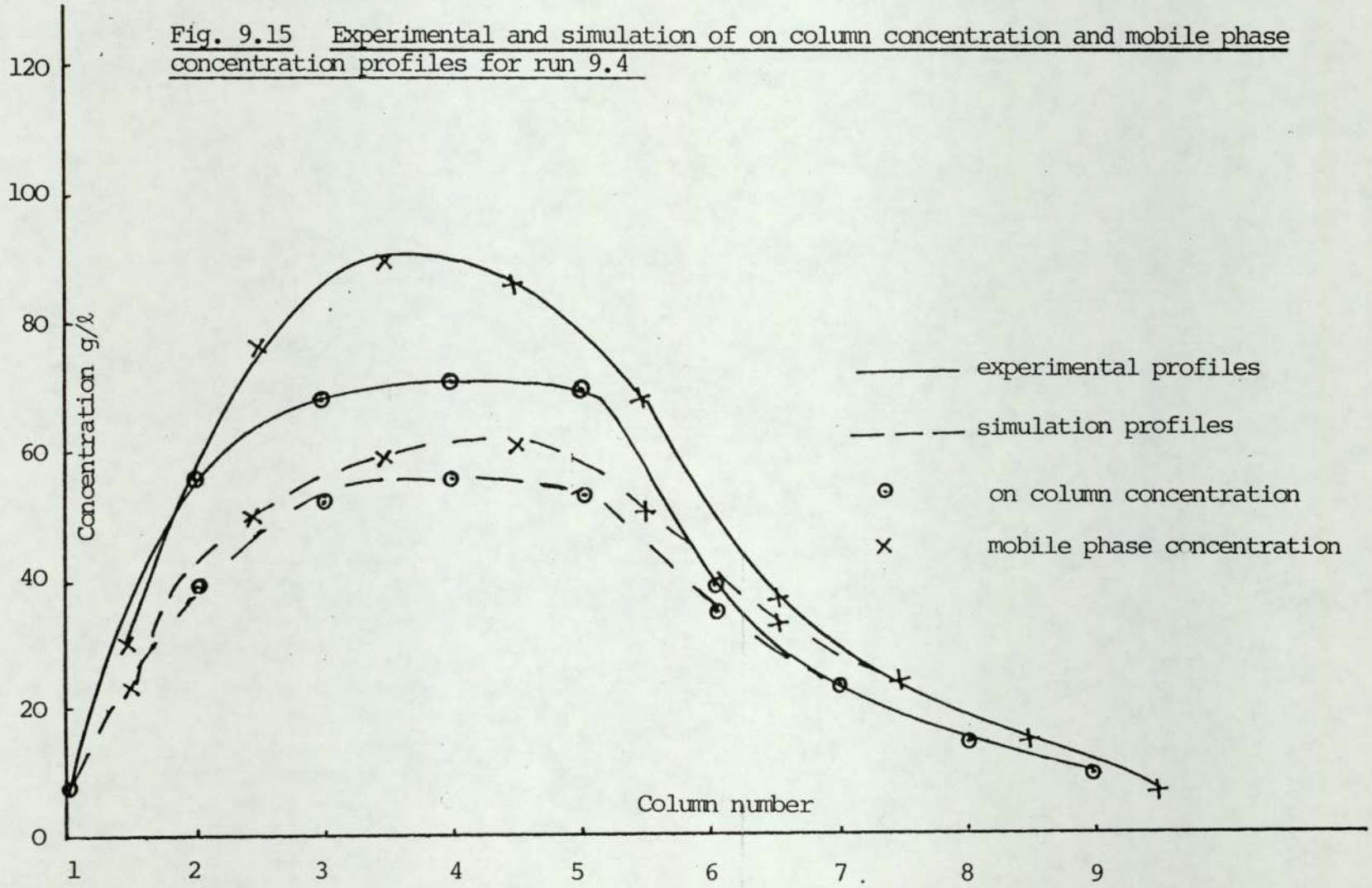


Fig 9.16 Results of simulation runs 8.1 and 8.1.1

Simulation Run	8.1	8.1.1
α, component 1	52	52
β, component 1(cm^3)	1302	1302
γ, component 1(cm^3)	1189	1189
α, component 2	40	48
β, component 2(cm^3)	997	1018
γ, component 2(cm^3)	911	911
Dextran in LMWP component 1(g)	16.0	16.0
Dextran in LMWP component 2(g)	11.5	12.9
Dextran in LMWP (%)	82.3	86.8
Dextran in HMWP component 1(g)	2×10^{-3}	2.2×10^{-3}
Dextran in HMWP component 2(g)	5.9	4.4
Dextran in HMWP (%)	17.7	13.2

Fig. 9.17 Simulation profiles, for simulation runs 8.1 and 8.1.1

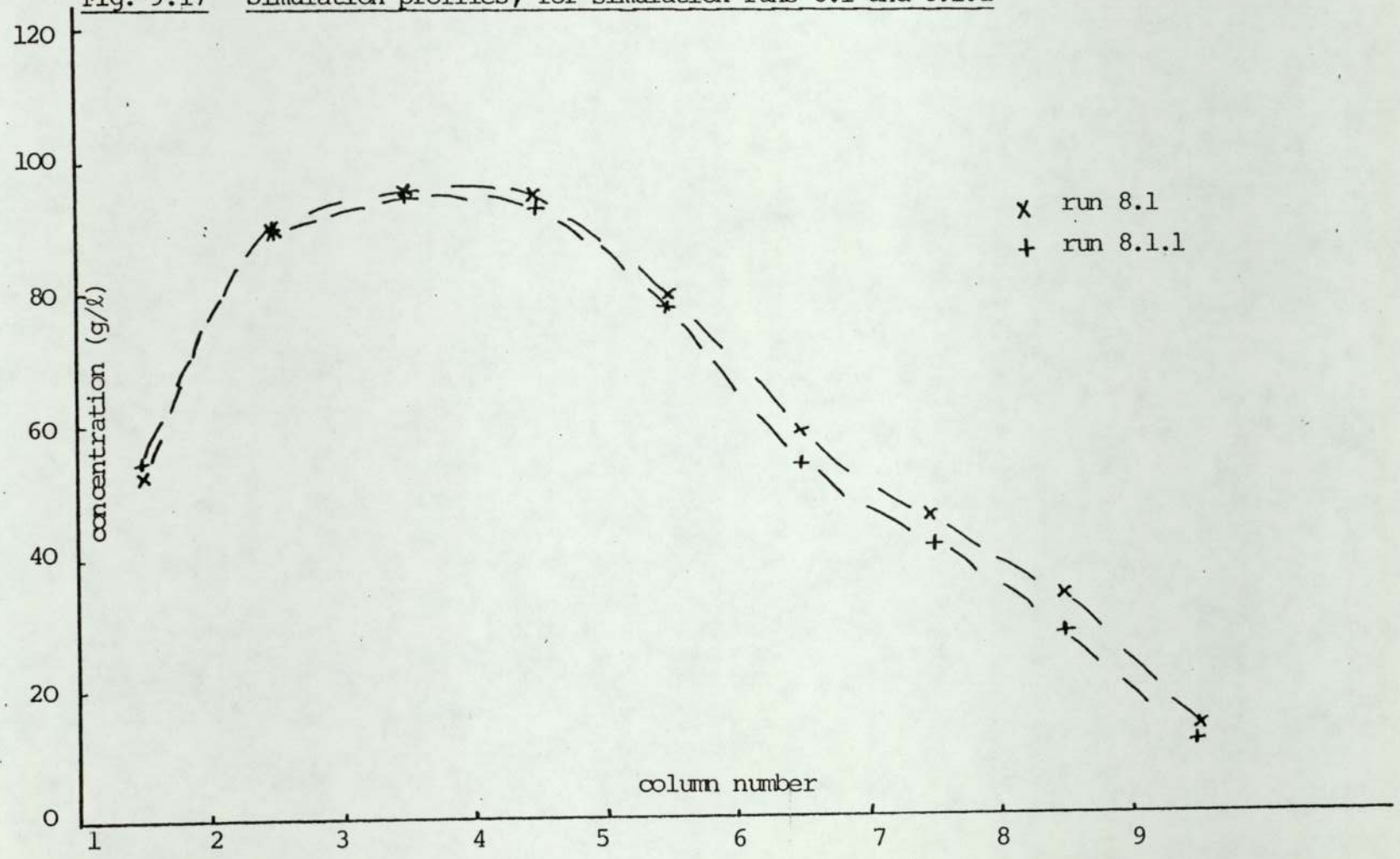


Fig. 9.18 Results of simulation runs 8.1 and 8.1.2

Simulation run	8.1	8.1.2
No. of columns in the SCCR5	10	12
Packing	XOB 030	XOB 030
Switch time (min)	7.5	7.5
Temperature (^{o}C)	60	60
Feed column no.	5	5
Feed rate (cm^3/min)	35	35
Feed conc. (g/ℓ)	131	131
Eluent rate (cm^3/min)	115	115
Dextran in LMWP component 1 (g)	16.0	15.9
Dextran in LMWP Component 2 (g)	11.5	11.4
Dextran in LMWP %	82.3	83.5
Dextran in HMWP Component 1 (g)	2×10^{-3}	3×10^{-4}
Dextran in HMWP Component 2 (g)	5.9	5.4
Dextran in HMWP %	17.7	16.5

to have 10 columns in run 8.1 and 12 columns in run 8.1.2. These runs (Fig. 9.18) showed that the amount of the most retained component, i.e. component 1, removed with the high molecular weight product is reduced. Therefore it is believed that by increasing the number of columns in the SCCR5, the GPC fractionating efficiency will increase.

9.5 CONCLUSIONS

The model describes an idealised picture of the GPC process taking place in the SCCR5. The inclusion of a concentration and temperature dependent distribution coefficient in the model provided a reasonable agreement between simulation and experimental results but it failed to describe the process completely. The model probably can be further improved by considering other effects that take place in the GPC, such as diffusion. Also by taking into account the dead volume of the valves and piping between columns (approximately 1.5% of the column volume) that help back-mixing, and by obtaining more experimental data for the concentration and molecular weight effects. To evaluate these effects it is preferable to carry out the experimental work on the SCCR5 under actual operating conditions instead of by batch mode techniques.

10.0 CONCLUSION AND RECOMMENDATIONS

10.0 CONCLUSIONS AND RECOMMENDATIONS

During the research project the suitability of GPC, UF and ion exchange for the production of clinical dextran 40 from dextran hydrolysate has been considered.

From the runs on the SCCR5 it was decided that GPC could be used for the removal of the very high molecular weight dextran. Variations in the operating conditions of the SCCR5 showed that the product-split was controlled by:

(a) the pre-feed cut position

(b) the post-feed cut position

(c) the feed concentration

(d) the operating temperature

(e) the feed port location

and (f) the pore size of the packing

Also it was found that for every 100g of dextran removed by the SCCR5 as high molecular weight product, 40 to 65 g were useful material, and although the amount of this dextran in the high molecular weight product was slightly reduced by increasing the eluent to feed ratio and by changing the feed port location, a way of reducing this amount further was required. The change in the pore size of the packing, i.e. by replacing the Spherosil XOB 075 with XOB 030, did not have any significant effect on the efficiency of the GPC process. Probably the fractionating efficiency of the SCCR5 can be further increased by using a packing of narrower pore size distribution, by increasing the number of columns

in the SCCR5 or by using a smaller particle diameter packing. It was also predicted from simulation run 8.1.2 (Fig. 9.16) and simulation run 5.5.1 done by England (46) that an increase in the number of columns in the SCCR5 and using a packing of narrower pore size distribution will decrease the amount of useful dextran in the high molecular weight product.

UF runs on several membranes and hollow fibre cartridges showed that very low molecular weight dextran and silica, present in the dextran solutions due to the dissolution of the GPC packing, can be best removed from dextran solutions by using an H1P5 hollow fibre cartridge on an Amicon DC2A system. H1P2 cartridges were better in concentrating dextran solutions.

Using an H1P5 cartridge on an Amicon DC2A system it was found that for every 100g of dextran removed with the low molecular weight product only 25 to 40g were useful material. Also high molecular weight material removal can be achieved using an H1P100 cartridge on the DC2A system, but GPC was found more efficient than UF in the removal of high molecular weight dextran.

Also Millipore cartridges packed with mixed bed nuclear grade ion exchange resins were used to produce silica free dextran solutions.

Finally GPC, UF and ion exchange were combined into a process that produced clinical dextran 40 from dextran hydrolysate with similar yields to the existing ethanol precipitation process (272).

After the successful combination of the GPC, UF and ion exchange for the production of clinical dextran a new industrial process (Fig. 10.1)that used only aqueous solutions was proposed for the clinical dextran production that replaces the current industrial ethanol fractionation process (Fig. 10.2).

In the new process it was proposed that the dextran hydrolysate will pass first through the SCCR5 to remove the very high molecular weight dextran. Then it will pass through the UF unit, where the very low molecular weight material and most of the silica that has been dissolved in the dextran solution from the GPC packing of the SCCR5, will be removed and the final product concentrated. To achieve dextran concentrations suitable for spray drying the final product (250-300 g/ℓ), it is necessary to concentrate the final solution in the UF unit at elevated temperatures (30-50^{o}C). Then the concentrated dextran solution will pass through a nuclear grade, mixed bed, ion exchange resin to produce a completely silica free dextran and finally this solution will be spray dried to produce dextran powder.

The computer simulation runs for the SCCR5 showed good agreement with the experimental runs, but further improvement in the simulation model is probably possible by:

(a) considering other effects that take place in GPC such as diffusion

(b) taking into account the dead volume of the valves and piping between columns (1.5% of

Fig 10.1 A new process for the manufacture of clinical dextran

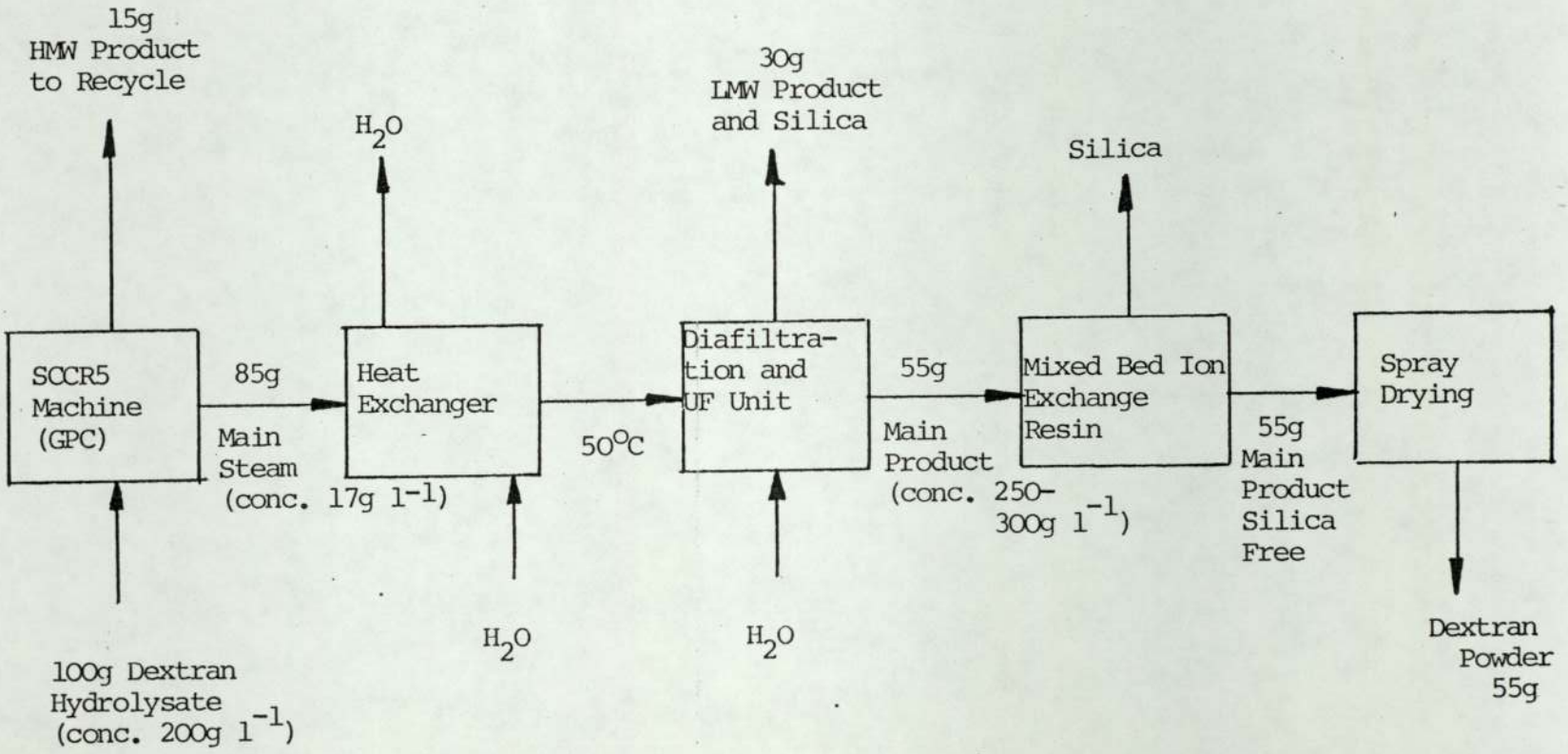
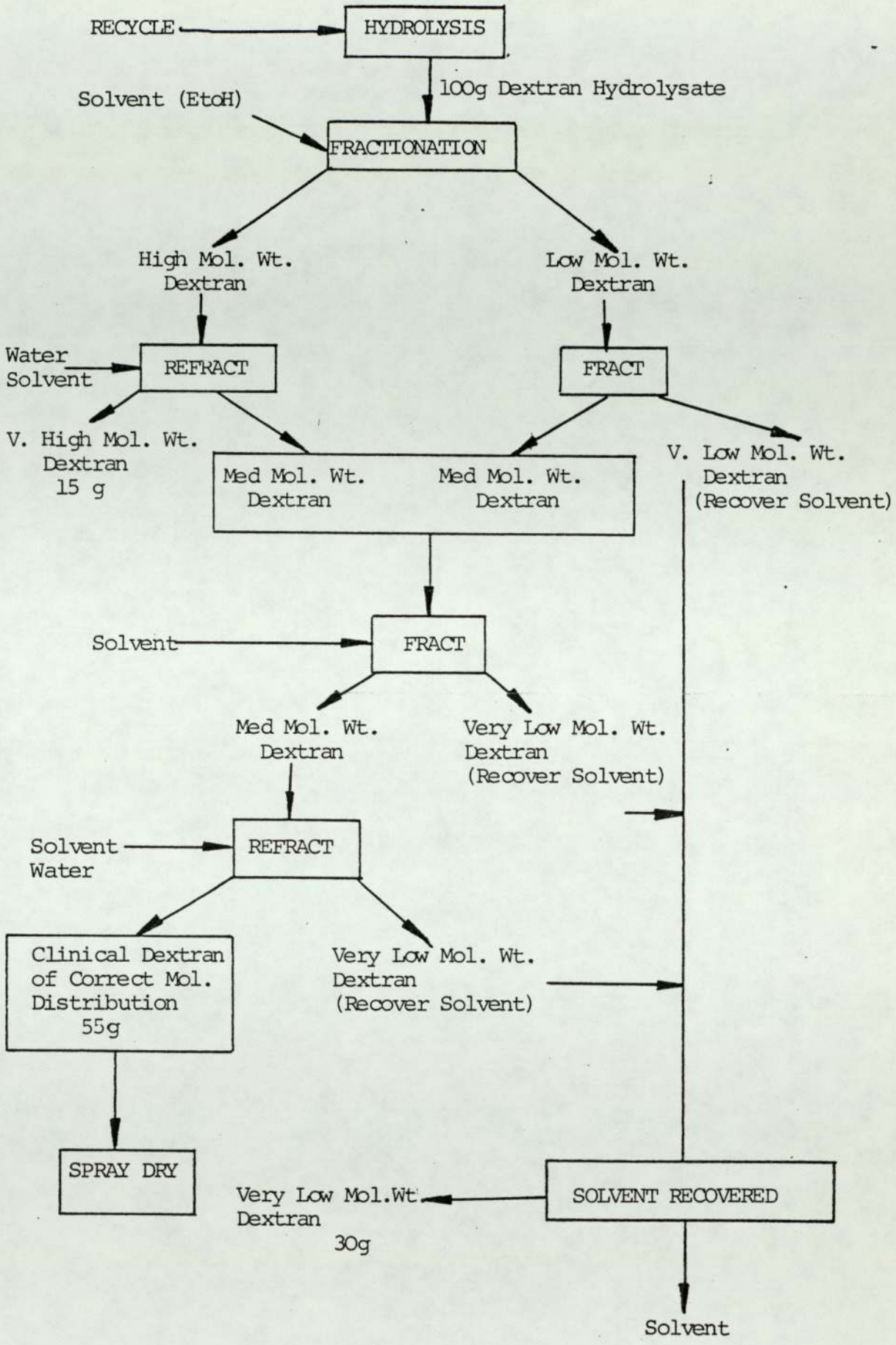


Fig. 10.2 The current industrial process for the manufacture of clinical Dextran by ethanol precipitation



a column volume) that create back-mixing

and (c) by obtaining more data on the SCCR5, under actual operating conditions, about the concentration, temperature and molecular weight effects. For these purposes, sample points must be fitted along and around the columns of the SCCR5.

The future work on this project can be divided into three categories.

(a) To improve the mathematical model of the SCCR5 by trying to understand more the complexity of the concentration, temperature and molecular weight effects on GPC.

(b) To study more fully the UF process and try to develop a mathematical model that will describe this process.

(c) To improve the fractionating efficiency of the SCCR5 and to use UF units, equipped with heat exchangers or heaters, that can be operated at elevated temperatures in order to achieve higher concentrations of the final solutions (approximately 300 g/ℓ). Also to carry out an economic evaluation of the new process and to compare its capital and operating costs against the corresponding costs for the existing ethanol fractionation process.

APPENDIX A1

COMPUTER PROGRAMMES FOR CALIBRATING THE ANALYTICAL COLUMNS AND CALCULATING THE AVERAGE MOLECULAR WEIGHTS

```
          GPC CALIBRATION PROGRAM
C     THIS PROGRAM CARRIES OUT THE CALIBRATION OF GEL-
C     PERMEATION CHROMATOGRAPHIC COLUMNS USING "BROAD"
C     FRACTIONS OF KNOWN WEIGHT AVERAGE MOLOECULAR WEIGHTS
      IMPLICIT DOUBLE PRECISION(A-H,O-Y)
      DOUBLE PRECISION M1,M2,KD,Z
      DIMENSION NY(32),KD(32,60),M1(32),M2(32),Y(32,60),S
     SUM(32,5), B(5),C(5),RO(5),R1(5),R2(5),XMAT(25),Q
     Q(4),ZZ(4)
      READ (1,102) (B(K),K=1,5)
      READ (1,105) NNI
      WRITE (2,200)
      WRITE (2,255)
      WRITE (2,265) (K,B(K),K=1,5)
      WRITE (2,210)
      NP=0
      T=10000.0
      B(4)=B(4)-DLOG(T)
      B(5)=B(5)/T
C
C     DATA INPUT
C
      DO 30 I=1,15
      READ (1,110) M1(I),P,VO,VT,VE,VDE,DIFF,VM,YM,SD
      NY(I)=P
      READ (1,120) (Y(I,J),J=1,NY(I))
      WRITE (2,557) (Y(I,J),J=1,NY(I))
  557 FORMAT(84F0.0)
      M1(I)=M1(I)/T
      VP=VT-VO
      SY=0.0
      IF (DIFF.NE.0.0) YG=-1/(2*SD**2)
      DO 25 J=1,NY(I)
      IF (DIFF.EQ.0.0) GO TO 20
      IF (VE.EQ.VM) GO TO 20
      YA=YG*(VE-VM)**2
      YE=DLOG(Y(I,J)/YM)
      YE=YM*EXP(YA*YE/(YA-YE)
      IF (YE.LT.Y(I,J)) Y(I,J)=YE
   20 SY=SY+Y(I,J)
      KD(I,J)=(VE-VO)/VP
      VE=VE+VDE
       WRITE(2,765)SY,KD(I,J),VE
  765 FORMAT(3F20.6)
   25 CONTINUE
      DO 28 J=1,NY(I)
      Y(I,J)=Y(I,J)/SY
      WRITE(2,876)Y(I,J)
  876 FORMAT(F10.5)
   28 CONTINUE
      NP=NP+1
   30 CONTINUE
```

```
C
C       ITERATION PHASE
C
   32   IF (NNI.EQ.0) GOTO 85
        DO 34 K=1,3
   34   ZZ(K)=K*10.0E10
        DO 80 NI=1,NNI
        DO 40 I=1,NP
        M2(I)=0.0
        DO 35 K=2,5
   35   SUM(I,K)=0.0
        SUM(I,1)=1.0
        DO 40 J=1,NY(I)
        Z=DEXP(B(4)+B(1)*KD(I,J)+B(2)*KD(I,J)**2+B(3)*KD(I,J)**3)
        M2(I)=M2(I)+(B(5)+Z)*Y(I,J)
        SUM(I,2)=SUM(I,2)+Y(I,J)*Z
        SUM(I,3)=SUM(I,3)+Y(I,J)*Z*KD(I,J)
        SUM(I,4)=SUM(I,4)+Y(I,J)*Z*KD(I,J)**2
        SUM(I,5)=SUM(I,5)+Y(I,J)*Z*KD(I,J)**3
   40   CONTINUE
        DO 45 K=1,5
        RO(K)=0.0
        DO 45 I=1,NP
   45   RO(K)=RO(K)+(M1(I)-M2(I))/M1(I)**2*SUM(I,K)
        JK=0
        DO 50 K=1,5
        DO 50 J=1,5
        JK=JK+1
        XMAT(JK)=0.0
        DO 50 I=1,NP
        XMAT(JK)=XMAT(JK)+SUM(I,K)*SUM(I,J)/M1(I)**2
   50   COMTINUE
        CALL MATINV (XMAT)
        JK=0
        DO 55 K=1,5
        R1(K)=0.0
        DO 55 J=1,5
        JK=JK+1
        R1(K)=R1(K)+RO(J)*XMAT(JK)
   55   CONTINUE
        R2(5)=R1(1)
        R2(4)=R1(2)
        R2(1)=R1(3)
        R2(2)=R1(4)
        R2(3)=R1(5)
        DO 70 N=1,3
        Q(N)=0.0
        DO 60 K=1,5
        IF (N.EQ.1) C(K)=B(K)
        IF (N.EQ.2) C(K)=B(K)+0.5*R2(K)
   60   IF (N.EQ.3) C(K)=B(K)+R2(K)
        DO 70 I=1,NP
        M2(I)=0.0
```

```
      DO 65 J=1,NY(I)
      Z=DEXP(C(4)+C(1)*KD(I,J)+C(2)*KD(I,J)**2+C(3)*
     1KD(I,J)**3)
   65 M2(I)=M2(I)+(C(5)+Z)*Y(I,J)
      Q(N)=Q(N)+((M1(I)-M2(I))/M1(I))**2
   70 CONTINUE
   72 NII1=NI-1
      WRITE (2,250) NII1,B(1),B(2),B(3),B(4),B(5),Q(1)
      Q(4)=0.5+0.25*(Q(1)-Q(3))/(Q(3)-2.0:Q(2)+Q(1))
      DO 75 K=1,5
   75 B(K)=B(K)+Q(4)*R2(K)
      DO 77 KK=2,4
      K=6-KK
   77 ZZ(K)=ZZ(K-1)
      ZZ(1)=SNGL(Q(1))
      IF (ZZ(4).NE.ZZ(3)) GOTO 80
      IF (ZZ(3).NE.ZZ(2)) GOTO 80
      IF (ZZ(2).NE.ZZ(1)) GOTO 80
      NI=NI+1
      GOTO 85
   80 CONTINUE
C
CC       FINAL RESULTS OUTPUT
C
   85 Q(1)=0.0
      DO 95 I=1,NP
      M2(I)=0.0
      DO 90 J=1,NY(I)
      Z=EXP(B(4)+B(1)*KD(I,J)+B(2)*KD(I,J)**2+B(3)*KD
     1(I,J)**3)
   90 M2(I)=M2(I)+(B(5)+Z)*Y(I,J)
   95 Q(1)=Q(1)+((M1(I)-M2(I))/M1(I))**2
      NII1=NI-1
      WRITE (2,250) NII1,B(1),B(2),B(3),B(4),B(5),Q(1)
      B(4)=B(4)+DLOG(T)
      B(5)=B(5)*T
   98 WRITE (2,260)
      WRITE (2,265) (K,B(K),K=1,5)
      WRITE (2,270)
      WRITE (2,275)
      DO 3 I=1,NP
      M1(I)=M1(I)*T
      M2(I)=M2(I)*T
    3 CONTINUE
      WRITE (2,280) (M1(I),M2(I),I=1,NP)
      STOP
C
CC       FORMAT STATEMENTS
C
  102 FORMAT (5F0.0)
  105 FORMAT (I3)
  110 FORMAT (12F0.0)
  120 FORMAT (140F0.0)
  200 FORMAT (' GPC CALIBRATION PROGRAM')
  210 FORMAT(//11X,'B1',8X,'B2',8X,'B3',8X,'B4',
     18X,'B5',9X,'RES SS'/)
```

```
  250 FORMAT(I3,3X,5F10.5,D15.6)
  255 FORMAT (//'INITIAL VALUES OF CALIBRATION
     1CONSTANTS :-')
  260 FORMAT (//'FINAL VALUES OF CALIBRATION
     1CONSTANTS :-')
  265 FORMAT (12X,'B',I1,'=',F11.3)
  270 FORMAT (//' COMPARISON OF MOLECULAR WEIGHTS:-')
  275 FORMAT (11X, 'MW(LS)',4X,'MW(GPC)')
  280 FORMAT (7X,F10.0,1X,F10.0)
      END
      SUBROUTINE MATINV (A)
C     MATRIX INVERSION ROUTINE
      DOUBLE RPECISION A,R,AA,AH
      DIMENSION A(25),L(5),M(5)
      R=1.0
      N=5
      NK=-N
      DO 80 K=1,N
      NK=NK+N
      L(K)=K
      M(K)=K
      KK=NK+K
      AA=A(KK)
      DO 20 J=K,N
      IJ=N*(J-1)
      DO 20 I=K,N
      II=IJ+I
      IF (DABS(AA).GE.DABS(A(II))) GOTO 20
      AA=A(II)
      L(K)=I
      M(K)=J
   20 CONTINUE
      J=L(K)
      IF (J.LE.K) GOTO 35
      KI=K-N
      DO 30 I=1,N
      KI=KI+N
      JI=KI-K+J
      AH=-A(KI)
      A(KI)=A(JI)
   30 A(JI)=AH
   35 I=M(K)
      IF (I.LE.K) GOTO 45
      JJ=N*(I-1)
      DO 40 J=1,N
      JN=NK+J
      JI=JJ+J
      AH=-A(JN)
      A(JN)=A(JI)
   40 A(JI)=AH
   45 IF (AA.NE.0.0) GOTO 50
      R=0.0
      GOTO 150
   50 DO 55 I=1,N
      IF (I.EQ.K) GOTO 55
      IK=NK+I
```

```
      A(IK)=A(IK)/(-AA)
   55 CONTINUE
      DO 65 I=1,N
      IK=NK+I
      AH=A(IK)
      IJ=I-N
      DO 65 J=1,N
      IJ=IJ+N
      IF (I.EQ.K)GOTO 65
      IF (J.EQ.K)GOTO 65
      KJ=IJ-I+K
      A(IJ)=AH*A(KJ)*A(IJ)
   65 CONTINUE
      KJ=K-N
      DO 75 J=1,N
      KJ=KJ+N
      IF (J.EQ.K) GOTO 75
      A(KJ)=A(KJ)/AA
   75 CONTINUE
      R=R*AA
      A(KK)=1.0/AA
   80 CONTINUE
      K=N
  100 K=K-1
      IF (K.LE.0.0) GOTO 150
      I=L(K)
      IF (I.LE.K) GOTO 120
      KK=N*(K-1)
      II=N*(I-1)
      DO 110 J=1,N
      JK=KK+J
      JI=II+J
      AH=A(JK)
      A(JK)=-A(JI)
      A(JI)=AH
  110 CONTINUE
  120 J=M(K)
      IF (J.LE.K) GOTO 100
      KN=K-N
      DO 130 I=1,N
      KN=KN+N
      JN=KN-K+J
      AH=A(KN)
      A(KN)=-A(JN)
      A(JN)=AH
  130 CONTINUE
      GOTO 100
  150 RETURN
      END
      FINISH
```

MWDC PROGRAM

```
      MASTER MOLWTDISTRIB
      DIMENSION H(200),AMINT(200),HNORM(200),VINT(200)
     1,S(200),S4(200)
    1 READ(1,1001) IUSE,INDIC1,INDIC2,INDIC3,INDIC4,NMAX
      IF(IUSE.EQ.0)GOTO 9999
      IF(INDIC1.EQ.6)GOTO 10
C
C     INPUT  OF  DATA  FOR PRODUCT/RECYCLE/FEED
C
      READ(1,1020) D1,D2,D3,CSPEED,CONCMA,DILSAM,PVOL,
     1TEMP,PRESS,STROKE,RANGE,RESPON,P
      READ(1,1020) (H(I), I=1,NMAX)
      GOTO20
C
C     INPUT  OF  DATA  FOR  STANDARD
C
   10 READ(1,1020) D1,D2,D3,CSPEED,CONCST,TEMP,PRESS,
     1STROKE,RANGE,RESPON,P
      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
      CALL MWDC(H,AMINT,VINT,VI,VH,NMAX,S,AVMW,AVMN,
     1SPREAD,S4)
      CALL OUTPUT(INDIC1,INDIC2,INDIC3,INDIC4,NUMBER,
     1CSPEED,RATE,TEMP,PRESS,STROKE,RANGE,RESPON,CONCST,
     1NMAX,AVMW,AVMN,SPREAD,VI,VH,H,HNORM,AMINT,VINT,
     1S,S4,P)
      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)
      CALL MWDC(H,AMINT,VINT,VI,VH,NMAX,S,AVMW,AVMN,
     1SPREAD,S4)
      CALL OUTPUT(INDIC1,INDIC2,INDIC3,INDIC4,NUMBER,
     1CSPEED,RATE,TEMP,PRESS,STROKE,RANGE,RESPON,CONCEN,
     1NMAX,AVMW,AVMN,SPREAD,VI,VH,H,HNORM,AMINT,VINT,S,
     1S4,P)
      GOTO1
 7999 WRITE(2,213)
  213 FORMAT(////  ,20X,22H READINGS OUT OF RANGE,////)
 9999 CALL DEVEND
      STOP
 1001 FORMAT(613)
 1020 FORMAT(200F0.0)
      END
```

```
      SUBROYTINE MWDC(H,AMINT,VINT,VI,VH,NMAX,S,AVMW,
     1AVMN,SPREAD,S4)
      DIMENSION H(200),VINT(200),AMINT(200),S(200),
     1S4(200)
      T=10000.0
      B1=-16.249
      S1=0.0
      S2=0.0
      S3=0.0
      B2=18.389
      B3=-13.454
      B4=16.370
      B5=23.422
      DO 25 I=1,NMAX
      VINT(I)=VI+VH*FLOAT(I-1)
      IF(VINT(I).GT.1.0)GO TO 28
      IF(VINT(I).LT.0.0)GO TO 31
      AMINT(I)=B5+EXP(B4+B1*VINT(I)+B2*(VINT(I)**2)+
     1B3*(VINT(I)**3))
      GO TO 26
   28 AMINT(I)=B5+EXP(B4+B1+B2+B3)
      GO TO 26
   31 AMINT(I)=B5+EXP(B4)
   26 S1=S1+H(I)
      S2=S2+AMINT(I)*H(I)
      S3=S3+H(I)/AMINT(I)
   25 CONTINUE
      AVMW=S2/S1
      AVMN=S1/S3
      SPREAD=AVMW/AVMN
      S4(1)=H(1)
      DO 37 I=2,NMAX
      S4(I)=S4(I-1)+H(I)
   37 CONTINUE
      DO 36 L=1,NMAX
      I=NMAX+1-L
      S(I)=(S4(I)/S1)*100.0
   36 CONTINUE
      RETURN
      END
      SUBROUTINE CONCENTRATE(CONCMA,DILSAM,SAMPA,STANDA,
     1ACTCON,R)
C     ACTCON- ACTUAL CONCENTRATION OF SAMPLE
C     CONCMA- WEIGHT OF CONCENTRATED SAMPLE
C     DILSAM- WEIGHT OF DILUTE SAMPLE
C     SAMPA- AREA OF SAMPLE
      REAL MODSAM
      MODSAM=(CONCMA*SAMPA)/DILSAM
      ACTCON=(MODSAM/STANDA)*R
      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=(57.376*CSPEED)/(600.*D3)
   VIE=(D1*RATE*600.)/CSPEED
   VFE=(D2*RATE*600.)/CSPEED
   VI=(VIE-29.375)/28.001
   VF=(VFE-29.375)/28.001
   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.NE.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
   SUBROUTINE OUTPUT(INDIC1,INDIC2,INDIC3,INDIC4,
  1NUMBER,CSPEED,RATE,TEMP,PRESS,STROKE,RANGE,RESPON,
  1CONCEN,NMAX,AVMW,AVMN,SPREAD,VI,VH,H,HNORM,AMINT,
  1VINT,S,S4,P)
   DIMENSION H(200),HNORM(200),VINT(200),X(99),
  1SX(99),AMINT(200),S(200),S4(200),HM(200),HDM(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,
  1RESPON,CONCEN
   WRITE(2,2030) AVMW,AVMN,SPREAD
   WRITE(2,2035)
   V=VI
   DO 30 I=1,NMAX
   X(I)=HNORM(I)*P
   S(I)=100.0-S(I)
```

```
      SX(I)=S(I)*P
      HM(I)=(H(I)*AMINT(I))/100.
      HDM(I)=(H(I)/AMINT(I))*100.0
      WRITE(2,2040)I,VINT(I),AMINT(I),H(I),HNORM(I),
     1HM(I),HDM(I)
   30 CONTINUE
      WRITE(2,2045)
      DO 40 I=1,NMAX
      J=NMAX+1-I
      WRITE(2,2046)J,VINT(J),AMINT(J),H(J),S4(J),S(J),
     1SX(J)
   40 CONTINUE
 2001 FORMAT(1H1,///,10X,'THE ANALYSIS FOR THE FEED
     1OF RUN NO.')
 2002 FORMAT(1H1,///,10X,'THE ANALYSIS OF PRODUCT ONE
     1FOR RUN NO.')
 2003 FORMAT(1H1,///,10X,'THE ANALYSIS OF PRODUCT TWO
     1FOR RUN NO.')
 2004 FORMAT(1H1,///,10X,'THE ANALYSIS OF RECYCLE ONE
     1FOR RUN NO.')
 2005 FORMAT(1H1,///,10X,'THE ANALYSIS OF RECYCLE TWO
     1FOR RUN NO.')
 2006 FORMAT(1H1,///,10X,'***STANDARD SOLUTION***',//,
     110X,'THE DATE OF THE ANALYSIS IS :--')
 2010 FORMAT(42X,I4,'-',I4,'-',I4)
 2011 FORMAT('+',42X,I5,'-',I5,'-',I5)
 2020 FORMAT(//,10X,'THE CONDITIONS WERE  :-',//,10X,
     1'FLOWRATE, ML/MIN=',F10.6,'  ;     PERCENTAGE
     1STROKE ',F10.2,//,10X,'TEMPERATURE DEG.C ',F10.
     12,'    ;   PRESSURE  LBS/SQ.IN.  ',F10.2,//,10X
     1,'CHART-SPEED ',F10.1,'  ;    RANGE  SETTING',
     1F10.1,//,10X,'RESPONSE   MILLI-VOLTS',F10.1,'  ;
     1  CONCENTRATION ;GMS/100 ML',F10.6)
 2030 FORMAT(//,10X,'WEIGHT AVERAGE MOL. WT. ',  F20.0,
     1/,10X,'NUMBER     AVERAGE MOL. WT.',F20.0,/,16X,
     1'MW/MN RATIO',0PF10.6)
 2035 FORMAT(//,11X,'INPUT  DATA',
     1   ///,6X,'POINT',3X,'K.D. VALUE',6X,'MOL. WT.
     1',4X,'CHROMATOGRAM',4X,'NORMALISED',3X,'Y*M/100'
     1,3X,'Y*100/M',/,54X,7HEIGHT',8X,'FRACTION')
 2040 FORMAT(I6,5X,F10.4,5X,F12.0,5X,F10.5,1X,F12.6,
     13X,F8.0,3X.F6.3)
 2045 FORMAT(//,15X,'CALCULATED DATA',///,1X,'POINT',
     15X,'K.D. VALUE',4X,'MOL. WT.',4X,'HEIGHT',4X,
     1'SUM OF Y',8X,' % ',4X,'%OF%')
 2046 FORMAT(I6,F15.6,F15.5,F12.2,F12.2,F12.2,F8.4)
      IF(INDIC1.NE.6)GO TO 13
      CALL OPENGINOGP
      CALL DEVPAP(9999.0,249.0,1)
      CALL SHIFT2(60.,60.)
   13 CALL SHIFT2(300.,0.)
      CALL AXIPOS(0,0.0,0.0,250.0,1)
      CALL AXIPOS(0,0.0,0.0,175.0,2)
      CALL AXISCA(2,10,0.,1.,1)
      CALL AXISCA(2,7,0.,7.,2)
      CALL AXIDRA(2,1,1)
```

```
CALL AXIDRA(-2,-1,2)
CALL MOVTO2(-20.0,30.0)
CALL CHAANG(90.)
CALL CHAHOL(15HNORMAL HEIGHT*.)
CALL MOVTO2(50.,-20.)
CALL CHAANG(0.0)
CALL CHAHOL(5HKDS*.)
CALL GRACUR(VINT,HNORM,NMAX)
CALL GRACUR(VINT,X,NMAX)
RETURN
END
FINISH
```

Input Variables in the Calibration Program

B1,5	input constants
M1	average molecular weight of a sample
NNI	no. of chromatograms
P	number of data points in a chromatogram
VDE	step-change in elution volume
VE	elution volume at the start of a chromatogram
VO	void volume
VT	total liquid volume
Y	height of chromatogram

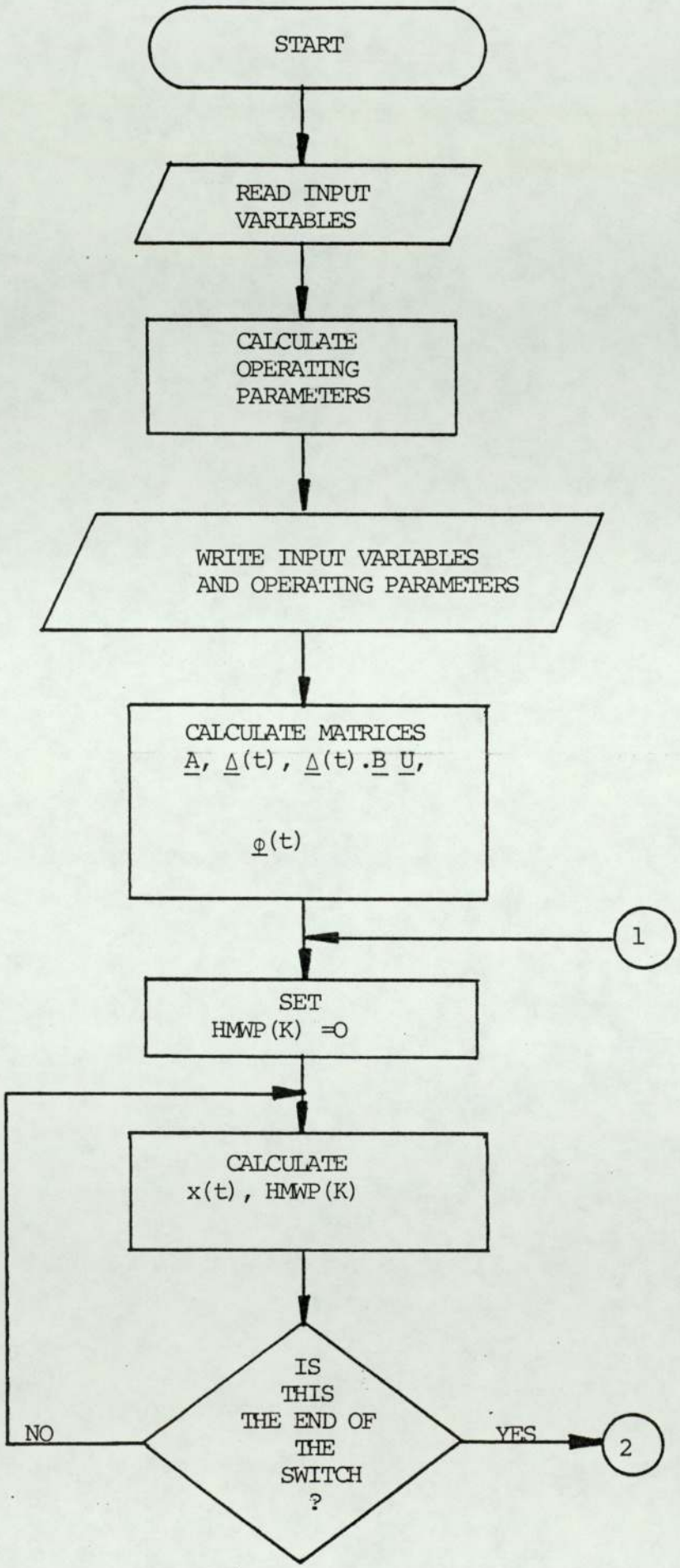
Input Variables in Program MWDC

CONCMA	mass of concentrated sample
CONCST	concentration 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 chromatogram
INDIC1,4	numbering integers
NMAX	no. of sample points
PRESS	column pressure
RANGE	detector range
RESPON	detector response
STROKE	pump stroke
TEMP	column temperature

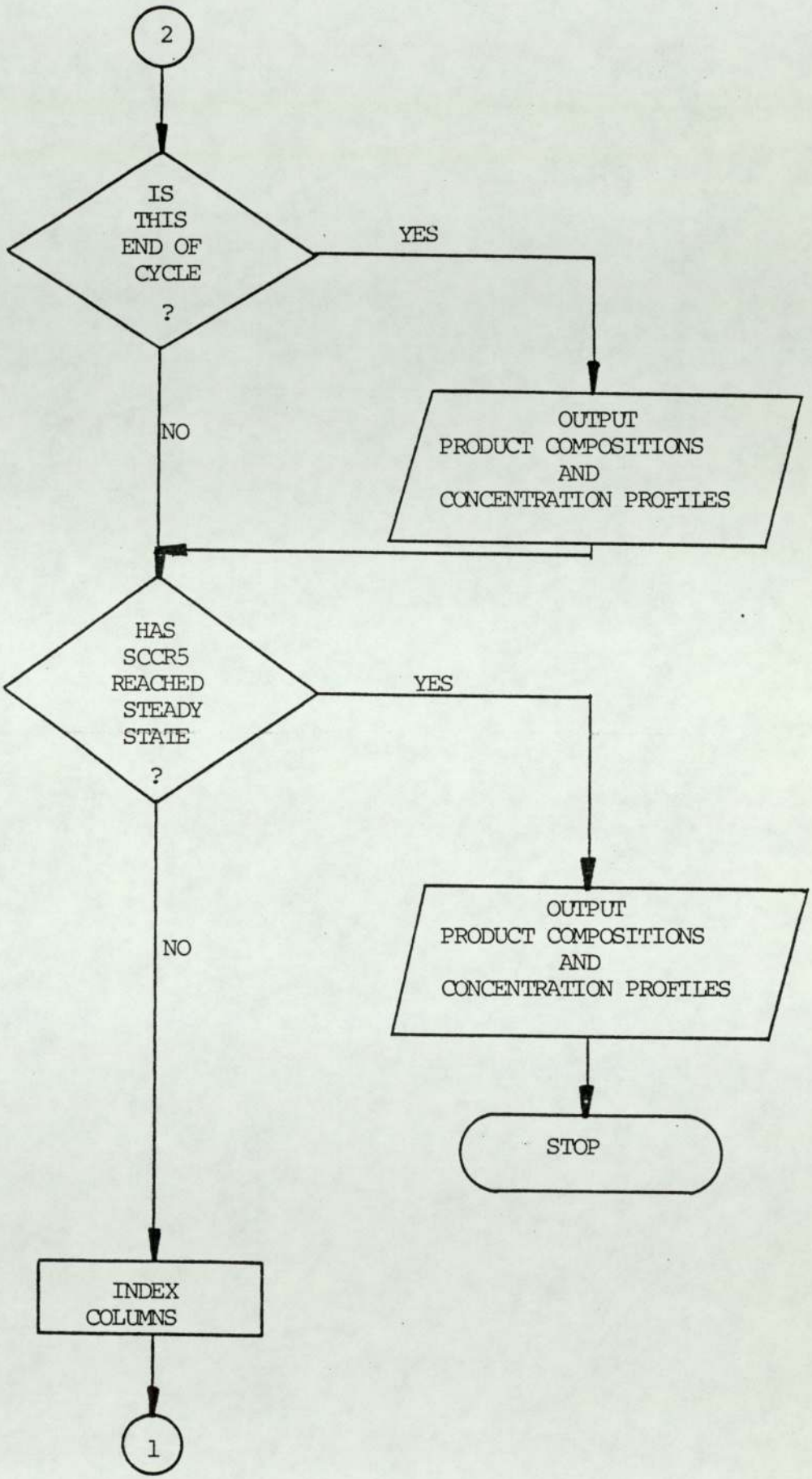
APPENDIX A2

SIMULATION PROGRAM

Flow diagram for the SCCRS simulation program PROFILE



Flow diagram (Cont..)



SIMULATION PROGRAM

```
      PROGRAM PROFILE(GD2,OUTPUT,TAPE1=GD2,TAPE2=OUTPUT)
      DIMENSION XFEED(9),BU(96,9),QVPREFD(9),QVPOSFD(9)
      DIMENSION DELTFRC(96,10,9),PHIFRAC(96,10,9),
     1DELTABU(96,9),HMWP(9),XO(96,9)
      DIMENSION SUMXO(96),ALPHA(9),BETA(9),GAMA(9),
     1OUTCON(20,9)
      DIMENSION AFRAC(96,2,9),XT(96,9),PHIXO(96,9),XP
     1(10,9),RKD(9)
      DIMENSION COLMAS(20,20),SUMPURG(20),TOTCOL(20),
     1AVCNC(20),TOTCON(20)
      EQUIVALENCE (DELTFRC(96,10,9),PHIFRAC(96,10,9))
      AAA=0.5
      IS=1
      VO=537.0
      VI=605.0
      VO=515.0
      V1=624.0
      INDEXNO=0
      NUMSWIT=0
      AINPUT=0.0
      READ(1,8001)TEMP
      READ(1,8000)NUMTSFR,NUMTSPG
      READ(1,8000)KOMPON,NFDPLT
      READ(1,8000)ITIME,LIMIT
      READ(1,8000)JMAX,NOFCOLS
      READ(1,8001)DELTAT
      READ(1,8002)(XFEED(K),K=1,KOMPON)
      READ(1,8003)QELUENT,QFEED
      READ(1,8004)DAY,DATE,YEAR
      READ(1,8002)(RKD(K),K=1,KOMPON)
      DO 123 K=1,KOMPON
      DO 123 I=1,NUMTSFR
      XO(I,K)=0.0
  123 CONTINUE
      NUMPLS1=NUMTSFR+1
      NCOLM1=NOFCOLS-1
C     CALCULATE CUT POINTS
      CUTPRE=((QELUENT*ITIME)-VO)/VI
      CUTPOS=((QELUENT+QFEED)*FLOAT(ITIME)-VO)/VI
      ITIMSTP=ITIME/IFIX(DELTAT)
      VO=VO/FLOAT(NUMTSPG)
      VI=VI/FLOAT(NUMTSPG)
      WRITE(2,9200)DAY,DATE,YEAR
      WRITE(2,9201)QELUENT,QFEED
      WRITE(2,9202)CUTPRE,CUTPOS
      WRITE(2,9203)ITIME,NUMTSPG,NUMTSFR, NFDPLT,DELTAT
      WRITE(2,9204)
      DO 201 K=1,KOMPON
  201 WRITE(2,9205)K,RKD(K)
  500 DO 515 KR=1,KOMPON
      HMWP(KR)=0.0
  515 CONTIMUE
      DO 510 ISTEP=1,ITIMSTP
C     CALCULATE PHI(T)*XO
```

```
      NTIMES=JMAX
      DO 501 IR=1,NUMTSFR
      II=NUMTSFR+1-IR
      L=NUMTSFR-IR+1
      DO 112 K=1,KOMPON
      DO 112 I=1,NUMTSFR
      PHIXO(I,K)=0.0
      DELTABU(I,K)=0.0
      DO 112 JJ=1,JMAX
      DELTFRC(I,JJ,K)=0.0
      PHIFRAC(I,JJ,K)=0.0
  112 CONTINUE
      SUMXO(L)=0.0
      DO 117 KJ=1,KOMPON
      SUMXO(L)=SUMXO(L)+XO(L,KJ)
  117 CONTINUE
      DO 114 K=1,KOMPON
      IF(K.EQ.1)GO TO 19
      ALPHA(K)=36.5
      BETA(K)=905.0
      GAMA(K)=827.0
      GO TO 17
   19 ALPHA(K)=51.0
      BETA(K)=1258.0
      GAMA(K)=1150.0
   17 IF (SUMXO(L).GE.0.0066)GO TO 67
      VR(K)=GAMA(K)
      GO TO 28
   67 VR(K)=ALPHA(K)*ALOG10(SUMXO(L))+BETA(K)
   28 IF(TEMP.EQ.40.0)VR(K)=VR(K)*(1.0-0.015)
      IF(TEMP.EQ.60.0)VR(K)=VR(K)*(1.0-0.025)
      RKD(K)=(VR(K)-V0)/V1
  114 CONTINUE
C     SET THE INITIAL ARRAYS
C     CALCULATE CONSTANTS FROM INPUT VARIABLES
      DO 351 K=1,KOMPON
      QVPREFD(K)=QELUENT/(V0+RKD(K)*VI)
      QVPOSFD(K)=QELUENT+QFEED)/(V0+RKD(K)*VI)
  351 CONTINUE
C     SET ARRAYS B*U AND A
      DO 310 K=1,KOMPON
      DO 310 I=1,NUMTSFR
      BU(I,K)=0.0
      IF(I.EQ.NFDPLT)BU(I,K)=XFEED(K)*QFEED/(V0+RKD(K)*VI)
  310 CONTINUE
      DO 311 K=1,KOMPON
      DO 311 I=1,NUMTSFR
      DO 311 J=1,2
      IF((I.EQ.1).AND.(J.EQ.1))GO TO 319
      IF(I.GE.NFDPLT)GO TO318
      AFRAC(I,J,K)=QVPREFD(K)
      IF(J.EQ.2)AFRAC(I,J,K)=-1.0*AFRAC(I,J,K)
      GO TO 311
  318 AFRAC(I,J,K)=QVPOSFD(K)
      IF((J.EQ.1).AND.(I.EQ.NFDPLT))AFRAC(I,J,K)=QVPREFD(K)
      IF(J.EQ.2)AFRAC(I,J,K)=-1.0*AFRAC(I,J,K)
      GO TO 311
```

```
  319 AFRAC(I,J,K)=0.0
  311 CONTINUE
C       CALCULATE DELTA(T)
        CALL FRADELT(AFRAC,DELTAT,NUMTSFR,JMAX,DELTFRC,KOMPON)
C       CALCULATE DELTA(T)*B*U
        DO 420 K=1,KOMPON
        LTIMES=JMAX
        DO 420I=1,NUMTSFR
        IK=NUMTSFR-I+1
        LK=NUMTSFR-I+1
        IF(LK-JMAX)421,422,422
  421 LTIMES=LTIMES-1
  422 DO 430 N=1,LTIMES
        M=JMAX-N+1
        DELTABU(LK,K)=DELTABU(LK,K)+DELTFRC(LK,M,K)*BU(IK,K)
        IK=IK-1
  430 CONTINUE
  420 CONTINUE
C       CALCULATE PHI(T)
        DO 439 K=1,KOMPON
        CALL FRAXMAT(DELTFRC,AFRAC,NUMTSFR,JMAX,K)
  439 CONTINUE
        DO 440 K=1,KOMPON
        DO 440 I=1,NUMTSFR
        PHIFRAC(I,JMAX,K)=PHIFRAC(I,JMAX,K)+1.0
  440 CONTINUE
C       CALCULATE X(T)
        IF(L-JMAX)502,503,503
  502 NTIMES=NTIMES-1
  503 DO 501 KR=1,KOMPON
        DO 504 N=1,NTIMES
        M=JMAX-N+1
        PHIXO(L,KR)=PHIFRAC(L,M,KR)*XO(II,KR)+PHIXO(L,KR)
        II=II-1
  504 CONTINUE
C       CALCULATE X(T)=PHI(T)*XO+DELTA(T)*B*U
        XT(L,KR)=PHIXO(L,KR)+DELTABU(L,KR)
        PHIXO(L,KR)=0.0
        IF(KR.EQ.KOMPON)GO TO 501
        II=NUMTSFR-IR+1
  501 CONTINUE
C       CALCULATEHMWP
        DO 535KR=1,KOMPON
        HMWP(KR)=HMWP(KR)+XT(NUMTSFR,KR)*0.001*DELTAT*
       1(QELUENT+QFEED)
C       IS THIS END OF SWITCH
        IF((ISTEP.EQ.ITIMSTP).AND.(KR.EQ.KOMPON))GO TO 600
  111 DO 535 N=1,NUMTSFR
        XO(N,KR)=XT(N,KR)
  535 CONTINUE
  510 CONTINUE
  600 NUMSWIT=NUMSWIT+1
        INDEXNO=INDEXNO+1
        IF(INDEXNO.EQ.NOFCOLS)GO TO 610
C       CHECK FOR STEADY STATE BY INPUT-OUTPUT LT. ERROR
  609 IF(NUMSWIT.EQ.1)GO TO 750
        AOUTPUT= 0.0
```

```
      DO 710 K=1,KOMPON
      SUMPURG(K)=0.0
      DO 710 I=1,NUMTSPG
      SUMPURG(K)=SUMPURG(K)+XP(I,K)*(VO+VI*RDK(K))*0.001
  710 CONTINUE
      DO 751 K=1,KOMPON
      AOUTPUT=AOUTPUT+SUMPURG(K)+HMWP(K)
  751 CONTINUE
      AR=AAA*AINPUT
      IF(AOUTPUT.GT.AR)GO TO720
      GO TO 611
  720 IF(AAA.EQ.0.5)GO TO 721
      IF(AAA.EQ.0.75)GO TO 722
      IF(AAA.EQ.0.9)GO TO 723
      IF(AAA.EQ.0.95)GO TO724
      IF(AAA.EQ.0.99) GO TO 725
  721 WRITE(2,9721)AAA,NUMSWIT
      AAA=0.75
      GO TO 611
  722 WRITE(2,9721)AAA,NUMSWIT
      AAA=0.9
      GO TO 611
  723 WRITE(2,9721)AAA,NUMSWIT
      AAA=0.95
      GO TO 611
  724 WRITE(2,9721)AAA,NUMSWIT
      AAA=0.99
      GO TO 611
  725 WRITE(2,9721)AAA,NUMSWIT
      IS=0
      GO TO 800
  750 DO 756 K=1,KOMPON
      AINPUT=XFEED(K)*QFEED*FLOAT(ITIME)*0.001+AINPUT
  756 CONTINUE
      WRITE(2,9756)AINPUT
      DO 757 K=1,KOMPON
      XFO=XFEED(K)*0.001*FLOAT(NOFCOLS)*FLOAT(ITIME)*QFEED
      WRITE(2,9757)K,XFEED(K),XFO
  757 CONTINUE
C     INDEX THE COLUMNS
  611 DO 623 K=1,KOMPON
      DO 620 I=1,NUMTSPG
      XP(I,K)=XT(I,K)
  620 CONTINUE
      LUX=NUMTSFR-NUMTSPG
      DO 621 I=1, LUX
      IR=I+NUMTSPG
      XO(I,K)=XT(IR,K)
  621 CONTINUE
      NET=NUMTSFR-NUMTSPG+1
      DO 622 I=NET,NUMTSFR
      XO(I,K)=0.0
  622 CONTINUE
  623 CONTINUE
      GO TO 1000
C     END OF CYCLE OUTPUT RESULTS
```

```
  610 INDEXNO=0
      DO 612 K=1,KOMPON
      SUMPURG(K)=0.0
      DO 612 I=1,NUMTSPG
      SUMPURG(K)=SUMPURG(K)+XP(I,K)*(VO+VI*RDK(K))*0.001
  612 CONTINUE
      DO 629 I=1,NCOLM1
      DO 629 K=1,KOMPON
      COLNAS(I,K)=0.0
  629 CONTINUE
      III=1
      DO 630 I=1,NCOLM1
      DO 631 K=1,KOMPON
      INK=NUMTSPG+III-1
      OUTCON(I,K)=XT(INK,K)
      DO 631 II=III,INK
      COLMAS(I,K)=COLMAS(I,K)+XT(II,K)*(VO+VI*RDK(K))*0.001
  631 CONTINUE
      III=III+NUMTSPG
  630 CONTINUE
      NN=NUMSWIT/NOFCOLS
      WRITE(2,9631)NN,(I,I=1,NCOLM1)
      DO 632 K=1,KOMPON
      DO 640 I=1,NCOLM1
      TOTCOL(I)=0.0
      TOTCON(I)=0.0
      DO 641 KK=1,KOMPON
      TOTCOL(I)=TOTCOL(I)+COLMAS(I,KK)
      TOTCON(I)=TOTCON(I)+OUTCON(I,KK)
  641 CONTINUE
  640 CONTINUE
      WRITE(2,9632)K,(COLMAS(I,K),I=1,NCOLM1)
      WRITE(2,9632)K,(OUTCON(I,K),I=1,NCOLM1)
  632 CONTINUE
      DO 642 I=1,NCOLM1
      AVCONC(I)=(TOTCOL(I))/((VO+VI)*0.001*FLOAT(NUMTSPG))
  642 CONTINUE
      WRITE(2,9843)
      WRITE(2,9844)(TOTCOL(I),I=1,NCOLM1
      WRITE(2,9844)(TOTKON(I),I=1,NCOLM1)
      WRITE(2,9845)
      WRITE(2,9846)(AVCONC(I),I=1,NVOLM1)
      WRITE(2,9847)
      DO 643 K=1,KOMPON
      WRITE(2,9848)K,SUMPURG(K),HMWP(K)
  643 CONTINUE
      GO TO 609
C     CHECK FOR PSEUDO-STEADY STATE
 1000 IF(IS.EQ.0)GO TO 800
      IF(NUMSWIT.EQ.LIMIT)GO TO 1099
      GO TO 500
 1099 WRITE(2,9999)
  800 CONTINUE
C     OUTPUT RESULTS
      WRITE(2,9801)NUMSWIT
      WRITE(2,9802)(I,I=1,NCOLM1)
```

```
      DO 829 I=1,NOFCOLS
      DO 829 K=1,KOMPON
      COLMAS(I,K)=0.0
  829 CONTINUE
      III=1
      DO 830 I=1,NCOLM1
      DO 831 K=1,KOMPON
      INK=NUMTSPG+III-1
      OUTCON(I,K)=XT(INK,K)
      DO 831 II=III,INK
      COLMAS(I,K)=COLMAS(I,K)+XT(II,K)*VO+VI*RDK(K))*0.001
  831 CONTINUE
      III=III+NUMTSPG
  830 CONTINUE
C     OUTPUT PSEUDO-STEADY STATE PROFILE
      DO 832 K=1,KPMPON
      WRITE(2,9632)K,(COLMAS(I,K),I=1,NCOLM1)
      WRITE(2,9632)K,(OUTCON(I,K),I=1,NCOLM1)
  832 CONTINUE
      DO 840 I=1,NCOLM1
      TOTCOL(I)=0.0
      TOTCON(I)=0.0
      DO 841 K=1,KOMPON
      TOTCOL(I)=TOTCOL(I)+COLMAS(I,K)
      TOTCON(I)=TOTCON(I)+OUTCON(I,K)
  841 CONTINUE
  840 CONTINUE
C     AVERAGE COLUMN CONCENTRATIONS
      DO 842 I=1,NCOLM1
      AVCONC(I)=(TOTCOL(I))/((VO+VI)*0.001*FLOAT(NUMTSPG))
  842 CONTINUE
      WRITE(2,9843)
      WRITE(2,9844)(TOTCOL(I),I=1,NCOLM1)
      WRITE(2,9844)(TOTCON(I),I=1,NCOLM1)
      WRITE(2,9845)
      WRITE(2,9846)AVCONC(I),I=1,NVOLM1)
      WRITE(2,9847)
      DO 848 K=1,KOMPON
      WRITE(2,9848)K,SUMPURG(K),HMWP(K)
  848 CONTINUE
      STOP
C     FORMAT STATEMENTS
 9200 FORMAT(////,5X,'MODEL FOR S.C.C.R.S.',/,
     15X,'INPUT INFORMATION :-',/,
     125X,'COMPUTER RUN NUMBER :-',3F10.0,///)
 9201 FORMAT(19X,'THE FLOWRATES WERE:   ELUENT',F15.5,3X,
     1'ML S-1',/,44X,'FEED ',F15.5,3X,'ML S-1')
 9202 FORMAT(19X,'THE THEORETICAL CUT POSITIONS WERE:',/,
     144X,'PRE-FEED',F12.5,/,
     144X,'POST-FEED',F11.5)
 9203 FORMAT(20X,'SWITCH-TIME                    ',5X,I8,8X,
     1'S',/, 16X,'NO. OF TANKS PER COLUMN',20X,I5,/,
     1 16X,'TOTAL NO. OF TANKS IN FRACTIONATING SECTION
     1 ',I5,/,  16X,'POSITION OF FEED TANK,TANK NO:',13X,
     1I5,/,16X,'STEP TIME INCREMENT',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 DEXRAN FOR
     1CYCLE',I4,2X,'  WAS:',//,15X,'COLUMN    COLUMN
     1COLUMN    COLUMN    COLUMN',7X,'COLUMN  COLUMN
     1COLUMN    COLUMN',//,
     19X,9I10,//,2X,'COMPONENT')
 9632 FORMAT(/,I7,8X,1P9E10.3)
 9721 FORMAT(////,5X,'THE OUTPUT MASS HAS REACHED ',2X,
     1F5.3,2X,' AS FRACTION OF THE INPUT MASS DURING
     1SWITCH NUMBER ',I5)
 9801 FORMAT(1H1,//,5X,'****  STEADY-STATE  ****',/,
     15X,'STEADY-STATE HAS BEEN REACHED AFTER ',I5,'
     1SWITCHES',///,7X,'THE DISTRIBUTION OF DEXTRAN
     1IN THE S.C.C.R.5. MACHINE IS',//,15X,'COLUMN
     1COLUMN    COLUMN    COLUMN    COLUMN    COLUMN
     1    COLUMN    COLUMN    COLUMN')
 9802  FORMAT(1H0,9X,9I10,/,2X,'COMPONENT',/)
 9843 FORMAT(//,4X,'TOTAL',/5X,'MASS',/,5X,'GMS.')
 9844 FORMAT(12X,1P9R10.3)
 9845 FORMAT(/,4X,'AVERAGE',/,4X,/,'COLUMN',/,5X,'CONC.
     1',/,7X,'-1,',/,4X,'G.L')
 9846 FORMAT(12X,1P9E10.3)
 9847 FORMAT(///,5X,'THE MASS OF DEXTRAN ELUTED FROM THE
     1PURGE',/,5X,'AND THE HIGH MOLECULAR WEIGHT OUTLET
     1PORTS',/,5X,'DURING THE FINAL SWITCH AS FOLLOWS:',
     1//,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
     1BEEN REACHED****',/, 10X,'THE NUMBER OF SWITCHES
     1HAS REACHED THE SET MAXIMUM')
 9915 FORMAT(1H0,5X,'THE MASS OF SOLUTE REMAINING IN THE
     1PURGE',1X,'SECTION IS  ',1PE10.3,' GMS.')
 9756 FORMAT(///,5X,'THE TOTAL INPUT PER SWITCH IS  ',1PE10
     1.3,'GMS.',//,5X,'THE INPUT OF EACH COMPONENT PER
     1CYCLE',/,5X,'IS AS FOLLOWS',/
     1,5X,'COMPONENT',5X,'CONCENTRATION',5X,'MASS',/
     1,32X,'-1?,/,28X,'GMS.L.',8X,'GMS.')
 9757 FORMAT(9X,I3,8X,1PE10.3)
 8000 FORMAT(2I10)
 8001 FORMAT(F10.2)
 8002 FORMAT(2F7.3)
 8003 FORMAT(2F10.5)
 8004 FORMAT(3F10.1)
      END
C     SUBROUTINES FOR THE CALCULATION OF
C           DELTA(T) IN THE STATE EQUATION
      SUBROUTINE FRADELT(A,DT,IMAX.JMAX,DELTA,KOMPON)
      DIMENSION A(96,2,9(,AMMD(96,10,9),DELTA(96,10,9)
      DO 43 K=1,KOMPON
      DO 22 I=1,IMAX
      DO 22 J=i,JMAX
      AMMD(I,J,K)=0.0
      IF(J.EQ.JMAX)AMMD(I,J,K)=1.0
   22 CONTINUE
```

```
      DO 30 N=1,JMAX
      IF(N.EQ.1)GO TO 32
      CALL FRAXMAT(AMMD,A,IMAX,JMAX,K)
   32 DO 33 I=1,IMAX
      DO 33 J=1,JMAX
      AMMD(I,J,K)=AMMD(I,J,K)*DT/FLOAT(N)
      DELTA(I,J,K)=DELTA(I,J,K)+AMMD(I,J,K)
   33 CONTINUE
   30 CONTINUE
      DO 42 I=1,IMAX
      L=JMAX-I
      IF(L)42,42,40
   40 CONTINUE
      DO 41 J=1,L
      DELTA(I,J,K)=0.0
   41 CONTINUE
   42 CONTINUE
   43 CONTINUE
      RETURN
      END
C     SUBROUTINE FOR THE MULTIPLICATION OF MATRICIES
      SUBROUTINE FRAXMAT(AA,A,IMAX,JMAX,K)
      DIMENSION AA(96,2,9),A(96,2,9),AAOLD(96,10,9)
      DO 1 I=1,IMAX
      DO 1 J=1,JMAX
      AAOLD(I,J,K)=AA(I,J,K)
    1 CONTINUE
      NT=JMAX
      DO 35 I=1,IMAX
      II=IMAX+1-I
      IJ=II
      IR=II-JMAX
      IF(IR)31,32,32
   31 NT=NT-1
   32 DO 35 N=1,NT
      NN=JMAX+1-N
      IF(NN.EQ.JMAX)GO TO 34
      NN1=NN+1
      IJ1=IJ+1
      AA(II,NN,K)=AAOLD(II,NN,K)*A(IJ,2,K)
     1+AAOLD(II,NN1,1,K)
      GO TO 33
   34 AA(II,NN,K)=AA(II,NN,K)*A(IJ,2,K)
   33 CONTINUE
      IJ=IJ-1
   35 CONTINUE
      RETURN
      END
```

Input Variables in Program PROFILE

ALPHA	slope of equation (9.9)
BETA	intersection of equation (9.9)
DATE	month of analysis
DAY	day of analysis
DELTAT	time increment
GAMMA	elution volume of components 1 and 2
ITIME	switch time
JMAX	terms in Δ matrix
KOMPON	no. of components in feed
LIMIT	maximum no. of switches
NFDPLT	feed point stage
NUMTSFR	total no. of stages
NUMTSPG	no. of stages per column
QELUENT	eluent flowrate
QFEED	feed flowrate
RKD	K_d of components
TEMP	operating temperature
VI	average liquid volume
Vo	average void volume
VO	void volume for the column that K_d was measured
Vl	liquid volume for the column that K_d was measured
YEAR	year of analysis

APPENDIX A3

CALCULATION OF THEORETICAL CUT-POSITIONS

Calculation of Theoretical Cut-Positions

e.g.

Conditions for run 3.1

Eluent rate, Q_e = 95 cm^3/min

Feed rate, Q_f = 40 cm^3/min

Purge, Q_p = 300 cm^3/min

Switch time, s = 7.5 min

V_o for SCCR5 = 537 cm^3

V_i for SCCR5 = 505 cm^3

(a) Pre-Feed Cut-Position

From equation (2.7)

$$K_d = \frac{V_R - V_o}{V_i} = \frac{(Q_e \times s) - V_o}{V_i}$$

$$= \frac{(95 \times 7.5) - 537}{605} = \underline{0.290}$$

(b) Post-Feed Cut-Position

$$= \frac{(95+50) \times 7.5 - 537}{605} = \underline{0.786}$$

(c) Purge Section Cut-Position

$$= \frac{(300 \times 7.5) - 537}{605} = \underline{2.831}$$

NOMENCLATURE

a	weight fraction of solute rejected
A	eddy diffusion term
$\underline{A}$	distribution matrix in the state equation
$b_{1,5}$	constants in the calibration program
B	longitudinal diffusion term
$\underline{B}$	feed flowrate vectors
c	stationary phase concentration
c_B	concentration in the bulk of the solution
c_f	final concentration of the filtrate
c_g	concentration in the gel formed on the membrane
c_m	concentration of the mobile phase
c_o	initial concentration
c_p	concentration in the pores of the membrane
c_s	concentration of the stationary phase
c_t	final concentration of the retentate
C'_m	resistance to mass transfer in the mobile phase
C'_s	resistance to mass transfer in the stationary phase
d	diameter
d_p	particle diameter
$\bar{D}$	polydispersity
D_m	solute diffusion coefficient in the mobile phase
D_s	diffusion coefficient
E	eluent rate
F	feed rate
$F(V_R)$	experimental chromatogram
$g_{1,2}$	geometric factors

$G(V_R-y)$	instrumental spreading function
h	reduced plate height
h_i	chromatogram heights
h_i'	normalised chromatogram heights
H	theoretical plate height
HETP	height equivalent to theoretical plate
HMWP	high molecular weight product
J_1	permeate flux
J_2	solute flux
K_d	distribution coefficient
K_m	mass transfer coefficient
K	partition coefficient
K'	capacity factor
ℓ	column length
L	column length
L'	effective flowrates
L_1	eluent rate
L_2	feed rate
L_3	eleunt plus feed rate
LMWP	low molecular weight product
$\bar{M}_N$	number average molecular weight
$\bar{M}_W$	weight average molecular weight
n_i	number of molecules
N	number of plates
P_B	pressure in the bulk of the solution
P_c	permeation constant
P_f	pressure in the filtrate side
Pu	purge rate
P_1	product 1 (HMWP) rate
P_2	product 2 (LMWP) rate

Q	flowrate
Q_e	eluent rate
Q_f	feed rate
r'	rate of movement
R	retention parameter
R_c	column radius
Re	Reynolds number
R_s	resolution
s	switch time
Sc	Schmidt no.
t	time
t_R	elution time
t_o	void time
u	reduced velocity
U	feed concentration
v	linear velocity
v'	average velocity
V_{GM}	volume of gel matrix
V_f	final volume of filtrate
V_i	pore volume
V_N	net retention volume
V_o	void volume
V_o	initial volume
V_p	volume of the packing
V_R	retentate volume and elution volume
V_s	volume of the stationary phase
V_t	final retentate volume
w	width of a chromatogram
W	weight fraction

$W(y)$	true chromatogram
x	mobile phase concentration
z	length along the column
α	separation factor
α	slope of equation (9.9)
β	intersection of equation (9.9)
γ'	tortuosity factor
$\dot{\gamma}$	shear rate
δ	thickness
Δ	difference
$\eta_{1,2}$	GPC efficiencies
$\eta_{3,4}$	UF efficiencies
λ_e	eddy diffusion constant
π_B	osmotic pressure of the bulk solution
π_f	osmotic pressure of the filtrate
σ	rejection
σ_{ret}	rejection derived from retentate
σ_{uf}	rejection derived from filtrate
σ_z	length based second moment
ϕ	no. of peaks resolved in a column

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Note

Suitability of TSK-gel Toyopearl packing for the gel permeation chromatographic analysis of dextran

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The molecular weight characterisation of dextran was one of the first applications of gel permeation chromatography (GPC). Several GPC packings suitable for the analysis of molecular weight distribution (MWD) of dextran are available[1]. In our laboratory several of these packings have been tried with dextran and the results of these trials were reported[2,3].

The TSK-gel PW-type (AMTO, Amsterdam, The Netherlands) has been used successfully for dextran analysis by Fisons Pharmaceutical[4] for fifteen months. This type of packing shows high efficiency (*ca.* 13,000 plates/m), it has been used with aqueous solutions with no significant loss in efficiency during this period. However, analysis times are typically around 40 min.

Recently samples of the TSK-gel Toyopearl (Toyo Soda, Tokyo, Japan) packing material, very similar to the TSK-gel PW type but of larger size, were obtained to be tried as an analytical packing for dextran.

PROPERTIES OF THE PACKING

TSK-gel Toyopearl[5], which is produced by the polymerisation of hydrophilic vinyl monomers, is a completely new packing material for GPC. It is a semi-rigid, mechanically and chemically stable, spherical gel and it can be used under low pressure drop (less than 10 kg/cm^2) conditions.

Toyopearl is produced in seven types[6], each with a different pore size distribution, covering a wide range of molecular weights. Each type is produced in three sizes, ranging from 20 to 100 μm.

The samples of packing examined were: (1) Toyopearl HW 55S, having, according to the manufacturer[6], a fractionation range for dextran from $1 \cdot 10^3$ to $2 \cdot 10^5$ daltons. The size of the particles was between 20–40 μm, and (2) Toyopearl HW 65S, having a reported fractionating range from $1 \cdot 10^4$ to $1 \cdot 10^6$ daltons and the same particle size as the HW 55S packing.

EXPERIMENTAL

A relatively simple chromatography system was used, consisting of a pump,

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sample introduction device, column, detector and recorder. All the results were manually measured and calculated from the chromatographs.

The eluent was pumped with a positive displacement pump (MPL, Metering Pumps, London, Great Britain). The samples were applied with a 100-μl syringe (Field Instruments). The eluate was monitored with a Model 1107 LJ differential refractometer (Laboratory Data Control) and the chromatograph displayed on a Type 2 flat-bed recorder (Smiths, Venture Servoscribe).

The packing technique used two glass columns of 1 m × 4 mm I.D. each, (Corning, Corning, NY, U.S.A.) connected directly together with a funnel on the top of the upper column. The columns were filled with water to remove the air and a slurry of the packing was poured into the funnel; it was left for *ca.* 12 h to settle into the columns. The eluent was then pumped through the columns to compress the packing into the lower column. The eluent rate was controlled so that a pressure drop of around 7 kg/cm^2 was generated.

The column filled with the packing was then connected to the chromatographic system. Throughout the work, the eluent used was a solution (0.02%, w/v) of potassium hydrogen phthalate in distilled water.

The efficiency of each column was measured with glucose as the solute by the equation: $N = 8 \cdot (t_R/W_{h/e})^2$ where N is the number of theoretical plates, t_R is the peak retention time and $W_{h/e}$ is the peak width at the peak height (h) divided by e, the base of the natural logarithm.

The dextran used had a wide molecular weight range of between $2 \cdot 10^2$ and $2 \cdot 10^6$ daltons, to test the packing's performance over this range which is of interest to our work. This dextran had a weight average molecular weight, $\bar{M}_w$, of 70,000 daltons (batch 161 D40, Fisons Pharmaceuticals (Holmes Chapel, Great Britain). Dextran T2000 (Pharmacia, Uppsala, Sweden) and glucose were used to determine the void volume, V_0, and pore volume, V_i, respectively. When t_0 and t_i are the times taken for totally excluded and totally included molecules to be eluted from the column, respectively, and F is the flow-rate of the mobile phase, then $V_0 = t_0 \cdot F$ and $V_i = t_i \cdot F$.

RESULTS

HW 55S

This packing has a satisfactory efficiency of *ca.* 5500 plates/m for glucose and a good resolution for the dextran used (see Fig. 1).

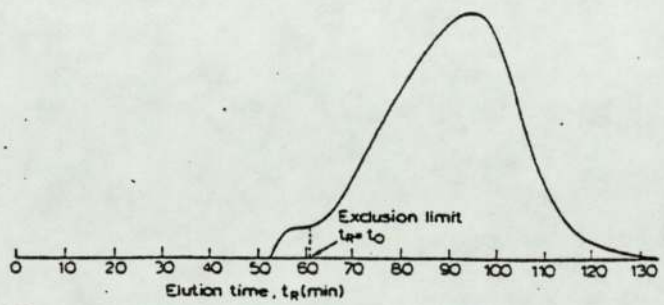

Fig. 1. Gel permeation chromatogram of Dextran B161 D40 on TSK-gel Toyopearl HW 55S (20–40 μm). Eluent 0.02% potassium hydrogen phthalate aqueous solution. Column: 100 cm × 4 mm I.D.

The disadvantages are: (a) it has a low exclusion limit, although higher than that reported by the manufacturer, and (b) the analysis time is too long (*ca.* 2½ h) (Fig. 1).

HW 65S

The HW 65S packing, although it has a high exclusion limit, gave poor resolution. It does not separate the chromatographic peaks of dextran ($\bar{M}_w \approx 70{,}000$ daltons) and of glucose (180 daltons) (see Fig. 2). It also has a low efficiency of approximately 575 plates/m for glucose.

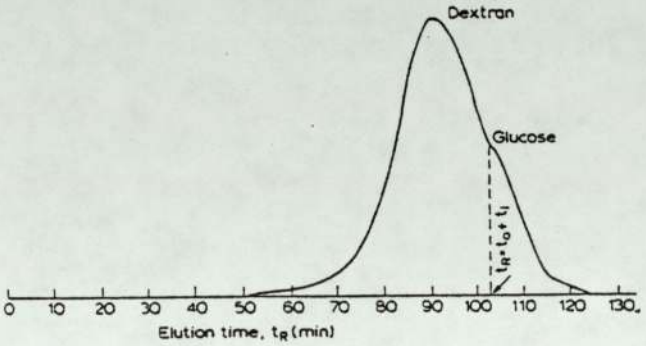

Fig. 2. Gel permeation chromatogram of Dextran B161 D40 and glucose on TSK-gel Toyopearl HW 65S (20–40 μm). Eluent 0.02% potassium hydrogen phthalate aqueous solution. Column: 100 cm × 4 mm I.D.

Mixtures of the HW 55S and HW 65S Packings

Since both packings have advantages and disadvantages we thought it worthwhile to investigate the effect of combining the high efficiency and good resolution of the HW 55S packing with the high exclusion limit of the 65S packing.

The following mixture combinations have been tried:

Mixture A: 26 cm^3 of HW 55S and 2 cm^3 of HW 65S.

Mixture B: 23.4 cm^3 of HW 55S and 10.6 cm^3 of HW 65S.

Mixture C: 24.3 cm^3 of HW 55S and 24.7 cm^3 of HW 65S.

Mixture D: 19.65 cm^3 of HW 55S and 25.3 cm^3 of HW 65S.

A shoulder appeared on the chromatographic peaks of dextran 70,000 for the first three mixtures. This may be due to the low exclusion limit, but as the percentage of HW 65S packing in the mixture was increased, the shoulder increased instead of decreasing, until it became a second peak (Fig. 3).

Mixture D has a suitable exclusion limit since no shoulder or exclusion peak appeared on the chromatographic peak of the dextran (Fig. 4). It also has good resolution for the dextran and its efficiency is satisfactory, *ca.* 1500 plates/m. The only disadvantage is the long analysis time ($\approx 2\frac{1}{2}$ h).

CONCLUSIONS

Although TSK-gel Toyopearl is mainly a laboratory preparative packing it is also suitable for analytical work on dextrans as it has a satisfactory efficiency, good resolution, and a suitable fractionation range.

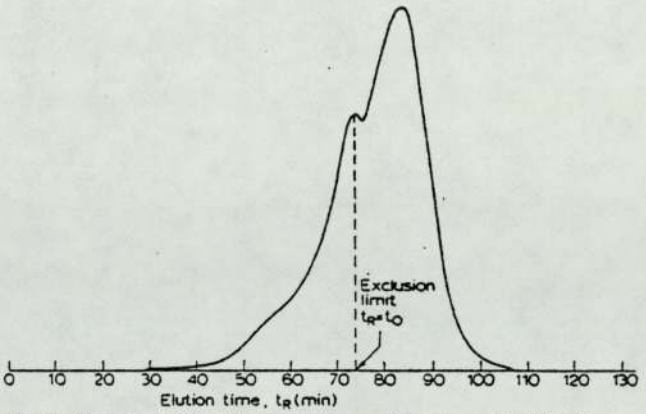

Fig. 3. Gel permeation chromatogram of Dextran B161 D40 on packing mixture C. Eluent 0.02% potassium hydrogen phthalate aqueous solution. Column: 100 cm × 4 mm I.D.

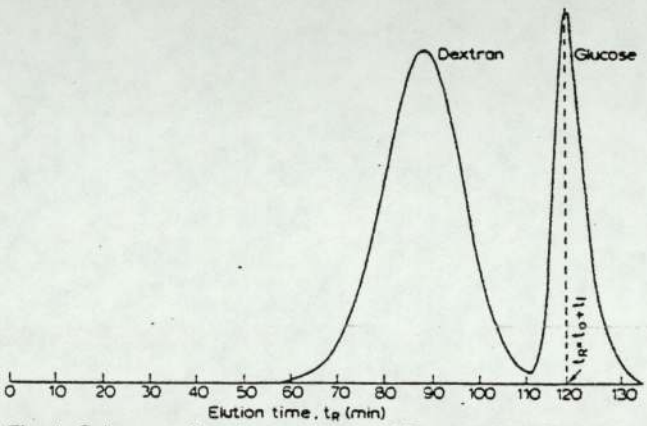

Fig. 4. Gel permeation chromatogram of Dextran B161 D40 and glucose on packing mixture D. Eluent 0.02% potassium hydrogen phthalate aqueous solution. Column: 100 cm × 4 mm I.D.

The main disadvantage with this packing is that the flow-rates are very low (3–6 cm^3/h) because of the low working pressure of the packing (< 7 kg/cm^2), and therefore the analysis time is long (2–3 h).

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