THE CHROMATOGRAPHIC SEPARATION OF CARBOHYDRATE MIXTURES

A thesis submitted

by

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SUMMARY

The separation performance of a semicontinuous counter-current chromatographic refiner (SCCR7), consisting of twelve 5.4 cm id x 75cm long columns packed with calcium charged cross-linked polystyrene resin (KORELA VO7C), was optimised. An industrial barley syrup was used containing 42% fructose, 52% glucose and 6% maltose and oligosaccharides.

The effects of temperature, flow rates and concentration on the distribution coefficients were evaluated and quantified by deriving general relationships. The effects of flow rates, feed composition and concentration on the separation performance of the SCCR7 were identified and general relationships between them and the switch time, which was found to be the controlling parameter, were developed.

Fructose rich (FRP) and glucose rich (GRP) product purities of 99.9% were obtained at 18.6% w/v feed concentrations.

When a 66% w/v feed concentration was used and a product splitting technique was employed, the throughput was 32.1 kg sugar solids/m^3 resin/hr. The GRP contained less than 4.5% fructose, the FRP was over 95% pure, and the respective concentrations were 22.56 and 11.29% w/v. Over 94% of the glucose and 95.78% of the fructose in the feed were recovered in the GRP and FRP respectively. By recycling the dilute product split fractions, the GRP and FRP concentrations were increased to 25.4 and 12.96% w/v; the FRP was 90.2% pure and the GRP contained 6.69% w/v fructose.

A theoretical link between batch and semicontinuous chromatographic equipments has been determined. A computer simulation was developed predicting successfully the purging concentration profiles at "pseudo-equilibrium", and also certain system design parameters.

An important further aspect of the work has been to study the behaviour of chromatographic bioreactor-separators. Such batch systems of 5.4cm id and lengths varying between 30 and 230cm, were used to investigate the effect of scaling up on the conversion of sucrose into dextran and fructose in the presence of the dextransucrase enzyme. Conversions of over 80% were achieved at 4 hr sucrose residence times.

The crude dextransucrase was purified using centrifugation, ultrafiltration and cross-flow microfiltration techniques. Better enzyme stability was obtained by first separating the non-solid impurities using cross-flow microfiltration, and then removing the cells from the enzyme immediately before use by continuous centrifugation.

KEY WORDS: Chromatography, production scale, continuous, High Fructose Corn Syrup, biochemical reactor-separator, dextransucrase.

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Dedicated to my family

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(2) "Development of a link between batch and semi-continuous liquid chromatographic systems". Paper accepted for publication in Chem Eng Science 375

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CHAPTER 1

1.0 INTRODUCTION

It is now 80 years since Tswett (1903, 1906) described a novel method for the separation of coloured plant pigments and named it "chromatography". The term is of Greek origin meaning "colour-writing". Chromatography can be defined as the unit operation where the separation of solutes occurs due to their differential migration rates through a system of two phases, the mobile and the stationary phase. A major contribution to the development of chromatography was the work by Martin and Synge (1), for which the former was awarded the Nobel prize. Chromatography has been used extensively as a powerful analytical tool. Over the last fifteen years or so, its separating capabilities have been appreciated by the industry and production scale processes have been used by the food, petrochemical and cosmetic industries.

Some representative examples are the FINNISH SUGAR ENGINEERING processes for the desugarisation of molasses, the Parex UOP process for the separation of p-xylene and the Sarex UOP process for the separation of carbohydrates. Economic, climatic, political and consumer related factors have reshaped the traditional sugar market, increased the use of alternative sweeteners such as saccharin and fructose, and increased the demand for High Fructose Corn Syrup (HFCS) produced from starch-based carbohydrate feedstocks such as corn, barley, rice and potatoes.

The increased sweetness of fructose, (approximately 1.8 times sweeter than the equivalent amount of sucrose in cold solution), has increased the demand for HFCS as a sucrose substitute by the food and beverage industries. This sudden change in the sugar market appeared in the early seventies in the USA, and currently 40% of the market in the States is supplied by HFCS. A similar market trend has appeared in Japan and the Scandinavian countries, but in Europe in general it has been delayed due to an EEC agricultural policy based on sugar beet.

The typical composition of a starch based syrup is 42% fructose, 52% glucose and the balance is maltose and other oligosaccharides. The HFCS market specification is for syrups containing either 55, 70 or 90% fructose.

Growth of chromatographic processes has been biased towards the batch mode, although recently a number of continuous counter-current systems have been developed. The continuous systems however make better use of the available mass transfer area, offer constant product quality and require no product recycling.

The development of the Semi-Continuous Chromatographic Refiners (SCCR) has been carried out successfully in this department and such a system (SCCR7) was used during the separation studies described in this work. It consisted of twelve columns, each 5.4cm id x 75cm long, and was packed with a cross-linked polystyrene resin in the calcium form (KORELA VO7C) supplied by FINN SUGAR ENGINEERING CO. The objective was to optimise the equipment's separating performance, evaluate the effects of various operating parameters and model its operation. A barley starch isomerose syrup, supplied also by FINN SUGAR ENGINEERING CO, was used, and the production of an HFCS meeting the strictest industrial specifications, was required. These specifications were:

- 90% fructose rich product;
- glucose rich product containing no more than 7% fructose;
- feed throughputs of over 30 kg sugar solids/m^3 resin/hr;
- and products having concentrations over 20% w/v.

The separation performance of this preparative scale system would allow an evaluation of the system's scaling up potential and also provide the basis for the derivation of a theoretical link between the batch and semicontinuous modes of operation.

The separation capabilities of the chromatographic equipments and their potential as reactor-separators has been recognised world-wide over the last twenty years. A number of researchers have carried out the modelling of such reactor-separators but their actual applications are limited. In this work it was decided to investigate the application of a batch chromatographic reactor-separator in carrying out the biochemical synthesis of dextran from sucrose using the dextransucrase enzyme. Dextran is a glucose polymer and for many years it has been used in the field of medicine as a blood plasma volume expander, for the production of iron dextran used in the treatment of anaemia, and for other industrial uses (2). During its biosynthesis fructose is produced as a valuable by-product. Fructose has been known to act as an acceptor, therefore by removing it from the reaction mixture the dextran yield can be improved (3) and also the fructose marketed separately. A number of 5.4cm id batch chromatographic reactor-separator systems were used made from glass or stainless-steel and having lengths varying from 30 to 230 cm.

The objectives of this part of the work were to investigate the effect of scaling up on the sucrose conversion, and to identify factors affecting it. Because of the increased enzyme requirements in the large scale reactor-separators, it was decided to investigate and choose appropriate separation processes that would purify the crude dextransucrase enzyme (supplied by FISONS Pharmaceuticals plc) from the cells and non-solid impurities. It would also be necessary that the chosen enzyme purification processes should keep the activity losses to a minimum, offer good process integration and scaling up potential.

CHAPTER 2

2.0 THEORY OF CHROMATOGRAPHY

The relevant literature to this research work is reviewed in the following three chapters.

This chapter provides a general introduction to the basic concepts of chromatography and to the development and scaling up of liquid chromatographic processes. In Chapter 3 the chromatographic separation of carbohydrates is described, and some industrial applications are reported.

Chapter 4 is devoted to the development of chromatographic reactor-separators, the theory and principles involved in the use of such a reactor-separator for the dextran synthesis, and the application of downstream processes in enzyme purification.

2.1 Principle of Chromatography

Chromatography can be defined as a separation method based on the differential migration of solutes through a system of two phases, the mobile and stationary phase.

The mechanism that determines the regularities of the movement and spreading of the chromatographic zone can be considered as a continual convective disturbance of the equilibrium distribution of a solute between the phases and simultaneously compensating for the deficiencies by diffusion. Separation in chromatography is due to the different migration velocities of the coponents resulting from the relative distribution of each component between the mobile and stationary phases. This can be expressed for each component with the distribution coefficient, K_{di}, defined by:

$$K_{di} = \frac{p_i}{c_i} \qquad \text{..... 2.1}$$

where: p_i = concentration of component i in the stationary phase
c_i = concentration of component i in the mobile phase

Although the chromatographic process involves inseparably the processes of convective transport, diffusion and sorption equilibration, it is the difference in the distribution coefficients of the components that determines the possibility of the differential migration along the system and hence the separation. Therefore, convective transport, diffusion and differences in the distribution coefficients are inseparable factors in the chromatographic processes, and although they constitute conditions for separation they also imply a limitation in the separation efficiency. Geometrical irregularities of the chromatographic bed and the hydrodynamic properties of fluids result in uneven mobile phase flow velocities and hence to the formation of velocity profiles.

Due to a finite rate of lateral solute diffusion, the front (sorption) part of the chromatographic zone during migration contains a certain excess of solute in the mobile phase, where as the rear part of the zone shows a deficiency in the solute concentration in the mobile phase. Also, longitudinal diffusion proceeds in both phases during chromatography. All these factors cause a spreading of the chromatographic zone. Chromatographic separation however is feasible because while the spacing between two zones increases linearly with migration length, the broadening increases only with the square root of migration (4).

Therefore, the efficiency of chromatographic separation depends on the distribution coefficient differences, the uniformity of the bed and mobile phase, the speed in establishing a sorption equilibrium and on how slow is the longitudinal diffusion of the solute in both phases.

2.2 Classification of Chromatographic Methods

2.2.1 Subdivision according to phases employed

In chromatography, the components of the mixture to be separated are distributed over two phases: the stationary phase and the mobile phase. According to the nature of these phases we have the general four systems as follows:

I - Solid stationary phase	1)	Liquid mobile phase - "Liquid-solid chromatography" (LSC)
	2)	Gaseous mobile phase - "Gas-solid chromatography" (GSC)
II - Liquid stationary phase	1)	Liquid mobile phase - "Liquid-liquid chromatography" (LLC)
	2)	Gaseous mobile phase - "Gas-liquid chromatography" (GLC)

In this work the liquid-solid chromatographic principle is employed.

2.2.2 Subdivision according to techniques employed

A further subdivision can now be made owing to the fact that each of these four methods may, in theory, be carried out by three different techniques, ie.

(a) elution development

(b) frontal analysis

(c) displacement development

A short description is given below, to avoid duplication each case is described with respect to liquid-solid chromatography.

(a) Elution development - (Figure 2.1(a))

A small sample of the liquid mixture is introduced into the top of the column. For simplicity the sample is assumed to contain only two components A and B, where the B is the stronger adsorbed component. After the injection a liquid (eluent) is introduced, this eluent is not adsorbed or at all events is less strongly adsorbed than either A or B. The separation of the mixture depends on the relative component distribution between the two phases. The components migrate through the bed at different rates and separation occurs. This technique is the most commonly used and its only drawback is that the strongly retained components travel very slowly. This difficulty can be overcome by successively using eluents with increasing affinity for the adsorbant or by "gradient elution" (5).

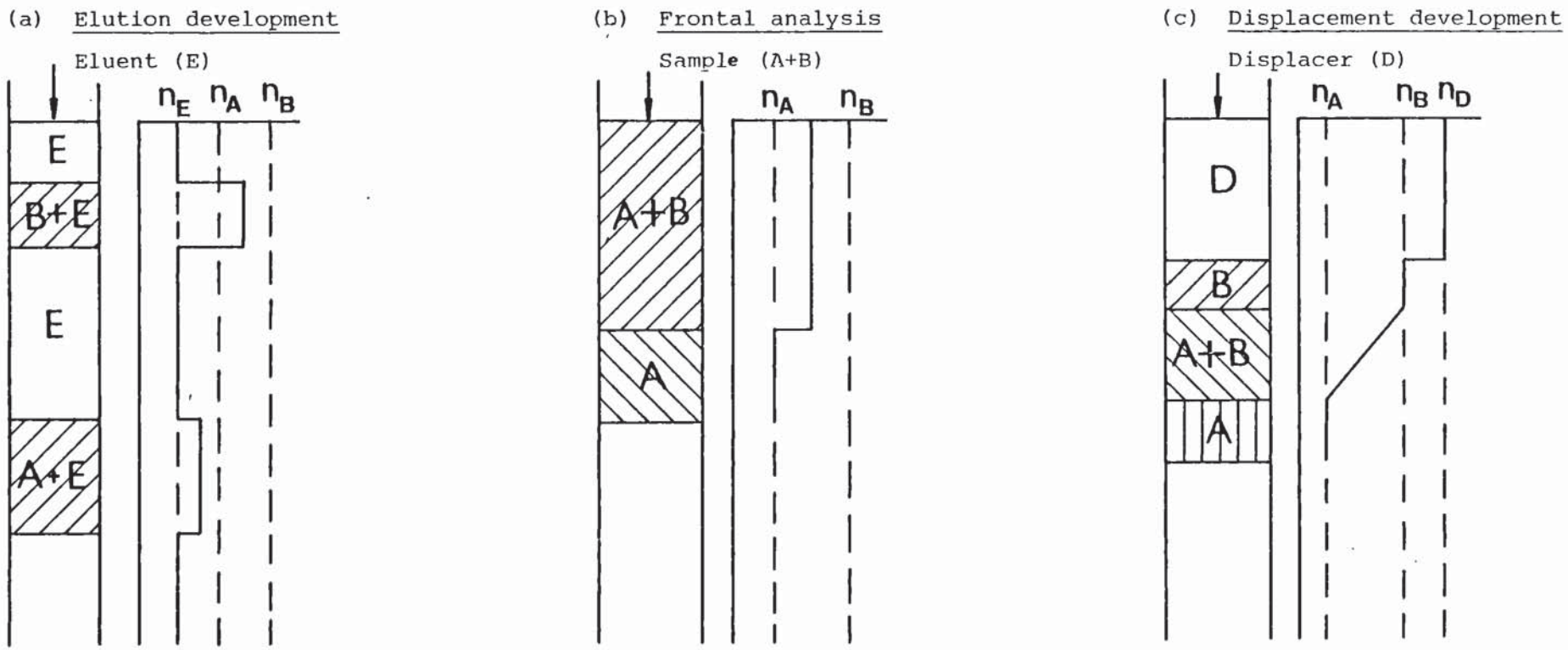
(b) Frontal analysis (Figure 2.1 (b))

A sample is introduced into the column continuously. The less strongly adsorbed A migrates faster and is eluted pure, until the accumulated strongly adsorbed B eventually breaks through together with some of the newly introduced A, after which there is no separation. Because of the obvious limitations of this principle, frontal analysis can be advantageous when the segregation of a powerfully adsorbed minor component from a bulk mixture is required. Tiselius (6,7) and Claesson (8) have used this technique for analysis by recording the changes in concentration as the fronts of material leave the column.

(c) Displacement development (Figure 2.1 (c))

In this technique the mobile phase is retained more strongly by the stationary phase than any of the components in the sample. The separations obtained

Figure 2.1: Schematic representation of the three chromatographic techniques



are purer than elution chromatography but increased sample loading can be applied (9, 10).

2.2.3 Modes of chromatographic operation

As described, the chromatographic separation is a result of the different migration velocities of the components. The means of achieving this differential migration is referred to as the "mechanism of retention", and there are generally four such types as follows.

(a) Exclusion chromatography, where separation is based on a non-ionic molecular-sieve effect and the solute separation depends on differencies in their molecular size, (otherwise known as gel permeation chromatogaphy). The smaller molecules penetrate the porous matrix of the packing and are retarded, while the larger are excluded and flow in the void volume around the packing particles and are eluted first. The origin of the method dates back to the early 1950s (11, 12), however, the main incentive for its rapid development was the publication of a fundamental paper by Porath and Flodin (13).

(b) Ion exchange chromatography, involving a continuous reversible exchange of ions between electrolytes and the ion exchangers. Separation is achieved through the different affinities of the solute ions in the multicomponent sample for the charged resin. Ion exchangers and their properties have been described in thousands of publications and a selection of the most important contributions since the 1960s is by Dorfuer (14), Helfferich (15), Paterson (16), Samuelson (17) and Genge (18).

The ion-exchange chromagraphic principle was employed in the separation studies carried out in this work.

(c) Adsorption chromatography, where separation is effected by a physical or chemical association formed between the solute and the active sites on the stationary phase, and do not involve ion exchanging, ie. physical association (physi-sorption) if the heat of reaction is between 15-20 kcal $mole^{-1}$ (19), or chemical association (chemi-sorption) if the heat of reaction is between 20-30 kcal $mole^{-1}$.

(d) Partition chromatography, which is how liquid-liquid chromatographic processes are usually referred to. The separation relies on the absorption of solutes by an inert solid support coated with a liquid stationary phase.

Some chromatographic separations are achieved by employing two or three of the above mechanisms, which presents a difficulty in classifying such a separation. Such a separation is the "affinity" chromatography or more appropriately bioaffinity chromatography. This technique is employed for the isolation of biologically active substances, making use of their exceptional property of selective and reversible binding of other substances, for which Reiner and Walch (20) introduced the term "affinant". An affinant can therefore be considered as a special case of a ligand. If an insoluble affinant is prepared, usually by covalently attaching to a solid support, and if the extract containing the biologically active component to be purified is passed through this column containing the above material, then all substances which possess no affinity for the given affinant pass directly through the column with the eluent. The specifically adsorbed component can then be eluted either by a soluble affinant or by changing the solvent composition in such a way as to cause the dissociation of the complex of the isolated substance with the bound affinant. This is usually achieved by changing the pH, or ionic strength or temperature, or by adding appropriate reagents. This separation technique is developing fast and has great potential in Biotechnology.

2.3 Fundamentals of ion-exchange Chromatography

2.3.1 Basic principles and the characteristics of ion-exchangers

An ion exchange particle consists of a porous matrix and electrically charged covalently bound functional ionogenic groups. Ion exchangers can be divided into four main groups according to the composition of the matrix:

(a) inorganic exchangers, based on aluminium silicates and other suitable minerals;

(b) synthetic resins of many types;

(c) ion-exchange cellulose;

(d) ion-exchange polydextran

There are five principal classes of functional groups and hence exchangers can be subdivided further as follows:

(1) cation exchangers;

(2) anion exchangers;

(3) amphoteric and dipolar ion exchangers;

(4) chelating ion exchangers;

(5) selective (or specific) ion exchangers.

In this work a cation exchange synthetic resin (cross-linked polystyrene in calcium form) was used. The separation depended on the formation of weak complexes between the fructose molecules in the carbohydrate feedstock and the calcium ions of the resin. The principle of the ion-exchange process can be demonstrated by considering the migration of a solute ion (I^+) through a cation exchange bed. The mechanism is as follows:

$$R - H^+ + I^+ \rightleftharpoons R - I^+ + H^+$$

The forward process is called adsorption and the reverse desorption and the equilibrium is governed by the concentration of the solute ions and the relative affinities of the ions for the exchanger.

The ionic form of commercial exchangers is usually indicated by the manufacturer. Both cation and anion exchangers are classified according to the nature of the active groups, ie:

Ion Exchanger	Type	Usual Functional Group
Cation exchanger	Strongly acidic	Sulphonic
	Medium acidic	Phosphonic
	Weakly acidic	Carboxylic
Anion exchanger	Strongly basic	Quaternary ammonium
	Medium basic	Mixture of tertiary and quaternary ammonium
	Weakly basic	Amines, polyamines

Synthetic ion exchangers are the most commonly used in chromatographic processes. These resins are solid insoluble high molecular weight polyelectrolytes, consisting of a three-dimensional matrix with large numbers of attached ionizable groups as mentioned above. These matrices are produced by polymerization of styrene cross-linked with itself and with divinyl-benzene (21).

The degree of cross-linking is very important in chromatography and defines the average porosity of exchangers. The process of cross-linking is easily controlled and in industrial resins it varies from 1 to 16%. The more cross-links present the less an exchanger swells. Swelling is known to disturb the chromatographic operation especially when resins of low cross-linking are used. In addition to that, although the lower cross-linked resins exchange ions more rapidly, they are less selective.

Inorganic exchangers were the first materials used in the initial stages of the development of ion-exchange systems (21). Among the naturally occurring exchangers, increased interest is shown in the use of zeolites (aluminosilicates) due to their substantial mechanical, thermal and chemical properties. Currently zeolites with controlled properties are prepared synthetically thus increasing their applications. A more detailed description of the synthetically formed zeolites has been carried out by Abusabah (22).

2.3.2 Properties of Ion-exchangers

The choice of an ion-exchange resin for a particular application is influenced by a number of properties, as follows.

Capacity. The capacity of an ion exchanger is a measure of the total amount of ions the resin is able to bind and is usually expressed as milliequivalents (mequiv) per gram of dry resin (in the H^+ or Cl^- form), or as mequiv per millilitre of fully swollen wet resin (in the H^+ or Cl^- form) in the bed. Factors that influence the available capacity are concentration and ionic strength of the eluent, pH, temperature, the accessibility of functional groups and the nature of the counter-ions.

Affinity. Affinity is the degree of adsorption of a solute ion by the exchanger. Ion exchange is, generally, a reversible process and therefore an equilibrium is obtained. This equilibrium depends not only on the relative affinities of ions for the exchanger, but also on the relative ionic concentrations. Therefore, ions of a low affinity for the exchanger can regenerate it and replace ions of a greater affinity, if the former are present at a higher concentration.

The affinity of ions for an ion exchanger is sometimes called the "ion-exchange potential" and in dilute aqueous solutions it increases with the size of

ionic charge. Polyvalent ions are more strongly bound than monovalent ions, the affinity being proportional to the charge. For ions of the same charge, the exchange potentials are inversely proportional to the radius of the hydrated ions. Because the radii of many cations are inversely proportional to their atomic weights, the affinities of these cations can be arranged in order of atomic weights. The composite affinity sequence for some common cations is:

$Li^+ < Na^+ < NH_4^+ < K^+ < Ag^+$

$Mg^{2+} < Ca^{2+} < Sr^{2+} < Ba^{2+}$

$Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} < Zn^{2+}$

and for some common anions:

fluoride < acetate < chloride < bromide < iodide

A measure of the affinity is given by the affinity coefficient:

$$\beta = \frac{X_A/Y_A}{X_B/Y_B} \qquad \text{..... 2.2}$$

where X_A and Y_A are the concentrations of the ion A in the solute and resin respectively and X_B and Y_B are the corresponding ones for ion B.

Nature of the resin. This includes the extent of cross-linking and the particle sizes. The extent of cross-linking and the resulting swelling were mentioned earlier. Particle size and particle form are important characteristics of ion exchangers. The particle size determines how quickly equilibrium is established and influences the sharpness of a chromatographic separation; the smaller the particle the sharper the separation. If the particles however are too small the flow resistance increases and

higher pressures are required. The particle form is also important. The particle must offer good mechanical properties, increased resistance to deformation and attrition, and chemical stability.

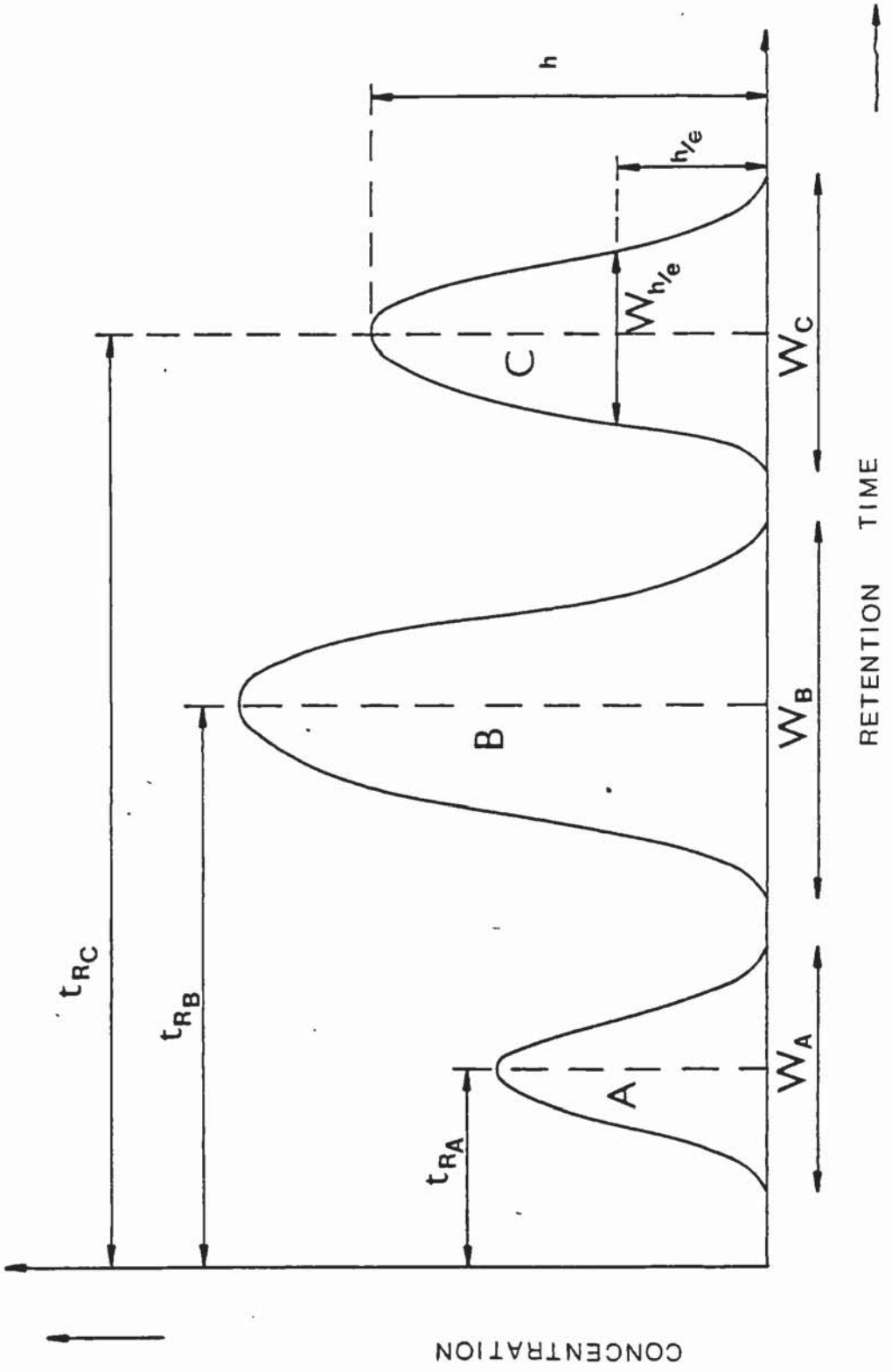
Ion-exchange reactions of simple ions are best described in terms of an ionic redistribution between the ion-exchange resin and the mobile phase. These reactions are always stoichiometric because the electroneutrality of the resin must be maintained. As no covalent bonds are formed or broken during this process, there is little heat evolution or absorption accompanying ion exchange. This is one of the strong advantages in employing the ion-exchange principle; the only exceptions are neutralization reactions involving a cation exchanger in the H^+ form or an anion exchanger in the OH^- form, in which the formation of low-dissociated water is the source of heat.

2.4 *Chromatographic Concepts and Definitions*

When a multicomponent mixture travels along a chromatographic column a gradual separation occurs according to the relative affinities of the components for the packing. If the concentration of the product stream is detected and plotted with time a profile similar to that of Figure 2.2 (for three-components) should be obtained. Such concentration-time profiles are usually called "chromatograms". In Figure 2.2 component A is the least retarded and C the one exhibiting the strongest affinity for the packing.

The retention time (or elution time): is the average time a component takes to migrate along the column and is measured to the mid-point of the elution curve, ie. t_{RA}, t_{RB}, t_{RC}.

Figure 2.2: A typical chromatogram for a three-component mixture obtained from a batch chromatographic column



The retention volume: is the volume of mobile phase required for the complete elution of a component, and is obtained by multiplying the retention time by the mobile phase flow rate.

The relation between the retention volume, V_i, and the distribution coefficient, K_{di}, for any component i is given by (23):

$$V_i = V_o + K_{di} V_s \qquad \text{..... 2.3}$$

where:

V_o = total void volume in column occupied by the mobile phase, and is usually defined with the elution volume of a totally excluded macromolecule.

V_s = volume of the stationary phase = $V_T - V_o$

V_T = total empty column volume

The model was developed initially for gel permeation chromatography and it has been generalised for other chromatographic processes by Kirkland (23).

Capacity factor: a measure of the time the solute i spends in the stationary phase relative to that in the mobile phase, and is defined by:

$$k_i = K_{di} \frac{V_s}{V_o} \qquad \text{..... 2.4}$$

Resolution: the resolution, Rs, is a measure of the degree of separation and is defined (for components B and C in Figure 2.2) by:

$$R_s = \frac{2 (t_{RC} - t_{RB})}{(W_C + W_B)} \qquad \text{..... 2.5}$$

where:

t_{Ri} = retention time of component i
W_i = peak width at the base of the elution curve of component i (in time units)

Alternatively equation 2.5 can also be expressed in terms of elution volumes.

It has been derived for symmetrical peaks, and for a Gaussian band the peak width can be taken as 4σ, where σ is the standard deviation of the Gaussian function. From this equation it is apparent that the separation efficiency increases with increased retention time differencies and with reducing band widths (ie. minimum broadening).

Purnell (24) developed a resolution relationship relating fundamental parameters as follows:

$$Rs = \frac{1}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_c}{1 + k_c}\right)(NTP)^{1/2} \qquad \text{..... 2.6}$$

where:

$$\alpha = \frac{K_{dC}}{K_{dB}} = \text{separation factor}$$

k_c = capacity factor of the most retarded component C
NTP = number of theoretical plates in the column

Since NTP is proportional to the column length, the extent of resolution is proportional to the square root of the migration distance. For R_s values over 1 adequate separation can be expected while for R_s over 1.5 it can be complete (25).

2.5 Chromatographic Models and the Theory of Band Broadening

2.5.1 General description

The important processes prevailing in a chromatographic column, such as adsorption or absorption equilibria, mass transfer between the two phases, diffusion and convection, can be expressed more or less accurately in mathematical terms. A general and exact treatment however leads to excessively complicated mathematics and models requiring the evaluation of a large number of parameters. By introducing simplifying assumptions various theories of arguable validity are obtained which nevertheless help in explaining the important chromatographic phenomena, such as the movement of bands of solute through the column or the dispersion of these bands, and have been proven valuable in the practical development of chromatography.

When a narrow feed band is introduced into a chromatographic column it is subjected to non-uniformities of flow, diffusion spreading, and variations in the rate at which the solute is redistributed between the two phases, which result in broadening. The extent of broadening has a direct effect on the separation and must be kept to a minimum. The nomenclature used in reporting chromatographic band broadening is quite varied, ie. a number of near synonyms are used such as peak broadening, zone spreading, and axial, longitudinal or column dispersion.

A review of various concepts and mathematical models developed to describe the band broadening has been reported by Giddings (26). The more popular of these are the "theoretical plate concept" and the "rate theory".

2.5.2 Classification of Chromatographic Theories

The simplified theories of chromatography can be classified according to the assumption that one or each of the two conditions mentioned below prevails (27, 40):

I) Linear or non-linear distribution isotherm;

II) Ideal or non-ideal chromatography. In ideal chromatography the exchange process is thermodynamically reversible, the equilibrium between the two phases is immediate, ie. infinitely high mass transfer coefficients, and longitudinal diffusion can be ignored. In non-ideal chromatography these assumptions are not made.

Therefore, there are four possiblities:

(1) Linear ideal chromatography. This is the simplest case where the separation depends upon the solute distribution in the two phases and the ratio of the amounts of these two phases present (28). The shape of the band during its migration therefore remains unchanged.

(2) Non-linear ideal chromatography. *It accounts for the effects of* non-linearity of the isotherm but assumes fast mass transfer and neglects the effects of longitudinal diffusion (28, 29). The band during its migration usually develops a sharp front and a long tail, making the theory less suitable for elution chromatography.

(3) Non-linear non-ideal chromatography. The elution bands are broader and distinctly asymmetric, without any sharp fronts or tails. A very involved mathematical modelling of this theory has been carried out by Klinkenberg and Sjenitzer (35).

(4) Linear non-ideal chromatography. In this case the band broadens during migration almost symmetrically in a Gaussian manner. This theory is usually dealt with in two ways:

- by assumng the chromatographic column as a series of large number of theoretical plates, as in extraction or distillation, ie. the "plate theory" or "theoretical plate concept" (1, 30, 31).
- by visualizing the column as a continuous medium where mass transfer and diffusion is accounted for, ie. the "rate theory" (27, 32, 33, 34).

A more detailed description of the "plate" and "rate" theories is given in the following sections.

2.6 The "Theoretical Plate" Concept

A continuous countercurrent process, such as distillation and extraction, may be carried out in a series of discrete stages, each constituting an elementary process where perfect equilibrium exists between the opposed phases. Such a stage is known as a "theoretical" stage or plate. In a packed column, however, the phases are continuously in motion and complete equilibrium is impossible to be established. In such processes, the length of the column over which the separation is achieved is equivalent to that of a theoretical plate is usually referred to as the "Height Equivalent to a Theoretical Plate" or "HETP".

In the "theoretical plate" concept the chromatographic column is visualised as being divided into a NTP number of adjoining separation zones of "plates", with each zone having such a length that there can be complete equilibrium of the solute between the two phases. The "plate" concept has been proven useful in chromatography where complete equilibrium cannot be achieved due to the continuous movement of at least one of the phases. This is a simplified phenomenological approach where the band broadening in a chromatographic column is explained by random fluctuations around the mean retention volume by a simulated partitioning model. The plate concept was first applied to liquid chromatography studied by Martin

and Synge (1), and because of its simplicity and relatively good accuracy it has been developed further and found useful in both liquid and gas chromatography (36, 37). The mathematical modelling of the "plate" concept indicates that the peak shape is that of a binomial distribution, and for the usual large number of plates in chromatographic columns (NTP > 50), it becomes indistinguishable from the Gaussian distribution function (38), ie.

$$C = C_o \exp\left[- \frac{(V_o - V_i)^2}{2\sigma^2} \right] \qquad \text{..... 2.7}$$

where:

C	is the solute concentration
C_o	is the concentration at the peak
V_o	is the mobile phase volume
V_i	is the retention volume of solute i
σ^2	is the peak variance in units of volume

Since the band width is represented by the variance, the square route of the variance (standard deviation, σ), is proportional to the peak's width, W_i, which is usually equal to 4σ, (Section 2.4). Therefore, equation 2.7 can be rewritten in terms of the number of plates, NTP, as:

$$C = C_o \exp\left[- \frac{NTP\,(V_o - V_i)^2}{V_i} \right] \qquad \text{..... 2.8}$$

Therefore, the relationship between the number of plates in a chromatographic column, NTP, the variance, column length, L, and solute retention volume is as follows:

$$NTP = \frac{V_i^2}{\sigma^2} \qquad \text{..... 2.9}$$

and

$$HETP = \frac{L}{NTP} = L\left(\frac{\sigma^2}{V_i^2}\right) \qquad \text{..... 2.10}$$

Glueckauf (31) using a similar approach has related the elution time, t_{Ri}, and the variance to the "apparent" number of theoretical plates, N*, (see Section 2.8) in the following form:

$$N^* = 8\left(\frac{t_{Ri}}{W_{h/e}}\right)^2 \qquad \text{..... 2.11}$$

where the band width, $W_{h/e}$, was measured at a height equal to the peak height, h, divided by the base of the natural logarithm, e, (Figure 2.2). Summarising, the predictions resulting from the general "plate" theory are:

- a Gaussian peak shape (eq. 2.7)
- the peak width increases linearly with retention volume, (eq. 2.9)
- a linear increse of NTP with column length (eq. 2.10).

Although the "plate" concept is widely accepted because of its relative simplicity, it does not account for the effects of mobile flow rates, the nature of the stationary phase, the uniformity of packing, and the kinematic processes occurring within the column.

2.7 The "Rate" Theory

For dispersion effects that involve simple flow and diffusion processes, exact expressions for the "height equivalent to a theoretical plate", HETP, otherwise

known as "plate height", H, can be derived from rigorous mass transfer differential equations from the rate theory approach. The initial version, incorporating in a combined form the effects of mass transfer and longitudinal diffusion, was developed by Lapidus and Amundson (32). Van Deemter et al (27) modified the model further to account, in addition to the rate of mass transfer between the phases and longitudinal diffusion, for the effects of flow behaviour on the history of a band in the column and for rate of adsorption or chemical reaction. For a general discussion of the effect of flow rate on plate height, H, the Van Deemter equation can be simply represented by (39):

$$H = A + \frac{B}{u} + Cu \qquad \text{..... 2.12}$$

Where:

u = mobile phase velocity
A = eddy diffusion term
B = longitudinal diffusion term
C = mass transfer term

A well known graphical representation of the parameters in equation 2.12 is shown in Figure 2.3. The solid line in Figure 2.3, which is the sum of all three dispersion processes, shows a minimum in plate height (H_{min}) which corresponds to the "optimum" velocity (u_{opt}); at this velocity the column has the maximum separation efficiency. The C term in the equation is the sum of the contributions from three possible processes:

(a) from the extraparticle effects (C_m term)

(b) from stagnant mobile phase effects (C_{sm} term)

(c) from conventional liquid chromatography stationary phase mass transfer effects involving the basic sorption processes (C_s term).

Therefore the expanded Van Deempter equation is:

$$H = A + \frac{B}{u} + C_m u + C_{sm} u + C_s u \qquad \text{..... 2.13}$$

This equation predicts a linear increase of plate height with increasing mobile phase velocity at high mobile phase velocity regions (Figure 2.3) while in practice it has been found to taper off (39). This could be explained by the Giddings coupling theory (26). The coupling theory (for Random Walk Theory) is in contrast to the assumed independence of the eddy and lateral-diffusion terms used in the classical plate height theory. Therefore the plate height equation incorporating the coupling theory is:

$$H = \frac{B}{u} + C_{sm} u + C_s u + \left(\frac{1}{A} + \frac{1}{C_m u} \right)^{-1} \qquad \text{..... 2.14}$$

This theory is generally considered theoretically more sound than the classical Van Deemter expressions. However, it should be noted that the focus of the liquid chromatography coupling theory is on extraparticle dispersion effects. A more detailed plate height equation with explicitly expressed dependence on packing particle size and solute-diffusion coefficients, is (26, 41):

$$H = b \frac{D_m}{u} + c_{sm} \frac{u d_p^2}{D_{sm}} + c_s \frac{u d_f^2}{D_s} + \left(\frac{1}{a d_p} + \frac{D_m}{c_m u d_p^2} \right)^{-1} \qquad \text{..... 2.15}$$

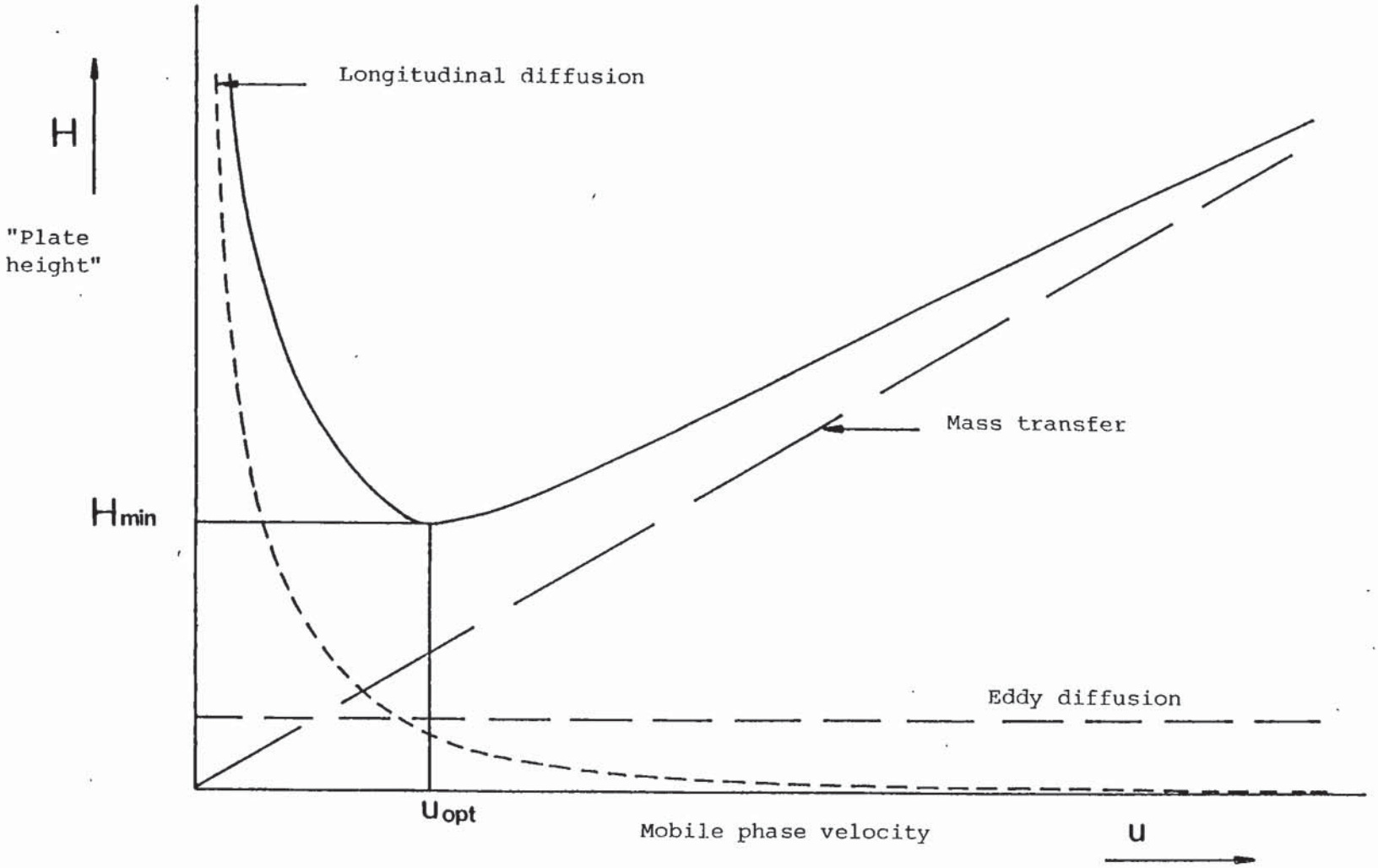
Where:

d_p = particle diameter of resin

d_f = film thickness of the liquid chromatography stationary phase

D_m, Dsm, D_s = solute-diffusion coefficients corresponding to extra-particle, stagnant mobile phase and stationary phase respectively.

Figure 2.3: Theoretical Van Deemter graph



a, b, c_m,
c_{sm} and
c_s = the coefficients of the respective terms in the plate height equation.

The rate theory is a proper theoretical approach for chromatographic design considerations, and is of increased accuracy since it incorporates the effects of the kinetic phenomena that exist in practice. However, its general application is limited because of the need to evaluate a number of parameters (ie. diffusion coefficients, and coefficients related with the terms A, B and C) for every application.

2.8 Evaluation of the Separation Efficiency, accounting for the Effect of Feed Band Broadening

Glueckauf (31), using the "theoretical plate" approach and assuming a linear isotherm, developed the following equation giving the "apparent" number of theoretical plates, N*, for a chromatographic column:

$$N^* = 8\left(\frac{t_{Ri}}{W_{h/e}}\right)^2 \qquad \text{..... 2.16}$$

where:

t_{Ri} = retention time for component i
$W_{h/e}$ = peak width evaluated at a height h/e (Section 2.6 and Figure 2.2)

This equation however is suitable only for narrow feed-bands. In practice, however, wider feed-bands exist and it is necessary to estimate the effect of the width, to calculate the "true" number of theoretical plates, N, for the actual conditions. The problem was dealt with previously by Van Deemter et al (27) but the Poisson distribution functions used did not lend explicitly to ways of obtaining the HETP and N values for wide feed bands.

Glueckauf (42) modified his initial theory to accommodate for wide feed bands and obtained the following relationship:

$$N = N^* \left[1 + \frac{P}{3} + \frac{7P^2}{45} + \frac{0.0765\,P^3}{1 - 0.565P} \right] \qquad \text{..... 2.17}$$

and

$$P = \frac{\alpha^2 N^*}{4} = \left(\frac{N_o}{N} \right)^2 \frac{N^*}{4} \qquad \text{..... 2.18}$$

Where:

P = is a system loading related parameter and is an indication of the applicability of equation 2.17. Equation 2.17 is accurate for up to $P \leq 1.6$.

$\alpha = \frac{N_o}{N}$, a feed band ratio

N_o = number of plates occupied by the feed band

N^* = "apparent" number of theoretical plates (obtained from equation 2.16)

As the width of the feed band increases it increases the band broadening and leads to band overlapping. Therefore to obtain the required separation more theoretical plates are needed than the ones found at narrow feed bands.

2.9 Factors affecting the Scaling up of the Chromatographic Systems

In this section attention is directed to experimental parameters that can affect peak broadening and hence the separation. Chromatography has been accepted as an invaluable analytical tool, and its great separation potential has led many workers into scaling up. Therefore the factors affecting its scaling up must be identified and accounted for. Pure packing techniques result in uneven velocity profiles that cause increases in the plate height, ie. the general Van Deempter equation becomes:

$$H = A + \frac{B}{u} + Cu + Hc \qquad \text{..... 2.19}$$

where Hc is the height contribution due to uneven velocity profiles.

Giddings (43) expressed the parabolic profile as:

$$Hc = G\frac{r^2u}{\gamma D_m} \qquad \text{..... 2.20}$$

Where:

r	=	column radius
G	=	constant
u	=	mobile phase velocity
γ	=	radial Labyrinth factor
D_m	=	solute diffusivity in mobile phase

This correlation is found to give good agreement with experimental results for 0.6 to 5.1cm (45) and 7.5cm (46) column diameters.

Bayer et al (44) assumed a concave profile and expressed Hc as:

$$Hc = 2.83\frac{r^{0.58}}{u^{1.886}} \qquad \text{..... 2.21}$$

Good agreement was obtained for columns between 1.3 and 10.2cm diameter.

Pretorious et al (47) suggested a W-shaped profile and developed:

$$Hc = \frac{1}{100}\exp\left[-\frac{d}{10d_p}\right]\left[\frac{d^2u}{2D_r d_p}\right] \qquad \text{..... 2.22}$$

Where:

d	=	column diameter
d_p	=	particle diameter
D_r	=	solute radial diffusivity

The effect of column diameter on separation is arguable. With increasing column diameter obtaining a well packed column (ie. uniform and of minimum voidage) becomes increasingly difficult which has a direct effect on separation efficiency. As the column size increases however, the "dead" volume and "wall" effects become less significant. Therefore, the subject is debatable, with the majority of opinion indicating that there is a loss in efficiency during scaling up.

The estimation of the number of theoretical plates required for a given separation (Section 2.8) has been carried out operating in a batch chromatographic column. In production scale operations however the necessity for increased throughputs requires the employment of techniques such as repetitive feed injections increased feed charges and concentrations, for batch systems, and increased flow rates and feed concentrations for continuous systems. These, in practice, result in overloading of the chromatographic system and have an adverse effect on the separation efficiency. The employment of repetitive injection (50) has an additional effect due to the band overlapping and should be accounted for. Equations 2.16 and 2.17 have been developed for a "once through" operation, and although they do not allow for repetitive injection, they provide a useful means of comparing the efficiency of various chromatographic systems. Their application has been extended in determining the separation efficiency of continuous systems by characterising each column in such systems in batchwise mode. This approach is considerably effective, however there is a strong need for developing similar relationships that apply directly to the continuous mode of operation.

The Van Deemter (27) and Giddings (26) models have been developed assuming a linear isotherm. In practice however as the concentration increases the solute distribution between the phases becomes dependent to the concentration changes and deviations from ideality occur. Helfferich (48) modified the retention volume equation (eq.2.3) to account for the concentration effects, ie:

$$V_i = V_o + V_s \frac{dp_i}{dc_i} \qquad \text{..... 2.23}$$

rearranging,

$$\frac{dp_i}{dc_i} = \frac{V_i - V_o}{V_s} \qquad \text{..... 2.24}$$

where:

p_i = solute concentration in stationary phase
c_i = solute concentration in mobile phase

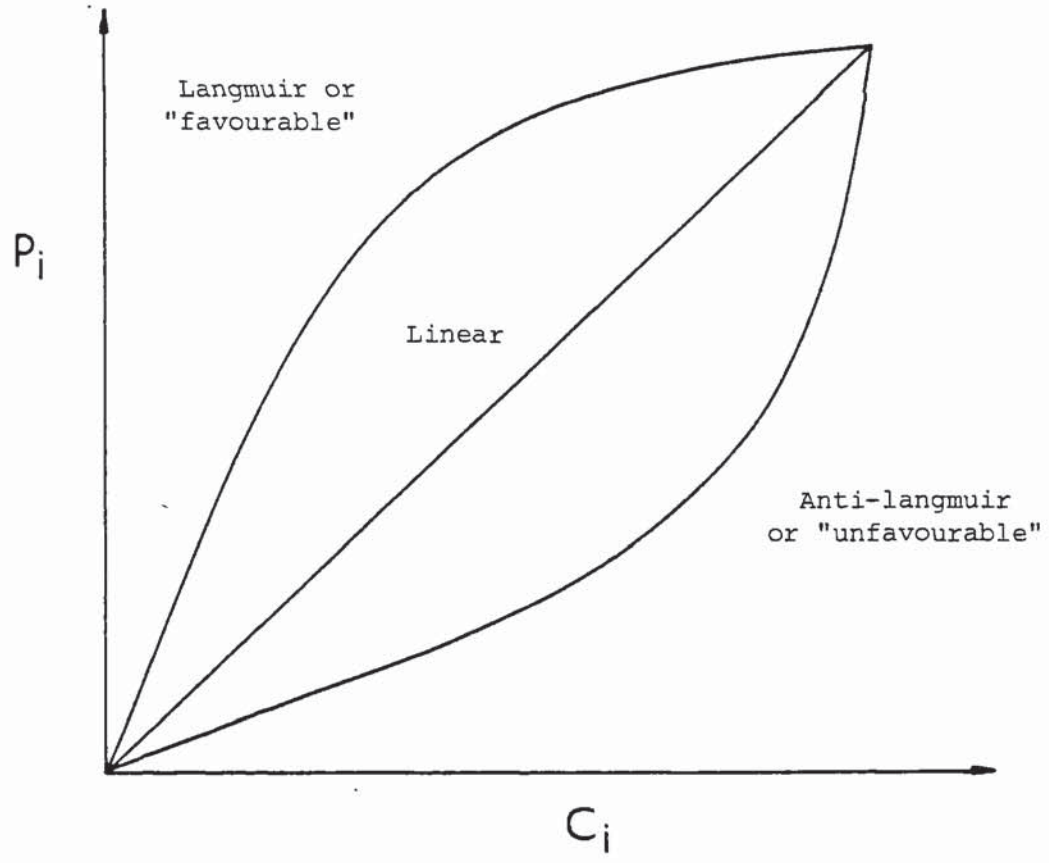
The curve representing this function is the absorption isotherm, applicable at constant temperature. Vermulen (49) describes the Langmuir or "favourable" and Anti-Langmuir or "unfavourable" isotherms (Figure 2.4). Because of the adverse effects of the operating conditions, such as feed charge size, flow rates and concentration, on the separation, in production scale chromatographic processes a comprcmise has to be reached based on an economic assessment.

2.10 Effects of Column and Stationary Phase related Parameters on separation Efficiency and Scaling up

Theoretically, optimum resolution is obtained with the smallest possible average particle size, of a minimum size range. The selection of the proper resin material for a given application is also very important. In practice however the increased pressure drops due to the small particle sizes, and resin availability lead to a compromise. The resin particles should also exhibit good mechanical properties and chemical resistance.

In chromatographic columns the force exerted on the lowest part of the bed is the sum of the weight of the packing and the drag force acting on it minus the friction force of the column wall. The drag force on the packing material is directly proportional to the total surface area, the eluent viscosity and the velocity of flow past

Figure 2.4: Absorption isotherms



the surface. In column diameters over 20cm (51) the stabilizing influence of the column walls may be neglected. Therefore, in large scale applications to minimise the forces exerted on the bed, and especially when soft gels are used, a series of shorter columns should be employed instead making up the total length, and interconnected with small bore pipes. This arrangement reduces the presure differences across any section of column, it reduces the problems associated with the location of tall columns, slow moving components can be removed at intermediate levels, permits better and easier packing of each column and independent repacking of any section of the system. This approach however also has its limitations. It requires a means of redistributing the mobile phase properly, and increases the "dead" volume of the system.

A number of alternative approaches have been proposed to overcome this problem of bed compression and attrition. One is to support the compressible particles by mixing them with incompressible inert ones (52). This however results in reduced volume capacities and resolution.

An alternative is to support the column packing material with various insertions such as coarse stainless steel meshes or extra walls as concentric tubes (53, 54). This however results also in reduced packing efficiencies. A further development is to divide the column into sections using porous plastic sheets, inserted at short intervals above each other (55). This however can lead to the formation of empty spaces beneath the meshes due to successive packing and compression of the bed material, leading to zone mixing and reduced resolution.

In large columns the wall effect is reduced; it has been found that it can influence the flow pattern of up to approximately ten particle diameters away from the wall. Increased difficulties however in obtaining a uniform packing of large columns, result in the formation of velocity profiles. A number of flow distributors and baffles (56, 57, 58) have been introduced in chromatographic columns to overcome this problem. These however, present similar problems with the packing support insertions

mentioned above, and present increasing difficulties in backflashing the system for cleaning and during regeneration.

Therefore, the best way to overcome the problem is by preventing it happening, ie. by employing an efficient packing technique, to give uniform packing and minimum void volume.

2.11 Column Packing Techniques

A number of alternative packing techniques have been developed classified as dry packing or wet (slurry) packing, with the second being more popular. A comprehensive description of various techniques employed in gas chromatography has been reported by Higgins and Smith (59). Bayer (44) introduced mechanical tapping and vibration to improve packing efficiency.

Slurry packing techniques include bulk pouring, pouring under vacuum and bulk pouring with vibration. In slurry packing segregation tends to occur, and the introduced particles tend to flow downwards as a unit, which can lead to separate layer formation. The packing of the column in practice is a rather empirical process, and different users use their own variations.

The columns used in this work were packed by employing slurry packing under vacuum. The resin was introduced at the top, as a 50:50 slurry, and some column vibration was applied.

2.12 Operating Techniques Employed in Continuous Chromatography

To increase the throughputs in production scale batch chromatographic systems the repetitive feed injection technique is employed. This results in band overlapping and reduced purities, and to overcome this, the overlapping fraction is

recycled. This recycling, which can be up to 40%, apparent in batch processes has directed interest towards the use of continuous chromatographic processes, which offer greater effective throughputs, and uniform product quality. Continuous processes fall into two broad categories, the cross-current and counter-current flow proceses; defined according to the relative movements of the mobile and "stationary" phases. Figure 2.5 shows a concentration profile for a two component mixtrure, B,C, where C is the retarded component.

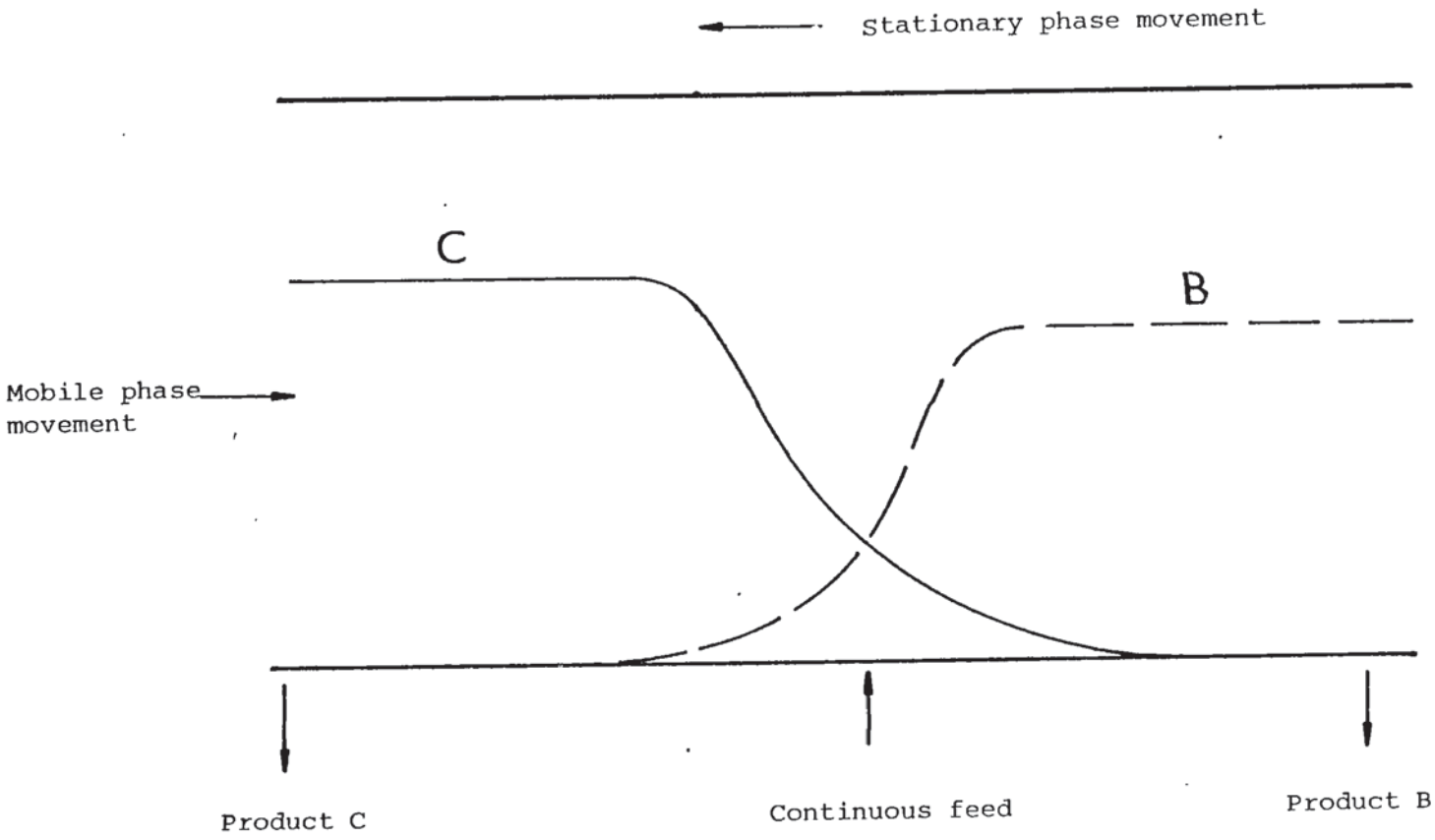
2.12.1 Cross-current Continuous Chromatography

In cross flow systems the "stationary" phases moves perpendicularly in the direction of the mobile phase. A number of such systems have been developed and can be subdivided into two categories.

2.12.1.1 Moving annulus systems

This type of process was first suggested by Martin (60) who proposed the use of a rotating annulus packed with chromatographic packing. The annulus rotates through a fixed inlet point. Mobile phase is introduced continuously at the top all the way around the annulus and leaves at the bottom. The components travel in helical paths around the annulus at different angles according to the relative affinities for the packing and are eluted at different points, with the strongly retarded component travelling along a longer helical path. Theoretically, this arrangement can provide continuous separation of a multicomponent mixture. Such systems have been constructed by Svensson (61) and Fox et al (62, 63). The Fox et al system was 30.5cm long and the inner and outer cylinder diameters were 27.3 and 29.2cm respectively.

Figure 2.5: Concentration profiles on a continuous chromatographic process



2.12.1.2 Moving Column - Open End Systems

Such systems consist of a circular array of parallel tubes rotating through a fixed top inlet and stationary product receivers at the open bottom ends. Toramasso and Dinelli (64) constructed such a system consisting of 100 tubes each of 6mm dia and 1.2m high. Similarly designed systems have also been reported by Turina et al (65). In these systems although the unit as a whole rotates, they are effectively a series of batch columns where in each one the mobile phase flows along a fixed stationary phase. The same effect can be achieved using a large batch column, a timer and a switching valve and collecting the eluted peaks separately over a number of short time periods. These systems are therefore of increased complexity with no practical significance.

2.12.2 Counter-current Continuous Chromatography

Although some cross-current systems have been found to operate satisfactorily on a small scale, their sealing up potential is limited due to mechanical difficulties associated with the rotary movement, problems in achieving perfect scaling between the moving parts (especially in the botton open ends), packing compressibility and attrition. Because of these limitations of the cross-current systems, a number of counter-curent processes have been developed and can be classified according to the principle they employ to obtain the counter-current movement of the two phases.

2.12.2.1 Moving Bed Systems

In these systems, the packing flows under gravity countercurrent to a stream of mobile phase flowing upwards in a column. When the feed mixture is introduced

into the centre of the column, the least strongly absorbed component is carried upwards and exits from the top, and the retarded component is carried down the column and stripped from the packing at the bottom column outlet. Barker and Critcher (66) used a 2.5cm dia x 2.79m long system to separate an azeotropic mixture of benzene and cyclohexane. Such an indusrial process is the Hypersorption Process developed by the Universal Oil Co, California, (67, 68) using a 26m high x 1.4m diameter column.

Moving bed processes however have been found to suffer from the following problems.

- difficulties in achieving solid control at increased scales
- low mass transfer efficiencies due to uneven packing
- packing attrition due to the increased shear forces, and packing entrainment
- relatively low mobile phase velocities to prevent fluidisation.

2.12.2.2. Moving Column Systems

To overcome the above problems various equipments have been proposed which employ a circular array of parallel columns interconnected to each other. The system rotates past fixed inlet and outlet ports. A number of such systems have been developed (69, 70, 71, 72) and there has been considerable success in the analytical field (small scale). Scaling up however is difficult because, like in the cross-flow systems, there are increased difficulties in achieving a reliable mechanical seal between the static ports and moving columns.

2.12.2.3 Moving Feed Point Systems

This technique is an intermediate one between conventional batch and simulated countercurrent systems. Wankat and Oritz (73) used such a process to separate dextran 2000 from cobalt chloride in water. The system consisted of a series of fixed columns. The mobile phase was pumped continuously through the inlet in the top column and the feed was introduced as a long pulse. The first feed pulse was introduced into the first column, then after a predetermined time period into the second column and so on. This continuous feed switching was controlled by a rotary valve and a timer. The switching period was chosen so that the average velocity of the feed ports advancement was between the migration velocities of the least and the more strongly adsorbed components. This technique has been found to be more efficient than the batch processes, but less than the simulated counter-current processes, since it only utilizes part of the total adsorbent packing in the system at any time.

2.12.2.4 Simulated Moving Bed Systems

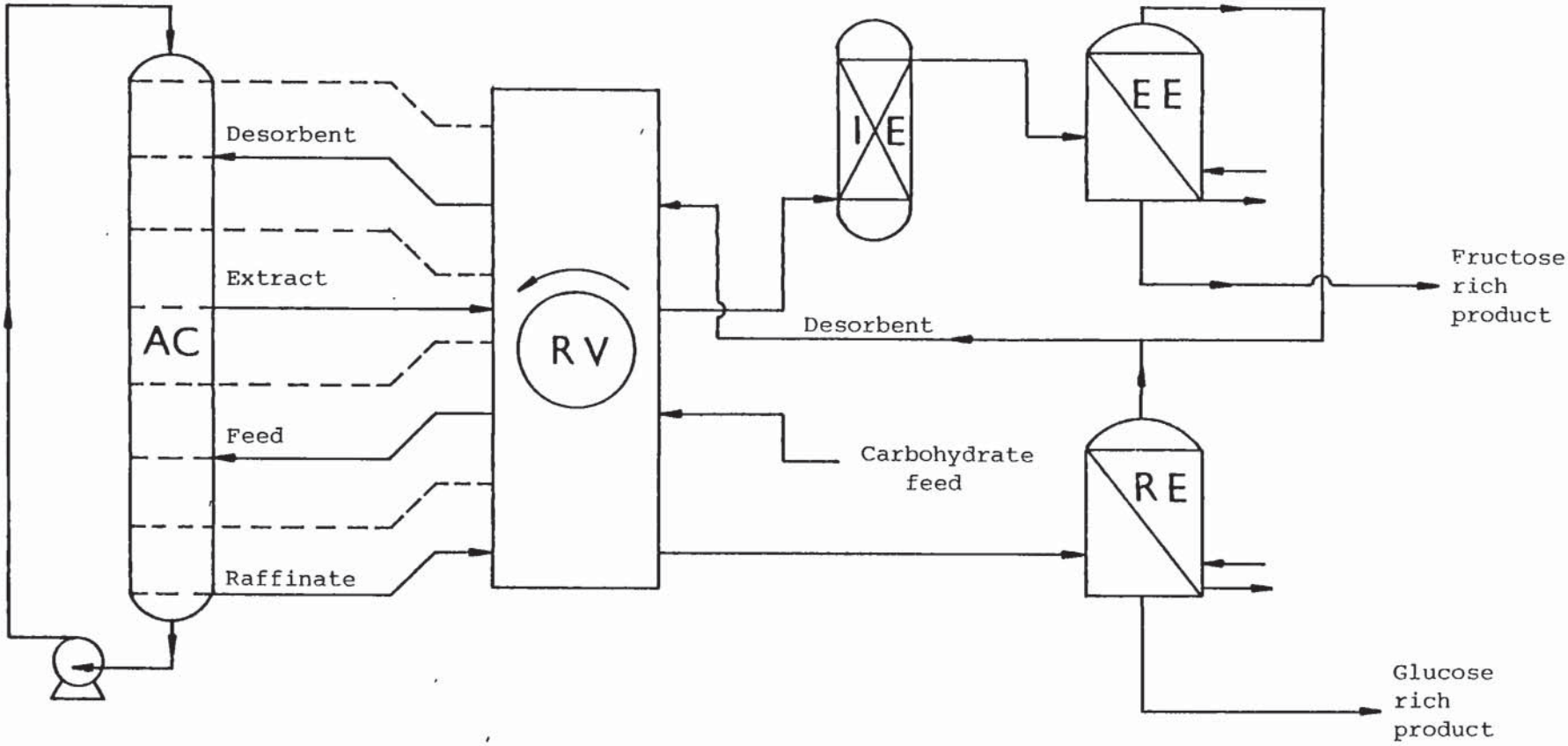
These systems offer the greatest potential since they make better use of the available mass transfer area, and therefore better separation efficiencies and throughputs could be achieved.

All such system employ a number of static interlinked columns or compartments and the countercurrent movement is effectively achieved by sequentially moving the inlet and outlet ports in the direction of the mobile phase.

There are two main approaches. The Universal Oil Products (USA) Sorbex Technique (74, 75, 76) and the Semi-Continuous-Chromatographic-Refiners (SCCR) (77, 78).

In a typical sorbex process the stationary phase is packed into the compartments of a static vertical column. Each compartment is connected to a specially designed rotary valve operating on the principle of a multiport stopcock.

Figure 2.6: Flowsheet of the Sarex Process



Key:

AC: Adsorbent chamber
RV: Rotary valve
IE: Ion exchanger
RE: Raffinate evaporator
EE: Extract evaporator

This technique has been applied for over fifteen years for hydrocarbon separations. Because of its design, extra care must be taken to overcome the problems associated with packing compressibility. It requires a very precise design of the main rotary valve and it does not offer the flexibility of repacking part of the system separately. A flow sheet of a variation of this process, applied in the separation of carbohydrates, is shown in Figure 2.6.

A similar technique was employed by Szepesy et al (79). They also employed a master rotary valve but used twelve glass tubes instead, each 30cm long and 1.4cm id, connected elliptically to each other by metal fittings.

Experience gained on the unreliability of large flat face moving seals, and the awareness of the limitations associated with the various alternative techniques mentioned above, led Barker and co-workers to develop an alternative technique to simulate the countercurrent movement of the two phases. They developed the "moving port" multicolumn system (SCCRs) whereby all moving parts were eliminated by using valves of proven commercial reliability. The countercurrent movement was simulated by the simultaneous closing and opening of the appropriate valves, connected to the ports of each column, in the direction of the flow of the mobile phase. These systems have been scaled up to ten columns, each of 10.8cm id and 75cm long.

A system employing this technique was used in this work and a detailed description is carried out in the following sections.

2.13 Production Scale Chromatographic Processes in Operation

The scaling up of the chromatographic process has been in progress since the early 1950s. During the first period the development was concentrated on the scaling up of batch gas chromatographic columns. Very soon though, increased interest was directed towards the scaling up of continuous counter-current chromatographic systems, employing the operating techniques described above. The

potential of chromatography has made the developers very secretive and comprehensive information about the individual developments is hard to come by. Some of the most popular large scale systems in operation are reviewed here, with the exception of the systems employed in carbohydrate separation which will be described in the next chapter.

2.13.1 Batch Systems

During the 1960s ABCOR (Boston, USA) pursued the scaling up of batch systems. Gas chromatography units of up to 2m id were used for the separation of alpha and beta pinenes (80), and there was talk of scaling up to 5m id and 50m long (81). After forming a partnership with Boehringer and Sohne (major German drug makers), they were considering also using similar systems in the liquid chromatographic mode for the purification and separation of biochemicals (82). Abcor and Boehringer columns have used packing materials such as aluminas, silicas and ion exchange (cross-linked polystyrenes) resins.

Pharmacia Fine Chemicals (Sweden) market purpose built chromatographic columns of various sizes. They offer a wide range of packing materials. They are promoting amongst others a cross-linked dextran polymer gel under the trade name of Sephadex with wide filed of application. Sephadex packed columns of up to 180cm dia have been used for the separation of milk protein from whey.

The French petrochemical company Elf-Aquainte has showed an active interest in batch chromatography. They have built a number of large scale gas chromatography and liquid chromatography plants. In 1979 they installed a large production scale gas chromatography plant in Jacksonville, Florida, USA. The system was used to manufacture perfume ingredients and flavour chemicals from terpene feedstocks. It consisted of a 40cm id x 150cm long column producing 10-15kg

of product per hour with claimed purities up to 99.995% (83). In 1982 a 100000 tons/year plant was to be built in Donges, France (85) producing a fuel base and an n-paraffin cut that serves as a feedstock for an isomerization unit converting normal paraffins to isoparaffins. The company also offers standard units, for the purification of flavours and fragrances, with capacities from 3 ton/yr to 300 ton/yr (86). Over the last decade a number of other companies (84), such as Waters Associates Inc (Mass, USA) and Instruments SA Inc, (NJ, USA) Market preparative liquid chromatography batch systems of different sizes and for a wide range of applications according to the customers requirements.

2.13.2 Continuous Systems

The inherent advantages and potential of continuous countercurrent chromatographic systems resulted in increasing their applications in production scale operations.

The Hypersorption Process. One of the largest earliest applications of large scale chromatography was by Universal Oil Products Co (California, USA) with its hypersorption process for the separation of ethylene from hydrocarbon vapour streams (67, 68) with production rates of up to 4.5×10^5 m^3 per day of ethylene.

The Sorbex Process. The next major industrial application of chromatography came again from Universal Oil Products (UOP) Co (USA), who were motivated by the limitations of the moving bed hypersorption process (Section 2.1.2.2.1) developed the sorbex process (Section 2.12.2.1). It is believed that over 40 UOP plants operate world wide employing the Sorbex technique with a total capacity approaching 4 million tons/year, and most of these plants are used for hydrocarbon separations. According to the particular application, the Sorbex process employed was given an alternative name, ie.

- Molex, used for the separation of normal paraffins from hydrocarbon mixtures.
- Olex, used for the separation of olefins from paraffins.
- Parex, employed in producing over 22% of the worlds p-xylene (1980).
- Sarex, used for the separation of carbohydrate mixtures and the production of High Fructose Corn Syrups (HFCS).

Oak Ridge National Laboratory's Chemical Division (Tenn, USA) have produced a "cross current" production scale system which is said to be ready for commercialisation (87). The system offers particular advantages for the continuous separation of components with close distribution coefficients; however, no further news on its progress has been reported.

CHAPTER 3

3.0 PROPERTIES AND SEPARATION OF SUGARS AND THE PRODUCTION OF HIGH FRUCTOSE CORN SYRUPS (HFCS)

The production and separation of carbohydrate mixtures is one of the major fields where chromatography has been applied. Because of the relative safety, in terms of toxicity, explosion and fire hazards, and specific industrial interest (by FINNISH SUGAR, Finland, and FISONS Pharmaceuticals, England) led us into using carbohydrate mixtures in this work to carry out the research and development of the chromatographic systems. This chapter considers the properties of glucose and fructose, their production and separation and a review of the large production scale chromatographic process employed.

3.1 The Chemistry of Glucose and Fructose

Carbohydrates in general are an important class of naturally occurring organic compounds universally found in plants, animals and microorganisms. The designation "carbohydrate" comes from their empirical formulas $(C.H_2O)_n$, where $n \geq 3$. The metabolism of carbohydrates is of central importance to organisms individually and collectively. They provide a major share of the body's energy requirements, act as energy reservoirs and serve architectural functions. Carbohydrates are divided into three basic categories: monosaccharides, oligosaccharides and polysaccharides. The monosaccharides have three to nine, usually five or six, carbon atoms and contain only one aldehyde or ketone function or are derivatives of molecules that do.

The oligosaccharides are oligomers of monosaccharides, linked by formation of glycosidic bonds, containing generally two to ten monomeric units. They are subdivided into disaccharides (such as maltose and sucrose), trisaccharides (such as raffinose), and so on, depending on whether they have two, three or more carbohydrate units.

Carbohydrate polymers containing more than ten monomeric units are called polysaccharides, and can have molecular weights up to many million. Starch, cellulose and glycogen are among the more important polysaccharides. Glucose is the most common monosaccharide occurring free in fruits, honey and other living material. Fructose is another common monosaccharide, a natural sugar frequently found in fruits with glucose. Glucose is dextrorotatory with a specific rotation of $(\alpha)^{20}_D = 52.7^o$ in water, while fructose is strongly laevorotatory with $(\alpha)^{20}_D = -132.2^o$ (88). Therefore, sometimes glucose is referred to as dextrose and fructose as laevoluse. Both glucose and fructose are soluble in water, and fructose is also soluble in ethers and alcohols while glucose is only slightly soluble in alcohols (89).

The formula of both sugars is $C_6H_{12}O_6$ and their open chain forms are shown in Figures 3.1 and 3.2. From these open chain forms it can be seen that glucose is an aldohexose, since it contains the aldehyde group (CHO), and fructose is a ketohexose, since it contains the carbonyl group (C = O). Glucose in solution exists with the isomeric ring structures shown in Figure 3.1 and an equilibrium exists between these forms, which is only slightly dependent on temperature and concentration (Table 3.1). The average equilibrium composition is 40% of α-D-glucopyranose and 60% of β-D-glycopyranose (90 to 92).

In solution fructose exists in the isomeric forms shown in Figure 3.2. The equilibrium between the α-D-Fructofuranose, β-D-Fructofuranose, β-D-Frucopyranose is affected by temperature (Table 3.2), but the effect of solution concentration is minimal (90 to 92).

Figure 3.1: Glucose forms in solution

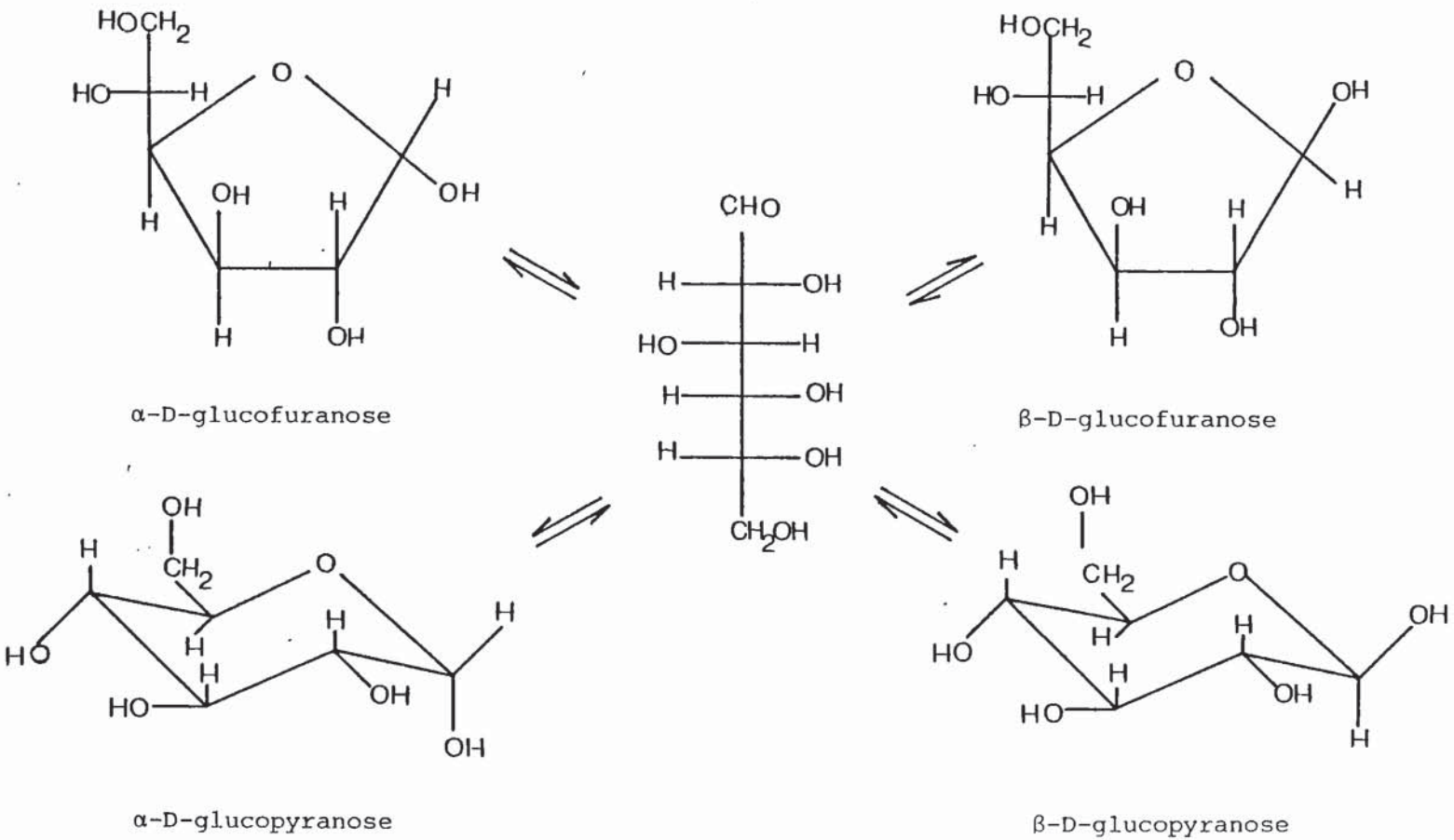


Figure 3.2: Fructose forms in solution

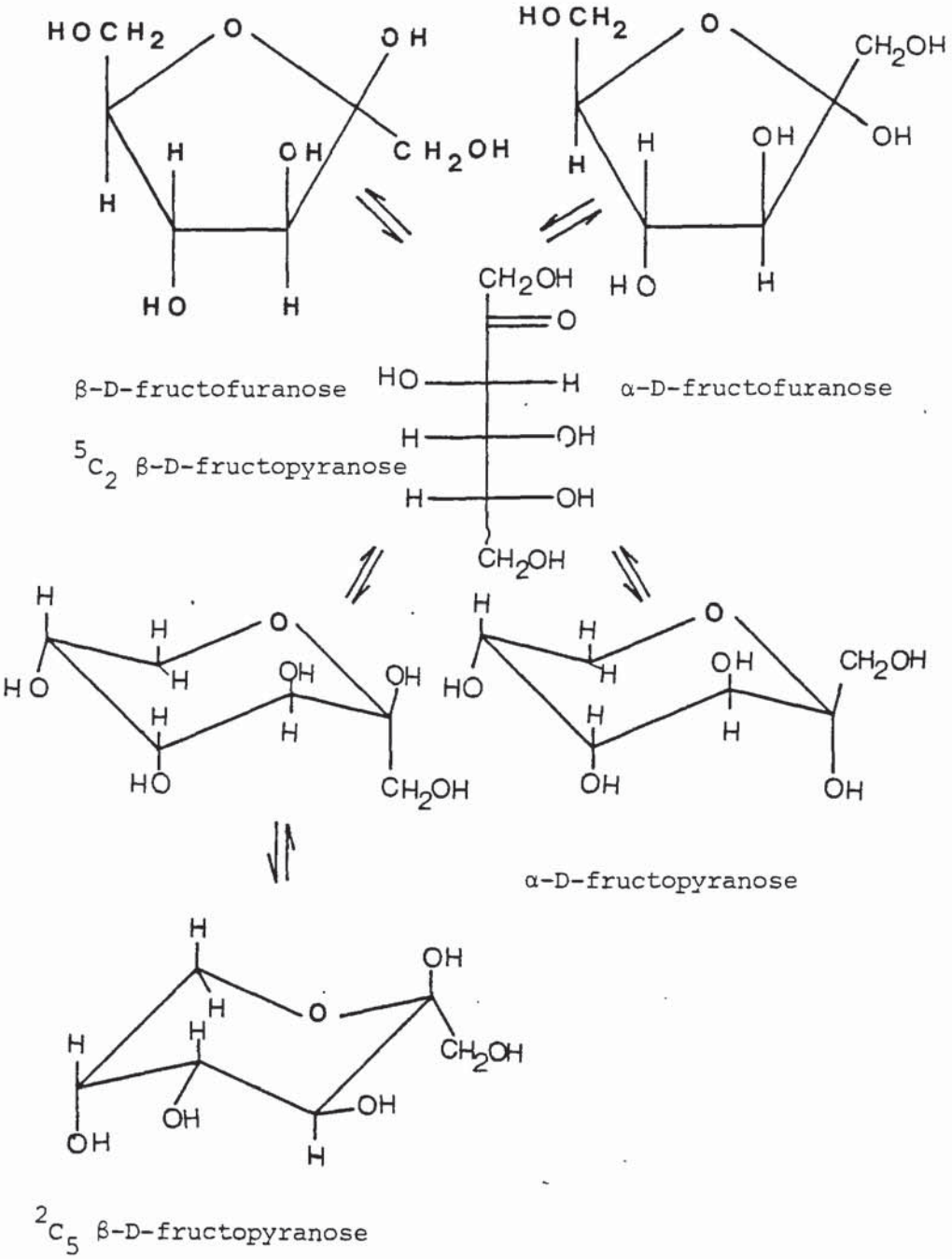


Table 3.1: Equilibria of α-and ß-D-glucose anomers in D_2O (91)

Glucose concentration (%)	α-D-glucopyranose (%)	ß-D-glucopyranose (%)
		At 5°C temperature
5	40	60
20	41	59
65	40	60
		At 23°C temperature
5	37	63
20	39	61
65	51	49
		At 80°C temperature
5	41	59
20	49	51
65	46	54

Table 3.2: Tautomeric Equilibria of Fructose in D_2O (91)

Fructose concentration (%)	Temperature °C	α-D-Fructo-furanose (%)	ß-D-fructo-furanose (%)	ß-D-Fructo-pyranose (%)
20	10	4	18	78
20	22	6	21	73
20	67	8	28	64
20	77	12	31	57
20	23	6	21	73
50	23	4	21	75
80	23	5	21	74

3.2 Fructose as a Sweetener

During the relatively short time that fructose has been industrially produced, it has become particularly established as a sweetener substitute to sucrose in beverages, and various dietetic food products. As part of the diabetic's diet, it offers metabolic and organoleptic advantages, and has minimal influence on the sugar and insulin levels in plasma. In Finland and Sweden it is used in baby foods and in some medicinal preparations intended for children. Fructose can be used in small quantities as a flavour enhancer in meat dishes and as a raw material in the industrial manufacturing of flavours. It enhances the inherent aroma of fruits, berries and vegetables more than any other sugar or sugar alcohol and its use in juices, jams and desserts offers particular advantages.

Fructose is a natural sugar and without toxic properties. Traditionally it has been used as an alternative to glucose in infusion therapy for patients who have reduced tolerance to conventional intravenous glucose. Its calorific value is slightly lower than sucrose, (ie. 3.7 and 4 kcal kg^{-1} respectively), but it is 1.8 times sweeter than sucrose in cold solution (93 to 95). When the minimum calories intake for the same sweetness is required fructose in the crystalline form should be used.

Some medical statistics have indicated that fructose causes less dental plaque formation than sucrose. Fructose is very soluble in water, and its solubility is almost 15% higher than sucrose.

Over the last years a number of synthetic sweeteners have been produced, such as saccharin and cyclamate which are entirely non-caloric. However, there is an increased speculation about their possible health side effects, and saccharin provokes a bitter after-taste. Sugar alcohols, sorbitol in particular, have gained some ground but have a laxative effect and are of lower sweetness. Therefore fructose is arguably the best alternative.

Fructose has been found to overcome the bitter after-taste of saccharin, and a 99.7:0.3% fructose: saccharin mixture has been found to be 3-4 times sweeter than a pure sucrose solution without any distinguishing difference in taste. Fructose has also been found to exhibit a similar synergistic sweetening effect with sucrose.

The β-D-Fructopyranose is believed to be the sweetest fructose form and the solution sweetness depends on the equilibrium between the above form and the β-D-Fructofuranose. As it was seen in the previous section, the equilibrium depends strongly on the temperature. At temperatures over 50°C the fructose solution's sweetness is reduced to that of sucrose. The equilibrium is also affected by concentration, pH and time. Tests have shown that the sweetness at room tempeature of a 5% fructose solution immediately after dissolution is about 1.49 times that of sucrose, and 24 hours later when complete equilibrium has been reached, it is only about 1.25 times. Therefore to provide maximum sweetness fructose is preferably used in neutral or slightly sour foodstuffs and beverages of relatively low sugar content, that are consumed cold.

Although fructose is not expected to substitute completely sucrose, its usage is expanding rapidly and it would become even more favourable when new and more economical production methods have been developed and utilised. The separation efficiency and low energy intensiveness of chromatography favour its application to this field and a large initiative has been commenced by the Scandinavian countries in particular.

3.3 Fructose Production

It is only during the last fifteen years or so that the use of pure fructose has become widespread and this is a result of the development of new more economical industrial manufacturing methods. These methods can be classified as follows:

- hydrolysis of inulin
- sucrose hydrolysis
- enzymatic isomerisation
- dextran synthesis

3.3.1 Hydrolysis of Inulin

Inulin is a polysaccharide occurring in many plants, but it was mainly obtained from Jerusalem artichokes and tubers of dahlias. It consists of about thirty D-fructofuranose units and can be easily hydrolysed to yield comparatively pure fructose. This has been the earliest method of fructose manufacture, but it was not economically viable and has been discontinued (96).

3.3.2 Sucrose Hydrolysis

The disaccharide sucrose consists of one fructose and one glucose molecule, and on hydrolysis it breaks into an equimolar mixture of the two monosaccharides. The hydrolysis (or inversion) of sucrose can be achieved either by "acid hydrolysis", where the sucrose solution is treated with dilute acids, or by "hydrogen ion resin treatment", where the inversion is carried out on a cation exchange resin in the H^+ form. The advantages of the hydrogen ion resin treatment is that it allows continuous sucrose inversion. The hydrolysis product mixture usually contains less than 50% fructose, and some sucrose is also present since it is very difficult to obtain 100% inversion. Lauer et al (117 to 119) have patented a process that inverts sucrose and separates the glucose-fructose mixtures in the same column which was packed with calcium charged DOWEX WX4 resin.

3.3.3 Enzymatic Isomerisation

The actual mechanism involves the rearrangement of the glucose molecule in the presence of an isomerase enzyme in an equilibrium reaction, where a transfer of H^+ takes place between adjacent carbon atoms.

The successful isomerisation of glucose into fructose was achieved as far back as the 1895 by Bruyn and Van Eckenstein (97) who used an alkaline catalyst at elevated temperatures. During the 1930s and 1940s (98), saccharifying enzymes were used to hydrolyse starch into glucose and the glucose was then partially isomerized in the presence of the enzyme isomerase. The breakthrough however took place in 1957 when Marshall and Kooi (99, 100) discovered the enzyme "xylose isomerase" that would isomerise glucose to fructose. The enzyme production however was expensive, at equilibrium the fructose was less than 35%, and the poisonous arsenate and fluoride were present. This however was enough to stimulate the interest of other workers who produced similar isomerases by other microorganisms thus eliminating the need of the above poisonous components, improving their stability and utilizing the fact that they are intra-cellular enzymes by immobilising the cells (101 to 105).

Based on the work of Takasaki and his associates, the American company Standard Brands Inc immobilized glucose isomerase by adsorption on DEAE cellulose. The isomerization was carried out in reactors having shallow beds of immobilized isomerase (115).

In 1975 Novo Industri A/S (Denmark) introduced an immobilized enzyme under the trade name of Sweetzyme suitable for continuous on-column isomerization (116).

The typical composition of the isomerization product mixture is 42% fructose, 52% glucose and the balance maltose and other oligosaccharides.

3.3.4 Dextran Synthesis

Sucrose can be converted to dextran and fructose using the dextransucrase enzyme. Although this technique is still under development if the successful control of the dextran synthesis and fructose separation are achieved the process offers promising commercial viability. This technique would be described in detail in Chapter 4 and it will be investigated in Chapters 4 and 11 by carrying the conversion out on a chromatographic reactor-separator.

3.3.5 Fructose Enrichment

The product compositions obtained by hydrolysis or by isomerisation indicate that there is a need for further separation to increase the fructose purity. This can be achieved by "chemical precipitation" or chromatography. Chemical precipitation is the conventional approach where fructose can be precipitated with an alkaline earth metal. For example fructose is precipitated by adding calcium hydroxide, and the precipitate is removed by filtration. The salt is then dicomposed using an acid, and the fructose is concentrated and crystalised from the solution. This technique however is somewhat outdated and worldwide the application of the novel chromatographic techniques is increasing fast.

3.4 Mechanism of Separation - Complex Formation

Saltman and Charley (107) reported that Ca^{2+}, Ba^{2+}, Mg^{2+} and Sr^{2+} ions form soluble compounds in aqueous alkaline solution with galactose, arabinose, maltose, lactose and fructose. Although their work has shown the existence of such complexes, the chemical formulae and explanation for such complex formations is still hypothetical. Angyal (108 to 113) suggested that in solution a sugar will form a complex with cations

if it contains a sequence of an axial, an equatorial and an axial hydroxyl group on a six-membered ring, or a sequence of three cis-hydroxyl groups on a five membered ring.

According to the Angyal hypothesis, only C^2_5 -β-D-fructopyranose is expected to form a complex with cations since it has an axial-equatorial-axial hydroxide group arrangement (Figure 3.2). Such an arrangement does not exist in any of the glucose forms (Figure 3.1) and therefore it is not expected to form a complex.

The percentage of the β-D-fructopyranose form in equilibrium has been found to be affected by various factors, as it was mentioned earlier (90 to 92). The most important factor is temperature, ie. as the temperature increases the amount of this fructose form is reduced. Since β-D-fructopyranose is also the sweetest fructose form, then during the chromatographic separation of fructose for the production of high fructose syrups, it is important to maintain the temperature low in order to have more of this form available for complexing. Alternatively, it has been found (114) that the use of strongly basic, ion-exchange resins in the bisulphite form results in a complex forming between the glucose and the anions.

If this approach is employed, the fructose becomes the "middle" component, ie.is eluted between the oligosaccharides and glucose peaks, and complete separation would require two "splits" and large recycling. Therefore, the above effect and the questionable stability of the anion resins (22) makes their application less favourable in the fructose production from multicomponent carbohydrate mixtures. In this work a cationic ion exchange resin (cross-linked polystyrene in Ca^{2+} form) was used. The fructose was retarded due to the complex formation and the glucose, maltose and oligosaccharides were carried with the mobile phase and were eluted first.

3.5 Production of High Fructose Corn Syrups (HFCS)

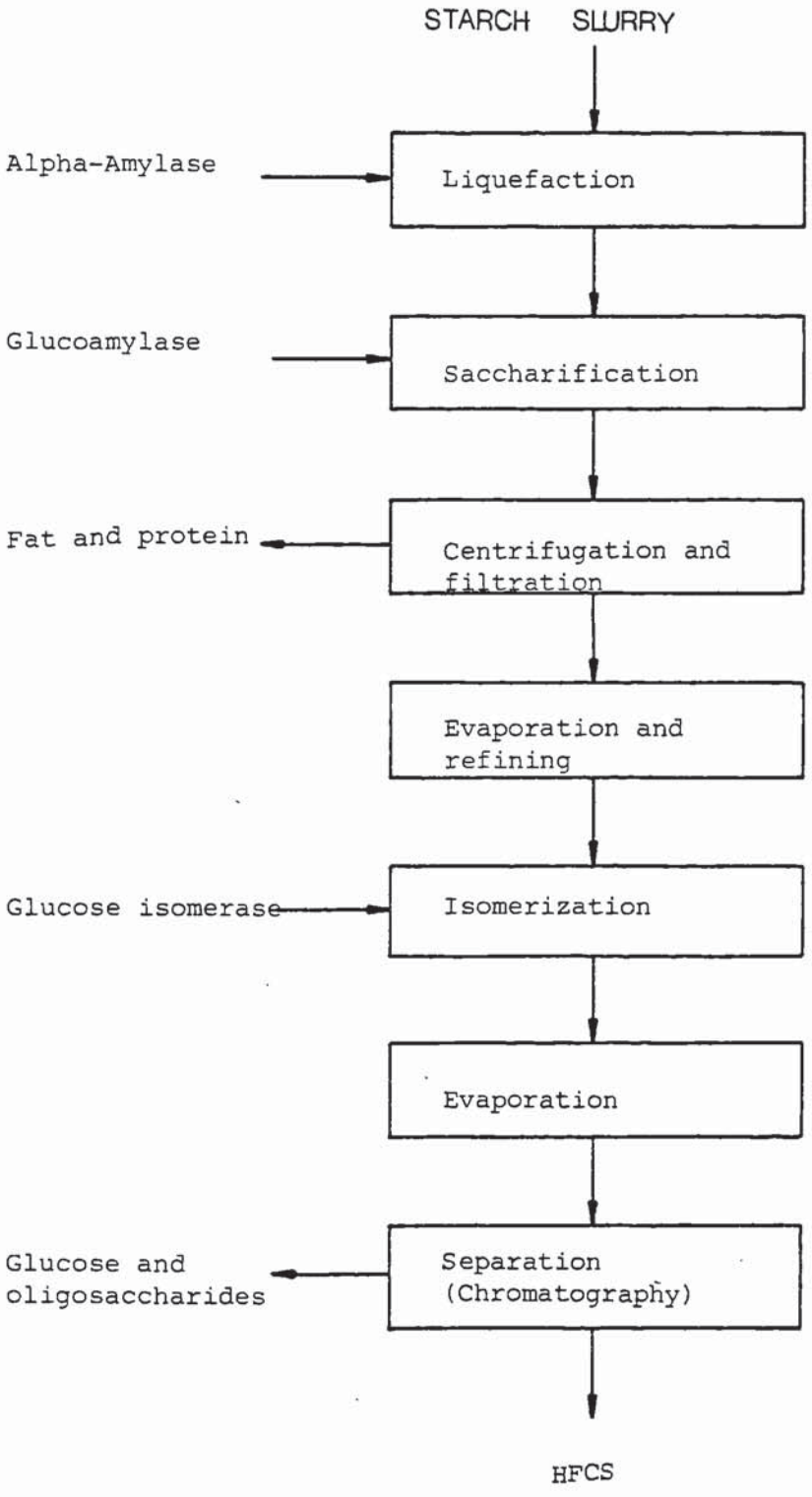
For many years corn was the only important source of sugar, then as a result of shortages during the Napoleonic wars the production of sugar from beets was developed. At the beginning of the nineteenth century, Kirchoff discovered that starch was converted into a sweet liquor containing glucose when boiled with acids. This discovery laid the foundation for further exploitation of starch-based sweeteners. The starch/wet milling industry today processes some 3×10^6 tonnes/year of raw starch in the conversion of cereal and root crops to a variety of syrups, used as energy sources in the fermentation of pharmaceuticals and bio-organic intermediates, as sweeteners, and as stabilisers in the food industry and beverages.

Commercial sources of starch are corn (maize), wheat, barley, potato, sago, cassava, rice, and the utilisation of one or more of the above is carried out according to the climatic conditions and agricultural output of the particular country. By-products such as oil and protein from the corn wet milling process are separated and marketed thus contributing to the overall economic feasibility of the operation.

Starch consists of amylose, a linear glucose polymer containing 1,4-alpha glucosidic links, and a branched polymer, amylopectin, in which linear chains of 1,4-alpha glucose residues are interlinked by 1,6-alpha glucosidic bonds. The glucosidic bonds can be hydrolyzed to produce syrups containing glucose, maltose and other oligosaccharides. This can be achieved by acid hydrolysis but undesirable by-products are formed which are not permitted for human consumption. The hydrolysis is therefore carried out using enzymes classified into Endo-Amylases, Exo-Amylases and debranching enzymes.

An aqueous starch slurry is obtained as a result of physical and chemical treatment of the cereal or root crop. The starch is still intact and requires further treatment before it is degraded to sugars. This process is generally referred as the "liquefaction step". This proces produces maltodextrins which are treated in the

Figure 3.3: A typical flowsheet for the production of HFCS



"saccharification step" to produce syrups containing glucose, maltose and other oligosaccharides. The "saccharification step" is currently carried out in batch processes in the presence of enzymes (such as Amyloglucosidase), but work has been carried out into developing immobilisable enzymes that would allow continuous operation and minimise the operating costs. The glucose in the product mixture of the "saccharification step" is then isomerised to fructose, and concentrated to around 71% w/v by evaporation. DDS Kroyer (Denmark) have built a plant in Pakistan (120) producing 150 ton/day of 42% fructose syrup from 130 tons of broken rice, by employing immobilised glucoisomerase enzymes.

In Hungary Naarden Internation co-operated in giving know-how for the processing of 400 ton/day of maize to produce 150 ton/day of 42% fructose syrup (121). The isomerisation was carried out continuously in columns filled with immobilised glucose isomerase (Taka Sweet, Miles, USA).

High fructose corn syrup is produced by separating the fructose from the isomerase mixture in an additional process which now is usually a chromatographic system packed with an ion-exchange resin. A typical flow sheet of a HFCS producing proces is shown in Figure 3.3. The glucose in the final byproduct stream of the separation step is usually recycled for further isomerisation.

The market specification for the HFCS (or second generation fructose syrup) is at either 55 or 70 or 90% w/v fructose content. The most dramatic plans to expand HFCS in the United States were announced in the early 1970s, and in 1977 50% of the beverage, 23.3% of the food and 22.7% of the baking industries sugar requirements were replaced by HFCS, worth over 2.2 million pounds. Worldwide, the share of the HFCS in the sugar is increasing, (especially in Japan and the Scandinavian countries). Because of the relatively low price of sucrose the HFCS processes have to become more economical in order to increse their share even more. This has motivated researchers into developing cheaper and more stable enzymes, improving the efficiencies of existing

separation techniques, utilising chromatography due to its low energy requirements and high separation potential, and also investigating the possibility of combining the isomerization and separation steps. Hashimoto et al (122) have developed a process for producing higher fructose syrup containing more than 50% fructose that involves a moving column continuous chromatographic system. The system combines selective adsorption of fructose and an immobilized glucose isomerase reaction. The countercurrent movements is simulated by advancing adsorption columns against the fixed inlet and outlet points, while the immobilized reactors are stationary.

3.6 Desugarisation of Molasses

The sugar industry produces approximately 90 million tons/year of molasses, with the UK producing over 100,000 tons/year. Molasses contain about 15% of the total sugars originally present in the beet or cane, and the total sugar content in molasses can be up to 60% w/w. Significant economic advantages can therefore be gained by recovering the sucrose from the molasses. The other impurities present are amino acids, colouring pigments and metallic ions such as Ca^{2+}, Mg^{2+} and K^{+}. This sucrose recovery can be achieved either by precipitation or by chromatographic separation. The later technique has found significant applications in the field and some large scale processes are described in the following section. The recovered sucrose can either be sold as sugar or can be inverted to glucose and fructose. Chromatography has also been used to combine in one step the invertion and product separation (Section 3.3.2), (123).

3.7 Large Scale Chromatographic Processes in Operation used in the Separation of Carbohydrate Mixtures

The existing large scale batch and continuous processes currently in operation are reviewed and a mention is given to other processes that are believed to offer such a potential in the near future.

3.7.1 Batch Processes

3.7.1.1 The FINN-SUGAR Process

The FINNISH SUGAR Co Ltd can be considered as the leader in the carbohydrate field. They have been developing batch chromatographic systems since the early 1950s and since the 1960s they have designed and built several production scale chromatographic systems in Europe and US used for the separation of sucrose from beet molasses and the recovery of crystalline fructose. Columns of 2 to 12m high and 0.5 to 4m in diameter have been used commercially. The columns are insulated to maintain an even temperature and inlet and outlet liquid distributors are used to minimise remixing of the fractions. Cation exchange resins are used having a lifetime of several years (124). The actual separation in the column takes place semicontinuously by employing the repetitive injection technique. The eluent flow is interrupted and a certain amount of pretreated molasses is injected. When the required amount of molasses has been injected the eluent flow is switched on again. During its migration along the column the sucrose and other carbohydrates are retarded by the resin but to a different extent according to their molecular size. The ionized components in the molasses mixture pass through the column faster due to the ion exclusion effect. That is, ionized components cannot penetrate the resin pores which are already highly ionized due to the ions present in the active groups that make up the resin. Therefore during the migration a number of bands are formed in the column. The next molasses slug is injected after a predetermined time interval so that its fastest travelling components will not overlap with the slowest ones of the previous injection by the time they are eluted. During operation however overlapping does take place and these overlapping fractions are recycled.

In 1975 they installed the then largest batch chromatographic molasses separation plant in the world (123) consisting of a 2.7m id column by 3 to 6m high resin, recovering up to 95% of the sugar in molasses and obtaining purities of up to

92%. In 1983 a new plant for the recovery of sucrose from beet molasses was constructed in Frellstedt (Germany) for Amino Gmb H at a reported cost of about $7.6 million (124). Seven resin-filled columns form the chromatographic separation step. The columns were 3.6m id x 12m high, processing 60,000 tons/year of molasses.

It has also been reported (124) that they have installed a plant for Xyrofin in Thomson (Ill, USA) for the production of 13,000 tons/year of crystalline fructose from corn syrup. The syrup is isomerized to a 42% fructose solution, using immobilised enzyme reactors containing glucose isomerase, and then separated in a total of 17 chromatographic columns. Finnish Sugar Co has also developed commercial-scale systems for the production of mannitol, xylose and betaine, and pilot units have been used for the separation of more specialized products such as raffinose, arabinose, lactulose, galactose, mannose, aldonic acids and some amino acids.

3.7.1.2 The Sudzucker Process

In the mid-seventies Munir of Suddeutsche Zucker AG (Germany) reported a new process for the separation of beet or cane molasses. Three columns each of 1m id x 6m high were used, packed with a 4% cross-linked Lewatit TSW 40 (Bayer AG) resin in the Ca^{2+} form. The water eluent was made slightly alkaline by the addition of calcium oxide and was pumped at a linear velocity of 3.4cm min^{-1}. The operation was carried out at 90°C. The main objective of the process was the recovery of sucrose but they claimed that the molasses can also be used as a source of recovery of amino-acids.

Molasses at 50% w/w were injected at feed charges equal to 6% of the total bed volume. An initial non-sugar fraction was removed from column 1 and the sugar fraction was loaded to column 2. Similarly, the non-sugar fraction of column 2 was removed and the sugar fraction was injected into column 3. The non-sugar fraction of column 3 was removed and the sugar fraction was the final product. This continuous

product splitting and redirection was controlled continuously by detector monitors. A 95% sugar recovery was claimed and the product contained up to 11% dry matter of 90% purity.

3.7.1.3 The Colonial Sugar Process

In 1967 the Colonial Sugar Refinery Co (Australia) (126), patented a process for the separation of fructose and glucose from inverted sugar feedstocks. A 1.8m high bed packed with a sulphonated polystyrene cation resin cross-linked with 4% divinylbenzene (DOWEX 50W) was used. The process was operating in a batchwise mode at 60^{o}C and was able to hydrolyse sugars and separate the products. The eluting stream was collected in different fractions of various purities and concentrations. Different fructose rich fractions were obtained, for example using a 50% w/w feedstock containing equal amounts of glucose and fructose, 95% fructose rich products at 24% w/w concentrations or 82% fructose rich products at 29% w/w concentrations were obtained. Although the process looked promising there are no further reports available about its industrial success.

3.7.1.4 The Boehringer Process

Also in 1967, Boehringer et al of the Mannheim-Waldhof Company (Germany) were granted a patent for a process for obtaining pure glucose and fructose from inverted sugars (127). Six glass columns, each of 15cm id x 2m high, were used packed with a low cross-linked polystyrene resin, (Dowex 50 WX 4). The operating temperature was 60^{o}C and the eluent linear velocity was between 1 to 2 cm min^{-1}. The resin was charged at 20^{o}C with calcium chloride solution to render it in the calcium form; the resin was allowed to retain 5 to 30% of its active sites in the hydrogen form. This permitted the

simultaneous hydrolysis of the sucrose present and the separation of the glucose and fructose. Shortly after the disclosure of this patent a number of European countries showed interest in constructing such plants under licence from the inventors.

3.7.2 Continuous Processes

3.7.2.1 The Sarex Process

The modified Sorbex process by UOP (Section 2.13.2) used in the production of HFCS was referred to as the Sarex process (128). A flow sheet of the process is shown in Figure 2.6.

A 50% w/v feed containing 42% fructose is fed continuously into the system. The fructose rich product stream contains over 90% of the fructose in the feed, and has a fructose purity of 90 to 94%. The glucose rich stream contains approximately 80% glucose. The product concentrations are around 20% w/v (129). In spite of the company's experience in large scale applications of the Sorbex process in the hydrocarbon industry, and the inherent advantages of the technique, the Sarex process seems to have missed the 1970s boom in HFCS producing plants in the USA.

3.7.2.2 The Odawara Process

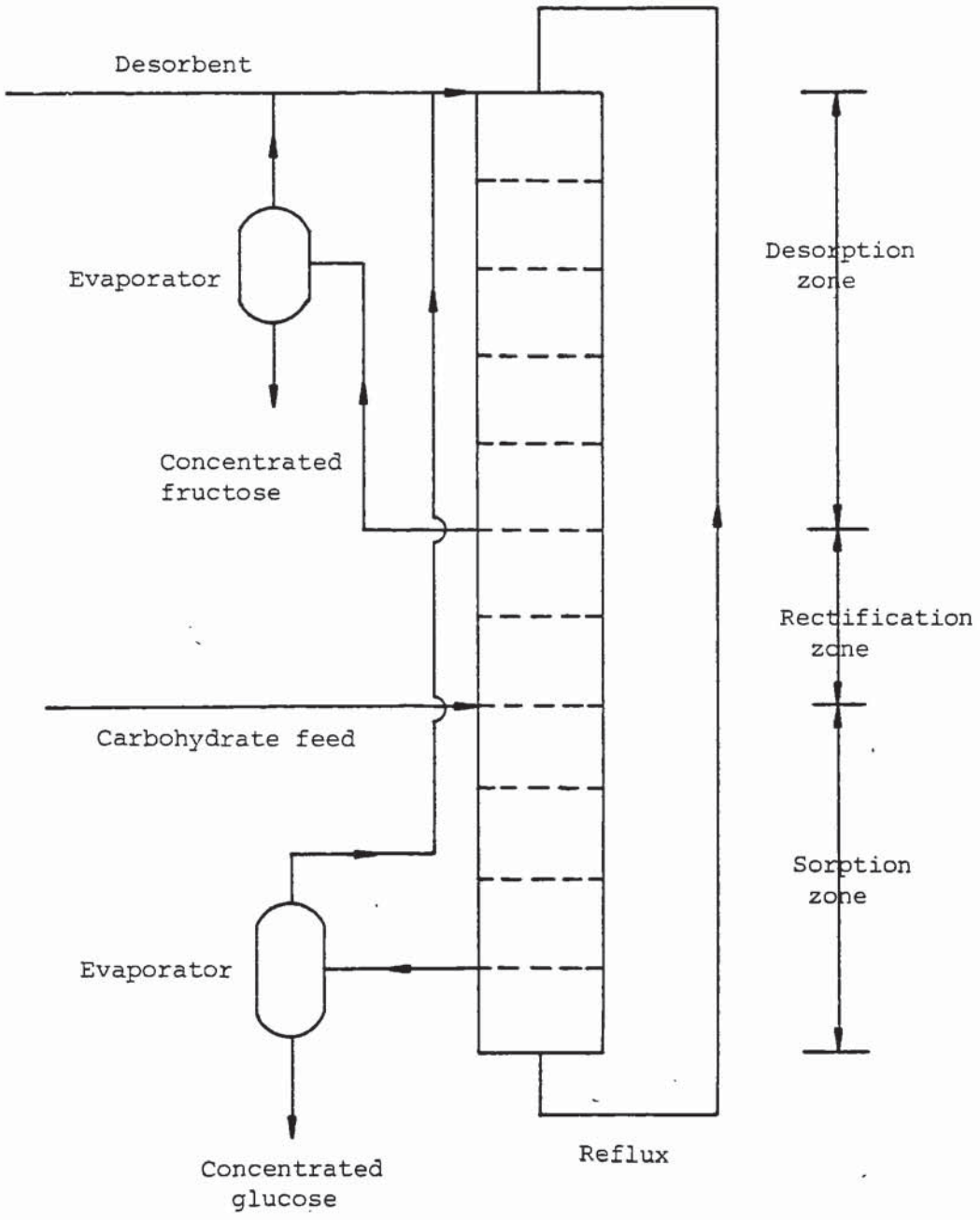
In the late 1970s Odawara et al of Toray Industries Inc (Japan) (130), patented a continuous process for separating fructose from glucose-fructose mixtures. The equipment consisted of eleven columns each of 2.5cm id x 1.5m long, packed to a height of 1.35m by barium zeolite which absorbs the fructose. The liquid streams flow through three serially and circularly inter-connected zones. The desorption, rectification and sorption zones consisting of 5, 2 and 4 columns respectively (Figure 3.4). A total of 66 valves and a timer were used to simulate the continuous operation by the advancement of

entry and exit ports around a closed loop. The system presents a close resemblance to the operation of the SCCR systems. Water was fed continuously as a desorbent at 2.9 kg hr^{-1}. A 7% w/v feed containing 57.5% glucose and 42.5% fructose was introduced at 1.4 kg hr^{-1} and another stream containing 1% w/v sugar solids was continuously refluxed at a rate of 8.5 kg hr^{-1}. The desorption effluent was withdrawn continuously at 12.7 kg hr^{-1}, had a concentration of 1% w/v and contained pure fructose. The raffinate effluent was withdrawn at a rate of 0.2 kg hr^{-1}, it had a concentration of 45% w/v and contained 3% fructose. The product streams were concentrated by evaporation and the desorbent was recycled.

3.7.2.3 The IWT Process

The Illinois Water Treatment Co (USA) has been active in the sweetner industry over the last 30 years. In conjunction with the Finnish Sugar Co they have used a continuous system for the desugarisation of molasses consisting of a number of vertical columns. They have developed their own way of switching the feed and eluent inlet and product outlet points to simulate the operation. Unfortunately, there is not enough information available about the system, its operation and its commercial viability.

Figure 3.4: Diagramatic representation of the Odawara process



CHAPTER 4

4.0 ENZYME BIOTECHNOLOGY

4.1 Introduction

The Spinks Report (1980) (131) defined Biotechnology as the application of biological organisms, systems or processes to manufacturing and service industries.

Microorganisms have many uses, including the production of chemicals such as ethanol by fermentation, brewing with yeast, production of enzymes and as a food stuff (single cell protein). One of the more important and possibly the largest application of biotechnology is in the pharmaceutical industry. The previous chapters highlighted the impact of chromatography in the separation field and gave an indication of its potential to perform a combined operation, ie. performing as a reactor-separator.

In this research work is was also desired to study the scaling up of chromatographic systems operating as biochemical reactors-separators. The validity of the products and specific industrial interest by FISONS Pharmaceuticals plc, led us into choosing the Dextran (a polyglucoside) synthesis from sucrose using the dextransucrase enzyme. Fructose is the other valuable byproduct of the biochemical reaction. One of the prime uses of Dextran is as a clinical product injected intravenously to act as a blood plasma volume expander. In the current industrial manufacturing process, the dextran synthesis takes place in a batch vessel, and then the native dextran is hydrolysed to reduce its molecular weight. The hydrolysate then undergoes a series of ethanol precipitation stages, first to separate the dextran from the reaction products, and to selectively obtain a clinical dextran product within a desired molecular weight range. The clinical specifiation for Dextran 40 requires at least 85% within 12000 and 98000 daltons. By employing a chromatographic reactor-separation as an alternative process the dextran yield will be improved (3) since more high

molecular weight dextran will be formed due to the continuous removal of the acceptor fructose from the reaction mixture. The subsequent employment of size exclusion chromatography and/or ultrafiltration as an alternative to ethanol precipitation (132, 133) is also expected to reduce the operating costs and provide a safer operation by eliminating the fire hazards associated with the use of ethanol. Due to the relatively large amounts of dextransucrase requirements in the scaling up studies of the chromatographic reactor-separators it was required to purify the enzyme in the fermentation broth. The choice of the purification process was based on their scaling up potential, ease of operation, successful integration and most importantly, their minimum contribution to enzyme denaturation.

This Chapter investigates the applicability of different downstream processes for the purification of proteins, describes the mechanism of the dextran synthesis and the dextransucrase enzyme and reviews various chromatographic reactor-separators.

4.2 Enzymes

The three major groups of biological polymers are polysaccharides, nucleic acids and proteins. Proteins are polyamides and their monomeric units are amino acids.

Proteins are one of the major classes of compounds found in living tissues. They are large polymers, usually between 9000 and 200000 daltons, and fulfill a wide range of functions. For example, as enzymes act as specific catalysts, as hormones act as messenger compounds, and as muscle fibres carry out mechanical work. Enzymes are proteins in nature and are produced by living cells. They are present in all living things and if sufficient care is taken to protect them, they can be isolated and purified

from any organism. The great bulk of enzymes used in industry are microbial in origin, but there are exceptions, such as various plant and animal proteases. An enzyme in its active state (the holoenzyme) is composed of a protein part (the apoenzyme) and occasionally, by ions or molecules of a different nature (cofactors). The apoenzyme is always the largest part of the molecule and its structure is most important for its catalytic effectiveness. The amino acids which form the enzymes are almost exclusively of the L-series and their sequence determines the primary structure of the protein. The secondary structure consists of those sections of the polypeptide chain that assume certain well defined structures such as the α helix. In addition to their secondary structure, proteins have higher orders of structure (tertiary, quarternary) due to interactions between side chains and between individual polypeptide chains. It is a universally accepted hypothesis, supported by many experimental facts, that biocatalysed reactions take place in well defined areas, or cavities, of the macromolecule forming the apoenzyme. These regions are called "active sites" and are of limited dimensions with respect to the whole protein surface. An enzyme can have one or many active sites, and a knowledge of the active site structure is essential for understanding the mechanism of enzyme catalysis. The nature and the relative position of the amino acid side chains contribute together to make a site active. All the three or four levels of protein organisation thus determine the catalytic function. Enzymes can be classified into the following groups according to the reaction they catalyse:

- Oxidoreductases, catalysing oxidation reduction reactions;
- Transferases, catalysing the transfer of atomic groups from a donor to an acceptor, (aldehydes, ketones etc);
- Isomerases, transforming the substrate into an isomer (ie. glucose to fructose);

- Lyases, catalysing the addition to or formation of double bonds;
- Ligases or synthetases, catalysing the union of two molecules by breaking a pyrophosphate bond.

As mentioned earlier, some of the enzymes are also composed of cofactors. This term indicates all those inorganic ions (eg. HPO^{2-}_4, or metallic ions such as Zn^{2+}, Mg^{2+}) and organic substances which co-operate with the enzyme in transforming the substrate. They are indispensable for the catalytic action of the enzyme.

4.3 Properties of Enzymes

Specificity. All *enzymes are biological catalysts*, that increase the rate of biochemical reactions without suffering any overall change of their own. The reactants in enzyme catalysed biochemical reactions are referred to as substrates. Each enzyme is specific in character acting on particular substrate(s) to give particular product(s). The specificity of enzymic reactions depends greatly on the structure of the active site. An initial description of the interaction between enzyme and substrate which still appears to be substantially valid is the "lock and key" hypothesis. According to this, the active site can be compared to a lock, and the substrate to the corresponding key. An improved description is the "inducet-fit" hypothesis, according to which, the site itself should undergo modification on interacting with the substrate, which in turn, would be altered in its molecular orbital structure during the binding with the enzyme. An enzyme-substrate complex is so formed and the precise electronic, steric and entropic situation favours the ensuing chemial reaction.

Activity. The enzymes are usually characterised according to the nature of the reactions they catalyse (Section 4.2) and their catalytic activity. The activity is a measure of the quantity of enzyme required to achieve a specified product yield. Since

most industrially used enzymes are marketed according to their activities, the activity provides a basis for estimating the prodution costs and also influences the plant size. The activity assay is usually carried out at conditions (ie. temperature and pH which correspond to the actual operating conditions of the biocatalysed reaction. These conditions under which the activity assay was carried out must always be stated when an activity figure is quoted. Another parameter which also needs to be stated is the method used in the activity assay. Usually the different activity determination assays are named after the detection procedures used, ie. manomeric, electrochemical, radiochemical, HPLC and spectrophotometric methods. During the 1960s, the International Union of Biochemistry defined an enzyme unit (U) as the amount of enzyme that will transform one micromole of substrate per minute under defined conditions. The activity sometimes is referred to as "specific activity", (ie. U/mg of enzyme) or "molecular activity" (ie. U/μmol of enzyme). Although these international activity definitions exist, various enzyme suppliers or users have their own definitions, therefore there is an extra need to state clearly the conditions under which the assay has been carried out.

Stability. Denaturation or destabilization are the generally used terms for inactivation of proteins. Proteins in general exhibit denaturation in aqueous solutions or suspensions and in air-liquid interfaces. Broadly speaking stability is also related to molecular size, large protein molecules being more unstable than peptides. The main factors that affect the stability of enzymes are temperature, pH and physical effects. Enzymes are usually very stable at temperatures below 4^{o}C and loose their stability at relatively high temperatures, with only a very small fraction of them being stable at temperatures of 80^{o}C or above. Usually temperatures around 15 to 30^{o}C give faster reaction rates. During operation a compromise should be made between reaction rate and stability. In general, enzymes are active over a limited pH range due to the pH's effect on the ionisation state of the amino acids, and the pH vs activity curve is of a

bell shape. The physical effects are the activity losses associated with heat generation and shear forces during the enzyme recovery and purification stages. Denaturation is believed to take place in two main steps. These are a reversible unfolding of the molecule, followed by irreversible changes leading to a permanent loss of biological activity. Denaturation is a chain process, once a few bonds have been broken for any reason, further disruption of the secondary and tertiary structure occurs rapidly.

During processing therefore, the following precautions should be taken:

- the temperature must be maintained as low as possible;
- the pH must be maintained within a close range (ie. 4 to 8);
- frothing and exposure to large surface areas must be kept to a minimum (134);
- during processing the usage of high shear pumps and other process equipment must be avoided (134);
- the process time should be minimised and storage should take place under controlled conditions;
- hygienic operation is necessary not only to avoid the biodegradation of proteins but also to prevent any contamination by other microorganisms.

4.4 Enzyme Kinetics

Kinetic and thermodynamic data on enzyme reactions generally indicate the following steps:

- formation of a complex between the active site of the enzyme and reagents (substrate(s) and cofactor(s)). This step is usually completed in a few seconds;

- reaction and subsequent formation of an enzyme-product complex;
- dissociation of the enzyme-product complex, and regeneration of the active site in the appropriate state for receiving the reagents again.

Many enzymic reactions can be described by the Michaelis-Menten equation:

$$r_o = \frac{r_{max}\,[S]_o}{K_m + [S]_o} \qquad \text{..... 4.1}$$

which holds within the following limitations:

- the concentration of only one substrate implied in the reaction can vary. Other substrates can participate in the reaction only if their concentrations remain constant;
- the initial concentration of the substrate must be much larger than that of the enzyme;
- the whole enzyme catalysed process should fit the following scheme:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad \text{..... 4.2}$$

where:

E	= enzyme
S	= substrate
ES	= enzyme-substrate
P	= product
k_i	= reaction constant
r_o	= initial rate of product formation
r_{max}	= maximum rate of product formation
K_m	= Michaelis constant
$[S]_o$	= initial substrate concentration

A good agreement with the Michaelis-Menten equation is found for the kinetics of enzymes such as hydrolases, many transferases, many isomerases and lyases. In other cases much more complicated kinetic equations must be used, ie. when a second enzyme-substrate complex (ES') is formed as an intermediate. The corresponding sequence is:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} ES' \xrightarrow{k_3} E + P_2 \qquad \text{..... 4.3}$$

$$ES' + P_1$$

The four most important factors which can influence the rates of an enzymic process are, temperature, pH, the presence of activators and the presence of inhibitors.

A temperature variation influences the rates of the single reaction stages as it either modifies Arrhenius activation energies, or, according to the transition state theory, the equilibrium between the reagents and the corresponding activated states. Almost all enzymes are extremely sensitive to the pH, and generally their activities decrease if the solution pH varies from the optimum pH for the particular enzyme.

Certain compounds can bind themselves to the enzyme without undergoing any chemical modification. Those compounds, which after binding to an enzyme, increse the rate of a corresponding enzyme reaction are called activators, whilst the ones which act in an opposite way are called inhibitors. Inhibition is by definition reversible, ie. once they are removed the enzyme regains its original activity. Inactivators are those substances which deactivate the enzyme irreversibly.

4.5 Enzyme Recovery and Purification

4.5.1 Downstream Processing

Downstream processing involves the separation and purification of a essential product or products from a fermentation process. Downstream processing used to be the most neglected part of biotechnology although it represents a major part of the overall costs of a process; almost as much as 40% of the total costs excluding the raw material costs (136).

The "biotechnological revolution" however will only be materialised when the laboratory bench concepts and processes are transferred to the market place and compete commercially with conventional technologies. Over the last ten years the significance of the downstream chemical engineering related processes has been appreciated by the biochemical industry and increased amounts of capital injections, and joint efforts have been initiated to achieve a successful "debottlenecking".

The first stage of purification is to release the enzyme into solution from the cells which produce it, although many microbial enzymes that degrade or sometimes synthesise polymeric substances are found extracellularly, and therefore only separation from the cell is necessary. Once the enzyme is recovered in solution it usually requires further purification to separate it from other impurities which might either be byproducts or substances added in the fermentation medium. During the downstream processing it is essential to try and prevent denaturation thus minimising the overall production costs. Summarising, the factors that need avoiding are: heat, shear, air-liquid interfaces (foam), large stainless steel surfaces (134), high velocity gradients, dilution, oxidation and loss of any stabilizing agents. The pH of the medium must be within the pH range of the particular enzyme, and also good

management practice must be employed. Any accidental spillage to the environment must be avoided since enzymes are known to cause allergies to humans. Therefore, there is a need to comply with the regulations set by the Genetic Manipulation Advisory Group (135).

Another important parameter in the downstream process selection is the rheology of the enzyme mixtures, since proteins in general exhibit Non-Newtonian behaviour.

4.5.2 Enzyme Recovery

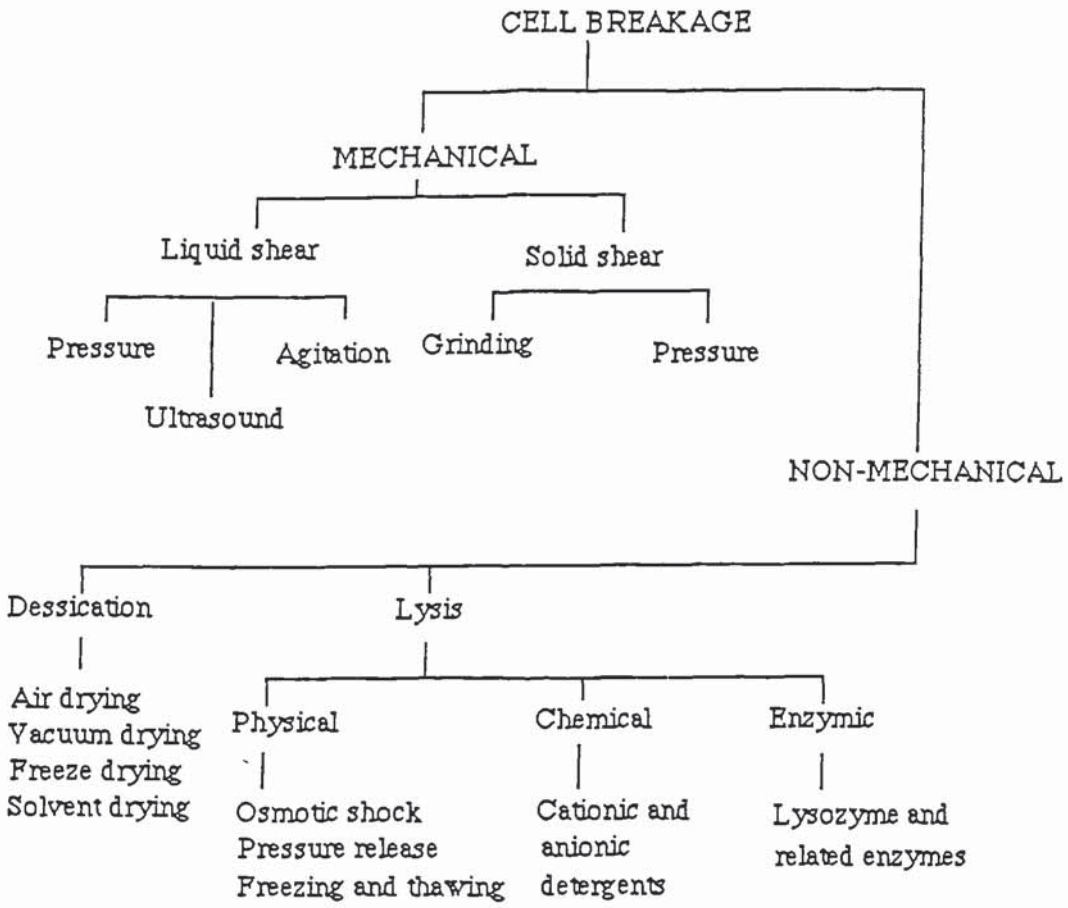
For intracellular enzymes, the enzyme first must be removed from the cell and recovered in the liquid phase. There are a range of operations that are available and are classified as mechanical and non-mechanical. A listing of the various alternative ways is shown in Figure 4.1.

4.5.3 Enzyme Purification

Once the enzyme has been recovered from the cells, for intracellular enzymes, or the fermentation broth, for extracellular enzymes, need further purification to give pure and more concentrated enzyme. The various purification processes applicable utilise one or more of the following properties:

- Electrical charge. Ion-exchange, two-phase aqueous partition, flocculation, fractional precipitation, chromatofocussing.
- Solubility. Isoelectrical precipitation, crystallization, precipitation with neutral salts, organic solvents and polymers.
- Molecular size. Membrane separation, size exclusion chromatography.

Figure 4.1: Cell Breakaging Techniques



- Hydrophobicity. Flotation, foam separations, two-phase aqueous partitioning, hydrophobic adsorption.
- Density. Flotation, centrifugation.
- Specific binding. Affinity precipitation, affinity chromatography, electrophoresis.

Some of the above processes are reviewed below. During the process selection, emphasis must be given on their scaling up potential, recovery efficiency, reliability, cost, process time, capability to accommodate large reductions in volume and to allow easy sterilisation.

4.5.3.1 Centrifugation

Centrifugation is probably the most widely used solid removal process in biotechnology. On the laboratory scale it is extremely efficient, but for large scale operations it looses some of its separation efficiency due to mechanical difficulties that limit the maximum centrifugal force obtainable. Efficient operation is favoured by large particle diameters, large density differences between the solid and fluid phases, and low viscosities. High angular velocity, large centrifuge diameter and thin sedimentation layers also assist. Biological materials however are usually small and of low density and relatively high viscosity.

Choosing the right centrifuge for a particular application is not too easy because the manufacturers provide a limited information and the highly competitive nature of the downstream purification industry results in process engineers being unwilling to reveal optimum operating parameters found from their experience. There are a number of centrifuge types available, ie:

- Tubular bowl centrifuge, consisting of a cylindrical tube driven by an overhead motor. Angular velocities (50000 rpm) can be obtained and the operation is continuous, although occasional stops are required to clean the bowl from the cell precipitate.
- Multichamber and disc centrifuges. They have relatively large radius and operate at low angular velocities. Batch and continuous discharge machines are available. The bowl is usually located above the gearbox, thus some heating takes place. Disk bowl centrifuges are widely used in industry.
- Solid bowl scroll centrifuge. This comprises an Archimedean screw inside a solid bowl. Continuous operation usually takes place, but due to the low rotation speeds only coarse solids can be removed efficiently.
- Perforate bowl basket centrifuge. This centrifuge resembles the domestic spin-driers. It is an efficient cell removing unit, but usually operates in a batch mode and safety considerations are very important.

Usually all centrifuges allow sterilisation and most of them have some means of refrigeration. Among its advantages are low process times, no need for additives, fairly good containment, and ease of sterilisation. Centrifuges however are generally efficient only for the removal of cells from biochemical feedstocks, generate substantial shear forces, and the high moving parts require additional safety precautions.

4.5.3.2 Membrane Processes

Membrane processes are increasingly gaining popularity and applications in downstream processing. They are used for cell removal, selective separation of the various soluble components in the fermentation broth, and concentration of final

enzyme mixture. In most of the commercial membrane processes the driving force is pressure and are classified as follows:

- Microfiltration. Microfilters have the most "open" membranes, and are used to reject suspended particles having molecular sizes over 0.1μm. Their rejection of proteins however is still under debate.
- Ultrafiltration. These membranes have a "finer" pore size and can reject smaller "dissolved" components, down to some macromolecules (ie. proteins) with molecular wights of a few thousand and molecular sizes of the order of 10nm.
- Reverse Osmosis. Consisting of the "finest" membranes rejecting not only macromolecules but also dissolved ionic salts and small molecules of a few hundred molecular weights.

Another membrane filtration mode is dialysis which is usually carried out using ultrafiltration type membranes but the separation is based on a concentration gradient across the membrane. A third category of membrane proceses includes electro-filtration and electrodialysis (137), where the driving force is an electrical potential gradient. Usually all the membrane units have the form of either flat disks or hollow cartridges. The hollow cartridges consist of a large number of membrane tubes placed inside the same holder. They have the greatest industrial potential since they combine large membrane surface area in relatively small overall sizes, and most importantly, because the fluid flow is in a "cross-flow" arrangement which helps to remove some of the "gel layer" formed on the membrane surface due to the concentration polarisation effect.

From the above processes microfiltration and ultrafiltration are the more established as industrial scale unit operations. Various figures have been published

forecasting potential growth rates in sales of membranes and associated plant over the next ten years (138). It was estimated that in 1985 the total value of membranes and associated plants sold worldwide were in the region of $2 billion, with membrane sales accounting for almost half of this figure. The old and most of the current generation of membranes are made of synthetic materials, and although they have been developed to present good chemical stability in both alkaline and acid conditions, they cannot be sterilised effectively. Some new ceramic based membrane allow sterilisation but since they are still under development there is some uncertainty about their separation efficiency. The process times are relatively long and the shear forces considerable, therefore low shear pumps should be used (134). By far the largest problem with membrane systems however is their "fouling" especially by proteins (139). By definition "fouling" is the accumulation of deposits on the surface of a membrane. The gel layer formation would be expected theoretically to be reversible. In practice the gel layer formed due to the gel polarisation effect is reversibly increased or decreased in thickness by altering operating conditions. Irreversible gel formation however can occur due to the precipitation of scaling materials and adsorption of certain feed solutes, such as proteins by the membrane which can even cause pores to become clogged (140, 141). This membrane fouling alters the membrane characteristics, ie. membranes with molecular weight cut-offs of 300000 daltons would even reject molecules with molecular weights below 50000 daltons. The mechanism of fouling however is still poorly understood. Membranes in general provide a very efficient means of separtion and concentration and it is believed that their applications would be increased further once the above problems have been understood and overcome.

4.5.3.3 Precipitation

Precipitation is one of the most commonly used methods in enzyme purification. Protein aggregation (precipitation) can be achieved by changing the

environment thus making the inter-protein attraction greater than that with the solvent. Such changes include changes in ionic strength, pH and dielectric constant changes.

One of the most interesting developments in the enzyme precipitation field has been the use of high molecular weight polymers as precipitants. Sternberg et al (142) have recently described the use of polyacrylic acids. Bergmeyer et al (143) and Boehringer have described and patented the use of polyethylenimine as a precipitant.

Polyethylene glycol (PEG) can also be used very satisfactorily to precipitate proteins (144). Unlike solvents, it has a protein-stabilizing effect and thus may be used at ambient temperatures and cause minimum activity loss. It is effective at relatively low concentrations, with most proteins being precipitated at PEG concentrations of 6-12%, and thus 50% w/w aqueous solutions of the precipitant (molecular weights from 1500 to 8000) may be used very conveniently.

4.5.3.4 Chromatography

This introduction of new efficient chromatographic techniques for the separation and purification of proteins over twenty years ago had a dramatic impact on the development of research in the biochemistry and molecular biology fields. The transfer however of the new technology from the bench to the production scale was very slow. The main problem seems to be design limitations to match the soft gels available for protein separations. This is probably the reason that only two large scale techniques were available in the 1960s, ie. adsorption on ion exchangers such as DEAE-Sephadex A-50 and desalting on gel filtration media such as Sephadex G-25. During the seventies new packing materials were developed, such as DEAE-Sepharose CL-6B, Ultro-gel and Sephacryl, which were more compatible for large scale operation. The various chromatographic processes employed in protein purification

can be classified in the following three groups. Gel Filtration, Ion Exchange and Affinity chromatography.

4.5.3.4.1 Gel Filtration Chromatography

This technique, which is well known on the laboratory scale as a method for determining the molecular weight of macromolecules, such as proteins, serves also for their purification. Many different names have been used for the same principle: gel permeation, molecular sieving, size exclusion. The materials most frequently used as the stationary phase are various grades of Sephadex (cross-linked dextrans), Sepharose (agaroses) and Bio-Rad gels (polyacrylamides), among others. The first industrial applications were group fractionations (desalting) where low molecular solutes were removed from protein solutions. Columns as big as 1.8m dia x 1m high have been used commercially in Sweden,producing 28kg of 75% protein powder per hour (145).

4.5.3.4.2 Ion Exchange Chromatography

Many types of ion exchange material are available which fall into two classes. The cellulose ion exchangers, which are cellulose combined with anionic or cationic charged groups. The most commonly-encountered ion exchange celluloses are diethyl-aminoethyl (DEAE) and carboxymethyl (CM) cellulose, anion and cation. Ion- exchange resins are water soluble polymers containing anionic or cationic groups such as $-SO_3^-$, $-COO^-$, or $-NH_3^+$. Ion exchange chromatography is a powerful purification technique, due to the high protein capacities which can be used (ie. 50g of protein per litre of ion exchanger) and due to the variation of elution procedures that are possible, (ie. combinations of pH, ionic strength and continuous and stepwise gradients). The most widely used large scale applications of ion-exchangers are

in human plasma protein fractionation (146), hormone (insulin) purification and the production of egg white lyosozyme (147).

4.5.3.4.3 Affinity Chromatography

This is a highly specific technique, as applicable to large scale use as it is to bench scale, which relies on biospecific interactions. Enzymes are capable of binding to their substrates, inhibitors, co-factors and specific antibody. If one of these, such as a monoclonal antibody, is covalently linked to supports suitable for use in chromatography, it will bind specifically to the appropriate enzyme. It is essential however that the binding is reversible, to enable the release of enzyme. Elution of the enzyme usually involves a change in pH, ionic strength or buffer composition. A comprehensive review of affinity separations has been carried out by Chase (148).

The high specificity and capacity of affinity chromatographic matrices means good flow rates and rapid separation and purification since column bed volumes are generally small. Large scale affinity chromatography is an area of growing importance in biotechnology. It is used for the purification of human interferon, removal of contaminants such as pyrogens, viruses, etc from medical substances, and for the purification of enzymes, such as phosphoglycerate kinase, alcohol dehydrogenase, malate dehydrogenase, and glycerokinase among others (149).

4.6 Dextran and its Applications

Dextrans are polysaccharides, which yield D-glucose on ultimate hydrolysis, and are characterized by predominance of α-(1—6)-linked anhydro-D-glucopyranosyl units. The early history of dextran goes as far as 1874 when Scheibler (150) gave its name.

The extracellular enzyme dextransucrase is able to synthesize dextrans from sucrose accordingly to the overall equation:

$$n \times \underset{\text{sucrose}}{(C_{12}H_{22}O_{11})} \longrightarrow \underset{\text{dextran}}{(C_6H_{10}O_5)n} + n \times \underset{\text{fructose}}{(C_6H_{12}O_6)}$$

The mechanism of the biosynthesis will be mentioned later on.

Probably the largest dextran usage is in the pharmaceutical industry as blood volume expander (average molecular weight of 70000) and blood flow improver (average MW of 40000). The molecular weight distributions of these fractions are controlled by various specifications and have been reviewed by Nilsson (151). The dextran and its derivatives which find commercial usage have been reviewed by Murphy (152), Baker (153) and Alsop (154). Some of these usages are: for the stabilisation of soil aggregates, as protective coating for seeds, as deflocculants in paper industry, in oil drilling muds, in the manufacture of packings for chromatographic columns etc.

4.7 The Dextransucrase Enzyme and the Dextran Synthesis

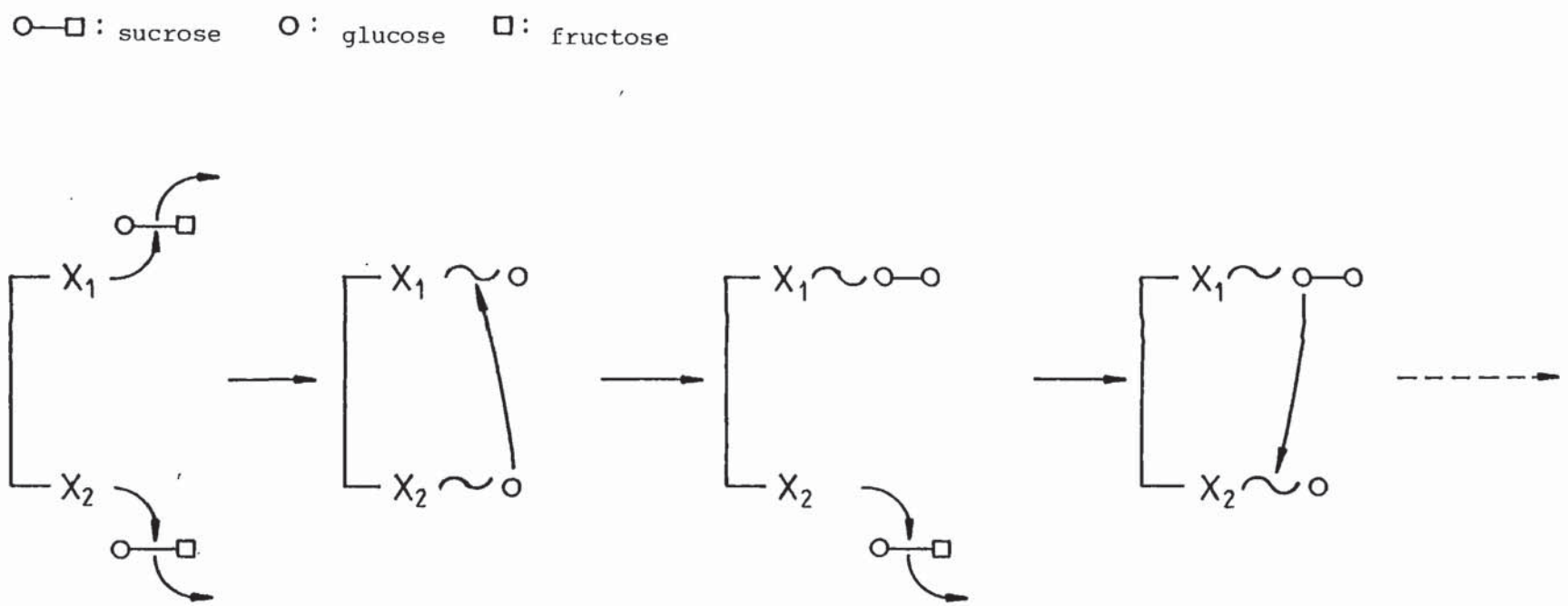
The extracellular dextransucrase is the enzyme that biocatalyses the conversion of sucrose to dextran and fructose. The main dextran synthesizing bacteria belong to the genus Leuconostoc, species Mesenteroides and dextranicum, tribe Streptococceae, and family Lactobacillaceae (155). In the western world the clinical dextran is produced from the Leuconostoc Mesenteroides NRRL strain B512F. This strain is non-pathogenic and produces mainly linear dextran, ie. containing 95% 1,6-and 5% 1,3-α-D-glucopyranosidic linkages (154).

After many years of controversy (156, 157, 158), it now appears that the reaction takes place according to the proposal by Robyt et al (159 to 162) which is:

two nucleophiles at the active site (X_1 and X_2 in Figure 4.2) displace fructose fro n two sucrose molecules, giving two β-glucosyl intermediates; these two intermediates rotate so that the C_6-hydroxyl of one is opposed to the C_1 of the other, a nucleophilic attack of the C_6 (wrt X_2) on the C_1 (wrt X_1) displaces the enzyme nucleophile a id forms an α-1,6 bond; the free active site nulceophile (X_2) attacks another sucr s molecule to give another β-glucosyl intermediate, then the cycle proceeds forming the growing dextran chain from the active site. The biosynthesis terminates when an acceptor product is added on the glucosyl or dextranosyl unit. If the acceptor is a low-molecular weight carbohydrate, the displacement of the glucosyl unit results in an oligosaccharide. Subsequent products may occur with the acceptor product acting also as an acceptor. Robyt (163) reports over 30 different acceptor products. The second reactor product fructose is a relatively strong acceptor, giving leucrose and isomaltulose (164). Theoretically, stoichiometry predicts that 100g of sucrose give 52.6g monosaccharides (fructose) and 47.4g of high molecular weight (HMW) dextran. In practice however disaccharides and lower molecular weight dextran is formed due to the acceptor reactions. Alsop (154) has reported the HMW dextran fraction to decrease sharply with increasing sucrose concentrations (ie. for sucrose concentrations 2,10 and 20% w/v the corresponding HMW dextran fractions were 45.9, 39 and 17.9%). Robyt and Walseth (162) proposed that the acceptor reaction proceeds by nuclecophilic displacement of glucosyl and/or dextranosyl groups from the enzyme-complex by a hydroxyl group of the acceptor. The acceptor reactions terminate polymerisation by displacing the growing dextran chain from the enzyme active site.

Therefore to obtain dextran having large proportions of the HMW fraction, the fructose in particular, must be removed immediately from the reaction mixture. This can be achieved using a chromatographic reactor-separator packed with a cationic

Figure 4.2: Reaction mechanism for the dextran synthesis (159 to 162)



resin which removes the fructose from the reaction mixture by forming a loose chemical complex. The scaling up evaluation of such a system will be carried out in Chapter 11.

4.8 Properties and Purification of Dextransucrase

During the enzyme production the optimum pH is between 6 7 and 6 9 while for enzyme action is 5.2. As an indication of the effect of pH on enzyme action, at pH 4.8 and 5.6 the enzyme has 90% of its maximum activity. For a pH below 4.8 and over 5.6 the activity drops sharply. Dextransucrase has its maximum activity at 30°C, but an operating temperature of 25°C is recommended since at higher temperatures a rapid decay occurs. At 4°C the enzyme was found to be stable for 3 weeks, at 35°C for 1 hour and at 40°C it was completely destroyed in 15 minutes (165). Miller and Robyt (166) found that the enzyme lost half of its activity after storage for 2 days at 4 or -15°C. A number of alternative activity assay methods have been reported (167 to 170), the method however employed in this work was the following. A unit of dextransucrase activity (DSU) was defined as the amount of enzyme which will convert 1mg of sucrose to dextran in 1 hour under the actual operating conditions, ie. pH of 5.2 and 25°C. The progress of the reaction was monitored by carrying out an HPLC analysis at set time intervals and tracing either the sucrose disappearance or the fructose formation.

Because of the instability problems of the enzyme, a large amount of research work has been done to identify the right stabilising agents. Robyt and Walseth (169) stated that during storage the enzyme stability increased by the addition of 4mg/ml of dextran; the activity decreased by the adition of ethylenedinitrilotetra-acetic acid (EDTA) and was restored by the addition of calcium ions. They also found that the presence of zinc, cadmium, lead, mercury and copper ions were inhibitory to various degrees.

The inhibitory effects of EDTA has also been reported by Kobayashi and Matsuda (171-173). Kaboli and Reilly (170) and Lopex and Monsan (174) have reported that approximately 0.05mM of Ca^{2+} are enough to give maximum stabilization. Miller and Robyt (166) reported that 2 μg/ml of HMW dextran or 10 μg/ml of PEG 20000 or 10 μg/ml of the nonionic detergent Tween 80 were enough to stabilize the dextransucrase. They found that calcium had no stabilizing action in the absence of other additions, but reduced the inactivation that occurred in the presence of 0.5% bovine serum albumin or high concentrations (> 0.1%) of Triton X-100. Table 4.1 contains a summary of the various reagents and metal ions on dextransucrase activity (165, 173).

The crude dextransucrase has a number of impurities present, such as leucrose, fructose, mannitol, other proteins and enzymes, dextran and oligosaccharides, which need purification. All the bench scale purification methods involve a combination of processes such as precipitation, gel chromatography, ion exchange chromatography, dialysis, ultrafiltration and electrophoresis.

Robyt and co-workers (166, 169) purified dextransucrase by cocentration and dialysis of the culture supernatant with a Bio-Fiber 80 miniplant, and by treatment with dextranase followed by chromatography on Bio-Gel A-5m. A 240-fold purification with a specific activity of 53 U/mg was obtained. Contaminating enzymes such as levansucrase, invertase, dextranase, glucosidase and sucrose phosphorylase were decreased to non-detectable levels. They reported that the purified enzyme lost activity at 25°C or on manipulation, in a same way the crude enzyme did but when it was diluted below 1U/ml.

Kobayashi and Matsuda (171, 172, 173) purified the enzyme by $(NH_4)SO_4$ fractionation, adsorption on hydroxyapatite, chromatography on DEAE-cellulose and gel filtration on Sephadex G-75. Enzyme activities reached up to 32 fold of the original, and the enzyme had a molecular weight between 64000 and 69000.

Table 4.1: Effects of Metal Ions and Chemical Reagents on the Dextransucrase Activity

Compound	Remaining activity* (173)	
	without Dextran	with Dextran
None	100	136
$CaCl_2$	111	124
$CuCl_2$	7	4
$MgCl_2$	97	125
$FeCl_2$	83	119
$FeCl_3$	20	0
$CoCl_2$	100	112
$MnCl_2$	38	52
Idoacetic acid	56	56
N-Ethylmaleimide	79	113
p-Chloromercuri-benzoate	88	110
Phenylmercuric-acetate	6	8
Sodiumdodecyl-sulfate	118	182
EDTA	8	96
Sodium thioglycolate	105	130

* Final concentrations of metal ions and chemical reagents were 1mM and 0.5mM except for p-chloromercuribenzoate which was 0.05mM.

Kaboli et al (170, 175) obtained enzyme of high stability by no using dextranase for hydrolysis. They used ultrafiltration (Amicon XM-100A) and gel chromatography (LKB ultrogel AcA34) to obtain a 247 fold purification with 54% yield. The specific activity was 58.6 U/mg. Bhatnagar et al (165) used dialysis ammonium sulphate fractionation, DEAE cellulase chromatography and Sephadex G-200 gel chromatography to obtain a 146 fold purification. The specific activity of the final enzyme was 3566 U/mg, and the enzyme had a molecular weight of 95000±5000.

Lopez and Monsan (174) concentrated the fermentation broth using ultrafiltration and purified the enzyme by gel chromatography on Ultrogel AcA-34. They obtained specific activities of 2500 DSU/mg and reported that the activity was enhanced greatly by the addition of calcium chloride. *Paul et al (176, 177)* recommended the repetitive PEG phase partitioning technique for the purification and reported a comparison with alternative purification techniques. Ultrafiltration, gel chromatography and phase partitioning gave enzyme recoveries of 75, 78 and 95% respectively and corresponding specific activities of 860, 2035 and over 3500 $DSU.mg^{-1}$. In 1982 workers from Fisons Pharmaceuticals (178) patented a purification method that involves ethanol precipitation and chromatography on DEAE A25 (Pharmacia) anionic resin. They claim 67% enzyme recovery with specific activity in excess of 3000 $DSU.mg^{-1}$. The resolubilisation difficulties associated with the ethanol precipitation led them into using PEG6000 precipitation instead, and a new patent has been applied for.

From the above review it is apparent that the dextransucrase purification is possible. In scaling up however, the process selection depends on the relative operating costs, ease of operation, process time and activity losses; therefore a compromise is required.

4.9 Immobilisation of Dextransucrase

To minimise process costs and excess enzyme usage, d[illegible]y t[illegible] immobilisation of the purified enzymes should be preferred. Some of the advant[illegible]es of using immobilised enzymes are:

- continuous operation and easy control;
- easy separation of the products;
- minimun effluent problems and material handling;
- reuse of enzyme.

The support used for the immobilisation should:

- have large mechanical stability;
- increase substrate binding;
- decrease product inhibition;
- discourage microbial growth;
- be reusable.

The employment however of immobilised enzyme reactors has a number of problems as well, such as:

- loss of activity due to denaturation or poisoning;
- reduced enzyme-substrate contact due to changes in flow pattern/enzyme distribution or fouling;
- microbial contamination;
- reduced productivity due to solubilisation of enzyme and support or attrition of the support.

The immobilisation of dextransucrase has been attempted by various researchers but unfortunately it has been proven very difficult. The dextransucrase immobilization on anion exchange resins (eg. DEAE-cellulose and DEAE-Sephadex) has been reported by Ogino (179) on Bio-Gel P2 beads by Robyt et al (15 , 161 and on polysulfone hollow fibers by Edwards and Drew (180). Kaboli et al (70 17 have carried out immobilisation work on Watman No 1 filter paper to Enzacryl materials, to DEAE-cellulose, DEAE-Sephadex, A25, A50, porous silica and to SP-sephadex C-25 and C-50 at pH conditions between 3 and 7.

From all 13 carriers they surveyed only silica allowed more than 10% retention, and even that was still below 20%. More encouraging results come from Lopez and Monsan (174), who immobilized dextransucrase on an amino porous silica (Spherosil), and obtained an immobilized activity of up to 830 DSU per gram of support. Because of the above difficulties in immobilizing the dextransucrase, in this work the enzyme was added in the eluent stream at the required strengths.

4.10 Chromatographic Reactor-Separators

Although increasing interest is shown to the application of chromatographic systems as reactor-separators, their employment in large scale operations has not been materialised fully yet. So far an increasing amount of work is carried out on the modelling of such systems to provide a better understanding of their operation. During the operation the stationary phase in the system can either act as a catalyst (ie. immobilized enzymes) and as adsorbent, or it can only act as adsorbent with the reaction carried out in the mobile phase. The combined operation of reactor-separators reduces the process costs (operating and capital) substantially, improves the product yield by removing acceptors from the reaction mixture, results in better conversions by removing the products and thus shifting the equilibrium in reversible reactions, and can provide better process control. Since the early sixties,

Roginskii et al (181 to 184) in USSR, and Magee et al (185, 186) in the USA reported the employment of chromatographic reactor separators and produced a theoretical basis to describe its operation. A comprehensive review has been carried out by Vermaux et al (187, 188). Langer et al (189, 190) employed a liquid chromatographic reactor to obtain kinetic rate constants. Mile et al (191) studied the catalytic dehydration of cyclohexane to benzene in a gas chromatographic reactor and reported an enhancement of product yield above equilibrium, verifying the equilibrium displacement. Urban and Rinker (192) carried out the exothermic ammonia synthesis beyond equilibrium limitations by pulsing nitrogen, with hydrogen as the carrier, through a packed bed of catalyst and adsorbant. Three columns were used of 0.63cm diameter and 95cm long packed with a mixed bed consisting of 50% catalyst and 50% adsorbant. They suggested that it might prove economical in industry to use the pulsed chromatographic reactor as a first stage in series with a conventional NH_3 synthesis reactor.

Takeuchi and co-workers (193, 194) studied the catalytic oxidation of CO on a 1.4 cm id x 55cm long moving bed chromatographic reactor. Aris et al (195, 196) used a similar system to model the hydrogenation of mesitylene with excess hydrogen over a P_t on alumina catalyst. The system used was 1.27 cm dia x 17.8cm long. Aris, Carr and Cho (197, 198) modelled the acid catalysed hydrolysis of methylformate in a continuous flow annular chromatographic reactor with a rotating feed point. The equipment was packed with activated charcoal and consisted of two concentric cylinders, an outer of 20cm od and an inner of 17.8cm od. The total length was 40.6cm. Hashimoto et al (199) used a continuous countercurrent system, for the production of higher-fructose syrup (45 to 65% fructose), involving selective adsorption of fructose and an immobilized glucose isomerase reaction. Continuous countercurrent contact of the liquid stream with the solid adsorbent was simulated by advancing adsorption columns against the fixed inlets and outlets of liquid streams

without actual movement of the solid adsorbent, while the immobilized enzyme reactors were stationary (also see Chapter 3.5). A total of 16 adsorption columns were used packed with Y zeolite (Ca^{2+} form). Each column was 1.38cm dia x 10.2 m long. Seven reactors containing the immobilized glucose isomerase were connected to the system, and were of 1.38 cm dia x 18cm long. Although fructose purities of up to 65% were obtained their prime objective was to model the operation.

The worlds first successful production of useful substances by bioreaction using an immobilized biocatalyst was the continuous production of L-methionine by asymmetric hydrolysis of acetyl-DL-methionine using immobilized amino acylase, which was industrialized by Tanabe Seiyaku in 1969 (20).

HFCS is the only example of large scale commercial production utilizing immobilized biocatalysts. The commercial success is attributed to two factors - the development of highly active and stable glucose-isomerase and the progress of immobilized technology. The list of companies employing or associated with the development of glucose-isomerase systems includes Clinton (US pat: 3788945), Corning (US pat: 3992329), Novo (Brit pat: 1362365) and ICI (US pat: 3645848, 3935068).

CHAPTER 5

5.0 THE SEMICONTINUOUS CHROMATOGRAPHIC REFINER (SCCR7)

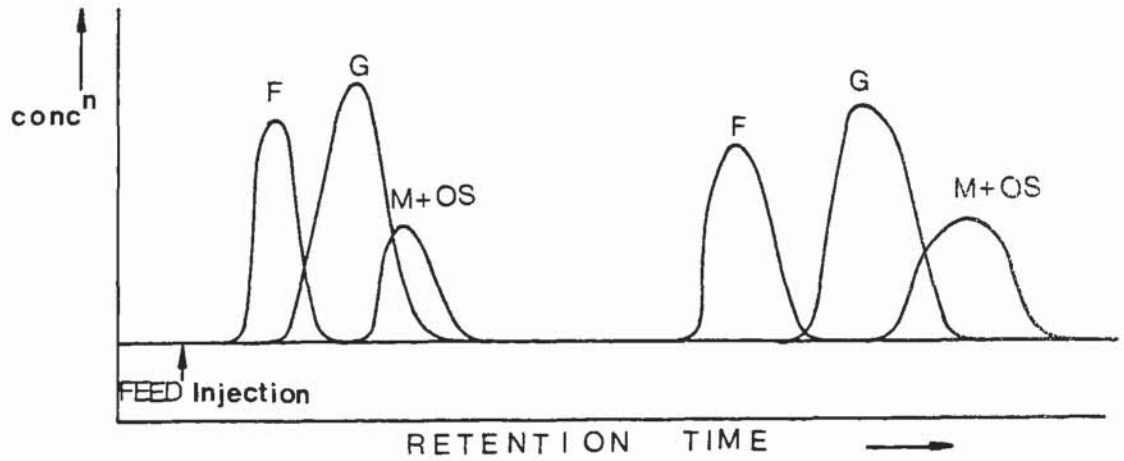
5.1 The Semicontinuous Principle of Operation

5.1.1 Introduction

When a multicomponent carbohydrate mixture containing fructose, glucose and other oligosaccharides is eluted through a cation chromatographic column packed with calcium charged resin, a separation occurs (127). Fructose forms a loose chemical complex with the calcium ions and is retarded, hence it travels through the column at a slower rate than the glucose and the oligosaccharides. The slightly different migration velocities of the glucose, (a monosaccharide), and the oligosaccharides is due to the size exclusion principle. The glucose molecules due to their smaller molecular size tend to diffuse into and out of the pores of the resin and are delayed while the larger oligosaccharides are excluded and are eluted faster. Although this results in an additional separation between the glucose and oligosaccharides, the ion exchange principle, ie. the fructose retention, is the more prominent one. A typical elution profile of such a batch operation is shown in Figure 5.1.

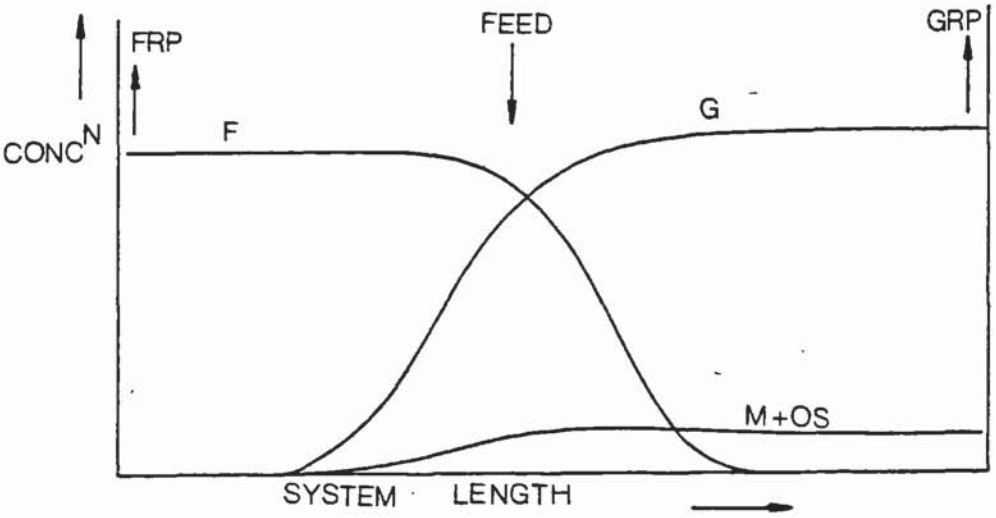
If however a continuous operation is employed, where the mobile phase and the stationary phase packing move countercurrently, and if the approprite operating conditions have been selected the resulting elution profile is as shown in Figure 5.2. The inherent advantages of continuous processes, such as product reproducibility, increased throughputs and continuous operation with minimum supervision, led Barker and Deeble (201, 78) into constructing and operating successfully a continuous gas-liquid chromatographic system. Since then, similar liquid-liquid continuous chromatographic systems have been developed in this Department with column diameters up to 10.8cm (22).

Figure 5.1: Concentration profile of a batch cocurrent separation



Key: F: fructose; FRP: fructose rich product; G: glucose: GRP: glucose rich product; M+OS: maltose + oligosaccharides

Figure 5.2: Concentration profile of a continuous countercurrent separation



5.1.2 Principle of Operation

The principle of operation of the SCCR system is shown schematically in Figure 5.3 where the whole system is illustrated as a closed loop. The multicomponent mixture is fed at port F. The less strongly adsorbed glucose and oligosaccharides move preferentially with the mobile phase towards the glucose rich product offtake P1. A section of the loop is isolated at any time by the two locks V1 and V2, and an independent purge fluid stream enters at port PU, strips the adsorbed fructose and exits from port P2 as the fructose rich product.

Figure 5.3(a) represents the component distribution within the system soon after "start-up". In Figure 5.3(b), all the port functions have been advanced by one position in the direction of the mobile phase flow. This port advancement results in a simulated movement of the stationary phase countercurrent to the direction of the mobile phase. In order to achieve separation and hence two enriched products, the rate of port advancement must be greater than the fructose migration velocity through the bed and lower than the glucose and oligosaccharides migration velocity, Figure 5.3(c). The frequency with which this port advancement occurs represents the "switch time".

Figure 5.4 demonstrates the counter-current operation mode of the SCCR7 system. The system is made up of twelve 5.4cm id x 75cm long stainless steel columns connected at the top and bottom to form a closed loop. Six pneumatic poppet valves are associated with each column, the feed, eluent and purge inlet valves, the glucose rich (GRP) and fructose rich (FRP) product valves and the transfer valve to the next column. Figure 5.4(a) represents the first switch period where column 1 is isolated and purged to give the FRP product. Feed and eluent enter columns 7 and 2 respectively and the glucose rich product is eluted from column 12. In the next switch period, Figure 5.4(b), all ports are advanced by one position; now column 2 is purged, feed and eluent enter

Figure 5.3: Principle of operation of the SCCR system

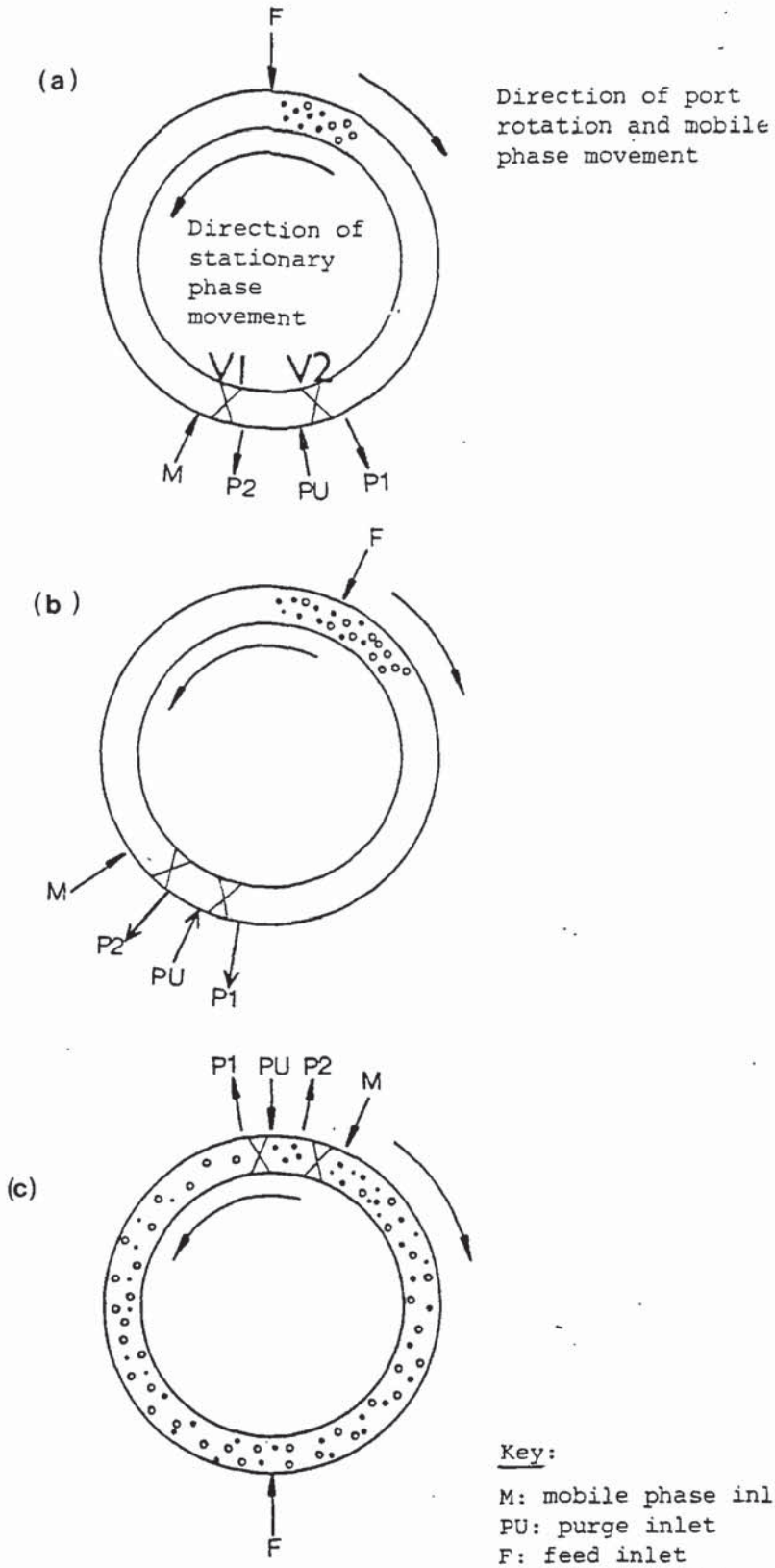
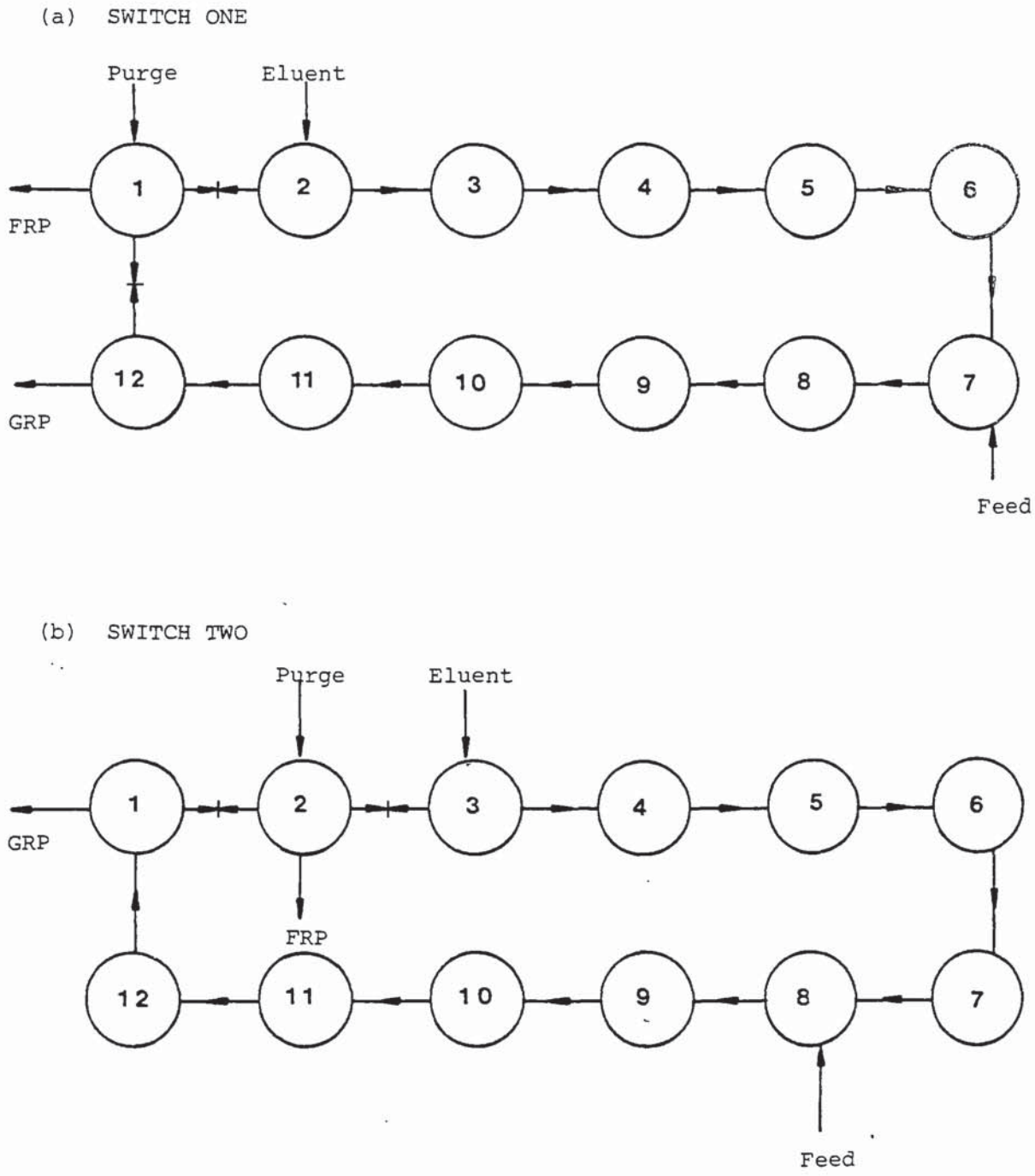


Figure 5.4: Sequential operation of the SCCR7 system.



columns 8 and 3 and the glucose rich product exits at column 1. After twelve such advancements the "cycle" is completed.

5.1.3 Idealised Operating Conditions

During the separation of a glucose-fructose mixture on the countercurrent semicontinuous system, glucose travels with the mobile phase and fructose with the stationary phase. An idealised model can be constructed relating mobile and stationary phase flow rates and component separation. A material balance on glucose about the feed point (Figure 5.5) gives:

$$L_2.f_g = Le.y_g + P.x_g$$

where: L_2 = feed flow rate ($cm^3\ min^{-1}$)

Le = mobile phase flow rate ($cm^3\ min^{-1}$)

P = stationary phase effective flow rate ($cm^3\ min^{-1}$) = total system volume/(total number of columns x switch time)

f_g = glucose concentration in feed ($g.cm^{-3}$)

y_g = glucose concentration in mobile phase ($g.cm^{-3}$)

x_g = glucose concentration in stationary phase ($g.cm^{-3}$)

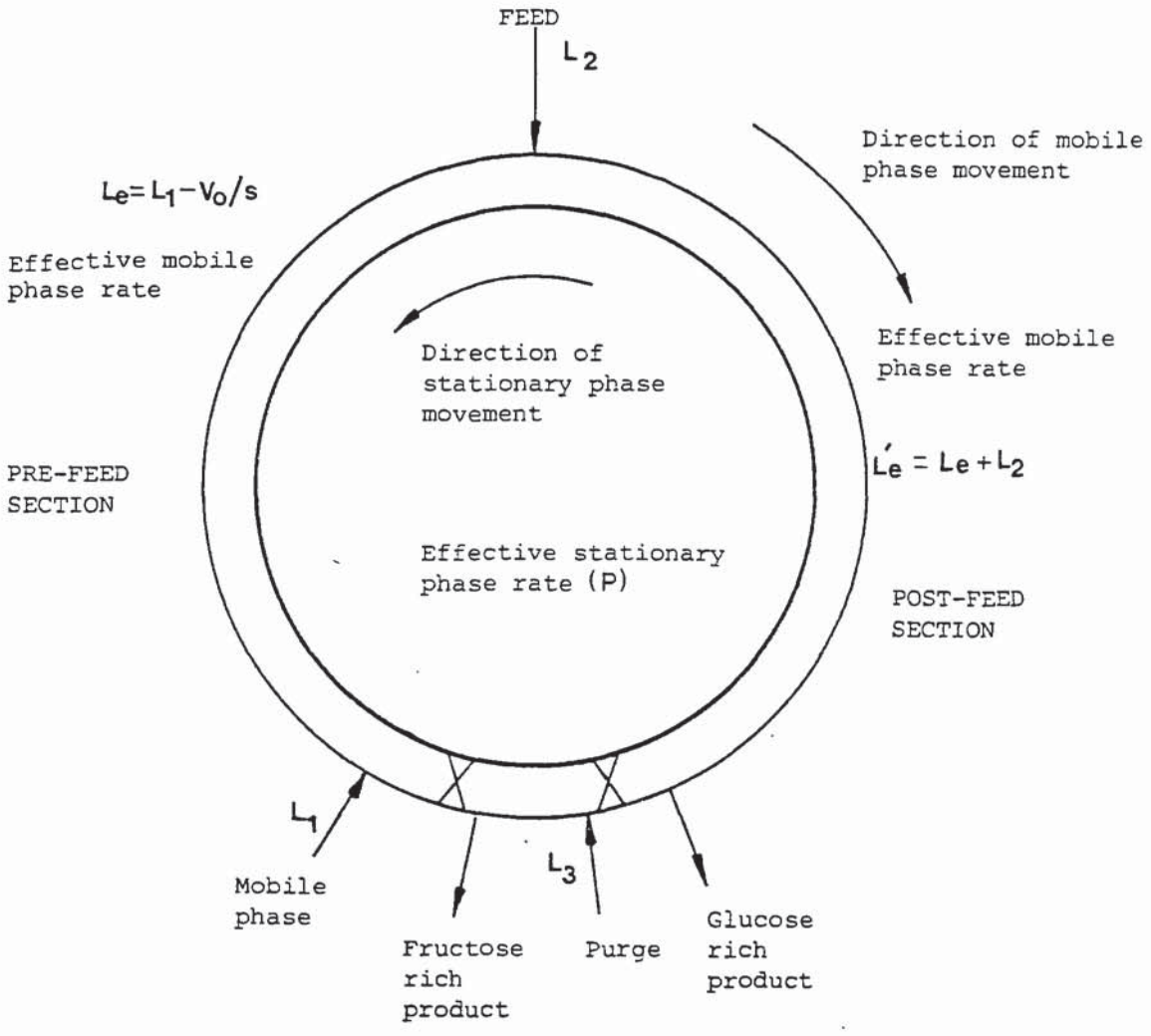
For a glucose molecule to move preferentially with the mobile phase

$$Le.y_g > P.x_g \qquad \text{..... 5.1}$$

Rearranging,

$$\frac{Le}{P} > \frac{x_g}{y_g} \qquad \text{..... 5.2}$$

Figure 5.5: Diagramatic representation of the semicontinuous principle of operation



and since by definition:

$$K_{dg} = \frac{x_g}{y_g} \qquad \text{..... 5.3}$$

Then

$$\frac{Le}{P} > K_{dg} \qquad \text{..... 5.4}$$

Similarly for fructose to move with the stationary phase,

$$\frac{Le}{P} < K_{df} \qquad \text{..... 5.5}$$

The theoretical limits of mobile and stationary phase to give separation of fructose and glucose are obtained by combining equations 5.4 and 5.5, ie.

$$K_{dg} < \frac{Le}{P} < K_{df} \qquad \text{..... 5.6}$$

As each column contains eluent phase in the void volume, Vo, the effective mobile phase flow rate is reduced to:

$$Le = L_1 - \frac{Vo}{s} \qquad \text{..... 5.7}$$

where L_1 = Mobile phase inlet flow rate ($cm^3\ min^{-1}$)

s = Switch period (min)

Because of the feed flow rate L_2, the effective mobile flow rate is different before and after the feed point. That means the effective mobile phase rate in the post feed section $\acute{L}e$, becomes:

$$\acute{L}e = Le + L_2 = L_1 + L_2 - Vo/s \qquad \text{..... 5.8}$$

Therefore equation 5.6 becomes:

$$K_{dg} < \frac{Le}{P} < \frac{\acute{L}e}{P} < K_{df} \qquad \text{..... 5.9}$$

and this equation now gives the true theoretical limits.

The purging flow rate L_3, in the isolated column is also governed by:

$$\frac{L_3}{P} >> K_{df} \qquad \text{..... .. 5.10}$$

5.2 Description of the SCCR7 System

5.2.1 Overview

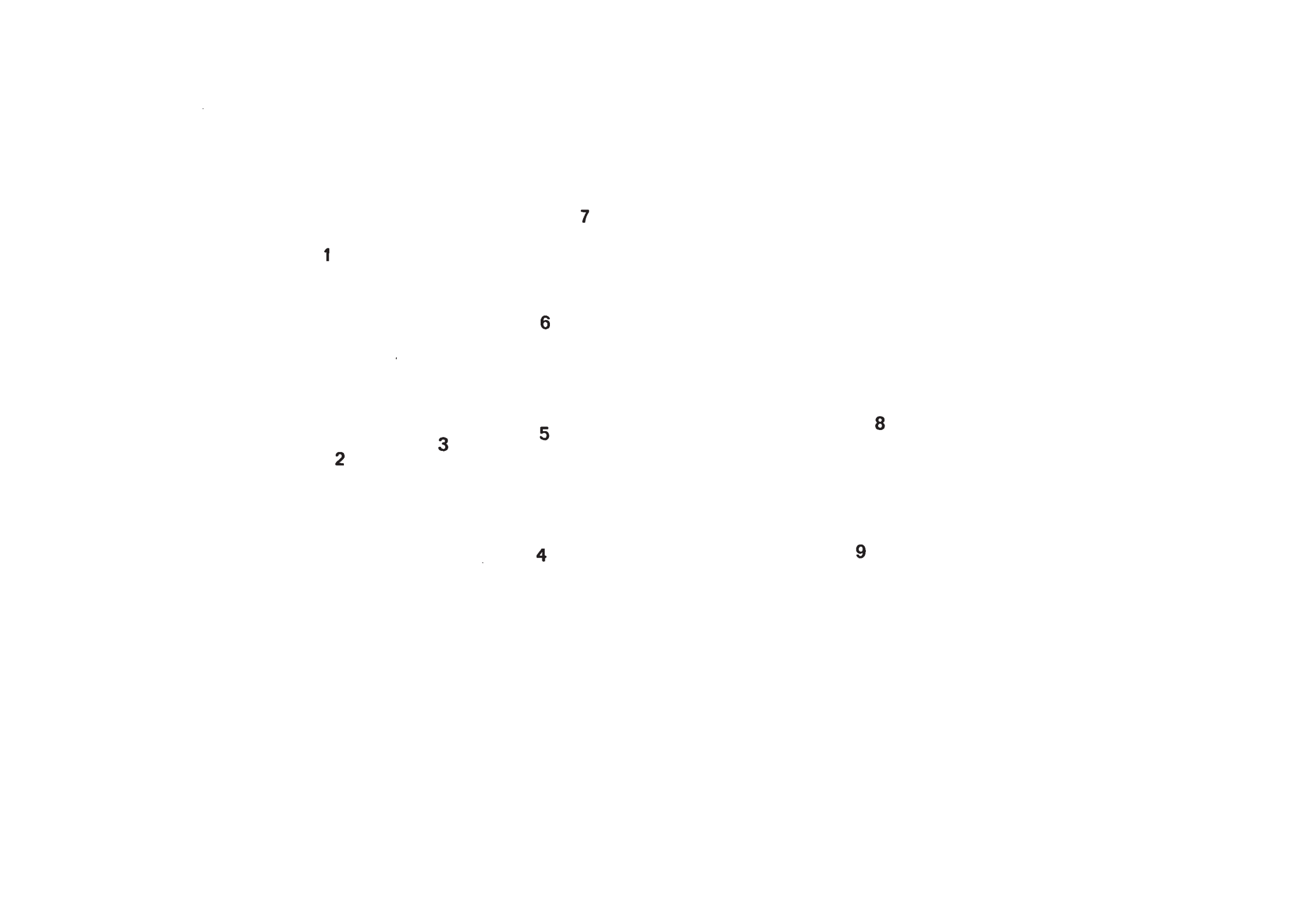
The separation section of the SCCR7 system consists of 12 stainless steel columns 75cm long and of 5.4cm internal diameter, Figure 5.5. The equipment was originally constructed by Ching (203), and modified by Chuah (204). Abusabah (22) modified it further by replacing the 2.5cm id columns by 5.4cm id stainless steel columns which were packed with an anion resin. The instability problems of the anion resin experienced by Abusabah (22) in conjunction with specific industrial interest led to the repacking of the columns with a calcium charged 7% cross-linked polystyrene resin. This cation resin was supplied by FINN-SUGAR Engineering, Finland, under the trade name KORELA VO7C and had a particle size range between 150 and 300 μm.

The equipment layout is shown in Figure 5.7. The columns are mounted on a mobile frame and are surrounded by a constant temperature enclosure. The inlet and outlet lines are arranged in a ring distribution network, connected to the supply pumps and product collection devices. The sequential functioning of the poppet valves is governed by a pneumatic controller. A description of the individual units of the equipment is given in the following sections. A more comprehensive description has already been reported by Ching (203) and Abusabah (22).

Figure 5.6: Picture of the SCCR7 System

Key:

1. Feed Tank
2. Heated aspirator for the eluent and purge
3. Automatic cut off
4. Pumps
5. Mains distribution board
6. Pneumatic controller
7. Product splitting timers
8. The SCCR7
9. Valves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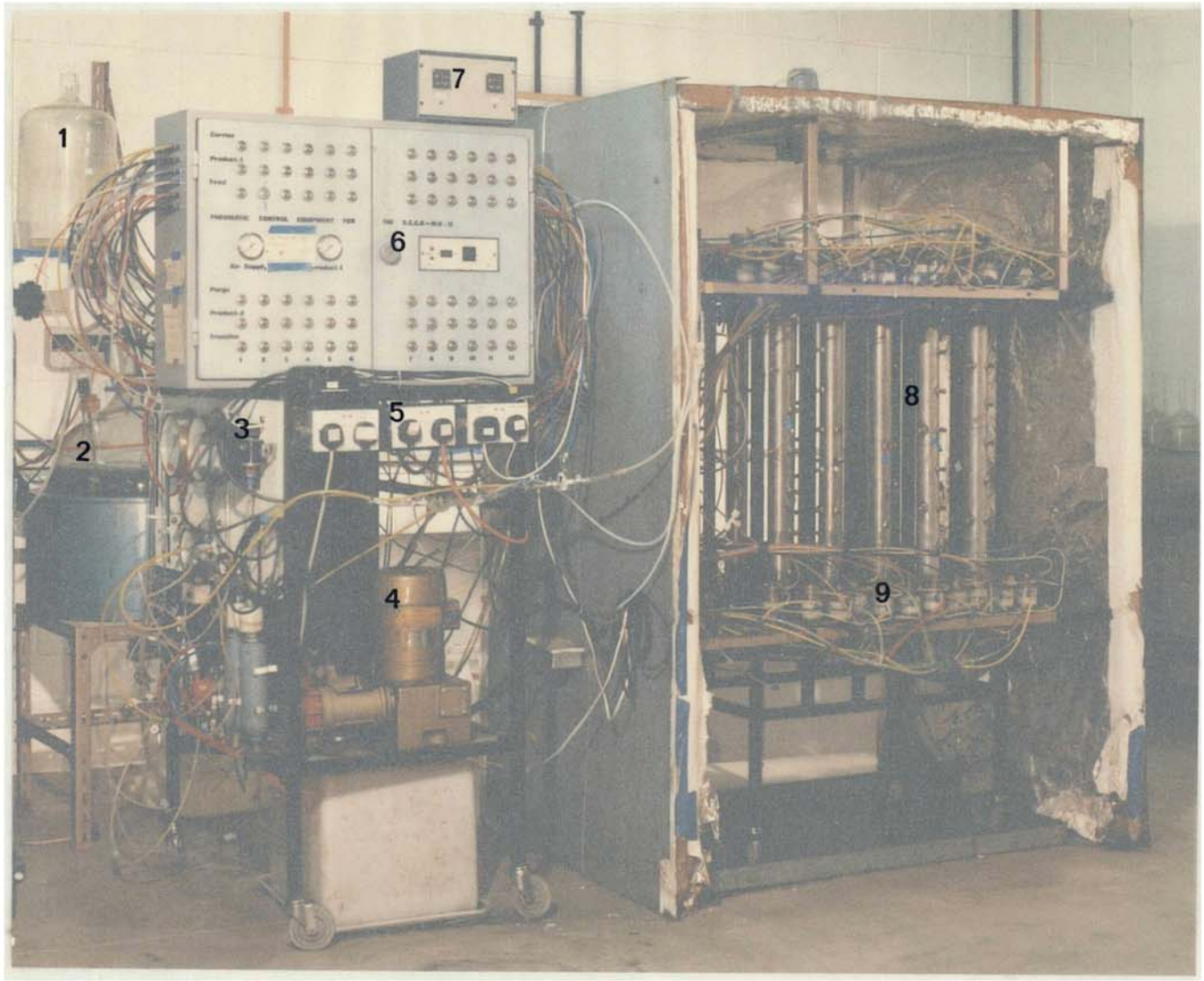
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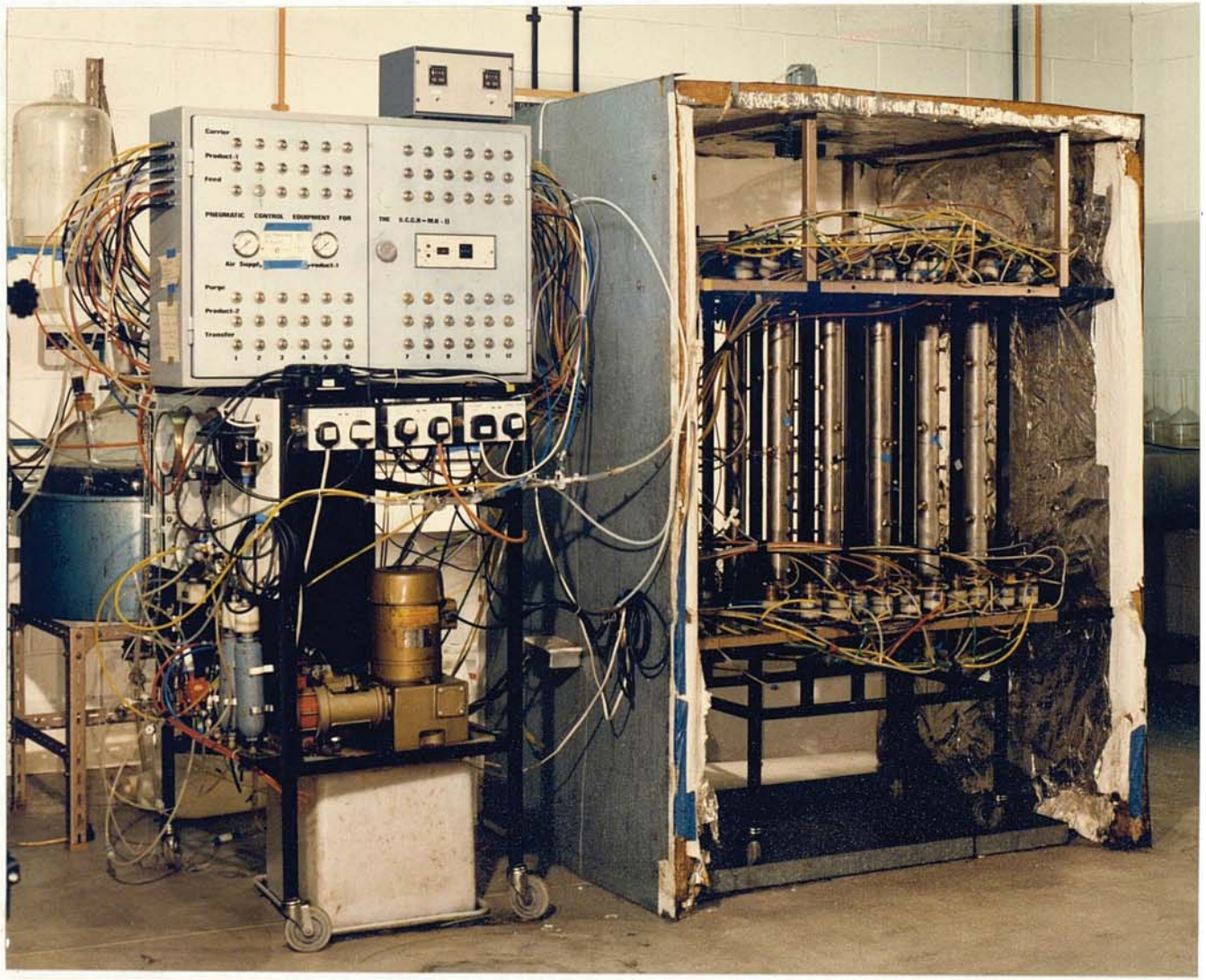
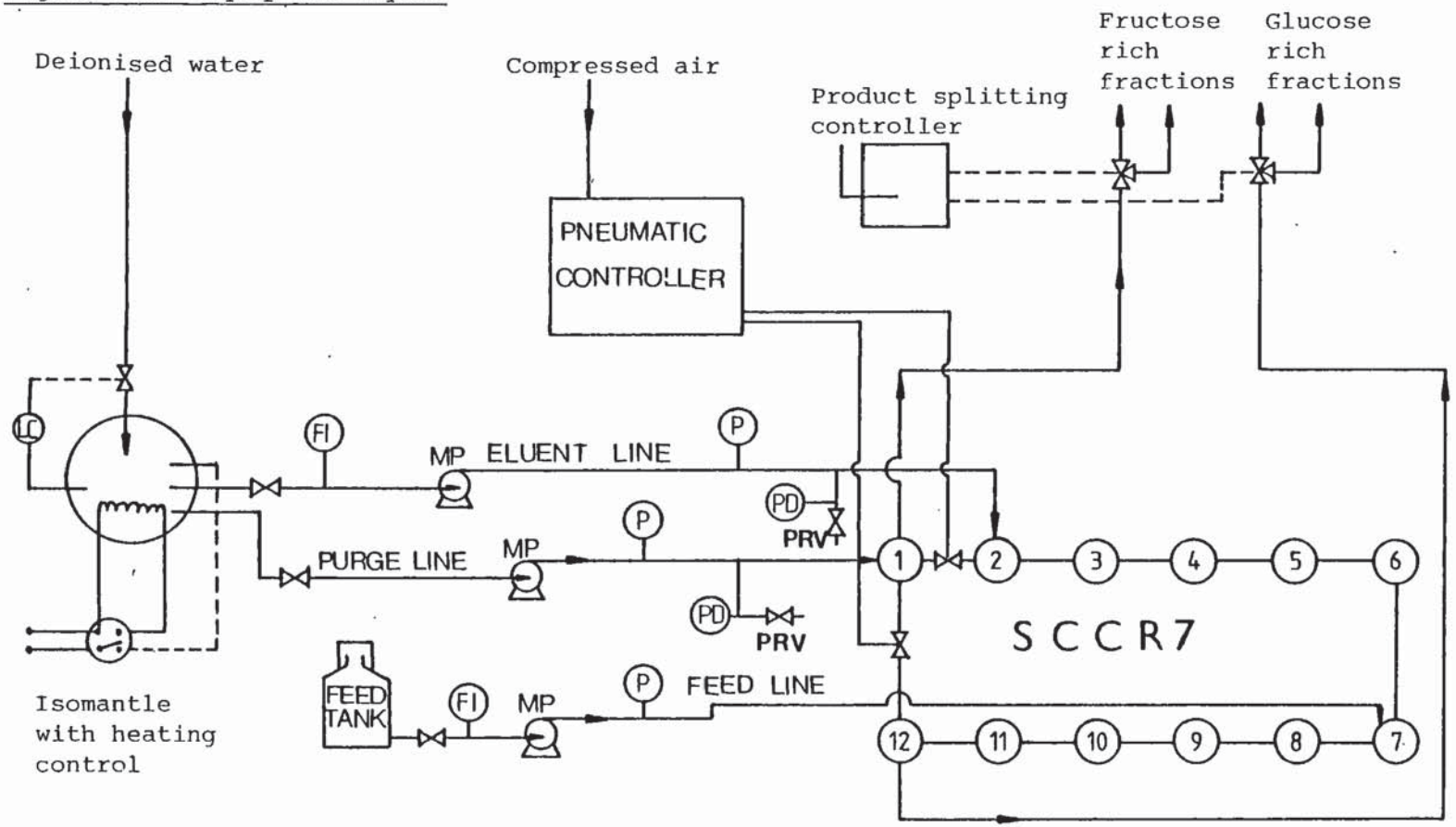

Carrier
Product-1
Feed
PNEUMATIC CONTROL EQUIPMENT FOR
Purge
Product-2
Transfer

Figure 5.7: Equipment layout



Key: P: pressure indicator
PD: pulsation dampener
PRV: pressure relief valve
LC: level controller
FI: flow indicator
MP: metering pump

5.2.2 The Columns and Fittings

The twelve columns were fabricated from type 321 seamless stainless steel tube, and were designed according to BS 5500: 1976 for pressure vessels. Each column is of 5.4cm internal diameter and 75cm long, and is fitted with ten 20mm x 1/8 BSP sampling points. These sampling points were 2 cm long stainless steel rods welded to the column and having 2mm holes drilled through them. The rod ends were plugged with silicone rubber septa which were held in position by simplifix nuts. Samples were taken by inserting hypodermic needles through the septa to the inside of the column. Two 130 x 10 mm mild steel flanges were fitted on each end of the columns and a 2 mmthick neoprene gasket was used to seal the two gaskets together. The inlet assembly consisted of a 65 x 40mm polypropylene inlet head, and a compression plunger to prevent the swelling and contraction of the resin. The plunger was made of a 130 x 19 mm stainless steel rod and a 54 x 50μm polypropylene piston. A polypropylene ring was fitted at the piston's end and it retained a 100μm polypropylene mesh and a polypropylene distributor which was used to ensure an even velocity profile at the colunn inlet. Two Dowty O-rings, no. 20-830-4470, were fitted around the polypropylene piston to ensure perfect sealing, and the first 10cm of the column's inlet was accurately machined.

Five 12.7 x 1/4 BSP ports were tapped on the inlet head to accommodate CK-1/4-PK4-KU type FESTO plastic connectors for the feed, eluent, purge and transfer lines and the piston rod. Inside these ports a 12mm x 2mm thick neoprene gasket, having a 6mm diameter central hole, was fitted to prevent leakages. Liquid channels of 3mm diameter were drilled through the head, the plunger and the piston.

The outlet assembly consisted of two parts, a stainless steel packing support and a 70 x 38mm polypropylene head machined to a T-shape to form a bottom 70 x

10mm flange. The packing support had two meshes of different sizes sandwiched together. The top mesh was a 50μm mesh used to prevent fine resin particles coming through and thus blocking the liquid ducts, and the bottom was a 100μm mesh which was used as bed supporting mesh.

Three 12.7mm x 1/4 BSP ports were tapped on the outlet head and were fitted with the same Festo fittings as the inlet head. The ports were connected to the fructose rich and glucose rich product lines and the transfer line. A 3^{o} cone was turned on the inside face of the outlet block to ensure uniform flow of liquid out of the column. One 2mm thick neoprene gasket was placed on each side of the support mesh to prevent leakages when the mesh, the outlet head and the mild steel flanges were clamped together.

5.2.3 Hydraulic Compression Facility

The packing was kept compressed by incorporating a dynamic hydraulic facility. The top flange in the column inlet was fitted with a 45 x 20mm stainless steel sleeve, a 6mm gas tap and a 1/8 BSP port fitted with a CK-1/8-PK4 Festo connector. Two Dowty O-rings, no. 200-113-4470 were fitted at the top of the piston to seal on to the stainless steel sleeve. Deionised water was introduced to fill the compression compartment and air was bled out via the gas tap. The compression compartments of all the columns were linked together with a 4mm id polyamide Festo PP4 tubing to form a closed loop.

5.2.4 Poppet Valves and Pneumatic Controller

The control of liquid flow into and out of the columns and hence the countercurrent operation was achieved by the pneumatic operation of a total of 72 double

acting poppet valves. Previous work on the SCCR systems (201, 203, 205) has led to the conclusion that this type of valve would offer a higher degree of reliability, smoother operation and a wider range of operating conditions. The valves were constructed by Aston Technical Services Ltd and the prime objective was to keep the internal volume to a minimum, thus minimising the system's dead volume. The sequence of the valve settings for every switch during a cycle is shown in Table 5.1. Compressed air was supplied by the University's main air copressor with an air cylinder as a stand by, which would be used if the main supply pressure had fallen below 580 KN m^{-2}. A pressure relief valve, set at 700 KN m^{-2}, was fitted on the main air supply line.

The supply was divided into bias and actuating streams whose pressures were controlled to 240 KN m^{-2} and 550 KN m^{-2} respectively by a pressure regulator. The bias supply line was branched and led directly to the poppet valves while the actuating line was led to the pneumatic controller. All the pneumatic lines were made from Festo PP3 polyamide tubing having a 3mm inside diameter. Figure 5.8 is a photograph of one of these valves with its parts. Normally the valves were closed due to the bias pressure which was applied constantly to the lower side of the diaphragm. The valve was opened by applying the actuating pressure to the upper side of the diaphragm.

After long continuous operation it was found that the nuts on the valve stems were becoming loose thus preventing the valve from closing fully. To avoid that happening again a locking nut was incorporated.

The operation of the poppet valves was governed by a pneumatic controller. The control mechanism consisted of a number of solenoid valves activated individually by a rotating Festo type PN-2OB camshaft mechanism. Twelve of these solenoid valves were set for the twelve columns and each valve would directly activate the appropriate transfer valve to isolate the corresponding column. The solenoid valves were each linked to double return valves which would activate the appropriate feed, eluent, and purge inlets and the product outlets. A digital timer programmable down to 10 seconds

Figure 5.8: Photograph of a Poppet Valve and its Parts

was incorporated to control the rotating mechanism which in turn opened and closed the solenoid valves at a fixed time period. Any change to the sequence of operation of the poppet valves can be achieved by altering the relative positions of the coloured "program carriers" on the rotating mechanism. The timer was set at a preselected fixed time period which corresponded to the switch time, and at the end of that period an electrical signal was triggered, activating the electric motor to rotate the camshaft unit to the next position.

5.2.5 Fluid Delivery and Pressure Control

5.2.5.1 Fluid Delivery

An Elgastat B224 deioniser was used to provide the deionised water for the eluent and purge streams. This water was passed through a GELMAN Acroflow II cartridge of 1.2μm pore size and a MILLIPORE carbon cartridge in order to remove any impurities and any ions present, and it was stored in an elevated 1.2×10^{-1} m^3 polypropylene reservoir. This reservoir was used to minimise any possible hazards during the overnight unattended operation. The deionised water was then fed by gravity through a 4mm id Festo tubing and a solenoid valve into a 7.5×10^{-2} m^3 heated glass aspirator. The water level inside the aspirator was governed by a FISONS level controller.

The feed mixture was filtered and fed into a 2×10^{-2} m^3 elevated glass aspirator. The feed then flowed under gravity, through a preheater and a flow measuring device, into one of the pumpheads of an MPL Series II double head metering pump. The feed was then pumped into the system through the feed supply network.

The eluent stream was pumped from the glass aspirator and through a flow measuring device to the second pump head of the above metering pump. Four different pump heads were available with different maximum flow rates, ie. $10cm^3$ min^{-1}, $20cm^3$ min^{-1}, $40cm^3$ min^{-1} and $55cm^3$ min^{-1}. The eluent was pumped into the system through

the eluent supply network. The purge was pumped from the glass aspirator into the system purge network by an MPL K-series metering pump. Two pump heads were available, a positive displacement piston head capable of high pressure drops and a maximum flow rate of up to 100cm^3 min^{-1}, and a diaphragm head for low pressure drops and flow rates of up to 2000 cm^3 min^{-1}. Whatman Gamma 20 type in line filter holders fitted with 25μm filter tubes were incorporated on each of the inlet lines to remove any fine particles and dirt, thus preventing any possible blockages. All the liquid supply lines were made of Festo PP4 polyamide tubing having an inside diameter of 4mm and were arranged in such a way to form an upper and lower ring. To minimise the system's dead volume the columns were mounted in such a way so that for two adjacent columns the inlet of one was at the top and for the other at the bottom. Because of this column arrangement, it was therefore necessary to have an upper and a lower pipe ring for each one of the inlet and outlet streams. Although the column interconnecting tubes were kept to a minimum, the total dead volume of the system taking into account the poppet valves was estimated at 6%. The collection of both products was into two 8 x 10^{-2} m^3 plastic containers.

5.2.5.2 Pressure Control

A Bourdon pressure gauge and a safety relief valve was fitted on each of the liquid supply lines, and were set at 1040 KN m^{-2} and 275 KN m^{-2} for the eluent and purge lines respectively.

Smooth liquid flow was ensured by incorporating a pulsation dampener on each of the eluent and purge supply lines. The dampeners were made of stainless steel and nitrile rubber diaphragms.

To allow safe continuous unattended operation a number of safety devices were introduced. These included the liquid level controller, high pressure relief valves and a low pressure sensor and cut-off device. This automatic cut-off device was constructed by the Department's electronic workshop. The pressure sensor was connected to the mains and to the eluent delivery network, and it was fitted with an adjustable pressure switch which was detecting minimum pressure. This minimum pressure was set at 10 $KN.m^{-2}$. When the operating pressure was above the minimum one, the electrical circuit from the mains, through the sensor and out to a secondary mains plug was completed. The electric terminals of the pumps, the liquid preheater and the level controller were connected to a distribution board which in turn was connected to the secondary mains plug. In case of an emergency, such as valve leakage or tube bursting, the system pressure would drop below the minimum set value. This would be detected by the pressure sensor and the electricity supply to the secondary mains plug will be broken.

During the start-up this safety cut-off was bypassed, by pressing manually a spring loaded overriding switch fitted on the pressure sensor, until the operating pressure was above the minimum set value.

5.2.6 Product Splitting Automation

As an attempt was made to increase the product concentrations, it was decided to separate the more dilute product fractions by fitting two product splitting devices. One device was fitted on each of the glucose rich and fructose rich product lines and they were operated independently. Each device consisted of a RS 346-390 digital timer and a 3-way solenoid value supplied by AJ Foster Ltd (Manchester). Each timer had six time ranges, set by front panel switches from 0 to 9.99/99.9/999 seconds and 0 to 9.99/99.9/999 minutes, and it was connected directly to the corresponding solenoid

valve. After a preselected time interval the solenoid valve was switched from one outlet position to the other, thus allowing the splitting of the corresponding product line into two separate lines.

5.2.7 Heating Facilities and Controls

The twelve columns, the valves and the fluid inlet and outlet networks were placed inside a heated enclosure constructed from galvanised steel sheets lagged with 50mm thick glass fibre pads, and covered by aluminium foil. The enclosure was heated by a U-shaped 5 kw fan heater which was controlled by a Diamond DH82 type temperature controller. Additional safety devices were incorporated which would cut-off the electricity supply if the temperature inside the enclosure exceeded 70^{o}C. The hot air was circulated by an electrical fan placed at the centre top of the enclosure. The temperatures of the enclosure, the surface of the individual columns, and of the liquid inside each column were measured by a network of Nickel-chrome thermocouples. The thermocouples were placed at the middle of each column, in the transfer lines and in all three inlet lines. The thermocouple terminals were connected to a multi-point switch, which displayed temperatures as millivolt potentials on a proportional digital controller supplied by Diamond Controls Ltd. The millivolt reading was converted into temperature using a callibration chart.

The feed solution was heated by passing it through a liquid preheater. The preheater was made of a 25.4mm id glass column, wrapped with a tape heater and insulating material. The tape heater was controlled by a temperature controller.

The deionised water used as eluent and purge solution was heated inside the 7.5 x 10^{-2} m^3 glass aspirator. The aspirator was jacketed with a 5 kw and 2 kw electrical heaters. During start-up both heaters were used to boost the temperature to the required

value. Then the 5 kw heater was switched off, and the 2 kw heater which was connected to a thermostat, was used to control the temperature.

Product Quenching Units

The SCCR7 system was to be used subsequently as a reactor-separator, and to stop the reaction carrying on outside the columns and inside the product lines, a quenching unit was installed in each one of the product lines. The quenching unit on the main product line was made up of a 0.5 kw immersion heater sealed inside a 45cm x 4cm glass tube fitted with 5mm inlet and outlet ports. Because of the higher flow rates of the purging stream a 1 kw immersion heater was used inside a 48cm x 7.5cm glass tube in a similar arrangement.

Both quenching units were insulated and the temperatures were controlled at 60^{o}C by a Diamond DH82 temperature controller. The glass tubes were constructed by the University's glass blowing department.

Table 5.1: Sequences of Valve Settings

Valves							Sequents
Transfer	Transfer	Purge	FRP	Feed	Eluent	GPR	
1	12	1	1	8	2	12	1
2	1	2	2	9	3	1	2
3	2	3	3	10	4	2	3
4	3	4	4	11	5	3	4
5	4	5	5	12	6	4	5
6	5	6	6	1	7	5	6
7	6	7	7	2	8	6	7
8	7	8	8	3	9	7	8
9	8	9	9	4	10	8	9
10	9	10	10	5	11	9	10
11	10	11	11	6	12	10	11
12	11	12	12	7	1	11	12

CHAPTER 6

6.0 COLUMN CHARACTERISATION AND THE EFFECT OF WIDE FEED BANDS

6.1 Introduction

A substantial amount of analytical work has been carried out by previous workers (22, 203) to investigate the separating capacity of a wide range of packing materials. The experiments were carried out in small batch chromatographic columns and the packing performance was investigated under different operating temperatures and flow rates. The overall findings were that, for fructose-glucose separations the cation exchangers were better at operating temperatures of up to 30^{o}C and comparable to anion exchangers at higher temperatures. The main advantage found for the anion exchangers was that higher fructose rich product concentrations could be obtained during semi-continuous chromatographic separation of sugar solutions, because fructose was the non-retarded component and was eluted with the mobile eluent phase. The application however of anion exchangers is limited because they are prone to mechanical fouling and auto-oxidation. In addition to packing stability and separating capacity other factors such as capital cost and pressure drop must be considered during the packing selection for large scale operations.

In this work the KORELA VO7C cationic packing was used because of specific industrial interest by sponsors of the research work. The packing was 7% cross-linked polystyrene, and it was supplied in the calcium form by FINN-SUGAR ENGINEERING, Finland.

For comparison purposes column packing and characterisation techniques similar to those adopted by previous workers were used. The column characterisation was carried out at inifinte dilution conditions, ie. very low sugar concentrations. In practice however high sugar concentrations and broad feed bands are encountered, and their effect was investigated.

6.2 Column Packing

6.2.1 Particle Size Analysis of the Resin

A particle size analysis was carried out using BS410 (1976) meshes. A sample of the resin was dried by leaving it in a 25°C oven for 12 hours, and then 100g of the dried resin were sieved. The results are shown on Table 6.1. It was found that over 99% was within the 150 to 300 μm size range, with over 94% being within 212 and 300μm. The results agreed well with those quoted by FINN-SUGAR, ie. 95% within 40% of a mean (270 μm).

A settling technique was employed to remove the very small amount of fines present to avoid any blockages in the valves and column inlet and outlet ports. Two volumes of water were added to one volume of resin. The mixture was shaked vigorously and it was left to settle for 20 seconds. The top layer containing most of the fine particles was decanted, and the technique was repeated twice more.

6.2.2 Column Packing Technique

The old packing was removed from the columns and new neoprene gaskets were fitted. Each column was filled with deionised water and its outlet was connected to a suction creating device.

The packing slurry, consisted of one volume of resin and one volume deionised water, and was added at the column inlet at the same rate as water was removed. This slurry packing method was used to minimize the segregation of particles apparent in gravitational settling. During the packing procedure deionised water was added continuously and the column was tapped at random. When the packing had reached a height of approximately 66cm the procedure was stopped, the inlet plunger fitted and the column reassembled. Deionised water was pumped through the packed column for two hours to remove any remaining fines.

Table 6.1: Sieve analysis results of the KORELA VQ7C packing

Size range μm	Weight collected %	Average mass fraction
> 355	0.45	0.0045
300-35	0.80	0.0080
250-300	72.00	0.7200
212-250	22.10	0.2210
180-212	3.55	0.0355
150-180	0.60	0.0060
125-150	0.35	0.0035
<125	0.15	0.0015
Total	100.0	1.00

6.3 Experimental Procedure

6.3.1 Equipment Set-up

The newly packed column was connected in an arrangement similar to the one shown in Figure 6.1. Eluent was delivered through a flow measuring device and through a T-piece using the same MPL metering pump used to deliver eluent into the SCCR7 system. The T-piece was connected as close as possible to the column inlet and a rubber septum was fitted to one of the T-piece inlets. The sample was loaded into the column by insering a needle through the septum.

A similar T-piece was connected at the column outlet. The main stream of product was collected in the product collection reservoir. A sampling needle was inserted into the rubber septum at the outlet T-piece allowing a small product stream to bypass through the sample detection arrangement. This arrangement consisted of a Technicon autoanalyser, proportioning pump, a Jobling laboratory refractive index detector linked to a Venture Servoscribe Type 2 chart recorder. The refractometer's outlet stream was also collected in the product collection reservoir.

The refractometer was connected as close as possible to the column's outlet to minimise any extra column dispersion.

6.3.2 Sample Preparation and Delivery

An eluent flow rate of 25cm^3 min^{-1} was chosen because it was predicted to be the appropriate operating flow rate of subsequent semicontinuous experiments and to keep similar superficial velocities to the ones used by previous workers (204,206), thus making a comparable performance evaluation of different size columns. Separate 10% w/v solutions of Dextran T40, glucose and fructose were made, and a 5cm^3 slug of these

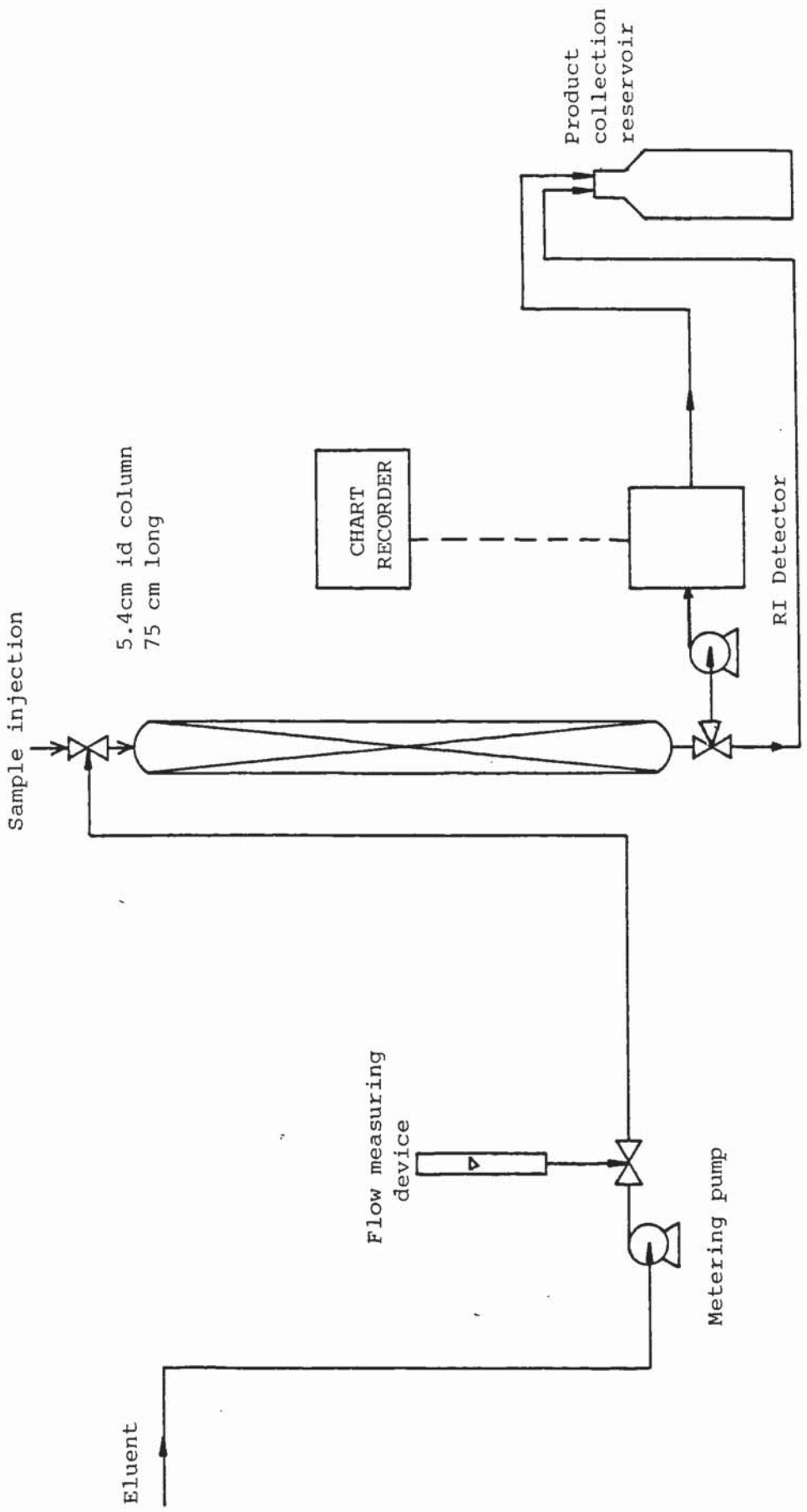


Figure 6.1: Equipment set up for column characterisation

solutions injected through the inlet rubber septum. The eluent flow rate was kept constant throughout the experiments by using the flow measuring device and adjusting the stroke length of the metering pump, and a double check was made by measuring the product volume collected at the end of the experiment. The experiments were carried out at ambient temperature (20°C).

6.4 Verification of Column Parameters

The column parameters were determined from the elution profiles of dextran, glucose and fructose as shown in Figure 2.2. The elution volumes, V_i, of the individual components were calculated by multiplying the eluent flow rate with the respective retention times, t_{Ri}.

The elution volume of the totally excluded dextran T40, was used as the column's void volume, Vo. The individual column volumes were expressed as voidage or void fraction, ie. column void volume over total empty column volume. A measure of the column's separation potential is the separation factor, α, and for a glucose-fructose mixture is:

$$\alpha = \frac{K^{\infty}_{df}}{K^{\infty}_{dg}} \qquad \text{..... 6.1}$$

where:

K^{∞}_{df} = distribution coefficient of fructose at inifine dilution
K^{∞}_{dg} = distribution coefficient of glucose at infinite dilution

The values of the distribution coefficients were determined using the fundamental retention equation (2.3):

$$V_i = V_o + K_{di} V_s \qquad \text{..... 6.2}$$

where:

V_i = retention volume of component i

V_o = column void volume = dextran retention volume

$V_s = V_T - V_o$

V_T = total column volume

K_{di} = distribution coefficient of component i

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

Rearranging equation 6.2:

$$K_{di} = \frac{V_i - V_o}{V_s} \qquad \text{..... 6.3}$$

Re-expressing equation 6.3 for glucose and fructose, their infinite dilution coefficients are given by:

$$K^{\infty}_{df} = \frac{V_f - V_o}{V_T - V_o} \qquad \text{..... 6.4}$$

$$K^{\infty}_{dg} = \frac{V_g - V_o}{V_T - V_o} \qquad \text{..... 6.5}$$

A widely used criterion for comparing the performance of a chromatographic column is the height equivalent to a theoretical plate, HETP. This is calculated by dividing the bed height by the number of theoretical plates in the column, N. The number of plates with respect to the individual component is calculated from the corresponding chromatogram and using the original Glueckauf equation (2.11):

$$Ni = 8 \left(\frac{t_{Ri}}{W_{h/e}} \right)^2 \qquad \text{..... 6.6}$$

Where:

N_i = number of theoretical plates based on component i

t_{Ri} = retention time of component i

$W_{h/e}$ = band width at a height h/e (Figure 2.2)

e = logarithmic base

The results for the individual columns and the average values are shown in Table 6.2.

6.5 Results and Discussion

The voidage is a measure of the packing density of a column and for a bed of spherical particles its theoretical value is about 0.4 (207). The average column voidage was 0.309. This lower value is because of the different packing geometry and because of the non-uniformity of the packing material; that is, the smaller particles (< 220 μm) were dispersed and filled the empty space between the larger ones (> 220 μm). This was also enhanced by the compression exerted by the piston plunger. Repeatable packing giving identical voidages becomes more difficult as the column size increases. The packing of the SCCR7 columns was very satisfactory since most of the voidages were close to the average one with a maximum variation of up to ± 8%. The average elution volumes were 686.2 and 953.4 cm^3 for glucose and fructose.

The glucose molecules diffuse in and out of the intra-particle space and hence are delayed with respect to the totally excluded dextran molecules. This results in the larger elution volume for glucose. In addition to that the fructose molecules are retained

Table 6.2: SCCR7 Column Characterisation, (packed with Korela VO7C of Packing Size 150-300μm)

Column 5.4cm id	Packing Height (cm)	Elution Volume (cm^3)			Distribution Coefficients		Number of Theoretical plates		HETP (mm)		Voidage
		V_R^D	V_R^G	V_R^F	K_d^G	K_d^F	Wrt Glucose	Wrt Fructose	Wrt Glucose	Wrt Fructose	
1	66	451.2	681.6	955.2	0.217	0.475	41.3	32.3	16.0	20.4	0.299
2	66	470.4	688.8	926.4	0.210	0.438	58.7	33.7	11.2	19.6	0.311
3	65	465.6	700.3	972.0	0.229	0.495	52.8	40.5	12.3	16.1	0.313
4	65	463.2	669.3	979.2	0.201	0.503	42.5	24.7	15.3	26.3	0.311
5	65	468.0	708.0	964.8	0.235	0.487	38.8	30.2	16.8	21.5	0.314
6	65	460.8	736.8	984.0	0.269	0.509	35.9	30.5	18.1	21.3	0.310
7	66	436.1	627.2	923.7	0.178	0.454	43.3	31.8	15.2	20.8	0.289
8	65	496.8	724.8	996.0	0.230	0.503	56.1	29.3	11.6	22.2	0.334
9	65	456.0	688.8	936.0	0.226	0.465	59.8	28.1	10.9	23.1	0.306
10	66	480.0	691.2	960.0	0.205	0.465	46.1	36.2	14.3	18.2	0.318
11	66	441.0	634.6	877.1	0.181	0.407	47.8	32.7	13.8	20.2	0.292
12	67	474.0	682.9	966.6	0.197	0.465	54.7	31.4	12.3	21.3	0.309
AVERAGE	65.5	463.6	686.2	953.4	0.215	0.472	48.2	31.8	14.0	20.9	0.309

Table 6.3: Comparison of Column Characterisation

Type of packing used	Column ID (cm)	Superficial velocity (cm/min)	Operating Temperature (^{o}C)	Voidage	Total System Length (cm)	Total No of Columns	Average K_d values		Average HETP (mm)		Separation Factor α
							K_d^G	K_d^F	Wrt Gluc	Wrt Fruc	
Korela VO7C resin Ca^{++} charged	5.4	1.09	20	0.309	787	12	0.215	0.472	14	20.9	2.20
Zerolit 225 SRC14 resin Ca^{++} charged	10.8	1.15	20	0.331	707	10	0.168	0.417	16.2	25.8	2.48
Zerolit 225 SCR14 resin Ca^{++} charged	2.54	1.05*	20	0.449	789	12	0.23*	0.66*	16*	24*	2.87

* Values read from graph

further due to the chemical complex formation with the calcium ions thus giving even higher fructose elution volumes. The average separation factor for a glucose and fructose mixture was found to be 2.2, and the average number of theoretical plates per column was 48 with respect to glucose and 32 with respect to fructose. This resulted in average HETP values of 1.4cm with respect to glucose and 2.09cm with respect to fructose. Variations in voidage, the distribution coefficients and HETP values from column to column are due to the non-uniformity of packing and different degrees of calcium charging of the individual columns. Ideally all these column parameters should be constant in order to ensure similar migration rates for the individual components through successive columns. However, these variations can be tolerated in the SCCR7 system because they are smoothed out along the 12 columns and the continuous cyclic operation ensures essentially constant component migration rates through the system's separation section.

Table 6.3 shows the characteristics of different size columns. All columns were packed with calcium charged resins and similar ZEROLIT 225 SRC14 resin was used for the 2.54cm id (204) and 10.8cm id (202, 206) systems. The results were comparable to the ones obtained by previous workers and the SCCR7, 5.4cm id, system had better separation potential, ie. lower HETP values. This is believed to be partly due to the more efficient KORELA resin and to the better packing density achieved, ie. lower voidage than the other systems.

6.6 Effect of Wide Feed Bands

The column characterisation was carried out using the initial Glueckauf equation (2.11) and at infinite dilution conditions; very low injection volumes and low concentrations, ie. 0.5 gram of carbohydrate (5cm^3 of 10% w/v solution) was injected once through a column having a total void volume of over 463cm^3 and containing only

deionised water. The distribution coefficient values and the "apparent" number of theoretical plates obtained under the above conditions are valid since infinite dilution conditions existed, and are used for comparing the SCCR7 performance against other similar systems. This equation (2.11) however does not account for the increasing separation difficulties encountered in reality due to increasing feed volumes.

Gluechkauf (42) modified his initial theory to account for wide feed bands and developed equation (2.17) which gives the "true" number of theoretical plates, N. This equation was rearranged in the following form:

$$B1 = \frac{N^*}{N}\left[1 + \frac{P}{3} + \frac{7P^2}{45} + \frac{0.0765\,P^3}{1 - 0.565P}\right] \qquad \text{..... 6.7}$$

Where:

P is a systems loading related parameter and is an indication of the applicability of equation 6.7, ie. its results are accurate for P less or equal to 1.6, and

$$P = \left(\frac{N_o}{N}\right)^2 \left(\frac{N^*}{4}\right) \qquad \text{..... 6.8}$$

N* is the "apparent" number of theoretical plates obtained from equation 2.11, and for the SCCR7 system its total value is 384 (based on fructose).

N_o is the "appartent" number of theoretical plates occupied by the feed band.

The solution to equation 6.7 for a given No is the N value for which the ratio B1 is very near or equal to 1. This N value represents the total "true" number of theoretical plates required to achieve complete separation, since it accounts for the actual operating conditions where feed band broadening is taking place. This equation has been obtained for batch columns, however it can be used for the semicontinuous operation since during any cycle the system can be visualised as a "static" column with the feed entering at the middle and the eluent at the inlet.

The equation 6.7 was solved by trial and error using a simple interactive Fortran 77 computer program for a wide range of No values. The calculated N and P values (Appendix A) applicable to the SCCR7 refiner are listed in Table 6.4 and are plotted on Figure 6.2. The number of plates based on fructose are used since this is the retarded component, and the component of main interest. This equation is applicable for No values of up to 240 plates. As can be seen from Figure 6.2 the values of N increase gradually for No up to 60 plates, and then show a sharper linear increase. To incorporate these results into the simulation program (Chapter 10) it was decided to approximate the N vs No curve by two straight lines as follows:

For No less than 60 plates:

$$N = 3.85 \times No + 339.40 \quad \text{..... 6.9}$$

and for No greater or equal to 60:

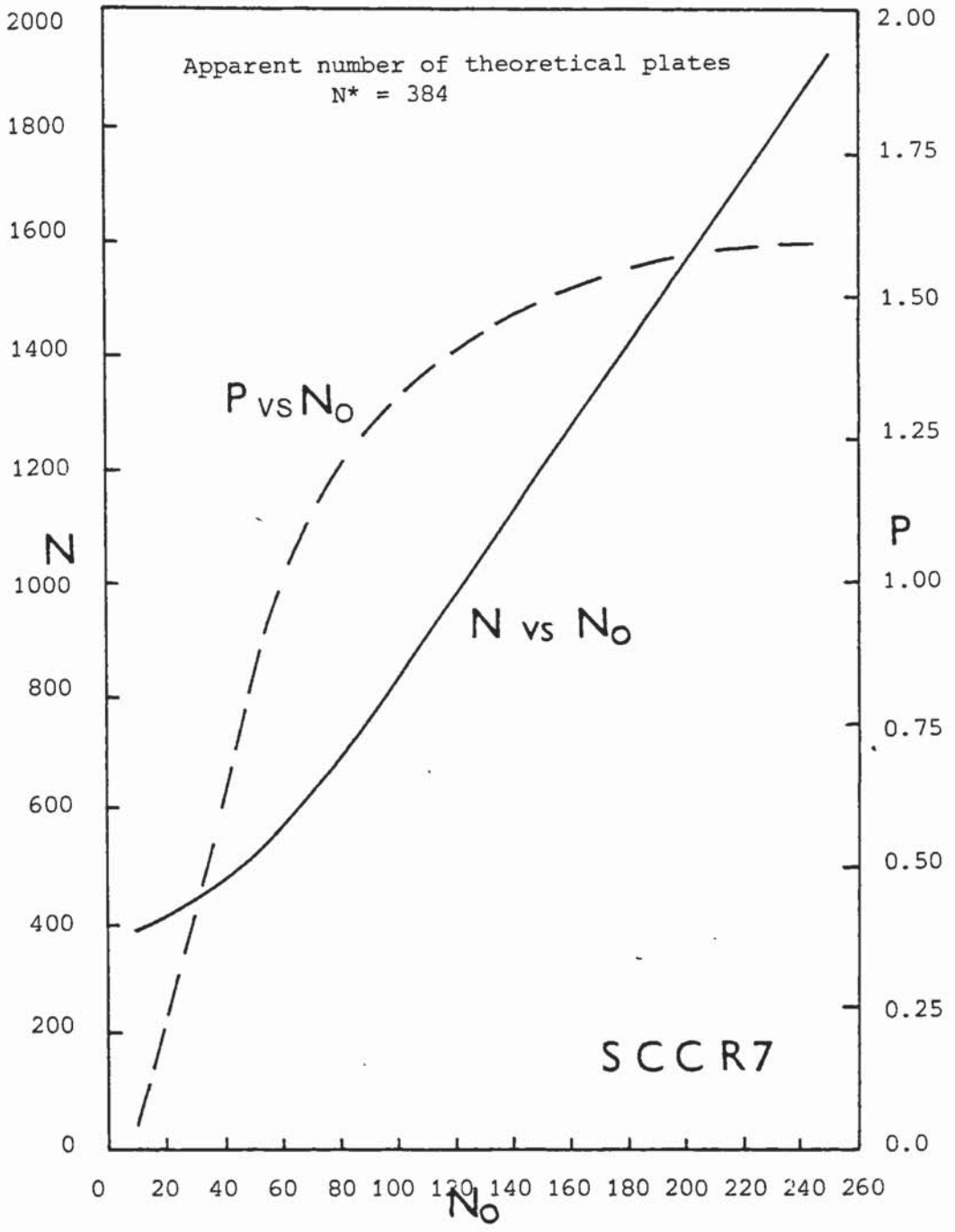
$$N = 7.13 \times No + 139.53 \quad \text{..... 6.10}$$

The "apparent" number of theoretical plates is useful during the comparison of different SCCR systems, however the "true" number of theoretical plates represents a better measure of the system's separation potential, and for the actual operating conditions it is expected to be greater than the "apparent" number.

Table 6.4: True number of theoretical plates (N), required for increasing feed bands (No) (SCCR7 System)

No	P	N
10	0.06	392
20	0.23	413
30	0.44	444
40	0.66	482
50	0.86	529
60	1.01	584
80	1.22	710
100	1.34	846
120	1.42	987
140	1.47	1130
160	1.51	1275
180	1.54	1420
200	1.57	1566
220	1.59	1712
240	1.60	1858

Figure 6.2: Plot of the "true" number of theoretical plates (N) and system loading related parameter (P), against the "apparent" number of theoretical plates occupied by the feed band (N_O)



CHAPTER 7

7.0 EXPERIMENTAL PROCEDURE AND EQUIPMENT DEVELOPMENT

7.1 Introduction

In this chapter the analytical systems used for the sugar analysis of the samples collected during the experimental work are described, and the performance and suitability of the main analytical carbohydrate columns available are evaluated. A detailed description of the experimental procedure, data collection and processing is carried out. A historical review of the up to date development of the SCCR systems is reported to justify the choice of the particular operating conditions used, and the effects of the various factors affecting the separating performance are examined. A set of general equations relating the effects of operating temperature, flow rates and concentration on the distribution coefficients of the principal components glucose and fructose has been developed, providing a better understanding of the separating operation and assisting in selecting the best operating conditions for any chromatographic system. Finally two commissioning runs are reported providing the basis for further development.

7.2 The Analytical HPLC Systems

7.2.1 System Description

Resin based High Performance Liquid Chromatography (HPLC) columns use the mechanisms of ion-exchange, liquid exchange, size exclusion, reversed and normal phase partitioning (222). The applicability of the above mechanisms depends on the compounds being analysed and the degree of selectivity required. Reversed phase and ion-pairing HPLC techniques require complex eluent conditions for effective separations, because they work on the principle of modifying the sample to be analysed until it is compatible with the column. In the resin based HPLC columns the column packing material is modified instead to be compatible with the compound chemical

structure. Therefore resin based columns usually allow the use of an isocratic HPLC system, and thereby simplify and speed up sample preparation and analysis.

Two isocratic HPLC systems were used for the analysis of the samples generated during the experiments, using deionised water as the eluent phase. The majority of the barley syrup experimental runs were analysed using the first and rather simpler HPLC system consisting of:

- an MPL Series II eluent delivery pump having an operating range of 0 to 2 $cm^3.min^{-1}$, (MPL Ltd, Feltham, Middlesex);
- a Waters Associates differential refractometer type R401, (Millipore UK Ltd, Harrow, Middlesex);
- a Hewlett-Packard 3390 A integrator (Hewlett-Packard Ltd, Altrincham, Cheshire);
- the analytical column was placed inside a glass jacket and it was kept at 85°C by recirculating hot water using a TECAM C400 Circulator (TECHNE, Cambridge);
- the eluent was degassed by keeping the eluent containing glass aspirator inside a hot water bath which was maintained at 85°C. The eluent contained 0.02 gl^{-1} calcium acetate;
- a 30 x 0.65cm SUGAR PAK1 (Waters Associates) column packed with calcium charged resin. The eluent flow rate was 0.5 $cm.min^{-1}$, and the pressure drop was approximately 6900 KN m^{-2};
- a pressure gauge was fitted between the pump and the HPLC column, acting as a pressure indicator and as a pulsation dampener.

On the later stages of the experimental work the second analytical HPLC system was acquired and was used in the analysis of the last three barley syrup experiments and in the reaction-separation work. The system consisted of:

- a BIO-RAD HPLC 1330 Pump (BIO-RAD UK Ltd, Watford). The pump is employing a two piston arrangement which operate out of phase by 180^{o} thus minimising any pulsations;
- a BIO-RAD column block heater;
- a BIO-RAD refractive index monitor model 1750;
- a SPECTRA-PHYSICS SP4270 Integrator (St Albans, Herts);
- and a TALBOT ASI-3 Autosampler (Alderley Edge, Cheshire);
- the eluent was effectively degassed by heating it to 80^{o}C. This was achieved using an isomantle and a Baird and Tatlock (Atherstone Warwickshire) temperature controller;
- an Anachem (Luton, Bedfordshire) debubbler was used to remove any bubbles that found their way inside the eluent delivery pipe;
- in addition to the SUGAR PAK1 column, a 30 x 0.78cm AMINEX HPX-87C calcium charged BIO-RAD and a 30 x 0.65cm ALLTECH 700 CH (Alltech UK Ltd, Carnforth, Lancashire) calcium charged HPLC columns were used, and the operating conditions were kept the same, ie. 0.5cm^{3}.min^{-1} eluent flow rate and 85^{o}C temperature. The pressure drops were approximately 6900 KNm^{-2} and 8300 KNm^{-2} for the BIO-RAD and ALLTECH columns repsectively.

7.2.2 Column Maintenance

The analytical HPLC columns are packed with fine particles, 5 to 10μm, and they effectively act as a depth filter. The resins although they are suitable for separating certain solutes, they also are capable of retaining other impurities. This decreases the column efficiency and selectivity and must be avoided. Therefore the following precautions were taken:

- fresh deionized water was used and it was filtered using a 10μm slip-on filter (BIO-RAD);
- all samples were filtered through a 0.45μm GELMAN (Brackmills, Northampton) disposable filter to eliminate insoluble particles;
- by heating the eluent to 80°C it was degassed and prevented from bacterial growth;
- 0.02 gl^{-1} of calcium acetate were added in the eluent to replace any calcium ions lost due to the presence of other ions and to prevent any on column inversion of the injected sucrose samples;
- deionized water was pumped for half an hour before switching the system off;
- when the column was not used for long periods it was flashed through with deionized water containing 0.02% sodium azide to prevent any microbial growth;
- after approximately 50 injections the column was reversed and cleaned by pumping deionized water at 0.2$cm^3.min^{-1}$ for over 6 hours;
- when a reduction in column performance was noted or the pressure drop was increased by more than 10%, the column was cleaned with a 30% acetonitrile solution at 0.1$cm^3.min^{-1}$ and then regenerated using 0.1M calcium acetate. The use of calcium chloride, calcium nitrate or other salt formed from a strong acid must be avoided since it may lead to column corrosion and irreversible bed poisoning (223);
- for additional protection a guard column was used. BIO-RAD Aminex HPX-87C Micro-Guard cartridges were used initially but they were later replaced with the more economical Hibar-Lichrocart 4-4 cartridges supplied by BDH Chemicals Ltd (Atherstone, Warwickshire). The use of a guard column was found to be essential because the insoluble matter present was sufficient to clog the cartridge after approximately 24 hours of continuous operation.

7.2.3 HPLC Column Selection

7.2.3.1 Introduction

Each column was evaluated in terms of life expectancy and resolution. All three HPLC columns were similarly priced and had similar characteristics, ie. 30cm long and having about 10000 theoretical plates. The experience gained from long term column usage and the results obtained from the separation of test solutions, were used to evaluate the merits of each column. The two test solutions were:

- test solution A, having a total sugar solids concentration of 10% w/v containing:
  2% w/v Dextran T40
  2% w/v Surcrose
  2% w/v Glucose
  2% w/v Fructose
  2% w/v Mannitol

- test solution B, having a total sugar solids concentration of 12% w/v, containing:
  2% w/v Dextran T40
  2% w/v Maltose
  2% w/v Sucrose
  2% w/v Glucose
  2% w/v Fructose
  2% w/v Mannitol

The test sample experiments were carried out on the second HPLC analytical system, operating at identical conditions, ie:

Eluent flow rate = $0.5cm^3.min^{-1}$

Operating temperature = 85^oC

Injection volume = 20μm

Chart speed = $1cm.min^{-1}$

Refractive index monitor range = 32

Integrator attenuation = 128

7.2.3.2 Column Life Expectancy

The SUGAR PAK1 column was used effectively for over 1800 injections, and then a large pressure increase was detected. This was believed to be because of the large pulsations created from the single head MPL pump, causing the collapse of the packed bed. The BIO-RAD and ALLTECH columns were used mainly on the second HPLC system (BIO-RAD) where the pulsations were kept to a minimum due to the double-head BIO-RAD pump, thus overcoming the above problem. Over 1300 samples have been analysed using the ALLTECH column which is still operating efficiently. The pressure drop over the new BIO-RAD column was increasing continuously after 24 hours of operation, caused by change of the ionic form. It was believed that the low pH (5.6 to 5.8) of the deionized water converted some of the resin into the sodium form resulting in a "swelling" of the packing. This problem was solved by using distilled water (pH 6.5) instead, and adding 0.02 gl^{-1} of calcium acetate as a precaution, and approximately a further 1200 samples have now been satisfactorily analysed.

7.2.3.3 Column Resolution Tests

The results of the analysis of the test samples are shown in Tables 7.1 and 7.2 and the chromatograms are shown in Figures 7.1 to 7.6. The retention times are similar for the SUGAR PAK1 and ALLTECH columns. The respective times are almost 50%

longer in the BIO-RAD column and the peaks are flatter and broader (Figures 7.3 and 7.4). The BIO-RAD column gives the best glucose-fructose resolution with the SUGAR PAK1 also being competitive. Disaccharides are separated from monosaccharides by size exclusion. The disaccharides are eluted together in both the SUGAR PAK1 and BIO-RAD columns thus making the separation of sucrose from maltose impossible (Figures 7.2 and 7.4). In the ALLTECH column the sucrose is eluted as two distinct peaks having retention times similar to glucose and fructose, (Figure 7.7) indicating that the sucrose has been inverted by the nature of the resin. Therefore samples containing sucrose, glucose and fructose could not be analysed using the ALLTECH column (Figures 7.5 and 7.6). This problem was reported to the supplier and the column was replaced. The new column did not exhibit the above behaviour, ie. there was no sucrose inversion, and glucose, fructose and sucrose were separated successfully (Figures 7.8 and 7.9 also Tables 7.1 and 7.2). As in the SUGAR PAK1 and BIO-RAD columns the disaccharides were eluted at the same time. The SUGAR PAK1 column was favoured in the enzyme studies because it was found to be more compatable with the nature of these samples (224). Towards the end of the experimental work a 25cm long Polypore CA (calcium charged) column supplied by PIERCE UK Ltd, (Cambridge), was evaluated. The recommended eluent flow rate was 0.3cm^3 min^{-1}, and the remaining operating conditions were the same. The resolution obtained at this eluent flow rate (Tables 7.1 and 7.2 and Figures 7.10 and 7.11) was inferior to the other columns and not satisfactory. There was no further improvement in separation when alternative eluent flow rates were used, ranging from 0.1 to 0.5 cm^3 min^{-1}.

7.2.4 Conclusions

The column is the heart of the HPLC system, and the success of the analysis depends on selecting precise operating conditions and on proper maintenance. Therefore the maintenance procedure described in Section 7.2.2 must be followed. Distilled deionised water must be used and the pH should be kept between 6.5 and 7.

Table 7.1: Analysis of Test Solution A

		HPLC Column				
				ALLTECH		
	Component	SUGAR-PAK1	BIO-RAD	Old	New	PIERCE
Retention time (min)	Dextran T40	4.66	8.17	4.53	4.35	4.86
	Sucrose	7.33	10.17	-	7.19	5.69
	Glucose	9.14	12.29	8.81	9.06	6.60
	Fructose	11.16	15.40	10.54	11.19	7.85
	Mannitol	13.89	20.40	12.88	14.05	9.87
Conc % w/v	Dextran T40	2	2	2	2	2
	Sucrose	2	2	-	2	2
	Glucose	2	2	3	2	2
	Fructose	2	2	3	2	2
	Mannitol	2	2	2	2	2

Table 7.2: Analysis of Test Solution B

		HPLC Column				
				ALLTECH		
	Component	SUGAR-PAK1	BIO-RAD	Old	New	PIERCE
Retention time (min)	Dextran T40	4.66	8.13	4.53	4.35	4.85
	Maltose	7.36	10.20	7.13	7.23	5.70
	Sucrose			-		
	Glucose	9.13	12.27	8.81	9.07	6.58
	Fructose	11.15	15.37	10.54	11.20	7.86
	Mannitol	13.88	20.33	12.88	14.05	9.88
Conc % w/v	Dextran T40	2	2	2	2	2
	Maltose	4	4	2	4	4
	Sucrose			-		
	Glucose	2	2	3	2	2
	Fructose	2	2	3	2	2
	Mannitol	2	2	2	2	2

Figure 7.1: Elution profile of test sample A using the SUGAR-PAK1 column

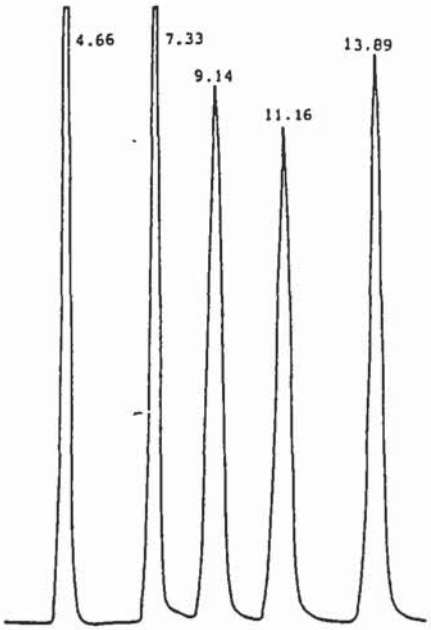


Figure 7.2: Elution profile of test sample B using the SUGAR PAK1 column

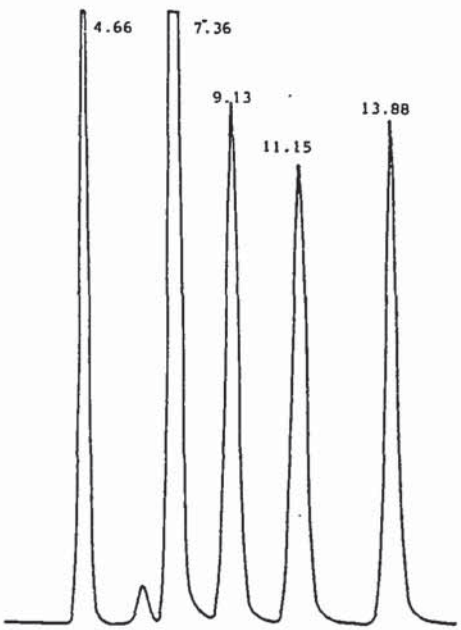


Figure 7.3: Elution profile of test sample A using the BIO-RAD column

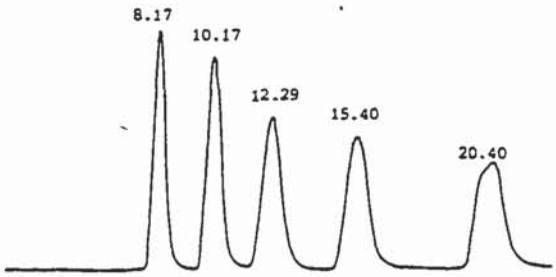


Figure 7.4: Elution profile of test sample B using the BIO-RAD column

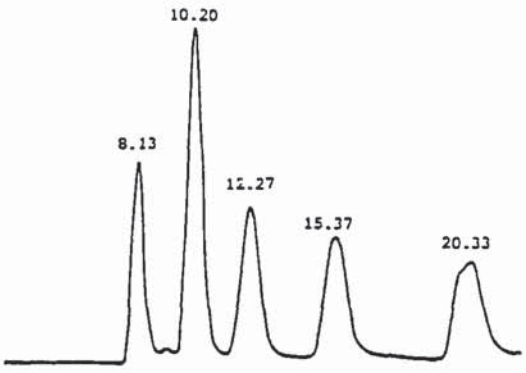


Figure 7.5: Elution profile of test sample A using the old ALLTECH column

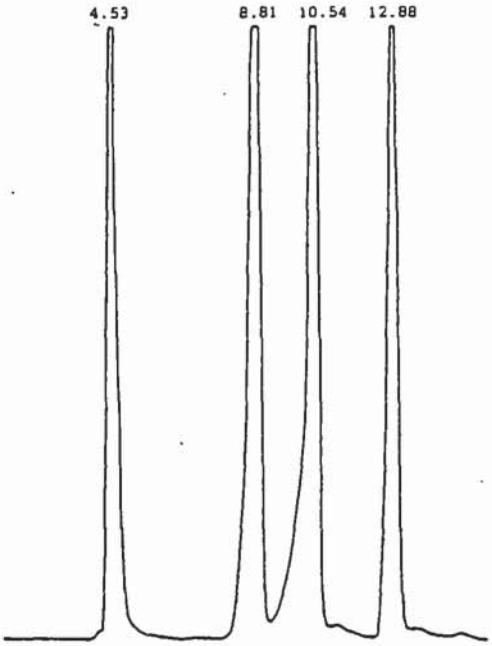


Figure 7.6: Elution profile of test sample B using the old ALLTECH column

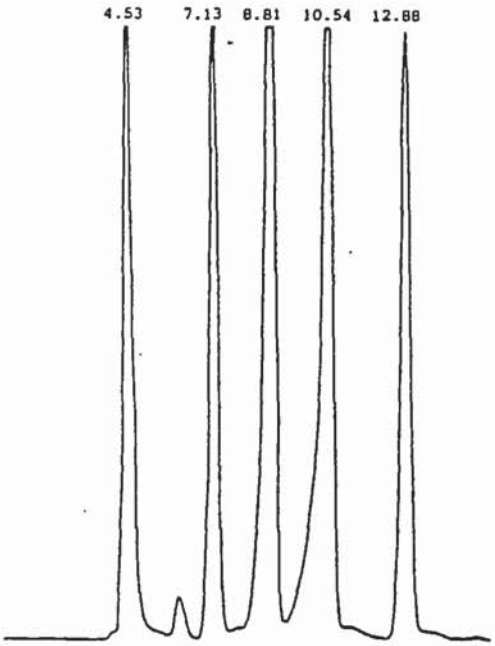


Figure 7.7: Standard sucrose elution profile from the old ALLTECH column

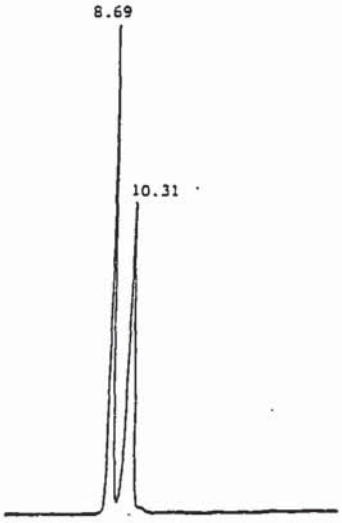


Figure 7.8: Elution profile of test sample A using the new ALLTECH column

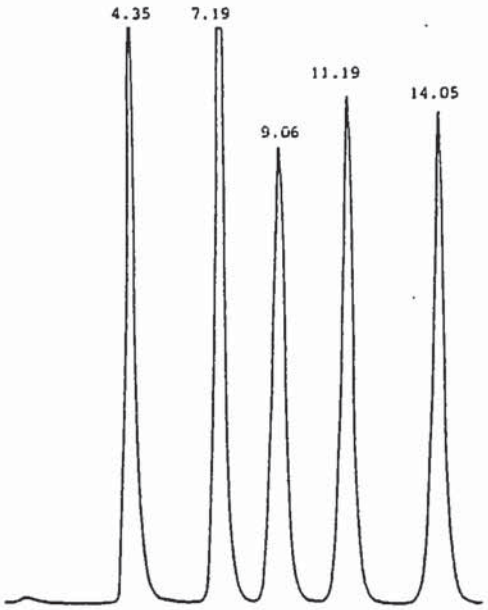


Figure 7.9: Elution profile of test sample B using the new ALLTECH column

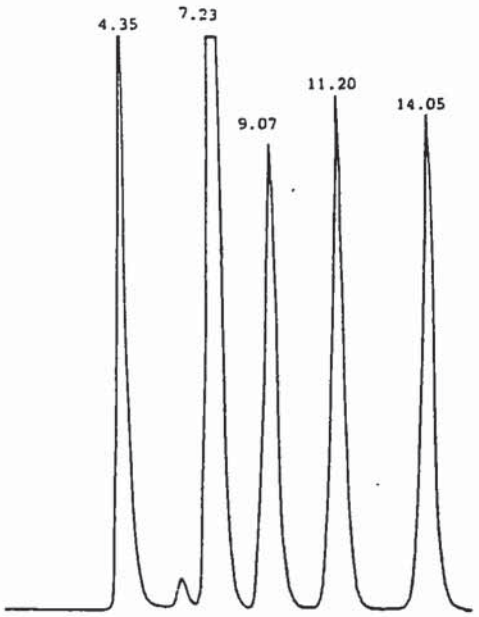


Figure 7.10: Elution profile of test solution A using the PIERCE column

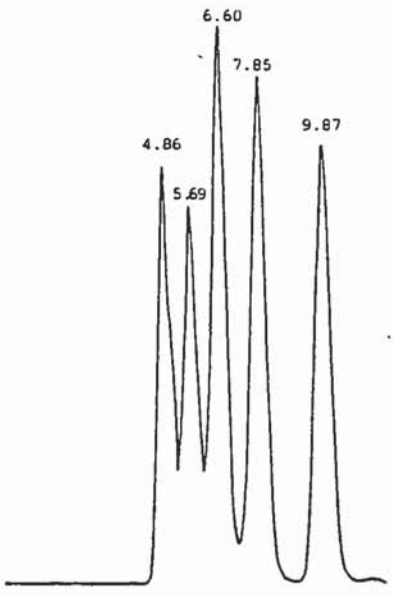
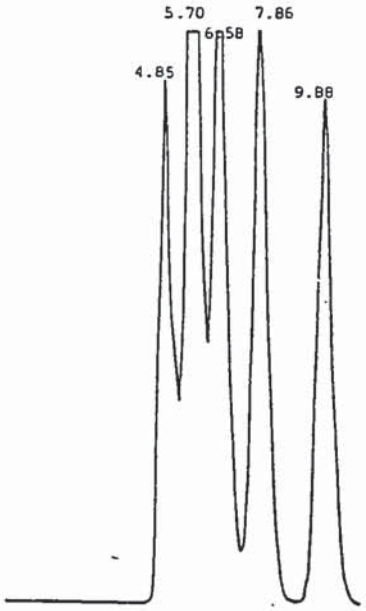


Figure 7.11: Elution profile of test sample B using the PIERCE column



When the glucose-fructose separation is of prime importance the BIO-RAD column offers the best resolution. No column can be used for the separation of disaccharides. The BIO-RAD column offers a slightly better resolution, while the stability of the Alltech's resin is questionable. The SUGAR PAK1 column is more compatable with samples containing enzymes and proteins, gives a good resolution of the basic carbohydrates and offers a long life expectancy and is therefore strongly recommended.

7.3 Experimental Procedure for the SCCR7

7.3.1 Feed Preparation

The industrial syrup was provided by FINN-SUGAR ENGINEERING, Finland, and had a 70% w/v total sugar solids concentration. It contained approximately 42% fructose, 52% glucose and 6% impurities. The impurities were mainly maltose and other oligosaccharides which were not identified by us or by the suppliers. The syrup was diluted with deionised water down to the desired concentration for the particular experiment. The new solution was analysed using the HPLC system and it was readjusted if needed. The results of the final HPLC analysis of the syrup provided the basis for the mass-balance calculations. When the experiments were conducted at low temperatures and concentrations 0.02% w/v of sodium azide, NaN_3, was used to prevent any biological growth. At high concentrations and temperatures the bacterial growth is inhibited, therefore the addition of sodium azide is not necessary and must also be avoided because at high temperatures (> $60^{o}C$) it can form a highly explosive salt with the copper, lead or brass metals present.

7.3.2 Start-up Procedure

Safe operation was ensured by operating in accordance with the following steps:

- the desired temperature settings were selected and the eluent-purge, feed, column oven heaters and the fan were switched on;
- each valve was checked by using a low switch time to ensure that they were operating properly;
- then, the digital timer controlling the switch time was set to the required value;
- all inlet valves were opened and the product collecting containers were positioned. When an automated product collection was used the appropriate timers were set to the proper values;
- the eluent, feed and purge pumps were set to the required flow rates, and were switched on;
- when the right operating pressure was reached the "automatic shut-off" mechanism was activated, and all the other controllers were checked
- finally, the experimental run started by switching on the digital timers simultaneously.

7.3.3 Procedures during the Experimental Run

7.3.3.1 Data Collection

The eluent, purge and feed flow rates were checked frequently during every cycle to ensure constant operating conditions. The pressure drop and the temperature inside the delivery network, at the column surface and inside the enclosure were recorded during each cycle. At the middle of each switch, samples were withdrawn from the same sample point on the same column and were analysed to produce the on-column concentration profiles for each cycle. This was possible because during the twelve switches of a cycle, each column served every function, ie. as a feed, eluent entry, purge column or any other column of the separting length.

At the end of the cycle the product collecting vessels were weighted and analysed and a mass balance was carried out. When a pseudo-equilibrium was reached, usually after 6 or 7 cycles, the results of the last two cycles were used to indicate the systems performance. At the end of the last cycle either a new run was commenced or the system was purged. If a new run was started, the new operating conditions were set, ie. new flow rates, feed stock or switch time were selected. By employing this technique it was found that four additional cycles were enough to reach the new "pseudo-equilibrium" corresponding to the new settings, because the on-column sugar concentrations were already built up.

If the purging of the system was selected, then at the end of the last cycle all pumps were switched off, and the feed heater was also switched off. The control system was switched to manual and the new purging period was set on the digital timer, usually 12 to 16 minutes.

The purge pump was switched on and the product was collected in a separate container and analysed. The procedure was repeated until all columns were purged out. The plotting of these data gave the purging concentration profiles and represent the average column concentrations for the particular operating conditions. This is the main difference between the purging and on-column concentration profile where the sugar concentrations are point and not bulk concentrations. The operating conditions and results of each experimental run were presented in a standard form and the run was defined with a set of five figures. For example, the set 55-13-39-24.5-50 corresponds to feed solid content (% w/v), feed flow rate ($cm^3.min^{-1}$), eluent flow rate ($cm^3.min^{-1}$), switch time (min), and operating temperature (^oC) respectively.

7.3.3.2 Establishing "Pseudo-Equilibrium"

Due to the semi-continuous nature of the SCCR7 only a "pseudo-equilibrium" state can be achieved and not a "true" equilibrium like in any other continuous counter-current mass transfer process, ie. distillation. When the on-column concentration profiles of two consecutive cycles were identical, within experimental accuracy, the "pseudo-equilibrium" state was deduced to have been reached, (Figures 7.12 and 7.13). The reference points were the relative concentrations in the corresponding columns and the position of the "cross-over" point, ie. the point of intersection of the glucose and fructose profiles.

7.3.4 Shut-down Procedure

The shut down procedure was as follows:

- all pumps were switched off;
- the eluent-purge, feed heaters, the enclosure heater and fun were switched off;
- the product lines were blocked to prevent the packing from drying out;
- the digital timers were switched off;
- the main valve in the deionised water supply line was closed;
- the compressed air supply was closed.

When the SCCR7 system was not to be used over long periods then 0.02% w/v sodium azide was pumped through to prevent any bacterial growth.

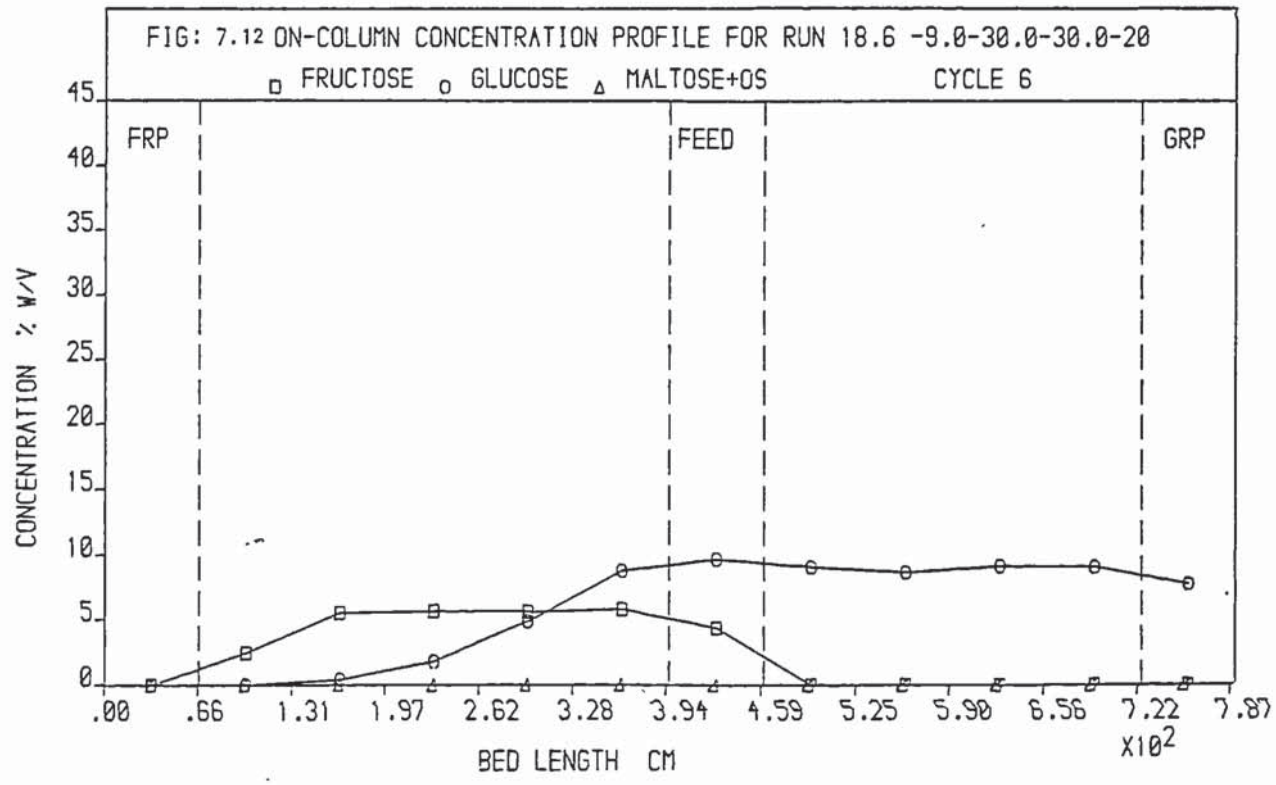


FIG: 7.12 ON-COLUMN CONCENTRATION PROFILE FOR RUN 18.6 -9.0-30.0-30.0-20
□ FRUCTOSE o GLUCOSE △ MALTOSE+OS CYCLE 6
FRP
FEED
GRP
CONCENTRATION % W/V
45
40
35
30
25
20
15
10
5
0
.00 .66 1.31 1.97 2.62 3.28 3.94 4.59 5.25 5.90 6.56 7.22 7.87
BED LENGTH CM
X10²

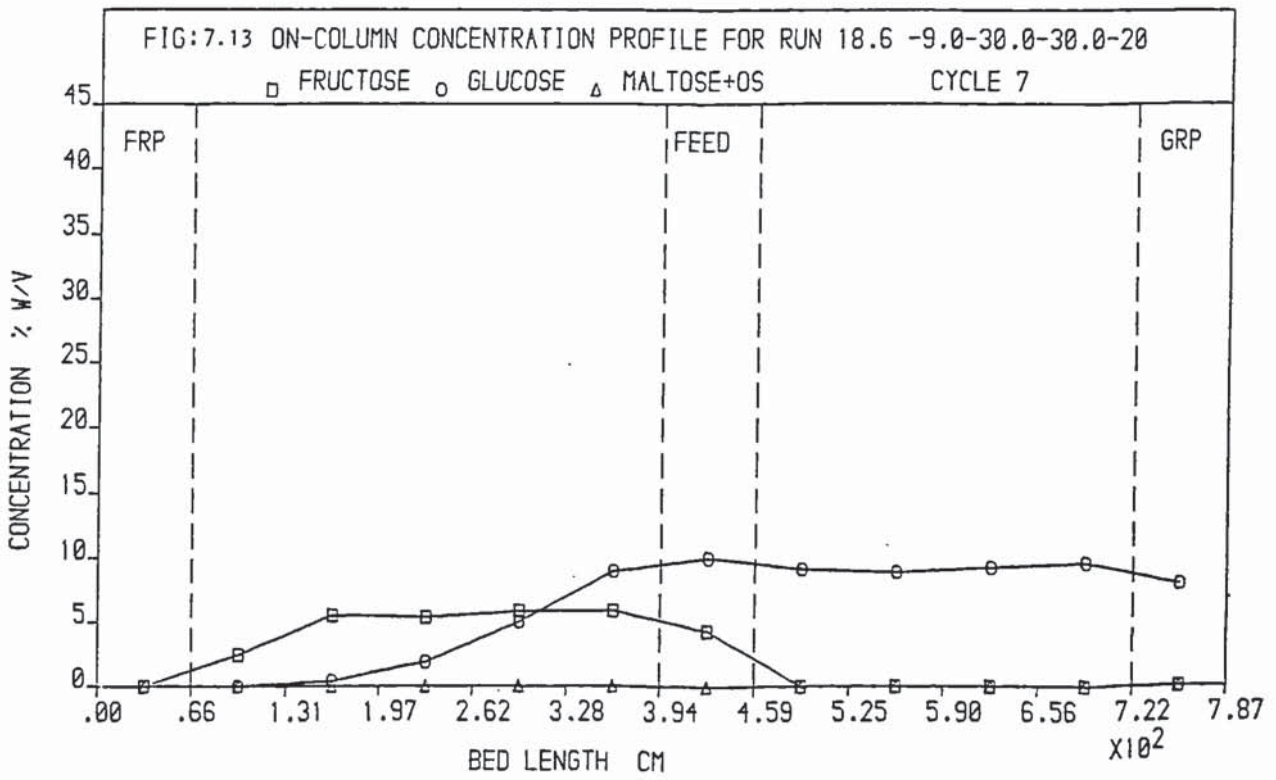


FIG:7.13 ON-COLUMN CONCENTRATION PROFILE FOR RUN 18.6 -9.0-30.0-30.0-20
□ FRUCTOSE o GLUCOSE △ MALTOSE+OS CYCLE 7
FRP
FEED
GRP
CONCENTRATION % W/V
45
40
35
30
25
20
15
10
5
0
.00 .66 1.31 1.97 2.62 3.28 3.94 4.59 5.25 5.90 6.56 7.22 7.87
BED LENGTH CM
X10²

7.4 The Development of the SCCR Systems

7.4.1 Equipment Development

The development of continuous counter-current chromatographic equipment has been carried out by Barker and co-workers over the last twenty years. In the prototype equipment (72) a packed torroidal bed was rotated past stationary feed, product and eluent ports and a mobile phase valve. Equipment based on this principle have been used with gaseous mobile phases (72) and liquid mobile phases (208) successfully on a small scale. The scaling up of systems with such a conformation was difficult because of practical limitations with regard to the length of the chromatographic column, and subsequent apparatus divided the packed bed into short interconnected column sections which were arranged parallel to one another to form a compact tube bundle (208, 209). The tube bundle was rotated in a similar fashion past fixed ports and a mobile-phase valve. While this type of chromatographic equipment has been successful with columns of up to 2.5cm diameter, direct scale-up for production scale purposes is not convenient due to the problems of achieving large moving face seals with the necessary sealing efficiency and the difficulty of rotating the heavy column arrangement at a constant speed.

These limitations together with useful experience gained from earlier work led to the construction of the first semi-continuous chromatographic refiner SCCR by Deeble and Barker (78) fifteen years ago. The major difference between the principle of operation of the SCCR unit and the earlier smaller scale CCR units lies in the fact that instead of the packed section rotating in a clockwise direction past fixed inlet and outlet ports, in the SCCR unit the inlet and outlet ports advance in an anti-clockwise direction past fixed columns (78). The equipment was operated as a Gas-Liquid chromatographic separator and the counter-current movement was achieved by operating a number of solenoid valves in a similar way as described in Chapter 5. The original system consisted of twelve columns, each having an effective height of 61cm and 7.6cm diameter (77, 78, 210).

The successful operation of the Gas-Liquid SCCR equipment (77, 210) and encouraging results obtained from the earlier Liquid-Liquid CCR units (208) led to the construction of the first Liquid-Liquid SCCR3 equipment in the mid-seventies (211). The special features incorporated into the design of the SCCR3 unit were based on experience gained with the earlier machines and the envisaged experimental program. The SCCR3 unit consisted of twenty 70cm long and 5.1cm id glass columns, packed with porous silica (Spherosil XOB075) and was operated in the gel permeation mode, GPC, investigating the continuous fractionation of dextran.

The successful operation of the SCCR3 system verified the scaling-up potential of the systems employing this technique. The next development step was the construction of the SCCR4 system (203), consisting of ten glass 70cm long columns of 2.54cm id, packed with Zerolit 225 SCR14 calcium charged resin, used for the separation of glucose-fructose mixtures by employing the ion-exchange principle. The main development was the replacement of the solenoid valves by pneumatic valves governed by a pneumatic controller. This was a direct result of the operating difficulties and structural weaknesses encountered in the solenoid valves and also to provide additional flexibility, safety and operational reliability. The development of the SCCR systems was carried out in parallel according to the operating principle employed. In one case the SCCR3 unit was replaced by the SCCR5 unit consisting of ten 70cm long by 5.4cm id stainless steel columns operating in the gel permeation principle. This unit was used for dextran fractionation, and it succeeded in separating efficiently both the high and low molecular weight dextran fractions, meeting the industrial specifications (206, 212). In the second case the emphasis has been on the use of the SCCR systems for the separation of carbohydrate mixtures containing in particular glucose and fructose. These separations were obtained using the appropriate ion exchange media.

Chuah (213), because of the pressure limitations of the glass columns and in order to get extra effective length, replaced the glass columns of the SCCR4 unit by twelve 2.54cm id and 65cm long stainless steel columns, also packed with Zerolit 225 SRC14 calcium charged resin, (SCCR4 Mk II). The next scaling-up step was the construction of the ten column SCCR6 unit having 75cm long columns of 10.8cm id (214, 215), packed with Zerolit SRC14 calcium charged resin. The glucose-fructose separation at production scale throughputs was investigated, and the system was operated in both batch and continuous modes to enable a comparison and a theoretical relationship to be determined between the two modes of operation (216).

Abusabah (217) investigated the separation of inverted sucrose feedstocks on the SCCR4 Mk II unit and then replaced the 2.54cm id columns with 5.4cm id stainless steel columns, and incorporated a safety shut-off device for continuous unattended operation (SCCR7). The columns were packed with Duolite A113 anion-exchange resin, and the production of High Fructose Syrups from glucose-fructose mixtures (218) was studied. These SCCR7 columns were repacked with calcium charged KORELA VO7C resin and used in this research work. The equipment was modified as described in Chapter 5 in order to minimise the dead volume, improve reliability and enable better operating flexibility with respect to better product quality and concentration.

7.4.2 Development of Operating Parameters

7.4.2.1 General

Deeble (77, 234) in his gas chromatography work investigated the effect of changing the operating conditions, such as feed and carrier flow rates, within the theoretical limitations as defined by equation 5.9. In his work he identified factors that restricted the separating potential, ie.

- the effect of solute concentration on the distribution coefficients;
- the variation of solute migration velocity due to variations of solute concentration, temperature and pressure gradients;
- system characteristics, namely: semi-continuous operation mode, and limited length of the separation section.

and he incorporated them into equation 5.9.

The same conclusions were shared by other workers (22, 211, 203) and the basic design equation 5.9 was modified as follows:

$$(K_{dg} + \delta_{1g} + \delta_{2g}) < \frac{Le}{P} < \frac{Le + Lf}{P} < (K_{df} - \delta_{1f} - \delta_{2f}) \quad \text{..... 7.1}$$

or

$$(K_{dg} + \delta_{1g} + \delta_{2g}) < \frac{Lm}{P} < (K_{df} - \delta_{1f} - \delta_{2f}) \quad \text{..... 7.2}$$

where:

$$\frac{Lm}{P} = \frac{\left(\frac{Le}{P} + \frac{Le + Lf}{P}\right)}{2}$$

where:

δ_{1g} and δ_{1f} = fractional changes due to the effect of concentration on the distribution coefficient K_{dg} and K_{df} respectively.

δ_{2g} and δ_{2f} = fractional changes due to the effect of system characteristics on the distribution coefficient K_{dg} and K_{df} respectively.

Additional terms could be inserted in the above equations to account for the effects of temperature changes, feed and eluent flow rate increases, feed variations, ie.

changes in glucose to fructose ratio, and presence of impurities, on the distribution coefficients and thus on the separation performance. Although this would provide an illustrative indication of the narrowing in the operating limits, Lm/P ratios, its practical usefulness would be limited since the physical values and the variations of each of these terms are not known. In the following part of this work the individual effect of the above mentioned factors has been investigated to establish its significance and hence assist in selecting the proper operating conditions.

7.4.2.2 Operating Temperature

It has been shown conclusively (22, 203, 213) that by increasing the operating temperature from ambient to 60°C the on-column concentration profiles are altered, the "cross over" point moves progressively towards the glucose rich product resulting in reduced GRP purities. An investigation of the individual concentration profiles (202) reveals that the glucose profile remains unaffected while the fructose profile shifts towards the GRP. This can be explained by the fact that the retention volume is generally reduced with an increase in temperature and therefore the solute bands are brought nearer to each other resulting in a greater overlap. In addition, β-D-fructopyranose is the sweeter fructose tautomer and is the only known form of fructose that forms a complex with the Ca^{2+} ions (Chapter 3). The proportion of this form at equilibrium to the other form is a function of temperature (91) and it decreases with increasing temperature (Table 7.3) thus reducing the fraction of fructose available for complexing and increasing the amount of fructose moving with the mobile phase. The concentration has been found not to affect the relative amounts of the fructose tautomers. To establish a relationship between temperature and distribution coefficients Chuah's (204) results were used (Table 7.4) and it was assumed that any chromatographic system would show analogous K_d changes. As expected, the

glucose distribution coefficient was not affected significantly and although its values were fluctuating they showed no clear pattern. The experiments were carried out in a 1.14cm id and 65.5cm long column packed with Zerolit 225 SRC14 resin in the calcium form, and operating at infinite dilution conditions. Because the actual distribution coefficient values change from system to system, due to different column dimensions and packing characteristics of the particular chromatographic system, it was decided to express the changes in the K_{df} values as a percentage, where the $^{25}K_{df}$ value at 25°C was taken as 100%. By assuming a linear change in K_{df} a regression line through the five points provides the general equation:

$$K_{df} = (114.55 - 0.563 \times TEMP) \times {}^{25}K_{df}/100 \qquad \ldots\ldots\ldots 7.3$$

Therefore, for each system its own fructose distribution coefficient value, derived at 25°C and at infinite dilution can be modified using equation 7.3 to give a better estimate of the actual K_{df} for the particular operating temperature. In this experimental work the operating temperature was kept at 60°C, with the exception of the commissioning runs, to prevent any microbial growth and to minimise the pressure drop along the system.

7.4.2.3 Operating Switch Time and Flow Rates

The selection of both the switch time and flow rates is discussed together because they are directly related (equation 5.9), ie. for constant Lm/P ratios, as the switch time increases the operating flow rates must decrease. Throughout the development of the SCCR systems the eluent, feed and purge flow rates were selected for an arbitrary chosen switch time, by using the constrains imposed by equations 5.9 and 5.10. Ching (203) initially used a 45 minute switch time but the long process times (about 9 working days per run), resulted in reducing the switch time to 30 minutes. This

Table 7.3: Equilibrium Concentrations of 20% Fructose at Different Temperatures (91), (the amounts of the Keto-fructose and α-D-fructopyranose present were very low)

Temperature °C	α-D-Fructo-Furanose %	β-D-Fructo-Furanose %	β-D-Fructo-Pyranose %
0	4	18	78
22	6	21	73
67	8	28	64
77	12	31	57

Table 7.4: Effect of temperature on the fructose distribution coefficient (204)

Temperature °C	Fructose distribution coefficient K_{df}	% variation in K_{df}
Ambient (~ 25)	0.875	100.0
33	0.827	94.5
40	0.825	94.3
50	0.762	87.1
65	0.674	77.0

Table 7.5: Effect of Eluent Flow Rate, expressed as a Linear Velocity, on the Distribution Coefficients (204)

Linear Velocity cm/min	Glucose Distribution coefficient K_{dg}	% variation in K_{dg}	Fructose distribution coefficient K_{df}	% variation in K_{df}
0.098	0.290	115.5	0.808	116.3
0.412	0.231*	-	0.735	105.8
0.588	0.247	98.4	0.722	103.9
0.813	0.252	100.4	0.712	102.5
1.092	0.251	100.0	0.695	100.0
1.372	0.303*	-	0.657	94.5
1.568	0.262	104.4	0.648	93.2
1.999	0.216	86.1	0.648	93.2

* data omitted during the calculations.

switch time was adopted by other workers. However, by operating at 30 minutes the production throughput is low and the fructose on-column profiles are found to be broader (202). In order to increase the throughput Gould (214) and others (22, 202) decreased the switch time to 15 minutes and doubled the feed and eluent flow rates so that the Lm/P ratio would be kept constant. This decrease in switch time, although increasing the throughputs, resulted in lower fructose rich product (FRP) purities and in a substantial shift of the cross-over point towards the FRP section. In this work it was decided as a compromise to operate at a switch time and flow rates in between the above mentioned values. It has been found that by increasing the eluent flow rate the separation potential is reduced mainly due to the decrease of the distribution coefficients. Chuah (204) carried out a series of experiments to evaluate this effect. A 1.14cm id 65.5cm long column packed with Zerolit 225 SRC14 was used and the results are shown on Table 7.5.

To generalise the application of these results, for any chromatographic system having different dimensions and characteristics, the K_d variations were expressed in percentage terms, the eluent flow rate was expressed as a linear velocity (flow rate over column cross-sectional area), and it was assumed that any system would exhibit analogous K_d changes at similar eluent flow rate variations. The fructose distribution coefficient decreased with increasing linear velocities (Table 7.5). The behaviour of the glucose distribution coefficient was less clear, and it was decided to omit the K_{dg} values at 0.412 and 1.372 cm/min linear velocities from the calculations due to their wide fluctuations from the general pattern. The K_d values in Table 7.5 were calculated at infinite dilution, therefore the derived equations relate the K_d variations to a reference K_d* value calculated for the particular chromatographic system at infinite dilution conditions and at a predetermined linear velocity. A column characterisation is always

carried out during the commissioning of all chromatographic systems, therefore these reference K^*_d values are one of the first derived sets of experimental data and represent the system's characteristics and configuration. The same reference K^*_d values are also used in the equations that relate the effect of temperature, and as it will be shown later, concentration. Therefore all these equations relate to the particular system they are used for and require a minimum set of experimental data. To simplify and also standardize the calculations a linear velocity of 1.09 cm/min was chosen, since it corresponded to the operating flow-rate used in the evaluation of the infinite dilution distribution coefficients during the column characterisation (25 cm^3/min, Chapter 6), and also because it was believed to be a moderate setting applicable to any chromatographic sytem. In Chuah's results there were no K_d values at 1.09 cm/min, and these values were calculated from the regression lines fitted to his results. These values were used as the basis (100%) during the rearrangement of the results as shown on Table 7.5. By assuming a linear change in K_d and fitting regression lines to the experimental data, the following equations were derived relating to the effect of flow rates (expressed as linear velocity) on the distribution coefficients:

- for glucose:

$$K_{dg} = (111.71 - 10.631 \times \text{Linear Velocity}) \times K^*_{dg}/100 \quad \text{..... 7.4}$$

and for fructose:

$$K_{df} = (112.68 - 11.593 \times \text{Linear Velocity}) \times K^*_{df}/100 \quad \text{..... 7.5}$$

Where:

K^*_{di} = distribution coefficient of component i calculated for the particular chromatographic system, at infinite dilution, at 25°C and at 1.09cm min^{-1} linear velocity.

If the operating temperatures are different, the K^*_{df} value must be modified for the particular temperature using equation 7.3 and then used in equation 7.5.

It has been found that the separating performance is also affected by changes in the eluent to feed flow ratios. A decrease in this ratio from 6:1 to 2:1 resulted in shifting the "cross-over" point towards the GRP and in decreasing the glucose rich product purities (203).

The experimental results of previous workers indicate that a 3:1 eluent to feed ratio is the most favourable, and this ratio was used in this work. The operating conditions used as a basis of the experimental program were chosen, for comparison purposes, to be analogous to the ones used in the SCCR6 unit (202, 214) bearing in mind the restrictions of equation 5.9.

These conditions were:

For a 30 minute switch time - eluent flow rate: 30 cm^3/min
- feed flow rate: 9 cm^3/min

For a 15 minute switch time - eluent flow rate: 50 cm^3/min
- feed flow rate: 18cm^3/min

Because of the factors mentioned previously, most of the experiments were carried out at intermediate operating conditions which were modified according to the needs of the particlar experiment. The basis of these conditions was:

- eluent flow rate: 39 to 41 cm^3/min
- feed flow rate: 13 to 14.6 cm^3/min
- reference switch time: 20.8 to 18.5 min

Table 7.6: FRP Concentrations during Purging. Run 55-13-39-24.5-60. Purge flow rate: 80cm^3/min

Time (min)	Total concentration (% w/v)
0-4	11.32
4-8	3.07
8-12	0.93
12-16	0.81
16-20	0.20
20-24.5	0.07

Figure 7.14: FRP concentration profile during purging
run: 55.1-13-39-24.5-60

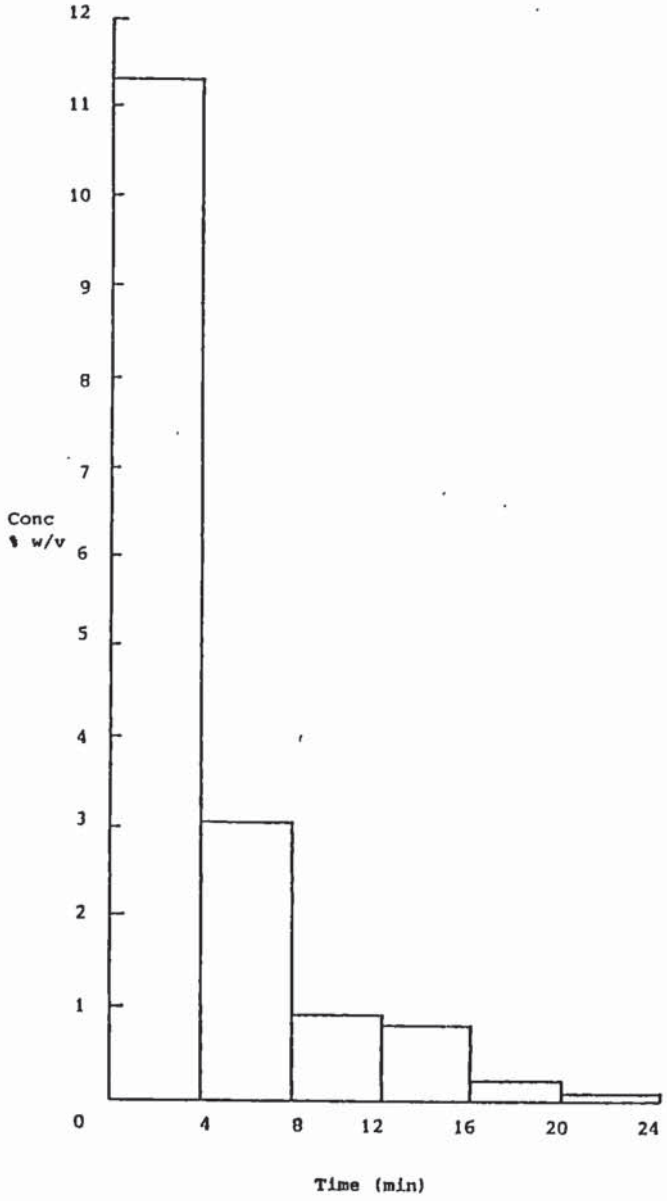
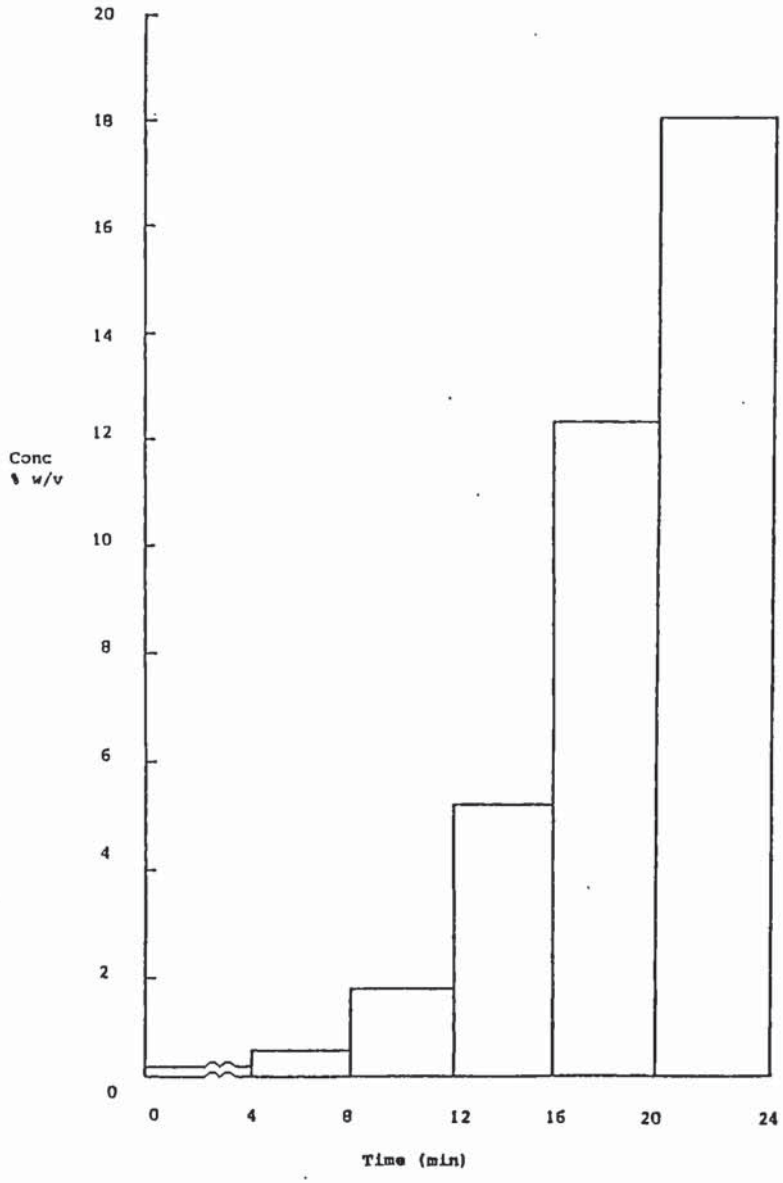


Figure 7.15: GRP concentration profile during a switch
run: 55.1-13-39-24.5-60



The actual operating conditions were modified according to the requirements of the particular experiment. During the early stages in the development of the SCCR systems the purging flow rate was chosen to be 35 to 50 times the eluent (203). This however was too excessive resulting in very dilute fructose rich products, and it was subsequently reduced to 3 to 5 times the eluent rate (22, 202). This was believed to be still too high and in this work it was reduced to about twice the eluent flow rate (80 cm^3/min). A subsequent analysis on the fructose rich product stream proved that it was sufficient (Table 7.6 and Figure 7.14). These results were obtained by collecting the fructose rich product at 4 min intervals during a switch, immediately after leaving the purging column.

7.4.2.4 Concentration Effects

From the early development work it became apparent that the separation performance was affected by increases in concentration. Ellison (211) in his GPC work demonstrated that as the dextran background concentration in the eluent stream was increased from 0% w/v to 15% w/v the elution profiles of the injected dextran samples were becoming broader and less sharp, and the corresponding distribution coefficients were increased approximately from 0.36 to 0.71. The same effect was also apparent in the results of other workers with ion-exchange resins. As the feed concentration was increased the on-column concentrations also increased, the profiles became broader (214), the "cross-over" point was moved towards the FRP and the product purities, especially the GRP purity, were reduced.

This was a direct result of the effect of increased background concentration on the distribution coefficients. Thawait (202, 219) quantified this effect by carrying out a set of experiments on a 0.5cm id and 50cm long column packed with Zerolit SRC14 calcium charged resin. An investigation of the experimental results (Tables 7.7 to 7.9) revealed that:

Table 7.7: Effect of Glucose Background Concentration on K_d (202)

Glucose concentration gr/cm^3	Distribution coefficients			
	K^G_{dg}	% increase in K^G_{dg}	K^G_{df}	% increase in K^G_{df}
0.0	0.120	100	0.380	100
0.1	0.196	163	0.400	105
0.2	0.300	250	0.510	134
0.3	0.330	275	0.560	147
0.4	0.450	375	0.630	166
0.5	0.500	417	0.690	182

Table 7.8: Effect of Fructose Background Concentration on K_d (202)

Fructose background concentration gr/cm^3	Distribution coefficients			
	K^F_{dg}	% increase in K^F_{dg}	K^F_{df}	% increase in K^F_{df}
0.0	0.120	100	0.380	100
0.1	0.160	133	0.40	105
0.2	0.180	150	0.42	111
0.3	0.200	167	0.45	118
0.4	0.230	192	0.43	113
0.5	0.240	200	0.46	121

Table 7.9: Effect of Dextran Background Concentration on K_d (202)

Dextran background concentration gr/cm^3	Distribution coefficients			
	K^D_{dg}	% increase in K^D_{dg}	K^D_{df}	% increase in K^D_{df}
0.00	0.120	100	0.380	100
0.05	0.190	158	0.520	137
0.10	0.210	175	0.570	150
0.20	0.280	233	0.620	163

- the distribution coefficients of glucose and fructose increase more rapidly in glucose background concentration than in fructose;
- all distribution coefficients increase most rapidly in dextran background concentration;

and the following conclusions were drawn:

Chemical Structure Effect

The chances of the β-D-Fructo-pyranose fraction of the fructose present been chemisorbed by the calcium ions are reduced at increased background concentrations. This causes a decrease in the fructose distribution coefficient.

Concentration Gradient Effect

A concentration gradient is created between the stationary and mobile phases, forcing the glucose to stay with the stationary phase either by diffusion or by osmosis. This effect is less prominent with fructose since it is already chemisorbed by the resin's calcium ions.

Viscosity Effect

The viscosity of the solution is increased with increased sugar background concentration, resulting in larger elution volumes. This causes all distribution coefficients to increase and also explains the rapid increase of K_d's in dextran background concentration increasing due to the comparably larger dextran viscosities.

Thawait (202, 219) used the experimental data and expressed these changes by a series of linear regression lines as follows:

Glucose Background Concentration:

$$K^{G}_{dg} = 0.77 \times c_g + 0.1237 \quad \text{..... 7.6}$$

$$K^{G}_{df} = 0.65 \times c_g + 0.3648 \quad \text{..... 7.7}$$

Fructose Background Concentration:

$$K^{F}_{dg} = 0.24 \times c_f + 0.129 \quad \text{..... 7.8}$$

$$K^{F}_{df} = 0.15 \times c_f + 0.386 \quad \text{..... 7.9}$$

Dextran Background Concentration:

$$K^{D}_{dg} = 0.7 \times c_d + 0.143 \quad \text{..... 7.10}$$

$$K^{D}_{df} = 1.1 \times c_d + 0.426 \quad \text{..... 7.11}$$

The general relationship between retention volumes and sugar concentrations in the stationary and mobile phases was derived in Chapter 2 and is of the form:

$$\frac{dp_i}{dc_i} = \frac{V_i - V_o}{V_s} \quad \text{..... 7.12}$$

Where:

p_i = concentration of component i in the stationary phase

c_i = concentration of component i in the mobile phase

V_i = elution volume of component i

$V_s = V_T - V_o$

V_T = total column volume
V_o = column void volume = dextran elution volume

To relate directly the effect of background concentration c_i equation 7.12 is approximated to:

$$\frac{dp_i}{dc_i} = \frac{V_i - V_o}{V_s} = Ac_i + B \qquad \text{..... 7.13}$$

For a linear system, $\frac{dp_i}{dc_i} = K_{di} \equiv \frac{p_i}{c_i}$, equation 7.13 becomes:

$$K_{di} = Ac_i + B \qquad \text{..... 7.14}$$

This is valid for very low concentrations of component i irrespective of the concentrations of the other components present. In a non-linear system where the K_{di} is dependent on concentration, equation 7.14 does not apply and the general equation 7.13 should be used instead (220). Equations 7.7, 7.8, 7.10 and 7.11 are still valid because a small amount of component i was injected in a solution of the other component j and hence it was effectively at infinite dilution. When, however, a sample of component i was injected in a solution containing the same component then equation 7.13 applies instead. This means that equations 7.6 and 7.9 must be re-interpreted as the derivatives, ie:

- equation 7.6 becomes

$$\frac{dp_g}{dc_g} = 0.77 \times c_g + 0.1237 \qquad \text{..... 7.15}$$

- and equation 7.8

$$\frac{dp_f}{dc_f} = 0.15 \times c_f + 0.386 \qquad \text{..... 7.16}$$

The glucose distribution coefficient in a glucose background concentration is derived after integrating and rearranging equation 7.15:

$$K^G_{dg} \equiv \frac{p_g}{c_g} = 0.385 \times c_g + 0.1237 \qquad \text{..... 7.17}$$

Similarly for the fructose distribution coefficient with a fructose background:

$$K^F_{df} \equiv \frac{p_f}{c_f} = 0.075 \times c_f + 0.386 \qquad \text{..... 7.18}$$

These modifications indicate that the distribution coefficient of component i is still increasing in increasing background concentrations of the same component but only half as fast. The results of equations 7.7, 7.8, 7.10. 7.11, 7.17 and 7.18, although they represent the variation of the distribution coefficients with concentration, are only applicable to the system they were obtained from. To generalise their application to any chromatographic system the following steps were followed:

- all K_d values were expressed as a percentage of the corresponding distribution coefficient, calculated at infinite dilution conditions, ie. aqueous mobile phase;
- a new set of linear regression lines was developed using the respective data from Tables 7.5 to 7.7.

The advantage of this development is that the K_d variations relate to the reference infinite dilution coefficients which have been calculated during the column characterisation of the particular system of interest. This clearly increases their application and also keeps the required experimental data to a minimum, since the same reference infinite dilution coefficient is also used in the K_d vs temperature, equation 7.3, and K_d vs flow rate, equations 7.4, and 7.5 relationships. The new general K_d vs concentration relationships were:

- For glucose background concentration:

$$\frac{dp_g}{dc_g} = (492.5 \times c_g + 164.83) \times K^{\infty}_{dg}/100$$

integrating

$$K^{G}_{dg} = (246.25 \times c_g + 164.83) \times K^{\infty}_{dg}/100 \quad \text{..... 7.19}$$

also

$$K^{G}_{df} = (173.1 \times c_g + 95.71) \times K^{\infty}_{df}/100 \quad \text{..... 7.20}$$

- For fructose background concentration:

$$K^{F}_{dg} = (198.3 \times c_f + 107.43) \times K^{\infty}_{df}/100 \quad \text{..... 7.21}$$

$$\frac{dp_f}{dc_f} = (38.9 \times c_f + 101.62) \times K^{\infty}_{df}/100$$

integrating

$$K^{F}_{df} = (19.45 \times c_f + 101.62) \times K^{\infty}_{df}/100 \quad \text{..... 7.22}$$

- For dextran background concentration:

$$K^{D}_{dg} = (627.4 \times c_d + 111.6) \times K^{\infty}_{dg}/100 \quad \text{..... 7.23}$$

$$K^{D}_{df} = (289.1 \times c_d + 112.2) \times K^{\infty}_{df}/100 \quad \text{..... 7.24}$$

Equations 7.19 to 7.24 were used in this research work.

Maltose and the other oligosaccharides, present in the industrial syrup used in this experimental work, are retained to a lesser extent than the glucose and fructose because they do not form any complex with the calcium ions and due to their larger molecular size, and they are effectively travelling with the mobile phase. It was therefore assumed that they exhibited the same behaviour with the totally excluded dextran molecules, also their very low concentrations minimise any inaccuracies caused by this assumption. For the above reasons and due to the lack of experimental data equations 7.22 and 7.23 were used to represent the effect of increasing maltose and oligosaccharide background concentration on the K_{df} and K_{dg} values.

7.4.2.5 Product Concentration Improvement Techniques

Because of the nature of the chromatographic operating principle reduced product concentrations are obtained. To improve these concentrations two techniques have been examined, namely: product splitting and recycling of dilute product fractions.

Product Splitting

It has been found that most of the retained fructose is removed during the early part of the purging period. The GRP exit column in the previous switch was the FRP column and since it was purged it contains water only. When it becomes therefore the GRP exit column the water is eluted at the beginning of the switch and the incoming glucose is eluted towards the end. This was confirmed by the results of an analysis of the FRP and GRP product streams. Each sample was collected directly at the outlet port of the respective column over a 4 minute interval. The results are shown in Tables 7.6

and 7.10 and are plotted on Figures 7.14 and 7.15. Therefore, by dividing the GRP and FRP product collection periods into two fractions and collecting each fraction separately, the concentrations of the desired products can almost be doubled without any significant decrease in purity. This was confirmed in Thawait's results (221) where by employing this technique to the FRP the concentration increased from 1.74% w/v to 3.4% w/v. The product splitting technique was applied to both FRP and GRP streams in this work and the SCCR7 equipment was modified in order to automate this process (Chapter 5).

Recycling of Dilute Product Fractions

In the operation of the SCCR system the eluent entry column during a switch n becomes the purging FRP column at the next switch n+1 (Figure 5.4). By using de-ionized water as eluent the sugar concentration in the eluent entry column is reduced, therefore resulting in a reduced fructose product concentration. By recycling the concentrated fructose rich product which was collected in the first half of the switch, the concentration in the eluent entry column was kept high. When this technique was employed on the SCCR6 (221) the FRP concentration increased from 1.74% w/v to 9.4% w/v, but the purity was reduced from 82% to 72%. Because of the lower purities and the reduced throughputs per cycle this technique was not pursued further. The recycling however of the dilute FRP and GRP fractions was considered instead.

Two other techniques, the use of two adjacent purging columns (204) and the use of a sidestream (221) have been suggested, but have been proved unsuccessful.

7.4.2.6 Effect of Other Parameters

It has been found (217) that when an inverted sucrose feedstock was used and the operating conditions were kept the same, the cross-over point shifted towards the FRP and the fructose rich purity was reduced. A close investigation of the synthetic and

Table 7.10: Results of the GRP Analysis over a Switch - Run: 55-13-39-24.5-60

Time (min)	Total concentration % w/v
0-4	0.21
4-8	0.42
8-12	1.84
12-16	4.93
16-20	12.71
20-24.5	18.39

inverted sucrose concentration profiles indicated that the glucose profile is broader and sharper for the inverted sucrose feedstock. This means the glucose from the inverted syrup is retained more than the glucose from the synthetic feedstock. No explanation can be given for this behaviour.

During the separation of industrial syrups, such as the barley syrup used in this work, the reduced fructose content in the feed (ie. glucose/fructose ~ 52%/42%), results in reduced fructose rich product purities, and in shifting the "cross-over" point towards the FRP. This is because by increasing the glucose to fructose ratio in the feed the overlapping of the peaks increases due to the greater glucose present hence the "over-shadowing" effect. Gould (214) using the results from his simulated work proposed that this effect can be counteracted by moving the feed inlet location towards the GRP thus allowing extra length in the pre-feed section for the separation. The simulated results showed the glucose profile increasing sharply from one or two columns before the feed column, and the fructose profile decreasing sharply immediately after the feed column. This however does not apply for the real situation because of the effects of temperature, flow rates and concentration on the K_d values. Although the initial simulated results were encouraging, they become less promising when the above factors are incorporated in the simulation program, and has also been confirmed experimentally (202).

Because of the limited length of the existing SCCR systems it is therefore believed that this technique can only be applied effectively with low (< 25% w/v) feed concentrations. An alternative technique is developed in this work which overcomes efficiently the above limitations and it is explained in Chapter 8.

7.4.2.7 The Selection of the Best Operating Conditions (Summary)

An alternative and more general way of arriving at equation 7.2 has been developed relating the effect of the principle operating parameters on the K_d values and thus on the SCCR system's performance.

The selection of the best operating parameters that represent the actual conditions can be carried out as follows:

- the glucose and fructose distribution coefficients K^*_{dg}, K^*_{df} for the chromatographic system in question are evaluated as shown in Chapter 6 by operating at infinite dilution, ambient temperature (20-25°C) and at a linear velocity of 1.09 cm/min;
- the fructose distribution coefficient K^*_{df} is modified using equation 7.3 in order to account for the effect of temperature;
- equations 7.4 and 7.5 are used to modify these glucose and fructose distribution coefficients further accounting for the effects of flow rates;
- these new K^*_{dg} and K^*_{df} values are used in the set of equations 7.19 to 7.24 to get a set of new K_d values that account for the effects of background concentrations. Then the weighted average K_{dg} and K_{df} values obtained by taking into account the above K_d values and the concentrations of the individual components can be used in the basic relationship 5.9 to derive the appropriate operating conditions. The advantage of this new approach is its general application, since all relationships are expressed in terms of the reference distribution coefficients K^*_{di} which are properties of the particular chromatographic system under investigation.

7.5 Commissioning of the SCCR7

7.5.1 Commissioning Run 56-10-30-30-20

The aim of this experiment was to provide a pressure testing of the newly packed and assembled SCCR7 system and also get an early evaluation of its performance with the industrial feedstock. Therefore, a high feed concentration (56% w/v) was used and the operating temperature was 20°C resulting in increased viscosities and h ncepressure drops. The system performed satisfactorily at these adverse conditions and no leakage was detected. The operating conditions were chosen to correspond to the ones used in the operation of the ten 10.8cm id column SCCR6 system (202, 214) and the results are shown in Tables 7.11 and 7.12. All the maltose and oligosaccharides were eluted in the glucose rich product (GRP) which was fructose free. The fructose rich product (FRP) however contained over 30% glucose, and the cross-over point had moved to the eluent entry column next to the FRP column. When the results were compared to the ones obtained on the ten column SCCR6 system at similar conditions but using a 50:50 synthetic glucose-fructose feed (run 60-35-105-30-20, (202)), it was found that the fructose rich product purity was even lower and the relative shift of the cross-over point towards the FRP was greater. This confirmed the increased difficulty in separating efficiently the industrial feedstock because of its reduced fructose content. An examination of the concentration profile, Figure 7.16 indicates that the multi-component nature of the feed did not affect the separation directly since the maltose and oligosaccharides were retarded less and were carried through in the glucose rich product. The small presence of these impurities however is thought to have an indirect effect since their background concentration would affect the values of the distribution coefficients. This effect has been evaluated in Section 7.4.2.4, but its actual contribution on the K_d values is small due to the very low concentrations involved.

7.5.2 Reference Run: 18.6-9-30-30-20

This experiment was carried out to provide a basis for comparison. A synthetic feed containing glucose and fructose at similar ratios to the industrial feed was used, and the operating temperature was 20°C. Both products were 99.9% pure because of the very low feed concentration (18.6% w/v total sugar solids). The results were similar in terms of purity to the ones obtained by Gould (214) on the ten column SCCR6 system using a 50:50 synthetic feed, but the cross-over point was shifted towards the FRP as a direct result of the reduced fructose content (Figure 7.17). The results of these commissioning runs (run 56-10-30-30-20 and 18.6-9-30-30-20) indicated that alternative operating techniques must be employed in order to achieve the desirable objectives and these techniques are discussed in the following chapter.

Table 7.11 Operating conditions in the commissioning runs

Experimental run	Average flow rates $cm^3.min^{-1}$			Feed concentration % w/v				F:G ratio in feed	Switch time min	Average K_d incorporating the effects of temp, flow rates & concentration	Lm/P ratio	cycle
	Feed	Eluent	Purge	G	F	M+OS	TOTAL					
56-10-30-30-20	10	30	75	29	24.5	2.5	56	44:52	30	0.528	0.391	8
18.6-9-30-30-20	9	30	75	10.78	7.82	-	18.6	42:58	30	0.447	0.381	7

G: glucose; F: fructose; M+OS: maltose + oligosaccharides

Table 7.12 Experimental results from the commissioning runs

Experimental run	Feed throughput (sugar solids) kgh^{-1}	GLUCOSE RICH PRODUCT					FRUCTOSE RICH PRODUCT				
		Glucose purity %	% of glucose in feed recovered	Total product conc %w/v	Impurities % F	M+OS	Fructose purity %	% of fructose in feed recovered	total product conc %w/v	Impurities % G	M+OS
56-10-30-30-20	0.336	87.5	67.4	3.98	-	12.5	69.7	106	5.07	30.3	-
18.6-9-30-30-20	0.100	99.9	97.1	2.12	0.1	-	99.9	98.4	0.93	0.1	-

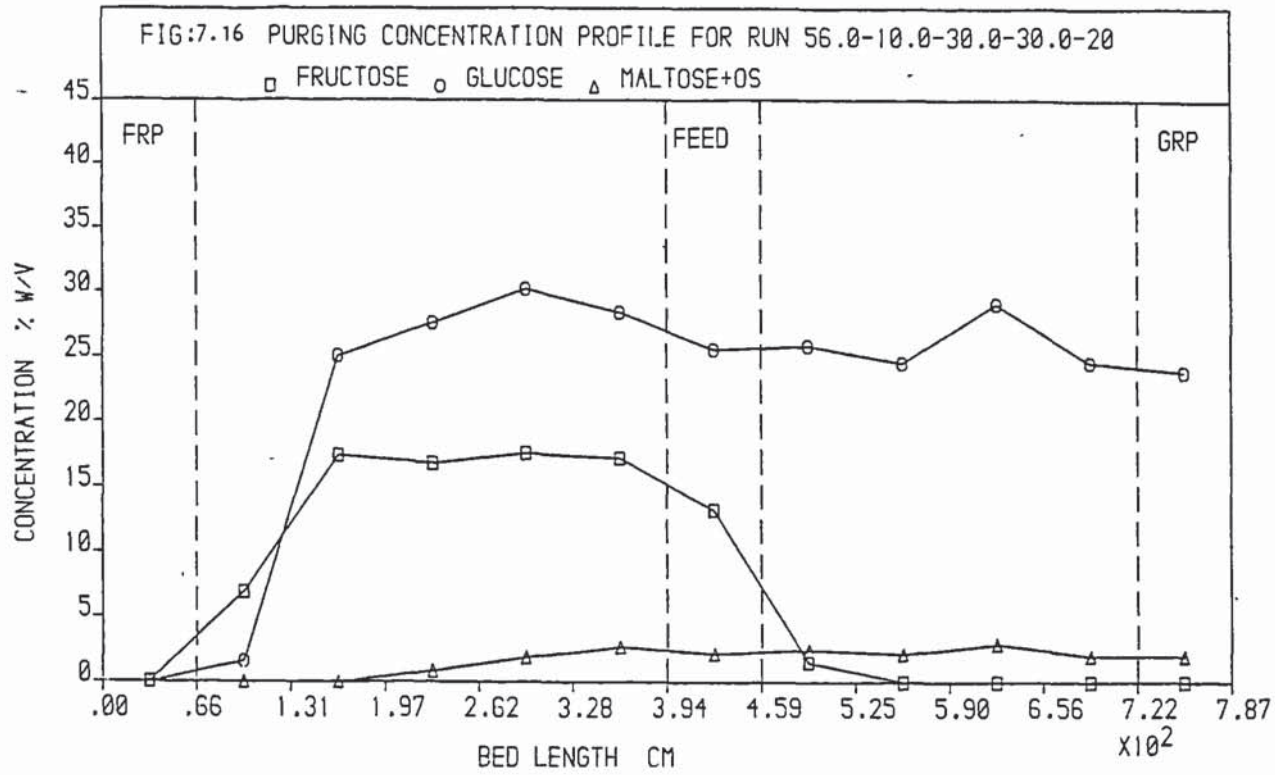


FIG:7.16 PURGING CONCENTRATION PROFILE FOR RUN 56.0-10.0-30.0-30.0-20
□ FRUCTOSE o GLUCOSE △ MALTOSE+OS
FRP
FEED
GRP
CONCENTRATION % W/V
45
40
35
30
25
20
15
10
5
0
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87
X10²
BED LENGTH CM

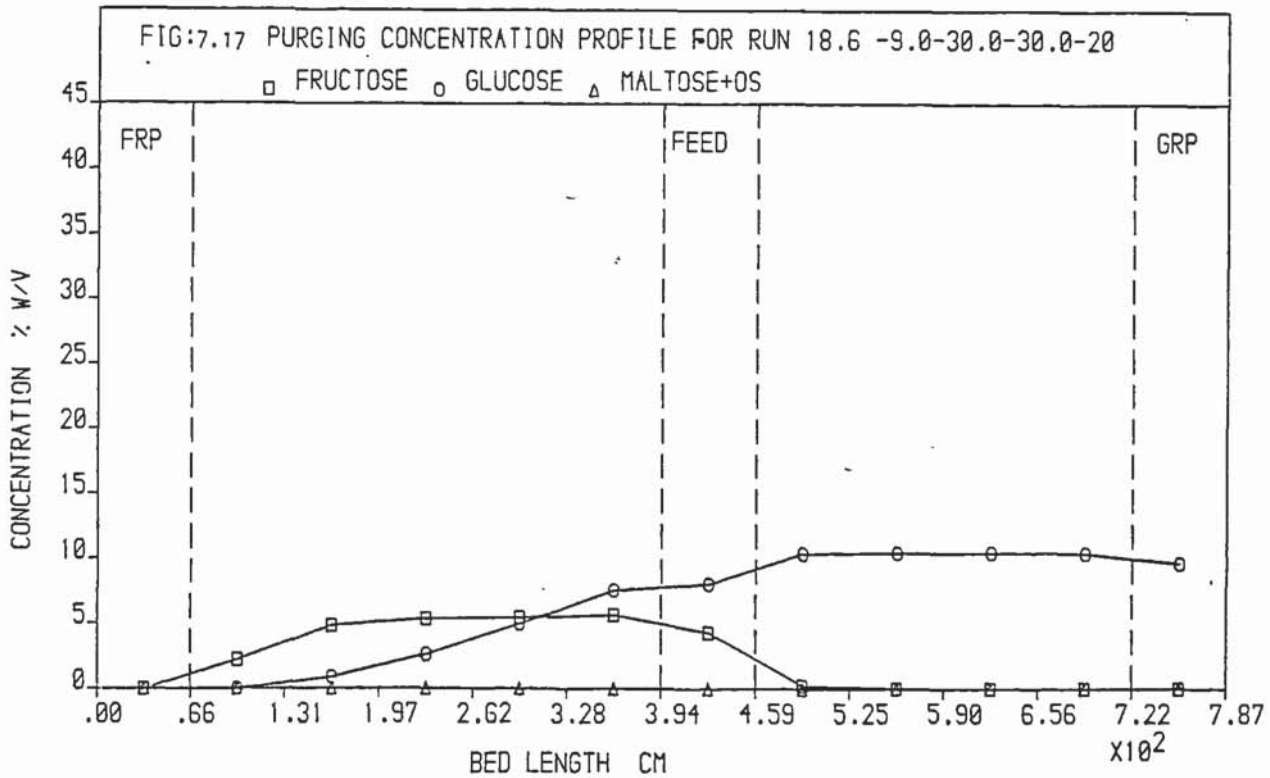


FIG:7.17 PURGING CONCENTRATION PROFILE FOR RUN 18.6 -9.0-30.0-30.0-20
□ FRUCTOSE o GLUCOSE △ MALTOSE+OS
FRP
FEED
GRP
CONCENTRATION % W/V
45
40
35
30
25
20
15
10
5
0
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87
X10²
BED LENGTH CM

CHAPTER 8

8.0 THE CONTINUOUS SEPARATION OF A BARLEY SYRUP USING THE SCCR7 EQUIPMENT

8.1 Introduction

The majority of the experimental runs carried out by previous workers on SCCR equipments were based on synthetic feed solutions having fructose to glucose ratios of 50:50 or higher. In this experimental program the industrial FINN-SUGAR isomerose syrup made from barley starch was used and the objective was to optimise the SCCR7's separation performance and produce high fructose corn syrup (HFCS) meeting the strictest industrial specifications. These specifications are:

- 90% fructose rich product;
- glucose rich product containing no more than 7% fructose;
- feed throughput of at least 30 Kg sugar solids/m^3 resin/hour;
- products having total sugar solid concentrations of over 20% w/v.

The lower fructose content and the multicomponent nature of the feed indicate a more difficult separation, and the results of the commissioning runs (56-10-30-30-20 and 18.6-9-30-30-20) highlighted the necessity of developing alternative operating techniques, and obtaining experimental relationships relating the effect of various operating parameters on the system's separating performance.

Over thirty successful experimental runs have been carried out, from which the appropriate runs have been selected to support the particular findings. By operating at the intermediate feed and eluent flow rates of 13 and 39 cm^3min^{-1} respectively, the experiment duration increased slightly, and the production throughput also increased without causing substantial overloading (Section 7.4.2.3). Although higher flowrates would have increased the throughput further and also reduced processing times, they were not used because limitations in storage capacities of feed, eluent and purge tanks

did not allow continuous overnight operation. All experiments were carried out at 60°C. The equipment was operating continuously and it was purged at the end of the week. When two runs were carried out during a week without purging the system out at the end of the first run, the cycle at which the experimental readings were taken in the second run is indicated with a plus (+) sign. In order to compare the system's performance with the theoretical relationship 5.9, the pre-feed and post-feed effective mobile flow rates and the K_{df} and K_{dg} values were calculated for every run. The actual values of the distribution coefficients were used taking into account the effects of temperature (equation 7.3), flow rates (equations 7.4 and 7.5) and concentration using a weighted average of the results obtained from equations 7.19 to 7.24 according to the feed composition.

8.2 Effect of Changing the Switch Time

8.2.1 Experimental Results and Discussion

An examination of the criterion for separation (relationship 5.9 and equation 5.7) reveals that the switch time is a critical operating parameter. By using high feed concentrations and reduced feed fructose to glucose ratios, the on-column concentrations increase, with the glucose concentration increasing relatively more, due to the greater amounts of glucose present. Therefore the distribution coefficients and thus the separation are directly affected. This is supported by the fact that the distribution coefficients increase linearly with concentration, and also their rate of change increases more with increasing glucose background concentration than fructose, (Section 7.4.2.4).

The effect of switch time on the equipment's performance was studied by using syrups containing 35 to 37% w/v total sugar solids, and the experimental conditions and results are shown on Tables 8.1 and 8.2. The feed syrup used in the first two runs (35-13-40-21-60 and 35-13-40-26-60) was drawn from the top of the

forty gallon drum supplied by Finn-Sugar and had a fructose to glucose ratio of 48.9 to 50.6%. This was due to crystallisation of some of the glucose and oligosaccharides at the bottom of the drum. For the subsequent experiments the syrup in the drum was mixed thoroughly before use. A 21 minute switch time was used in run 35-13-40-21-60 because it corresponded to the switch times used by previous workers (202, 214), and both product purities were expected, from previous experience with equimolar feeds, to be in the high nineties. The experimental results however were contradictory. A 98.2% pure glucose rich product was obtained containing no fructose, while the fructose rich product purity was reduced to 88.8%. For a successful separation, from relationship 5.9, the average Lm/P ratio must be very near the average distribution coefficient ratio. This however was not so for this run, and the pre-feed L/P ratio was well below the K_{dg} value (Table 8.3). Because of the low switch time the stationary phase effective movement was too fast, causing some of the glucose to be eluted in the fructose rich products. As a result, only 8 columns (Figure 8.1) were used effectively in the separation and the cross-over point was in the third column. To overcome that, the switch time was increased to 26 minutes in run 35-13-40-26-60, resulting in a broader fructose profile and shifting the cross-over point to column 5 (Figure 8.2). The post-feed L/P ratio was well above the K_{df} value causing fructose to travel with the mobile phase and contaminate the glucose rich product. Pure fructose rich product (FRP) was obtained at the expense of the glucose rich product (GRP) which contained 13.5% fructose. The results of these two runs using equimolar feed, indicated the significance of the switch time, and three further experiments were carried out to verify this. The well mixed barley syrup was used and the operating switch times were 21, 23 and 25 minutes, while the remaining operating conditions were kept the same, (Tables 8.1, 8.2, 8.3 and 8.4). A 21 minute switch time (run 37-13-40-21-60) resulted in shifting the cross-over point to column 2, causing some of the glucose to be eluted with the FRP reducing its purity to 70.6%,

(Figure 8.3). The simulated movement of the stationary phase was too fast (low switch time) and from Table 8.4 it is clear that the criterion for separation was not met since the pre-feed Le/P ratio was well below the actual K_{dg} value, (0.251 and 0.345 respectively). The glucose rich product was fructose free since there was no fructose present beyond column 8. All fructose was removed in the FRP, although the % recovery value of 115.4% shown in Table 8.2 is not correct due to dilution and analytical and weighing experimental errors. A 25 minute switch time (run 37-13-40-25-60) shifted the cross-over point to column 11, and almost half of the fructose was eluted with the GRP. As before the experimental results were also supported by the theory, where, this time, the (Le + Lf)/P value of 0.547 was well above the K_{df} value of 0.404. The general pattern of the results obtained from runs 35-13-40-21-60, 35-13-40-26-60 and 37-13-40-21-60, 37-13-40-25-60, is similar, while the concentration profile and FRP purity of run 37-13-40-21-60 are even worse than the corresponding run 35-13-40-21-60 due to the reduced fructose content. A comparison of the three concentration profiles (Figures 8.3, 8.4 and 8.5) shows that as the switch time increases the glucose concentration profile is gradually shifted to the right and the fructose profile becomes broader. This broadening in the fructose profile is a direct result of the switch time changes. As the switch time increases, the stationary phase effective flow rate, P, decreases resulting in increasing L/P ratios. These L/P increases are too high, especially for the post-feed L/P in comparison to the actual K_{df}, thus causing greater amounts of fructose to move with the mobile phase (Tables 8.3 and 8.4). A 23 minute switch time was found to be the right one (run 36-13-40-23-60), giving a 92.4% pure FRP and a GRP containing only 5.49% fructose.

The concentration profile (Figure 8.4) indicates that all the system's length was used effectively for the separation and the cross-over point was situated in column

5. Although the average K_d and Lm/P values were not identical they deviated by less than 9%.

8.2.2 Conclusion

The experimental results and a close investigation of the concentration profiles (Figures 8.3, 8.4 and 8.5) verifies clearly the effect of switch time. This new operating technique requires no location changes of the relative inlet feed or eluent positions and no equipment modification, and also can be easily applied at any stage during the operation to improve the systems performance according to the operator's requirements. Its approximate value can be obtained from the criterion of separation (relationship 5.9) using the actual distribution coefficients and the pre- and post-feed effective mobile phase flow rates. An experimental relationship has been developed in the following sections to predict the operating switch time for different feed concentrations.

8.3 Equipment's Sensitivity to Small Changes in Switch Time

The significance of the switch time on the equipment's separating performance was indicated in the previous section. The system's sensitivity and the importance of choosing the exact switch time is shown here from a set of six experimental runs with switch time changes as low as 10 seconds (Tables 8.5 and 8.6). The switch time was reduced from 24.5 to 23.5 and 23 minutes in the runs 35.2-13-39-24.5-60, 35.2-13-39-23.5-60 and 36-13-40-23-60. The magnitude of the effect on the concentration profiles is shown in Figures 8.6, 8.7 and 8.4 where a 1 minute reduction in switch time shifted the cross-over point by 3 column, from column 9 to 6, and a further 0.5 minute reduction from column 6 to column 5. In terms of product purities, the fructose content in the GRP was reduced from 17.88 to 11.5 and

5.49%, and the FRP fructose purity was also reduced from 99 to 92.85 and 92.4% respectively. The differerence in separating performance between runs 35.2-13-39-23.5-60 and 36-13-40-23-60 was not so great, due to the relatively low feed concentrations. When the feed concentration is increased the limits in the operating switch time are narrower and the exact value must be chosen. This is illustrated well in the following three experiments, (runs 45.7-13-39-24-60, 45.77-13-39-24.33-60 and 46-13-39-24.17-60). A 20 seconds increase in switch time, from 24 to 24.33 minutes, caused the cross-over point to move from the beginning of column 4 to column 5 and the FRP purity to increase from 89.5 to 95.37% (Figures 8.8 and 8.9), and consequently resulted in increased fructose contamination of the GRP, ie. from 2.4 to 11.76%. When an intermediate switch time, of 24 minutes and 10 seconds, was used (run 46-13-39-24.17-60) both product purities were within specification. It is interesting to note that a 10 second reduction or increase in the switch time from its correct value of 24.17 minutes, reduced or increased the FRP purity by almost the same amount (approximately 3%), and the fructose content in the GRP showed similar decreases or increases. The corresponding cross-over point locations were changed by less than a columns length (Figures 8.8, 8.9 and 8.10).

This is also supported by the corresponding changes in the actual distribution coefficients and L/P ratios as shown on Table 8.4. Any changes smaller than 10 seconds in switch time were not possible due to the limitations of the timer used.

8.4 Effect of Increasing Feed Concentration on Product Purities and Concentration

8.4.1 Introduction

By increasing the feed concentration, the production throughput and hence the equipment's utilization increases. This however has a detrimental effect on the

separating efficiency since the sugar concentrations increase and eventually lead to equipment overload. The drop in separating efficiency is a direct result of the increasing background sugar concentrations on the distribution coefficients as described in Section 7.4.2.4, namely the chemical structure, concentration gradient and viscosity effects. The resulting effect on the glucose distribution coefficient is relatively greater and is illustrated well in Figure 8.11. Figure 8.11 contains the variation in the glucose concentration profiles with increasing concentration, and was obtained by Thawait (202) using the SCCR6 and a 50:50% synthetic feedstock, operating at 20^{o}C and keeping the switch time constant at 30 minutes. In this work the barley syrup was used and the switch time was altered accordingly to obtain products within specification. The eluent to feed flow rate ratio was kept at approximately 3:1. The results are shown in Tables 8.7 and 8.8, and the purge concentration profiles are used since they offer a better representation of bulk composition for each column. Because of the continuous operation there was no purge concentration profile available for run 36-13-40-23-60 and the on-column profile (Figure 8.4) was used instead.

8.4.2 Results and Discussion

In run 18.6-9-30-30-20 a synthetic glucose-fructose mixture was used, and the experiment was carried out at ambient temperature (Section 7.5.2). As this run was to be used as a reference, a 30 minute switch time was chosen, similar to the ones used by previous workers (202, 214) and 99.9% pure products were obtained. Although this switch time, for the particular conditions, is believed to be slightly high the separation was not affected due to the comparatively low feed concentration used. As the feed concentration was increased (runs: 36-13-40-23-60, 46-13-39-24.17-60, 54-13-39-24.5-60 and 66-14.6-40-25-60) the product purities were kept within specification by increasing appropriately the corresponding switch times. These increases however resulted in increasing deterioration of the product purities, ie. as

the concentration increased from 18.6 to 66% w/v the FRP fructose purity decreased from 99.9 to 90.1% and the GRP fructose content increased from 0 to 6.9%. These switch time changes are ideally made gradually and relative to the concentration changes.

An investigation of the concentration profiles (Figures 7.17, 8.4, 8.12, 8.13 and 8.14) shows that the "cross-over" points were situated approximately 1 or 2 columns before the feed inlet column. Experience has shown that if the "cross-over" point is situated approximately one column before the feed inlet column both product purities would be within specification. The increases in the distribution coefficient values with increasing feed concentration are shown in Tables 8.3, 8.4 and 8.9, and the corresponding L/P ratios also increase due to the increasing operating switch times. The decreasing separation performance is also supported by the reducing separation factors.

In run 66-14.6-40-25-60 the feed flow rate was increased to 14.6cm^3 min^{-1} in order to increase the production throughput nearer to the specified one, reducing the eluent to feed flow rate ratio to 2.74:1.

In the development of the experimental switch time-feed concentration relationships the results of runs 18.6-9-30-30-20, 36-13-40-23-60, 46-13-39-24.17-60 and 54-13-39-24.5-60 were used due to the similar eluent to feed flow rate ratios (ie. 3:1). Because of the different flow rates used in run 18.6-9-30-20-20, the switch time was modified to correspond to a reference feed rate of 13 cm^3 min^{-1} (Table 8.10), thus providing a better basis of comparison. The results are plotted in Figure 8.15, whereby it can be seen that at relatively low feed concentrations, ie. less than 45% w/v, the switch time increases linearly with concentration and then its rate of increase is reduced. Most of the experiments have been carried out more than once thus eliminating the possibility of experimental error.

As the on-column sugar concentrations increase due to increasing feed concentrations, the concentration dependent distribution coefficients change significantly thus requiring similar switch time increases to maintain the separation efficiency. For feed concentrations over 45% w/v the system starts to become overloaded and the rate of change in the concentration effect of the distribution coefficient is reduced. It is therefore apparent that at approximately 45% w/v a "critical" point is reached, after which the operation is carried out at overloaded conditions. Successful separation can still be achieved but the selection of the right switch time is critical. Because of these different rates of change, it was decided to represent the switch time-feed concentration relationship with two regression lines. Also to generalise their application the switch times have been reexpressed as percentage change with respect to a reference switch time, (Table 8.10) therefore:

- for feed concentrations up to 45% w/v:

  $S' = (0.542 \times \text{feed conc} + 80.20) \times STR/100$ 8.1

- and for feed concentrations over 45% w/v:

  $S' = (0.175 \times \text{feed conc} + 97.05) \times STR/100$ 8.2

where: S' = predicted switch time (min)

STR = reference switch time for the particular SCCR system operating at a feed flow rate of 13cm^3 min^{-1}, feed concentration 36% w/v, eluent to feed rate ratio of 3:1 and a feed fructose content of 42%, (min).

The appropriate experimental relationships can therefore be used to predict the switch time for the particular feed concentration. The switch time corresponds to a feed flow rate of 13cm^3 min^{-1} and an eluent to feed flow rate of 3:1. If a different feed flow rate is chosen then the actual switch time can be obtained from:

$$ST = (FFRR/FFRN) \times S' \quad \text{..... 8.3}$$

where: ST = actual switch time (min)

S' = switch time obtained from relationships 8.1 and 8.2 (min)

FFRR = reference feed flow rate (13 $cm^3\ min^{-1}$)

FFRN = actual operating feed flow rate ($cm^3\ min^{-1}$)

8.5 Nature of the Feed and its Effects on the Separation Achieved

The effect of reducing the fructose content of the feed on the separation performance has been mentioned earlier in sections 7.5.2 and 8.2. The difference between the concentration profiles when using either synthetic or inverted feedstocks of similar composition has also been discussed in Section 7.4 .2.6. The results of three runs, carried out at 35 to 37.8 w/v feed concentrations and fructose contents from 48.9 down to 36.8%, are shown in Table 8.11. Different switch times were used to counteract the increased separation difficulty associated with decreasing fructose contents to obtain products within specification. As the fructose content was decreased the on-column concentration profiles became broader (Figures 8.1, 8.4 and 8.16) due to the increasing switch times. The FRP purities in runs 35-13-40-21-60 and 37.8-13-40-24.5-60 were slightly outside specification because the chosen switch times were somewhat smaller than the optimum ones, which from experience should have been 21.17 and 24.67 minutes respectively. The experimental switch times have been used to develop a relationship between switch time and feed fructose content. This relationship has been used in conjunction with relationships 8.1 to 8.3 to modify the predicted switch time according to the feed composition. Since these relationships (8.1 to 8.3) have been developed using feeds with approximately 42:52 fructose to glucose ratios, the actual switch times are re-expressed as a percentage with respect to the reference switch time corresponding to 42.2% fructose feed content,

(Table 8.11). A linear relationship is assumed given by the regression line:

$$S = (152.89 - 1.25 \times FFC) \times ST/100 \quad \text{..... 8.4}$$

where: S = switch time for actual feed composition (min)

FFC = fructose content in feed (%)

ST = actual switch time as obtained from relationships 8.1 to 8.3 (min)

The multicomponent nature of the feed did not have a direct effect on the separation since the maltose and oligosaccharides were retarded less and were carried through in the glucose rich product. The presence of these impurities however had an indirect effect since their background concentrations affects the distribution coefficients, and although its contribution is small due to the low concentrations involved, it is still included in the evaluation of the actual distribution coefficients (Section 7.4.2.4).

It has therefore been demonstrated that the SCCR7 equipment can be used effectively to separate more "difficult" feedstocks provided the right switch time has been chosen.

8.6 Effect of Reducing Eluent and Purge Flow Rates on Product Purities and Concentration

A reduction in the eluent and purge flow rates increases product concentrations but at the expense of the separation efficiency. This has been evaluated by previous workers and they recommended a 3:1 eluent to feed flow rate ratio. This ratio was kept in the majority of the experiments in this work. In run 66-14.6-40-25-60 the ratio was reduced to 2.74:1 (Tables 8.13 and 8.14) and the product purities were still within specification. The bulk product concentrations were

5.84 and 11.6% w/v for the FRP and GRP respectively, and the total length of the equipment was utilized effectively as illustrated by Figure 8.17. If the same run had been performed at a 3:1 eluent to feed ratio the switch time would have been 22.15 minutes (as predicted from relationships 8.2 and 8.3), instead of 25 minutes. Therefore, a 9.5% decrease in the eluent to feed flow rates ratio required a 13% increase in switch time.

In run 65.1-14.2-40.5-25-60 the purge flow rate was reduced further to $50cm^3\ min^{-1}$. From the concentration profile (Figure 8.18) it is apparent that the pre-feed fructose concentrations are higher than the corresponding ones of the previous run (Figure 8.17), indicating that the flow rate was not enough to purge the columns completely and some of the fructose was left behind. This is also supported by the increased fructose content and higher concentration of the GRP since the purge column becomes the GRP exit column after the column switching. This partial recycling of fructose alters the fructose-glucose background concentrations thus reducing the adverse effect of the reduced fructose content of the feed. This and the slightly higher eluent to feed ratio of 2.85:1 resulted in better FRP purities. The difference in product composition between the two runs also show the equipment's sensitivity to small changes in any one of the operating condition, and this should also be taken into account during the selection of the switch time.

Two additional runs were carried out at a $50cm^3\ min^{-1}$ purge rate and an eluent to feed ratio of 2.46:1, (runs 47.5-13-32-29-60 and 47.5-13-32-28.5-60). Run 46-13-39-24.17-60 is also included for reference. The reduced eluent and purge flow rates increased the product concentrations substantially but at the expense of their purities, (Table 8.14). A 30 seconds reduction in switch time, from 29 to 28.5 minutes, shifted the cross-over point by 3 columns (Figures 8.19 and 8.20), reduced the GRP fructose content from 16.7 to 9.4% and decreased radically the FRP purity

from 96 to 76.62%. It is therefore concluded that a 2.75:1 eluent to feed ratio is the minimum recommended one, and a purge flow rate of approximately 70cm^3 min^{-1} should be sufficient, although an even lower purge rate could be used because of the favourable results mentioned above.

8.7 Increasing Throughput and Product Concentrations

8.7.1 Production Throughput

The results obtained by previous workers show that higher throughputs and product concentrations can be achieved by increasing the feed flow rate and feed concentration but all the expense of product purities as the direct result of the increased on-column sugar concentrations. When an effluent of a Dextran plant (Fisons Pharmaceuticals plc, Holmes Chapel, UK) containing over 68% fructose, was used the maximum throughput achieved corresponded to 0.71 kg/hr sugar solids or 39.5 kg solids/m^3 resin/hr, ie. a feed flow rate of 17cm^3 min^{-1} and 70% w/v feed concentration (202). In an attempt to achieve the specified throughput of 30 kg sugar solids/m^3 resin/hour, run 66-14.6-40-25-60 was carried out with the feed flow rate increased to 14.6 cm^3 min^{-1} and the feed concentration to 66% w/v total sugar solids. The remaining experimental conditions and the results are shown in Tables 8.13 and 8.14. A throughput of 0.578 kg sugar solids per hour or 32.1 kg sugar solids/m^3 resin/hour was achieved, which was well above the specified one, and the product purities were within specification (ie. 90.1% pure bulk FRP, and bulk GRP containing only 6.4% fructose).

8.7.2 Means of increasing Product Concentrations

To improve the commercial viability of the SCCR system it is essential to improve the product concentrations, thus reducing the operating and capital costs of

any additional processes (ie. evaporation and/or crystallization). Therefore, the product splitting technique was employed and the corresponding product fractions were collected separately. This was achieved by using a 3-way valve and a timer in each product line, (Chapter 5). To investigate the elution profiles each one of the products was collected over 3 minutes as it was eluted at the end of the corresponding product line and it was then analysed, (Tables 8.15 and 8.16). The individual elution profiles were obtained by plotting the total sugar concentrations (Figures 8.21, 8.22). Both profiles are "delayed" with respect to the "actual" ones (Figures 7.14 and 7.15), which were obtained from the respective column exit ports. This was due to the relatively long length of the system's product line network between the column exit ports and actual product collection port. The GRP line network was filled with the very concentrated fraction of the previous switch which was therefore eluted with the product of the current switch, and similarly, the FRP network was filled with the dilute fraction of the previous switch thus diluting the initial fraction of the current switch. The exact "delayed" periods vary from system to system because of the different lengths of the respective product networks; therefore the elution profiles of the SCCR system in question must be obtained in order to select the exact splitting intervals.

The product splitting technique was employed on the FRP in the early commissioning run, 56-10-30-30-20, where the concentrated fraction was collected during the first 0^- to 15 minutes collection intervals (Tables 8.17, 8.18 and 8.19). This increased the product concentration from 5.07% w/v for the bulk product to 5.79% w/v, but the increase was not substantial due to the relatively long switch time. During the switch over, as the last column in the separating section becomes the purge column, there is a sudden drop in operating pressure (Table 8.29), this results in a sudden emission of the FRP at the beginning of the switch. To account for that the FRP collection started about 1 second before the switch, and this is indicated on the tables as 0^-.

As it can be seen from Tables 8.15 and 8.16, approximately half of the glucose in the FRP is eluted with the dilute FRP fraction (second half of the switch), and a substantial amount of the fructose in the GRP is also present in the dilute portion of the GRP (around the middle of the switch). Therefore, by employing the product splitting technique the purities of the concentrated fraction are also improved further. This was verified in the following experiments. In run 55.1-13-39-24.5-60 the fractional splitting of the FRP increased its purity to 98.87% and its concentration to 4.49% w/v. Most of the glucose present was removed in the dilute collection fraction, which contained only 2% of the total fructose present in the feed (Tables 8.17, 8.18 and 8.20). The analysis of the bulk products (Table 8.18) and the concentration profiles (Figures 8.13 and 8.23) of runs 54-13-39-24.5-60 and 55.1-13-39-24.5-60 should be similar. This however was not so and it is believed to be due to malfunctioning of the feed pumphead in run 55.1-13-39-24.5-60. To increase the throughput and product concentrations further, the feed concentration and flow rates were increased to 66% w/v and 14.6 cm^3 min^{-1}, the eluent to feed ratio was reduced to 2.74:1, and the purging flow rate was also reduced to 70 cm^3 min^{-1}, Table 8.17. The product splitting technique was employed to both products, (runs: 66-14.6-40-25-60* and 66-14.6-40-25-60). For both runs the concentrated FRP was collected from 0^- to 12.5 minutes during each switch and the results obtained were almost identical (Tables 8.18, 8.21, 8.22, 8.23 and 8.24). Over 95% of the fructose in the feed was recovered in the concentrated fraction, and its concentration increased from about 5.8% w/v (bulk product) to 11.3% w/v. The fructose purity also increased from 90.1 to over 95%. The diluted FRP fraction contained only 2.8% of the fructose fed into the system and also contained most of the glucose present in the FRP stream. In run 66-14.6-40-25-60* the GRP product split period was from 0 to 12.5 and 12.5 to 25 minutes during each switch. The concentration of the desired product (2nd collection period) increased from 11.7% w/v of the bulk GRP product, to 16.21% w/v. Over 68% of the glucose in the feed was recovered during this period and the fructose

content was reduced to 4.3%. Because of the "delayed" effect a substantial amount of glucose was not recovered in the concentrated GRP fraction. In the following run 66-14.6-40-25-60, the GRP product split periods were altered to 4 to 16.5 min (dilute fraction) and 16.5 to 4 minutes (concentrated fraction).

This increased the concentration of the desired product fraction (16.5 to 4 minutes) to 22.56% w/v, thus recovering over 94% of the glucose in the feed, and maintaiing the fructose content below 4.5%.

A significant amount of the fructose and maltose-oligosaccharides was removed in the dilute fraction which contained less than 2% of the glucose in the feed. These results showed that the concentrations and purities of the desired FRP and GRP products can be improved further by selecting carefully the timing of the product splitting. The introduction of a more sophisticated timer, with multiple and unequal time periods, would assist in a slightly larger increase of both product concentrations.

From the results of previous workers (202, 214), it is apparent that, when the feed flow rate is increased to the equivalent ot 17.5 cm^3 min^{-1} and the switch time is decreased to maintain similar L/P ratios, the fructose on column concentration profile is narrowed while the glucose profile is shifted towards the FRP. Therefore by increasing the feed flow rate to the above value and the feed concentration to 70% w/v, the product concentrations and throughputs would be increased further. However, the selection of switch time would be critical in order to maintain the product purities within specification.

A further increase of product concentrations can be achieved by altering the splitting periods to recover only the very concentrated fractions. This would be at the expense of the respective fructose and glucose recovery and an economic evaluation would determined the exact lengths of the splitting periods.

8.8 Recycling of the Dilute Product Fractions

By using the dilute FRP and GRP fractions as eluent and purge water, the eluent requirements would be minimised, all sugar solids would be recovered, and the product concentrations would increase slightly. This technique was evaluated in run 66.3-14.6-40-26.5-60, where the dilute GRP and FRP fractions were recycled into the eluent supply tank. The equipment was operating continuously and the results of cycle 19 are used (Tables 8.25, 8.26, 8.27). This prolonged operating period was used to allow extra time for the system to reach "pseudo-equilibrium" because of the recycling. The glucose present in the eluent and purge stream contaminated the FRP, and to overcome that, the switch time was increased to 26.5 minutes. The collection periods were from 18.25 to 5 minutes for the GRP and from 0^- to 13.25 minutes for the FRP. This increased the product concentrations to 12.96 and 25.4% w/v for the FRP and GRP respectively and the throughput to 0.581 kg/hour, ie. 32.3 kg sugar solids/m^3 resin/hour. Over 84% of the glucose in feed was recovered during this collection period and the GRP fructose content was 6.69%. The FRP fructose recovery was 87.8% and the product was 90.2% pure. The purging and eluent streams contained some maltose and oligosaccharides coming from the recycled GRP stream, hence some of these impurites were present in the FRP and also in all remaining columns. A comparison of the purging profiles of runs 66-14.6-40-25-60 (Figure 8.14) and 66.3-14.6-40-26.5-60 (Figure 9.24) verifies this, and also shows a one column shift of the cross-over point towards the FRP (Figure 8.24). It is therefore recommended that only the dilute FRP fraction is used for purging and the dilute GRP fraction can be used to dilute the industrial feedstock to the required concentration levels.

All fructose concentration profiles indicate that the fructose in the eluent entry column is diluted from the incoming eluent stream, thus the FRP product concentration would be increased if the fructose concentration in the eluent entry column is kept artificially high. It is therefore proposed that only the very concentrated FRP fraction is retained, ie.

from 0⁻ to 8 minutes during each switch, and the remaining is recycled and used to supplement the purge and eluent streams. This will reduce the fructose recovery per pass but it should increase the product concentration to the specified levels and above.

8.9 Additional Comments on the Equipments Performance

8.9.1 Actual Distribution Coefficients and Separation Factors

The extent of separation obtained by the SCCR systems is given by the relationship 5.9. When the infinite dilution distribution coefficients are used (K_{dg} = 0.125 and K_{df} = 0.472), the operating limits determined from this relationship and the separation factor of 2.2 indicate a relatively easy separation. This however does not represent the actual operating conditions because the effects of temperature, concentration and flow rates are not accounted for. In Tables 8.3, 8.4 and 8.9 the actual distribution coefficients and separation factors have been obtained for the actual operating conditions using the relationships 7.3, 7.4, 7.5 and 7.19 to 7.24. The fructose infinite dilution distribution coefficient is modified using relationship 7.3 to account for the different operating temperature. The new fructose coefficient and the glucose infinite dilution coefficient are modified further to account for the operating flow rates (feed plus eluent flow rate) using relationships 7.5 and 7.4 respectively. The resulting K_d values are then used in the corresponding relationships 7.19 to 7.24, and the final distribution coefficients are the weighted average of the above according to the feed content. For example, for a feedstock containing 42% fructose, 52% glucose and 6% maltose and oligosaccharides, the weighted average distribution coefficients are given by:

$$K_{dg} = 0.52\, K^{G}_{dg} + 0.42\, K^{F}_{dg} + 0.06\, K^{M}_{dg} \qquad \text{..... 8.5}$$

and

$$K_{df} = 0.52\,K^{G}_{df} + 0.42\,K^{F}_{df} + 0.06\,K^{M}_{df} \quad \text{..... 8.6}$$

Although the actual K_d values change along the system, as the respective concentrations vary from plate to plate, these weighted distribution coefficients can be considered as the average ones since they represent the actual feed composition. The accuracy in their values depends only on the accuracy of the experimental results and the validity of the assumptions made during the development of the relationships 7.3, 7.4, 7.5 and 7.19 to 7.24.

Tables 8.3, 8.4 and 8.9 also include the operating L/P ratios, which varied according to the chosen operating switch time. For complete separation (ie. 100% pure FRP and fructose free GRP), the average K_d and Lm/P ratios should be identical and the pre and post feed L/P ratios must be within the limits set by the K_{dg} and K_{df} values respectively. Identical sets of results cannot be expected because of the possible inaccuracies in the evaluation of the K_d values and since other factors, such as the semicontinuous principle of operation experimental errors and product recycling are not included. The results however show a similar variation with increasing feed concentration and flow rates, and the extent of disagreement indicates the expected degree of contamination. The K_d and L/P values can therefore be used as a guide for the suitability of the chosen operating parameters. The actual operating parameters, and in particular the switch time, must be chosen using the experimental relationships 8.1 to 8.4 and taking into account the effects of other parameters mentioned previously in this chapter such as purge flow rate, eluent to feed flow rate ratios and product recycling. The actual separation factors decreased from 1.53 to 1.13 as the feed concentration increased from 18.6 to 66.3% w/v, and these low values indicate the increased separation difficulty due to the actual operating conditions that were used.

8.9.2 System's Reproducibility

Table 8.28 contains the results of the analysis of the bulk products collected over various cycles during an experimental run (66-14.6-40-25-60). During the initial runs the purities are high and decrease gradually until they reach their final value after approximately six cycles. In cycle 2 the on-column sugar concentrations are still low and their effect on the K_d values is minimal, thus the high purities. In the following cycles, as the sugar concentration increases the purities decrease until the "pseudo-equilibrium" state is reached (cycle 7), when they become reproducible. This reproducibility is also verified from the results of runs 66-14.6-40-25-60* and 66-14.6-40-25-60 (Tables 8.7 and 8.8) which were taken five cycles apart (cycles 9 and 14), and their corresponding on-column concentration profiles (Figures 8.25 and 8.17) which were almost identical.

8.9.3 Calcium ion displacement

The fructose-glucose separation depends on the calcium charging of the packing. Any sodium ions entering the system could change some of the resin into the sodium form, especially at low pH, (less than 6). The presence of heavy or transition metals or organic acids can result in partial "inversion" of the packing (223). The above problems would affect the separation significantly and it would require the regeneration of the packing. This calcium displacement has been found to be very significant in the separation of molasses (225). Chuah (204) carried out an analysis of the product streams over 100 cycles, using an atomic absorption spectrometer. The FRP contained, in his experiments, 2 ppm of sodium and 10 ppm of calcium ions which was reduced to 3 ppm after 12 cycles. In the GRP stream, there was a continuous displacement of calcium ions of between 10 and 27 ppm, and 300 ppm of sodium were also present.

In this experimental program the SCCR7 has been operated for over 400 cycles, the eluent and purge water was deionised and filtered through a carbon filter. So far there is no apparent loss in separation efficiency due to calcium displacement. This is verified by the results of runs 45.7-13-39-24-60, 45.7-13-39-24.33-60 and 46-13-39-24.17-60. The later was carried out almost seven months later with over 100 cycles in between, and the results were as expected for the intermediate switch time of 24.17 minutes.

8.9.4 Operating Pressure Drop

The pressure drop, ΔP, through a packed bed under laminar flow conditions, and assuming no wall effects, is given by the Kozeny equation (207):

$$\Delta P = \frac{K'' S^2 (1 - \varepsilon)^2 \mu l u}{\varepsilon^3} \qquad \text{..... 8.7}$$

where: K'' = Kozeny's constant ($\simeq 5$)
S = Specific surface area of packing. If a spherical shape is assumed then $S = 6/dp = 22222\ m^2\ m^{-3}$
d_p = Particle diameter ($\simeq 270\ \mu m$)
μ = Fluid viscosity
l = Bed length ($\simeq 65.5$ cm)
u = Average fluid velocity, defined as $\frac{1}{A'}\left[\frac{dV}{dt}\right]$
V = Volume of fluid flowing in time t
A = Total cross-sectional area of bed
ε = Column voidage (0.309)

For comparison purposes the pressure drop at 20°C and 56% w/v feed concentration is calculated using the generating conditions of run 56-10-30-30-20. Since the fluid velocities are different in the pre and post feed sections the column pressure drop varies with position. A weighted average fluid viscosity is used as

follows:

Average fructose visocisty at 24% w/v (226) = 1.782×10^{-3} Ns m^{-2}

Average glucose visocisty at 28% w/v (226) = 2.176×10^{-3} Ns m^{-2}

Average maltose viscosity at 2.5% w/v (226) = 1.06×10^{-3} Ns m^{-2}

Therefore,

Weighted average viscosity at 20°C =

$0.52 \times 2.176 \times 10^{-3} + 0.44 \times 1.782 \times 10^{-3} + 0.04 \times 1.06 \times 10^{-3} = 1.958 \times 10^{-3}$ Ns m^{-2}

Therefore, for a pre-feed column:

$u = 0.218 \times 10^{-3}$ m.sec^{-1} and $\Delta P = 11.17$ kN m^{-2}

for a post-feed column:

$u = 0.291 \times 10^{-3}$ m.sec^{-1} and $\Delta P = 14.95$ kN m^{-2}

The total theoretical pressure drop over the eleven columns of the separation section of the SCCR7 is 145.6 kN m^{-2}. The actual pressure drops however are substantially higher due to the additional pressure drops over the valves, support meshes, sudden enlargements and contractions over the fittings and frictional pressure drops along the pipe network (133). The actual average pressure drops over the whole feed concentration range are shown in Table 8.29. Throughout the experimental work no pressure related problems were encountered.

Table 8.1: Effect of Switch Time on Product Purities - Operating Conditions

Experimental Run	Average Flow Rates $cm^3\ min^{-1}$			Feed concentration % w/v				F to G ratio in feed %	Switch Time min	Average K_d inc the effects of temp, flow rates & Concentration $\frac{K_{dg} + K_{df}}{2}$	Lm/P ratio	Cycle
	Feed	Eluent	Purge	G	F	M+OS	Total					
35-13-40-21-60	13	40	80	17.6	17.1	0.3	35	48.9/ 50.6	21	0.368	0.342	+5
35-13-40-26-60	13	40	80	17.6	17.1	0.3	35	48.9/ 50.6	26	0.368	0.497	+4
37-13-40-21-60	13	40	80	19.6	15.9	1.5	37	43/52.3	21	0.375	0.342	7
36-13-40-23-60	13	40	80	18.77	15.19	2.06	36.02	42.2/ 52.1	23	0.371	0.404	7
37-13-40-25-60	13	40	80	19.6	15.9	1.5	37	43/52.3	25	0.375	0.466	7

M+OS - maltose plus oligosaccharides
G - glucose
F - fructose

The + indicates the number of additional cycles carried out after making a change in the operating conditions without purging the system.

Table 8.2: Effect of Switch Time on Product Purities - Experimental Results

Experimental Run	Feed throughput (sugar solids) kg h^{-1}	GLUCOSE RICH PRODUCT					FRUCTOSE RICH PRODUCT				
		Glucose purity (%)	% of glucose in feed recovered	Total Product Conc (% w/v)	Impurities (%)		Fructose purity (%)	% of fructose in feed recovered	Total Product Conc (% w/v)	Impurities (%)	
					F	M+OS				G	M+OS
35-13-40-21-60	0.272	98.2	95	3.9	-	1.8	88.8	96.4	2.47	11.2	-
35-13-40-26-60	0.272	80.2	99	5.11	13.5	6.3	100	78.3	1.83	-	-
37-13-40-21-60	0.288	89.7	99	2.27	-	11.3	70.6	115.4	3.56	29.4	-
36-13-40-23-60	0.281	83.04	88.1	4.49	5.49	11.46	92.4	77.1	1.69	7.6	-
37-13-40-25-60	0.288	71.6	113	8.12	22.5	5.9	100.0	56.6	1.2	-	-

Table 8.3: Actual Distribution Coefficients and Effective Mobile Flow Rates for Feed Concentrations of 18.6 to 36% w/v

Experimental Run	Distribution coefficients incorporating the effects of temp, flow rates and concentration			Separation factor $\alpha = \frac{K_{df}}{K_{dg}}$	Pre-feed Le/P	Post-feed (Le + Lf)/P	Average Lm/P
	K_{dg}	K_{df}	Average K_d				
18.6-9-30-30-20	0.353	0.540	0.447	1.53	0.291	0.471	0.381
35-13-40-21-60	0.340	0.395	0.368	1.16	0.251	0.433	0.342
35-13-40-26-60	0.340	0.395	0.368	1.16	0.384	0.610	0.497
35.2-13-39-23.5-60	0.339	0.401	0.370	1.18	0.302	0.506	0.404
35.2-13-39-24.5-60	0.399	0.401	0.370	1.18	0.328	0.540	0.434
36-13-40-23-60	0.341	0.401	0.371	1.18	0.304	0.504	0.404

Table 8.4: Actual Distribution Coefficients and Effective Mobile Flow Rates for Feed Concentrations of 37 to 46% w/v

Experimental Run	Distribution coefficients incorporating the effects of temp, flow rates and concentration			Separation factor $\alpha' = \frac{K_{df}}{K_{dg}}$	Pre-feed Le/P	Post-feed (Le + Lf)/P	Average Lm/P
	K_{dg}	K_{df}	Average K_d				
37-13-40-21-60	0.345	0.404	0.375	1.17	0.251	0.433	0.342
37-13-40-25-60	0.345	0.404	0.375	1.17	0.358	0.574	0.466
37.8-13-40-24.5-60	0.355	0.416	0.386	1.17	0.344	0.557	0.451
45.7-13-39-24-60	0.361	0.420	0.391	1.16	0.315	0.523	0.419
45.7-13-39-24.33-60	0.361	0.420	0.391	1.16	0.324	0.534	0.429
46-13-39-24.17-60	0.361	0.419	0.391	1.16	0.319	0.529	0.424
47.5-13-32-28.5-60	0.409	0.479	0.444	1.17	0.299	0.546	0.423

Table 8.5: Sensitivity of Product Purities to Small Changes in Switch Time - Operating Conditions

Experimental Run	Average Flow Rates $cm^3\ min^{-1}$			Feed concentration % w/v				F to G ratio in feed %	Switch Time min	Average K_d inc the effects of temp, flow rates & Concentration $\frac{K_{dg} + K_{df}}{2}$	Lm/P ratio	Cycle
	Feed	Eluent	Purge	G	F	M+OS	Total					
35.2-13-39-24.5-60	13	39	80	18.38	14.63	2.23	35.24	41.5/ 52.2	24.5	0.370	0.434	7
35.2-13-39-23.5-60	13	39	80	18.38	14.63	2.23	35.24	41.5/ 52.2	23.5	0.370	0.404	+6
36-13-40-23-60	13	40	80	18.77	15.19	2.06	36.02	42.2/ 52.1	23	0.371	0.404	7
45.7-13-39-24-60	13	39	80	24.01	18.83	2.88	45.72	41.2/ 52.5	24	0.391	0.419	9
45.7-13-39-24.33-60	13	39	80	24.01	18.83	2.88	45.72	41.2/ 52.5	24.33	0.391	0.429	+6
46-13-39-24.17-60	13	39	80	23.98	19.22	2.8	46	41.8/ 52.1	24.17	0.391	0.424	10

Table 8.6: Sensitivity of Product Purities to Small Changes in Switch Time - Results

Experimental Run	Feed throughput (sugar solids) kg h^{-1}	GLUCOSE RICH PRODUCT					FRUCTOSE RICH PRODUCT				
		Glucose purity (%)	% of glucose in feed recovered	Total Product Conc (% w/v)	Impurities (%)		Fructose purity (%)	% of fructose in feed recovered	Total Product Conc (% w/v)	Impurities (%)	
					F	M+OS				G	M+OS
35.2-13-39-24.5-60	0.275	71.75	92.87	5.48	17.88	10.44	99.0	69.33	1.57	1.0	-
35.2-13-39-23.5-60	0.275	75.36	81.28	4.47	11.50	13.13	92.85	95.38	2.11	7.14	-
36-13-40-23-60	0.281	83.04	88.1	4.49	5.49	11.46	92.4	77.1	1.69	7.6	-
45.7-13-39-24-60	0.357	83.3	74.2	4.76	2.4	14.3	89.5	81.5	2.30	10.5	-
45.7-13-39-24.33-60	0.357	77.24	88.6	5.84	11.76	10.98	95.37	80.2	2.08	4.62	-
46-13-39-24.17-60	0.359	80.84	83.1	5.17	6.1	13.06	91.78	82.8	2.19	8.22	-

Table 8.7: Effect of Increasing Feed Concentration on Product Purities and Concentrations - Operating Conditions

Experimental Run	Average Flow Rates $cm^3\ min^{-1}$			Feed concentration % w/v				F to G ratio in feed %	Switch Time min	Average K_d inc the effects of temp, flow rates & Concentration $\frac{K_{dg} + K_{df}}{2}$	Lm/P ratio	Cycle
	Feed	Eluent	Purge	G	F	MIOS	Total					
18.6-9-30-30-20	9	30	75	10.78	7.82	-	18.6	42/58	30	0.447	0.381	7
36-13-40-23-60	13	40	80	18.77	15.19	2.06	36.02	42.2/ 52.1	23	0.371	0.404	7
46-13-39-24.17-60	13	39	80	23.98	19.22	2.8	46	41.8/ 52.1	24.17	0.391	0.424	10
54-13-39-24.5-60	13	39	80	28.08	22.73	3.19	54	42.09/ 52	24.5	0.405	0.433	9
66-14.6-40-25-60*	14.6	40	70	34.3	27.8	3.9	66	42.1/ 52	25	0.428	0.479	9
66-14.6-40-25-60	14.6	40	70	34.3	27.8	3.9	66	42.1/ 52	25	0.428	0.479	+5 (14)

Table 8.8: Effect of Increasing Feed Concentrations on Product Purities and Concentrations - Results

Experimental Run	Feed throughput (sugar solids) kg h^{-1}	GLUCOSE RICH PRODUCT					FRUCTOSE RICH PRODUCT				
		Glucose purity (%)	% of glucose in feed recovered	Total Product Conc (% w/v)	Impurities (%)		Fructose purity (%)	% of fructose in feed recovered	Total Product Conc (% w/v)	Impurities (%)	
					F	M+OS				G	M+OS
18.6-9-30-30-20	0.1	99.9	97.1	2.12	0.1	-	99.9	98.4	0.93	0.1	-
36-13-40-23-60	0.281	83.04	88.1	4.49	5.49	11.46	92.4	77.1	1.69	7.6	-
46-13-39-24.17-60	0.359	80.84	83.1	5.17	6.1	13.06	91.78	82.8	2.19	8.22	-
54-13-39-24.5-60	0.421	81.11	86.58	6.94	6.35	12.54	91.35	88.61	3.12	8.65	-
66-14.6-40-25-60*	0.578	83.1	95.7	11.77	6.9	10.0	90.1	89.5	5.76	9.9	-
66-14.6-40-25-60	0.578	82.9	94.13	11.6	6.4	10.7	90.07	90.72	5.84	9.93	-

Table 8.9: Actual Distribution Coefficients and Effective Mobile Flow Rates for Feed Concentrations of 47.5 to 66.3% w/v

Experimental Run	Distribution coefficients incorporating the effects of temp, flow rates and concentration			Separation factor $\alpha = \frac{K_{df}}{K_{dg}}$	Pre-feed Le/P	Post-feed (Le + Lf)/P	Average Lm/P
	K_{dg}	K_{df}	Average K_d				
47.5-13-32-29-60	0.409	0.479	0.444	1.17	0.310	0.561	0.435
54-13-39-24.5-60	0.377	0.433	0.405	1.17	0.328	0.540	0.434
55.1-13-39-24.5-60	0.377	0.433	0.405	1.15	0.328	0.540	0.434
56-10-30-30-20	0.427	0.628	0.528	1.47	0.291	0.491	0.391
65.1-14.2-40.5-25-60	0.399	0.452	0.426	1.13	0.366	0.603	0.484
66-14.6-40-25-60	0.401	0.454	0.428	1.13	0.358	0.601	0.479
66.3-14.6-40-26.5-60	0.402	0.455	0.429	1.13	0.398	0.656	0.527

Table 8.10: Operating Switch Times at Increasing Feed Concentrations

Feed Concentration (% w/v)	Feed flow rate ($cm^3\ min^{-1}$)	Switch time (min)	Switch time at a reference feed flow rate of 13 $cm^3\ min^{-1}$ (min)	Switch time expressed as a percentage
18.6	9	30	20.77	90.3
36	13	23	23	100.0
46	13	24.17	24.17	105.1
54	13	24.5	24.5	106.5

Table 8.11; Effect of Reducing the Fructose Content of the Feed on the Separation - Operating Conditions

Experimental Run	Average Flow Rates $cm^3\ min^{-1}$			Feed Concentration % w/v				F to G ratio in feed %	Switch time		Average K_d in the effects of temp, flow rates & Concentration $\frac{K_{dg}+K_{df}}{2}$	Lm/P ratio	Cycle
	Feed	Eluent	Purge	G	F	M+OS	Total		Min	as % change			
35-13-40-21-60	13	40	80	17.6	17.1	0.3	35	48.9/ 50.6	21	91.3	0.368	0.342	+4
36-13-40-23-60	13	40	80	18.77	15.19	2.06	36.02	42.2/ 52.1	23	100	0.371	0.404	7
37.8-13-40-24.5-60	13	40	80	21.75	13.91	2.17	37.83	36.8/ 57.5	24.5	106.5	0.386	0.451	+5

Table 8.12: Effect of Reducing the Fructose Content of the Feed on the Separation - Results

Experimental Run	Feed throughput (sugar solids) kg h^{-1}	GLUCOSE RICH PRODUCT					FRUCTOSE RICH PRODUCT				
		Glucose purity (%)	% of glucose in feed recovered	Total Product Conc (% w/v)	Impurities (%)		Fructose purity (%)	% of fructose in feed recovered	Total Product Conc (% w/v)	Impurities (%)	
					F	M+OS				G	M+OS
35-13-40-21-60	0.272	98.2	95	3.9	-	1.8	88.8	96.4	2.47	11.2	-
36-13-40-23-60	0.281	83.04	88.1	4.49	5.49	11.46	92.4	77.1	1.69	7.6	-
37.8-13-40-24.5-60	0.295	83.79	92.61	5.5	5.99	10.21	89.15	75.05	1.61	10.84	-

Table 8.13: Effect of Reducing the Eluent and Purge Flow Rates on Product Concentrations and Purities - Operating conditions

Experimental Run	Average Flow Rates $cm^3\ min^{-1}$			Feed concentration % w/v				F to G ratio in feed %	Switch Time min	Average K_d inc the effects of temp, flow rates & Concentration $\frac{K_{dg} + K_{df}}{2}$	Lm/P ratio	Cycle
	Feed	Eluent	Purge	G	F	MHOS	Total					
66-14.6-40-25-60	14.6	40	70	34.3	27.8	3.9	66	42.1/52	25	0.428	0.479	14
65.1-14.2-40.5-25-60	14.2	40.5	50	33.8	27.4	3.9	65.1	42.1/ 51.9	25	0.426	0.484	+6
46-13-39-24.17-60	13	39	80	23.98	19.22	2.8	46	41.8/ 52.1	24.17	0.391	0.424	10
47.5-13-32-29-60	13	32	50	25.2	19.7	2.6	47.5	41.5/53	29	0.444	0.435	9
47.5-13-32-28.5-60	13	32	50	25.2	19.7	2.6	47.5	41.5/53	28.5	0.444	0.423	+4

Table 8.14: Effect of Reducing the Eluent and Purge Flow Rates on Product Concentrations and Purities - Results

Experimental Run	Feed through-put (sugar solids) kg h^{-1}	GLUCOSE RICH PRODUCT					FRUCTOSE RICH PRODUCT				
		Glucose purity (%)	% of glucose in feed recovered	Total Product Conc (% w/v)	Impurities (%)		Fructose purity (%)	% of fructose in feed recovered	Total Product Conc (% w/v)	Impurities (%)	
					F	M+OS				G	M+OS
66-14.6-40-25-60	0.578	82.9	94.13	11.6	6.4	10.7	90.07	90.72	5.84	9.93	-
65.1-14.2-40.5-25-60	0.555	76.7	104	12.1	9.9	13.2	94.5	82.3	5.4	5.5	-
46-13-39-24.17-60	0.359	80.84	83.1	5.17	6.1	13.06	91.78	82.8	2.19	8.22	-
47.5-13-32-29-60	0.371	72.1	76.6	8.19	16.7	11.2	96	74.1	3.26	4.0	-
47.5-13-32-28.5-60	0.371	77.6	73.7	7.44	9.4	13	76.62	73.74	4.07	23.38	-

Table 8.15: GRP Composition over a Switch (Run 66-14.6-40-25-60)

Time period (min)	Composition % w/v			
	Glucose	Fructose	M + OS	Total
0-3	27.80	4.60	2.80	35.20
3-6	4.30	1.80	0.40	6.50
6-9	0.70	0.52	0.03	1.25
9-12	0.30	0.20	0.0	0.50
12-15	0.14	0.11	0.42	0.67
15-18	2.30	0.20	1.30	3.80
18-21	12.60	0.40	2.20	15.20
21-24	23.60	1.50	2.90	28.00
24-25	26.50	3.00	3.20	32.70

M + OS = Maltose and oligosaccharides

Table 8.16: FRP Composition over a Switch (Run 66-14.6-40-25-60)

Time period (min)	Composition % w/v			
	Glucose	Fructose	M + OS	Total
0-3	0.42	11.47	-	11.90
3-6	0.08	17.61	-	17.70
6-9	0.00	9.84	-	9.84
9-12	0.00	2.90	-	2.90
12-15	0.01	0.99	-	1.00
15-18	0.08	0.52	-	0.60
18-21	0.09	0.33	-	0.42
21-24	0.16	0.21	-	0.37
24-25	0.22	0.11	-	0.33

Table 8.17: Increasing Throughput and Product Concentrations - Operating Conditions

Experimental Run	Average Flow Rates $cm^3\ min^{-1}$			Feed concentration % w/v				F to G ratio in feed %	Switch Time min	Average K_d inc the effects of temp, flow rates & Concentration $\frac{K_{dg} + K_{df}}{2}$	Lm/P ratio	Cycle
	Feed	Eluent	Purge	G	F	M+OS	Total					
56-10-30-30-20	10	30	75	29.0	24.5	2.5	56	44/52	30	0.528	0.391	8
54-13-39-40-60	13	39	80	28.08	22.73	3.19	54	42.09/52	24.5	0.405	0.434	9
55.1-13-39-24.5-60	13	39	80	28.22	23.47	3.42	55.12	42.6/51.2	24.5	0.405	0.434	+6
66-14.6-40-25-60*	14.6	40	70	34.3	27.8	3.9	66	42.1/52	25	0.428	0.479	9
66-14.6-40-25-60	14.6	40	70	34.3	27.8	3.9	66	42.1/52	25	0.428	0.479	+5 (14)

Table 8.18: Results of the Analysis of the BULK GRP and FRP Products

Experimental Run	Feed throughput (sugar solids) kg h^{-1}	GLUCOSE RICH PRODUCT					FRUCTOSE RICH PRODUCT				
		Glucose purity (%)	% of glucose in feed recovered	Total Product Conc (% w/v)	Impurities (%)		Fructose purity (%)	% of fructose in feed recovered	Total Product Conc (% w/v)	Impurities (%)	
					F	M+OS				G	M+OS
56-10-30-30-20	0.336	87.5	67.4	3.98	-	12.5	69.7	106	5.07	30.3	-
54-13-39-24.5-60	0.421	81.11	86.58	6.94	6.35	12.54	91.35	88.61	3.12	8.65	-
55.1-13-39-24.5-60	0.430	80.92	90.1	6.87	4.16	14.92	95.4	82.1	2.75	4.6	-
66-14.6-40-25-60*	0.578	83.1	95.7	11.77	6.9	10.0	90.1	89.5	5.76	9.9	-
66-14.6-40-25-60	0.578	82.9	94.13	11.6	6.4	10.7	90.07	90.72	5.84	9.93	-

Table 8.19: Results of run 56-10-30-30-20 when Product Splitting was Employed on the FRP

0⁻-15 min collection					15 to 30 min collection				
Fructose Purity %	% of fructose in feed recovered	Total product conc (% w/v)	Impurities (%)		Fructose purity %	% of fructose in feed recovered	Total product conc (% w/v)	Impurities (%)	
			G	M+OS				G	M+OS
68	58.6	5.79	32	-	80	5	0.37	20	-

Table 8.20: Results of run 55.1-13-39-24.5-60 when Product Splitting was Employed on the FRP

0⁻-12.5 min collection					12.5 to 24.5 min collection				
Fructose Purity %	% of fructose in feed recovered	Total product conc (% w/v)	Impurities (%)		Fructose purity %	% of fructose in feed recovered	Total product conc (% w/v)	Impurities (%)	
			G	M+OS				G	M+OS
98.87	70.7	4.49	1.13	-	73.03	2.0	0.20	26.97	-

Table 8.21: Results of run 66-14.6-40-25-60* when Product Splitting was Employed on the GRP

0-12.5 min collection					12.5 to 25 min collection				
Glucose Purity %	% of glucose in feed recovered	Total product conc (% w/v)	Impurities (%)		Glucose purity %	% of glucose in feed recovered	Total product conc (% w/v)	Impurities (%)	
			F	M+OS				F	M+OS
80.2	27	7.02	13.31	6.47	83.8	68	16.21	4.3	11.89

Table 8.22: Results of run 66-14.6-40-25-60* when Product Splitting was Employed on the FRP

0-12.5 min collection					12.5 to 25 min collection				
Fructose Purity %	% of fructose in feed recovered	Total product conc (% w/v)	Impurities (%)		Fructose purity %	% of fructose in feed recovered	Total product conc (% w/v)	Impurities (%)	
			G	M+OS				G	M+OS
95.3	95.7	11.32	4.7	-	75.5	2.80	0.43	24.5	-

Table 8.23: Results of run 66-14.6-40-25-60 when Product Splitting was Employed on the GRP

4 to 16.5 min collection					16.5 to 4 min collection				
Glucose Purity %	% of glucose in feed recovered	Total product conc (% w/v)	Impurities (%)		Glucose purity %	% of glucose in feed recovered	Total product conc (% w/v)	Impurities (%)	
			F	M+OS				F	M+OS
47.2	1.97	0.87	26.4	26.4	84.83	94.23	22.56	4.49	10.67

Table 8.24: Results of run 66-14.6-40-25-60 when Product Splitting was Employed on the FRP

0⁻-12.5 min collection					12.5 to 25 min collection				
Fructose Purity %	% of fructose in feed recovered	Total product conc (% w/v)	Impurities (%)		Fructose purity %	% of fructose in feed recovered	Total product conc (% w/v)	Impurities (%)	
			G	M+OS				G	M+OS
94.8	95.78	11.29	5.2	-	75.6	2.82	0.45	24.4	-

Table 8.25: Operating Conditions when the Recycling of the Dilute Product Fractions was Employed

Experimental Run	Average Flow Rates $cm^3\ min^{-1}$			Feed concentration % w/v				F to G ratio in feed %	Switch Time min	Average K_d inc the effects of temp, flow rates & Concentration $\frac{K_{dg} + K_{df}}{2}$	Lm/P ratio	Cycle
	Feed	Eluent	Purge	G	F	M+OS	Total					
66.3-14.6-40-26.5-60	14.6	40	60	34.57	27.82	3.9	66.3	42/52.1	26.5	0.429	0.527	19

Table 8.26: GRP Results after Recycling the Dilute Fraction, Run: 66.3-14.6-40-26.5-60

Collection period from 18.25 to 5 minutes				
Glucose purity %	% of glucose in feed recovered	Total product Conc (% w/v)	Impurities %	
			F	M + OS
86.22	84.1	25.4	6.69	7.19

Table 8.27: FRP Results after Recycling the Dilute Fraction Run: 66.3-14.6-40-26.5-60

Collection period from 0' to 13.25 minutes				
Fructose purity %	% of fructose in feed recovered	Total product Conc (% w/v)	Impurities %	
			G	M + OS
90.2	87.8	12.96	8.87	0.93

Table 8.28: Bulk Product Composition over a Number of Cycles. (Run: 66-14.6-40-25-60)

Cycle No	Fructose Rich Product			Glucose Rich Product		
	F %	G %	M + OS %	F %	G %	M + OS %
2	98.2	1.8	-	3.15	86.2	10.65
4	93.6	6.4	-	5.4	85.31	9.29
5	91.8	8.2	-	6.19	83.8	10.01
6	90.28	9.72	-	6.27	83.5	10.23
7	90.09	9.91	-	6.61	83.0	10.39
9	90.1	9.9	-	6.9	83.1	10.0
14	90.07	9.93	-	6.4	82.9	10.7

Table 8.29: Operating Pressure Drops

Feed Conc (% w/v)	Feed flow rate $cm^3\ min^{-1}$	Temp °C	Average Pressure drop $kN\ m^{-2}$		
			Eluent	Feed	Purge
56	10	20	280	140	20
18.6	9	20	138	62	20
36	13	60	186	90	10
46	13	60	200	103	10
54	13	60	227	110	10
66	14.6	60	250	120	10

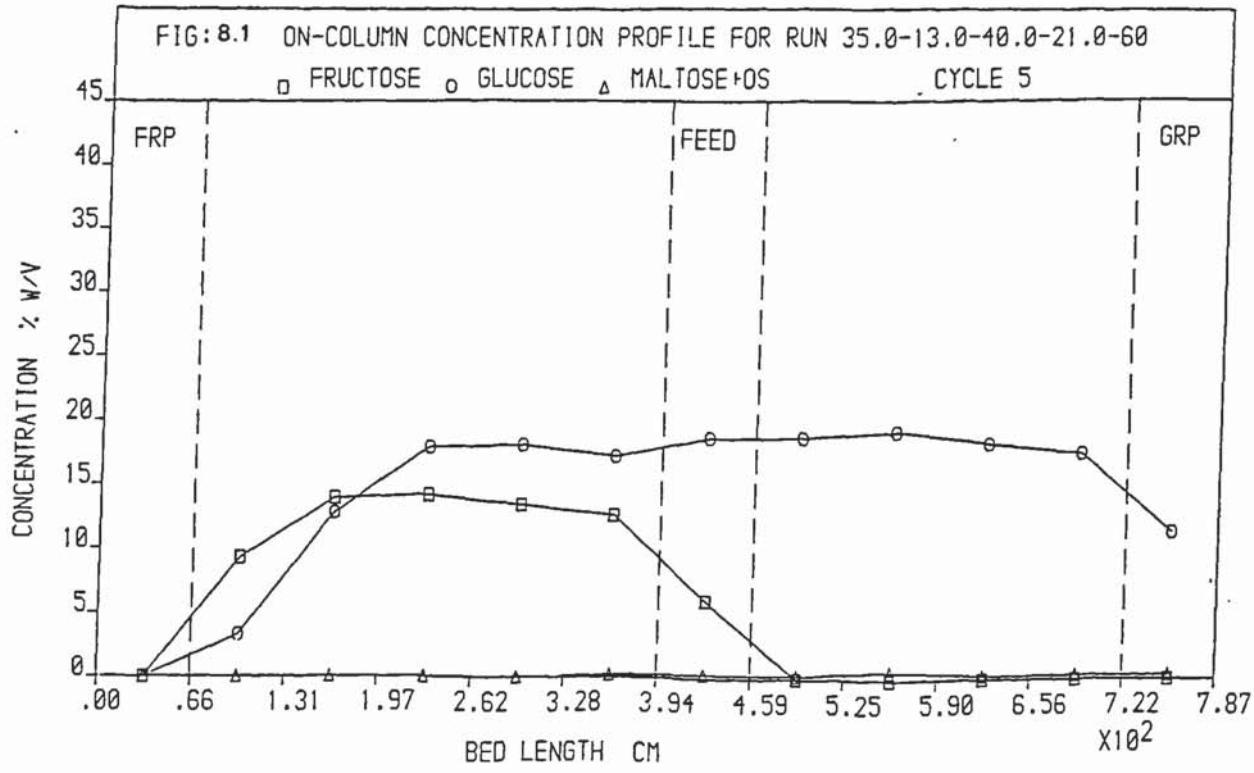


FIG: 8.1 ON-COLUMN CONCENTRATION PROFILE FOR RUN 35.0-13.0-40.0-21.0-60
□ FRUCTOSE o GLUCOSE Δ MALTOSE+OS CYCLE 5
FRP
FEED
GRP
CONCENTRATION % W/V
BED LENGTH CM
X10²
0
5
10
15
20
25
30
35
40
45
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87

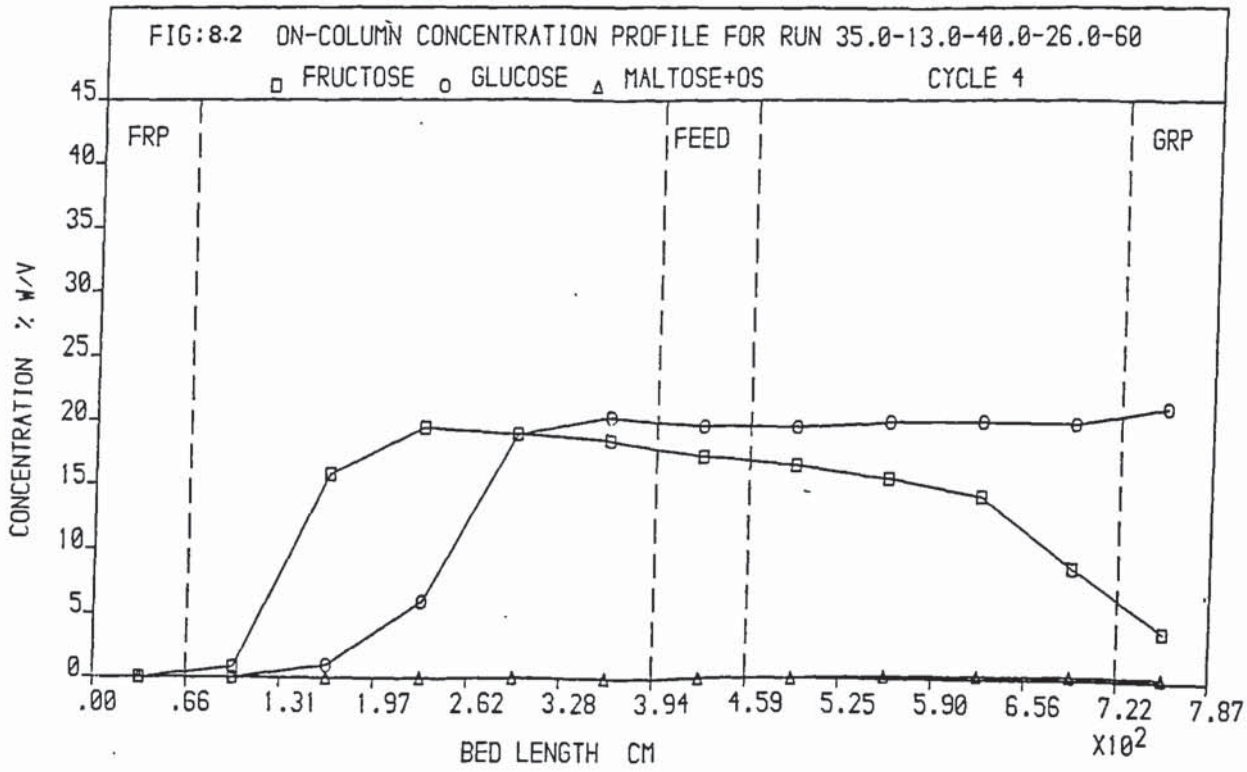


FIG: 8.2 ON-COLUMN CONCENTRATION PROFILE FOR RUN 35.0-13.0-40.0-26.0-60
□ FRUCTOSE o GLUCOSE Δ MALTOSE+OS CYCLE 4
FRP
FEED
GRP
CONCENTRATION % W/V
BED LENGTH CM
X10²
0
5
10
15
20
25
30
35
40
45
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87

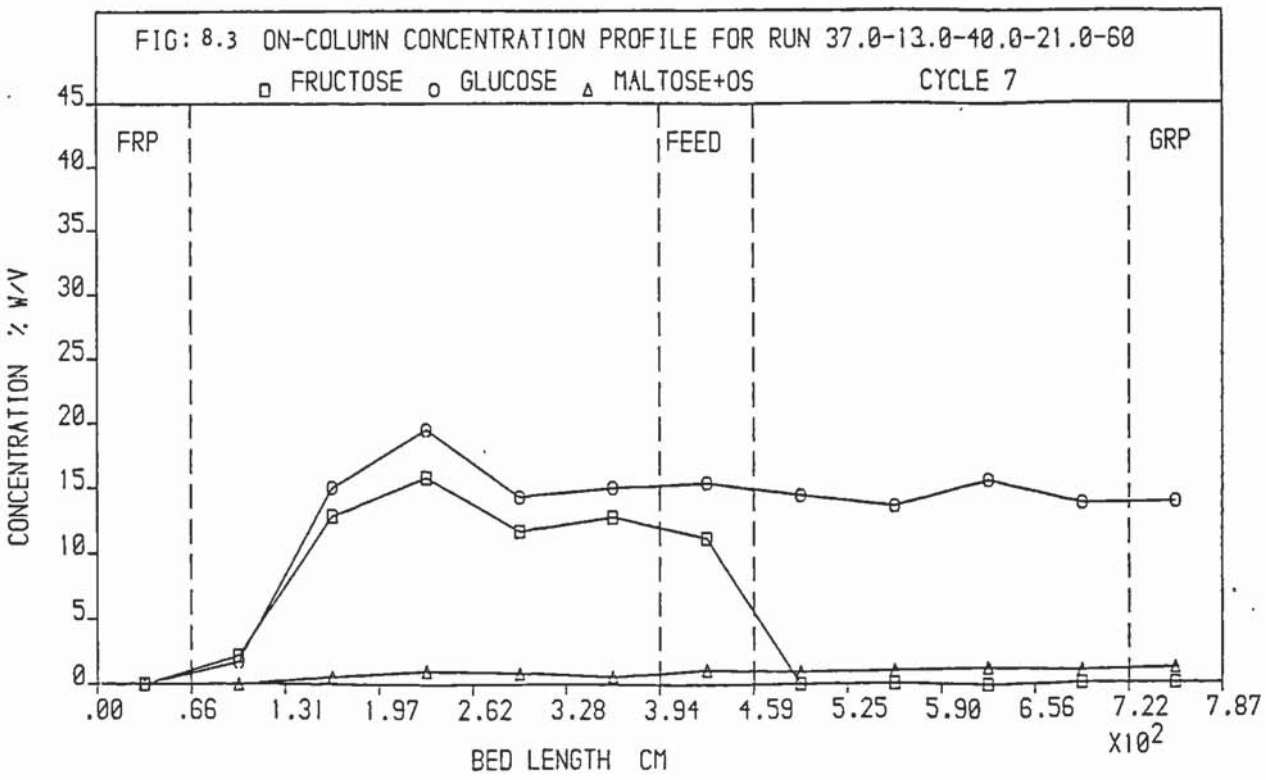


FIG: 8.3 ON-COLUMN CONCENTRATION PROFILE FOR RUN 37.0-13.0-40.0-21.0-60
□ FRUCTOSE o GLUCOSE △ MALTOSE+OS CYCLE 7
FRP
FEED
GRP
CONCENTRATION % W/V
45
40
35
30
25
20
15
10
5
0
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87
X10²
BED LENGTH CM

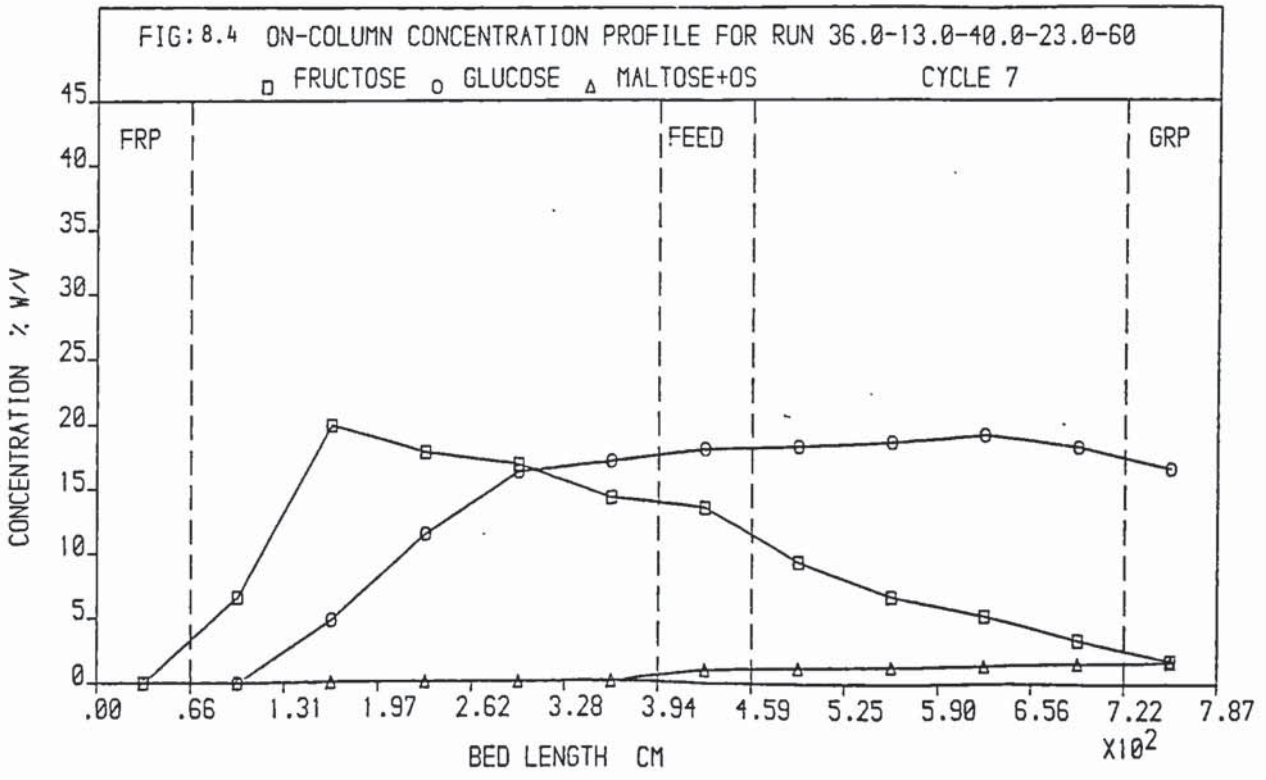


FIG: 8.4 ON-COLUMN CONCENTRATION PROFILE FOR RUN 36.0-13.0-40.0-23.0-60
□ FRUCTOSE o GLUCOSE △ MALTOSE+OS CYCLE 7
FRP
FEED
GRP
CONCENTRATION % W/V
45
40
35
30
25
20
15
10
5
0
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87
X10²
BED LENGTH CM

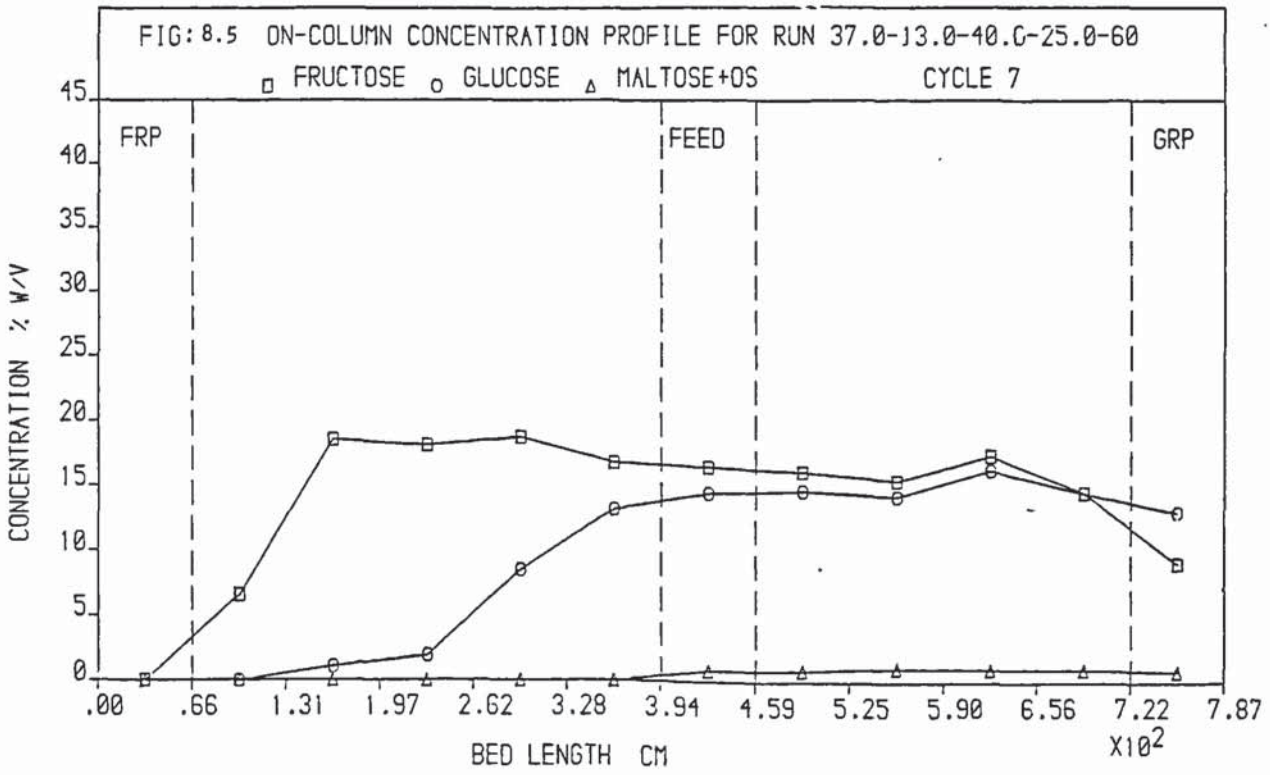


FIG: 8.5 ON-COLUMN CONCENTRATION PROFILE FOR RUN 37.0-13.0-40.0-25.0-60
□ FRUCTOSE o GLUCOSE △ MALTOSE+OS CYCLE 7
FRP
FEED
GRP
CONCENTRATION % W/V
0
5
10
15
20
25
30
35
40
45
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87
X10²
BED LENGTH CM

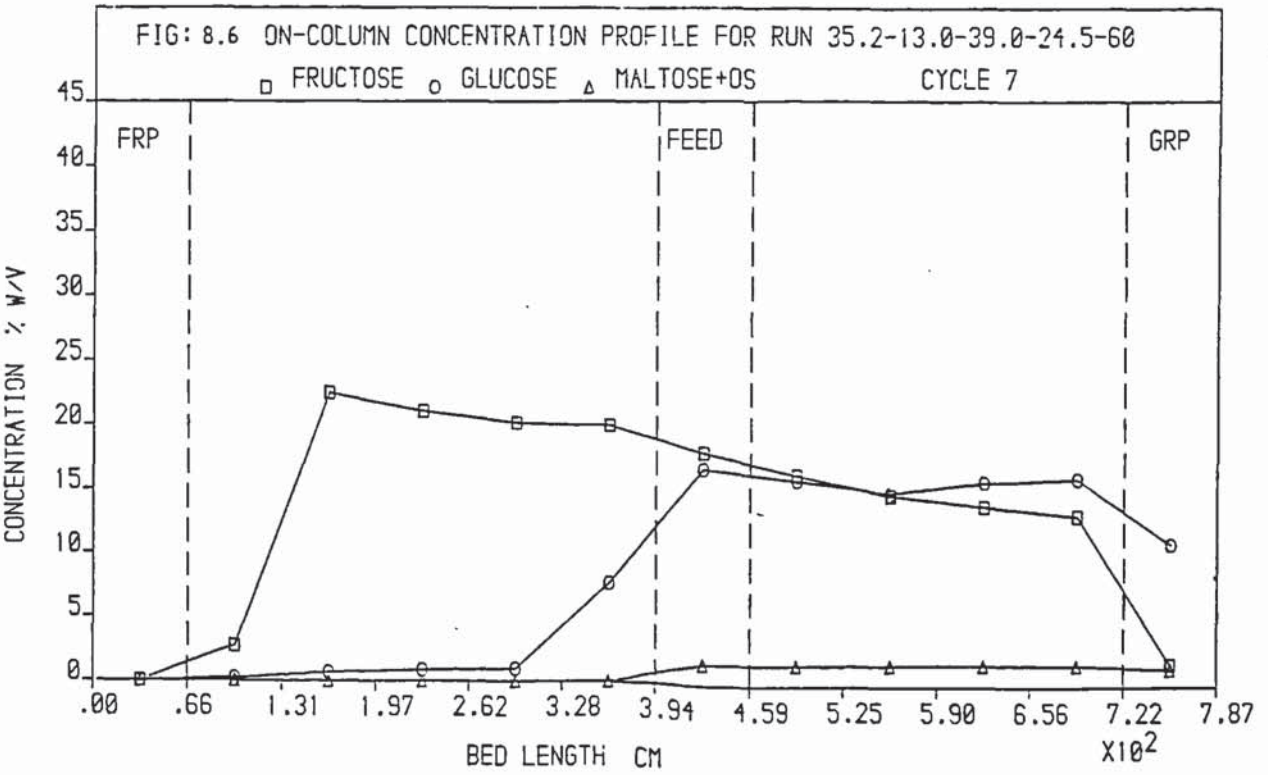


FIG: 8.6 ON-COLUMN CONCENTRATION PROFILE FOR RUN 35.2-13.0-39.0-24.5-60
□ FRUCTOSE o GLUCOSE △ MALTOSE+OS CYCLE 7
FRP
FEED
GRP
CONCENTRATION % W/V
0
5
10
15
20
25
30
35
40
45
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87
X10²
BED LENGTH CM

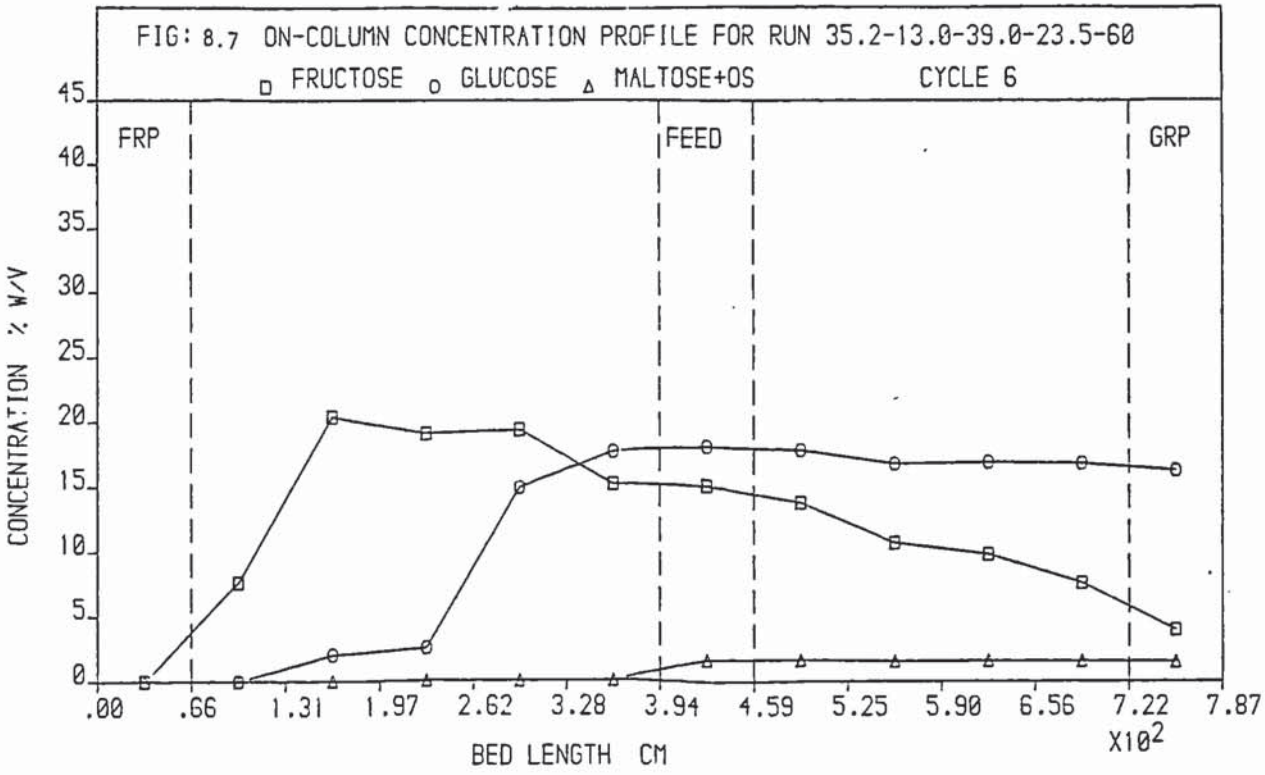


FIG: 8.7 ON-COLUMN CONCENTRATION PROFILE FOR RUN 35.2-13.0-39.0-23.5-60
□ FRUCTOSE ○ GLUCOSE △ MALTOSE+OS CYCLE 6
FRP
FEED
GRP
CONCENTRATION % W/V
BED LENGTH CM
X10²

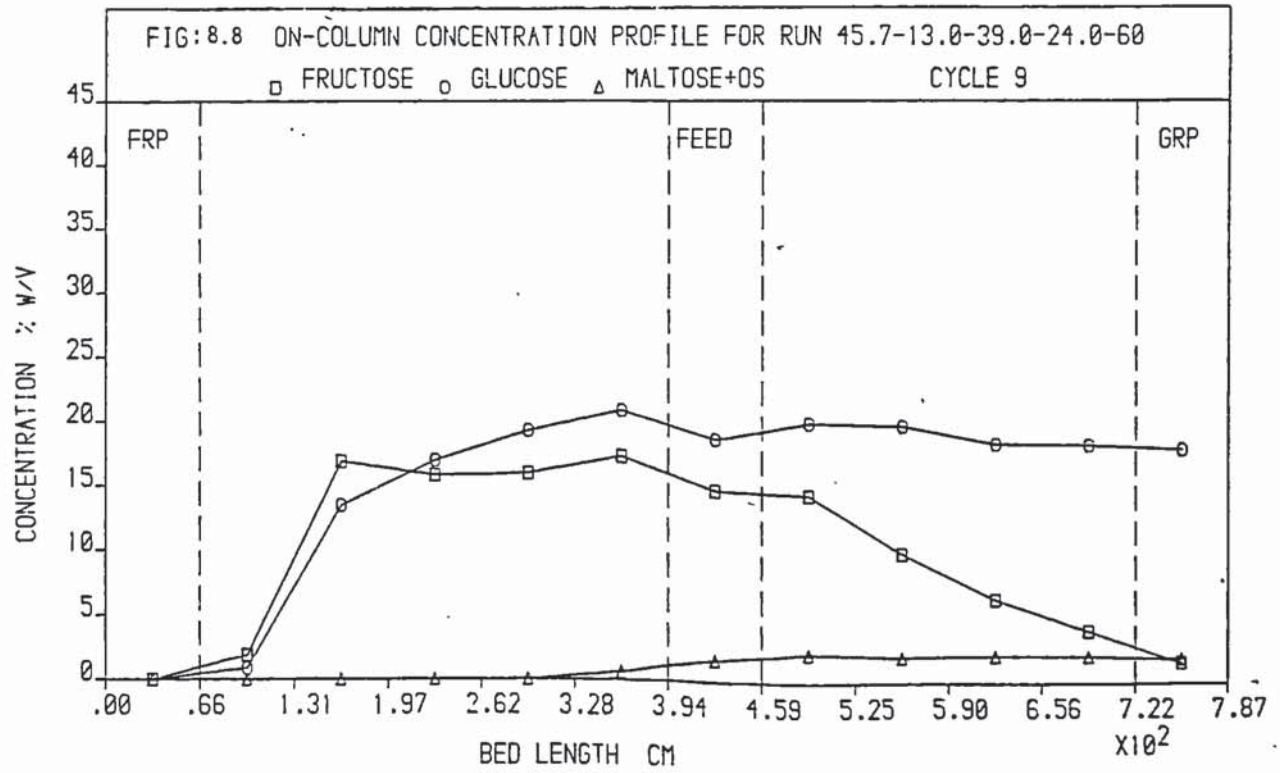


FIG: 8.8 ON-COLUMN CONCENTRATION PROFILE FOR RUN 45.7-13.0-39.0-24.0-60
□ FRUCTOSE ○ GLUCOSE △ MALTOSE+OS CYCLE 9
FRP
FEED
GRP
CONCENTRATION % W/V
BED LENGTH CM
X10²

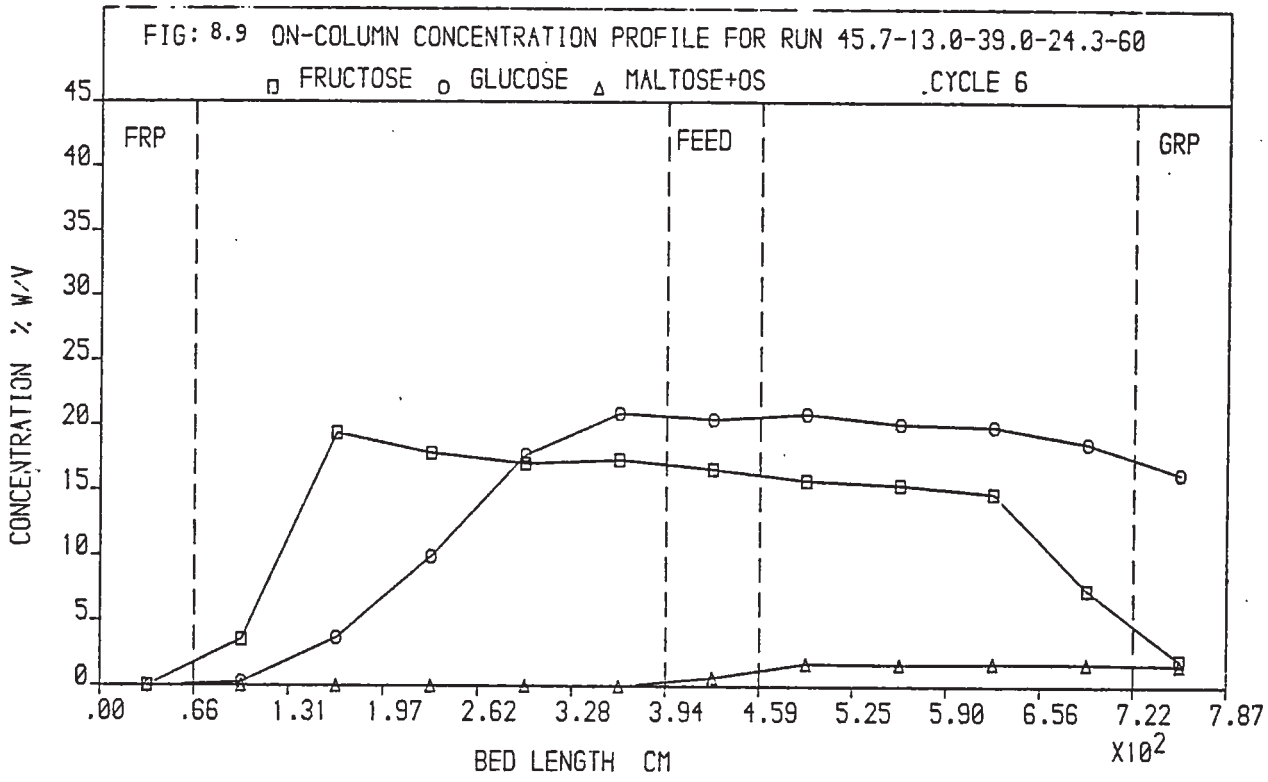


FIG: 8.9 ON-COLUMN CONCENTRATION PROFILE FOR RUN 45.7-13.0-39.0-24.3-60
□ FRUCTOSE o GLUCOSE △ MALTOSE+OS .CYCLE 6
FRP
FEED
GRP
CONCENTRATION % W/V
45
40
35
30
25
20
15
10
5
0
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87
BED LENGTH CM
X10²

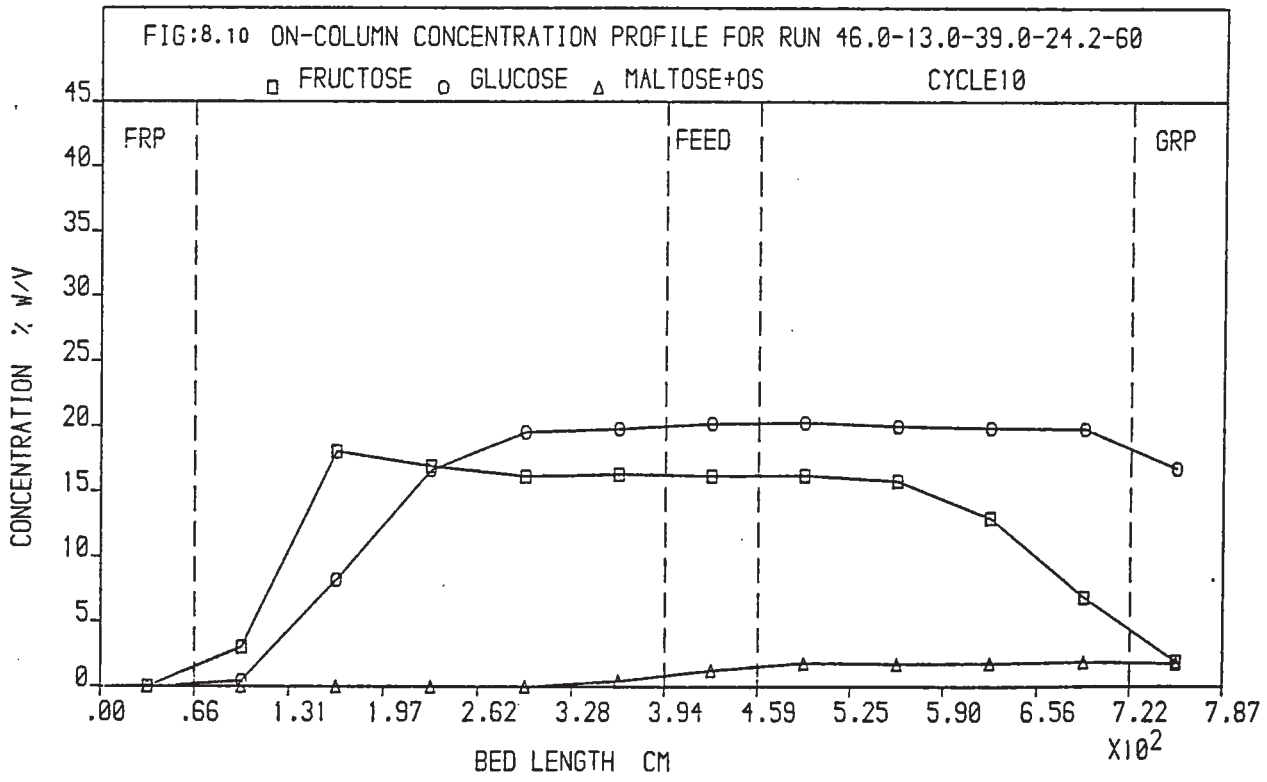
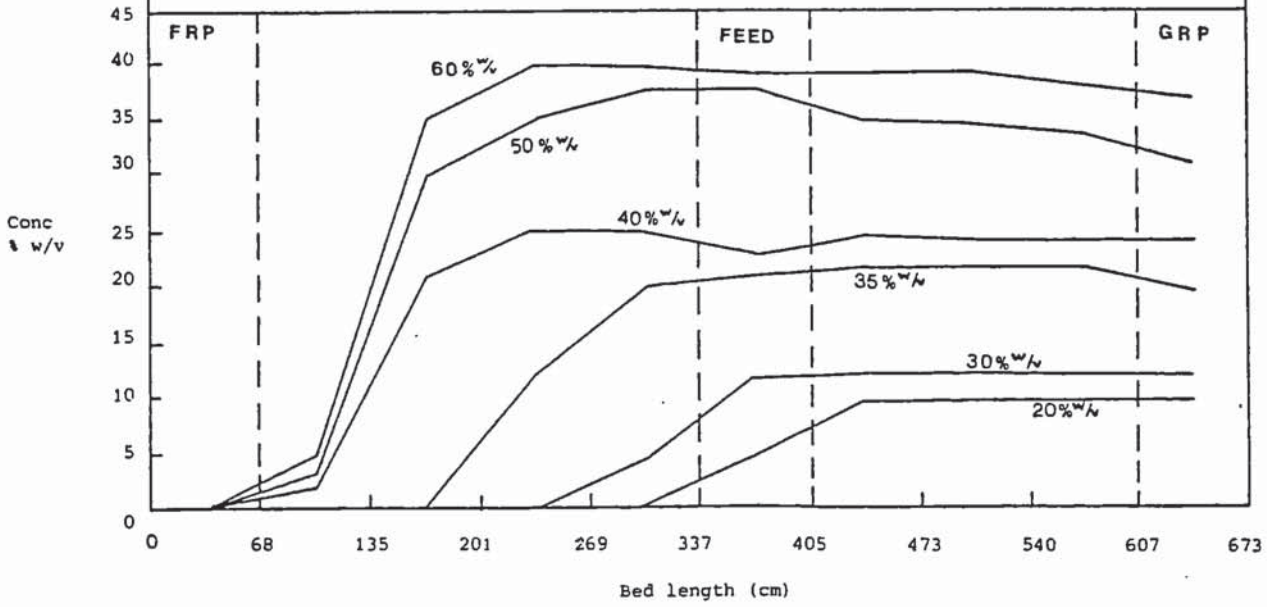
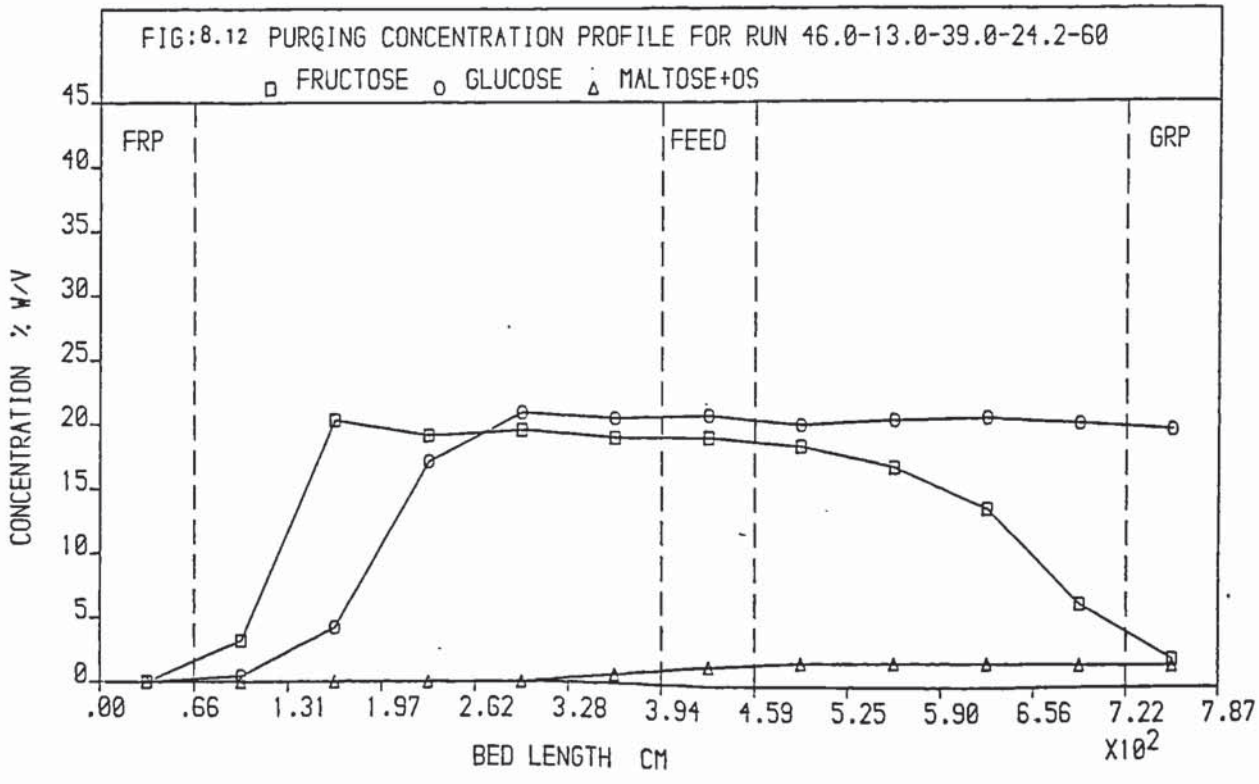


FIG:8.10 ON-COLUMN CONCENTRATION PROFILE FOR RUN 46.0-13.0-39.0-24.2-60
□ FRUCTOSE o GLUCOSE △ MALTOSE+OS CYCLE10
FRP
FEED
GRP
CONCENTRATION % W/V
45
40
35
30
25
20
15
10
5
0
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87
BED LENGTH CM
X10²

Figure 8.11: Glucose concentration profiles for increasing feed concentrations (202) Obtained on the ten column SCCR6 equipment, at 20°C, operating at 30 min switch time, 105 cm³ min^{-1} eluent flow rate and 35cm³ min^{-1} feed flow rate. A synthetic 50:50% feed was used





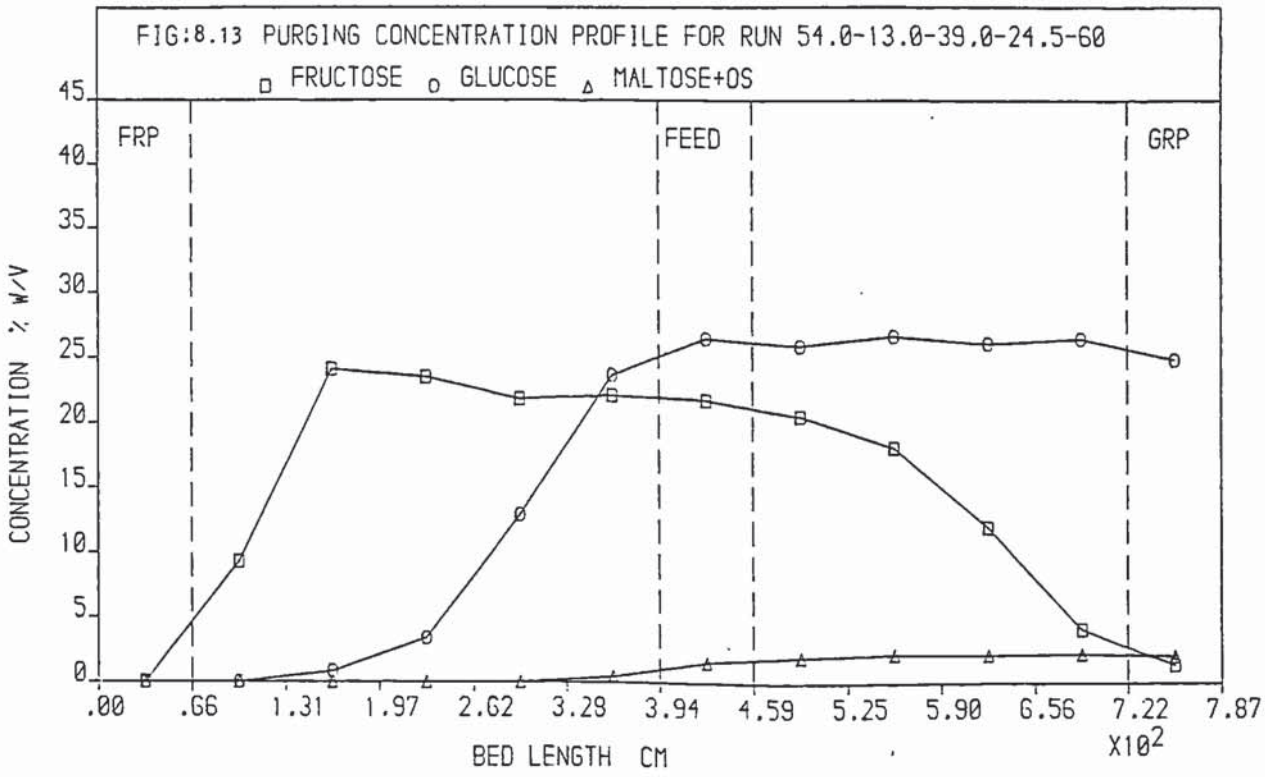


FIG:8.13 PURGING CONCENTRATION PROFILE FOR RUN 54.0-13.0-39.0-24.5-60
□ FRUCTOSE o GLUCOSE △ MALTOSE+OS
FRP
FEED
GRP
CONCENTRATION % W/V
0
5
10
15
20
25
30
35
40
45
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87
BED LENGTH CM
X10²

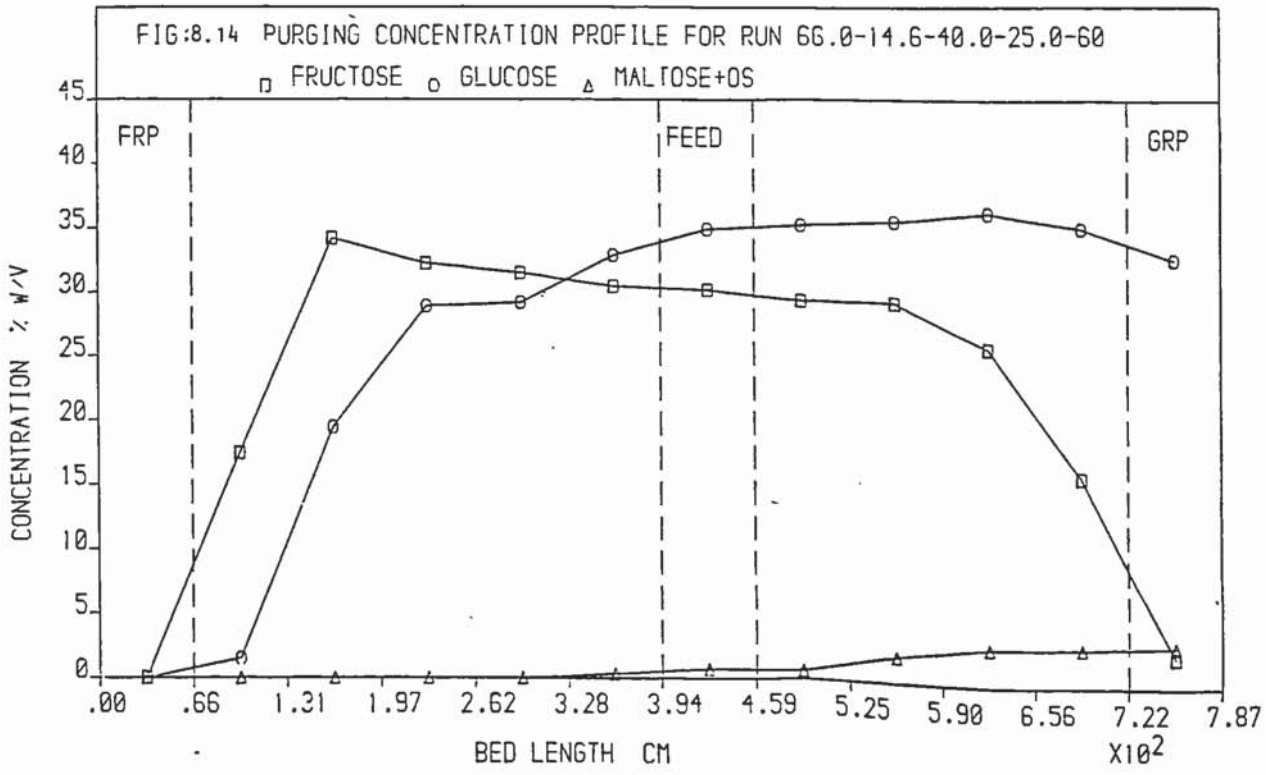
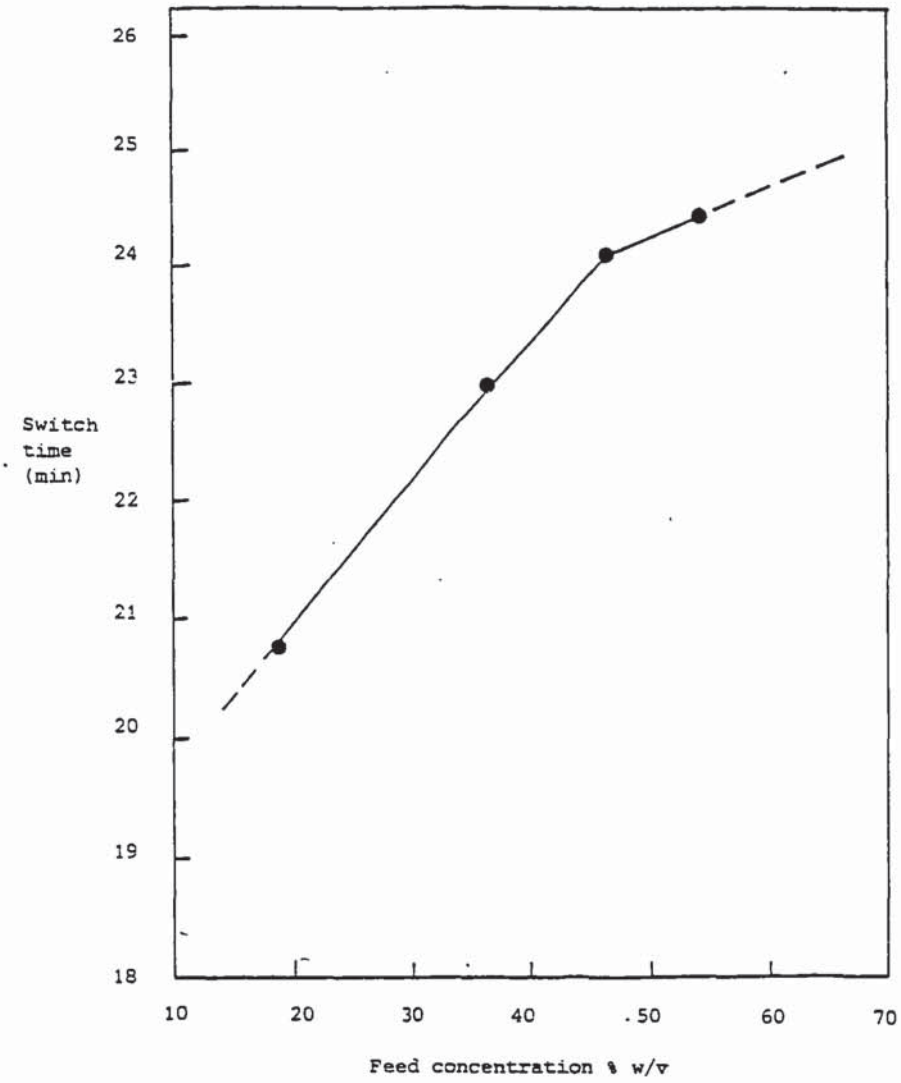
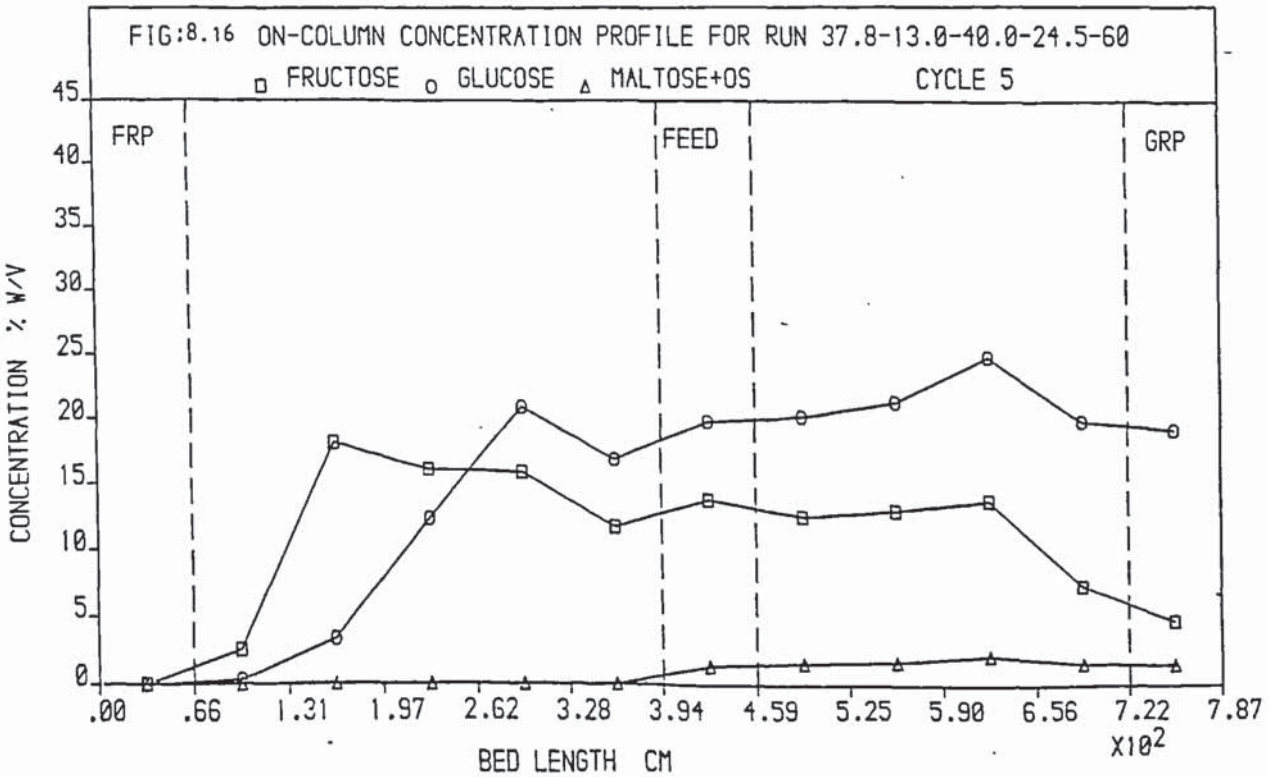


FIG:8.14 PURGING CONCENTRATION PROFILE FOR RUN 66.0-14.6-40.0-25.0-60
□ FRUCTOSE o GLUCOSE △ MALTOSE+OS
FRP
FEED
GRP
CONCENTRATION % W/V
0
5
10
15
20
25
30
35
40
45
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87
BED LENGTH CM
X10²

Figure 8.15: Prediction of correct switch time to obtain the specified purities at a particular feed concentration. The experimental switch times have been modified to correspond to a reference feed flow rate of 13 cm^3 min^{-1}





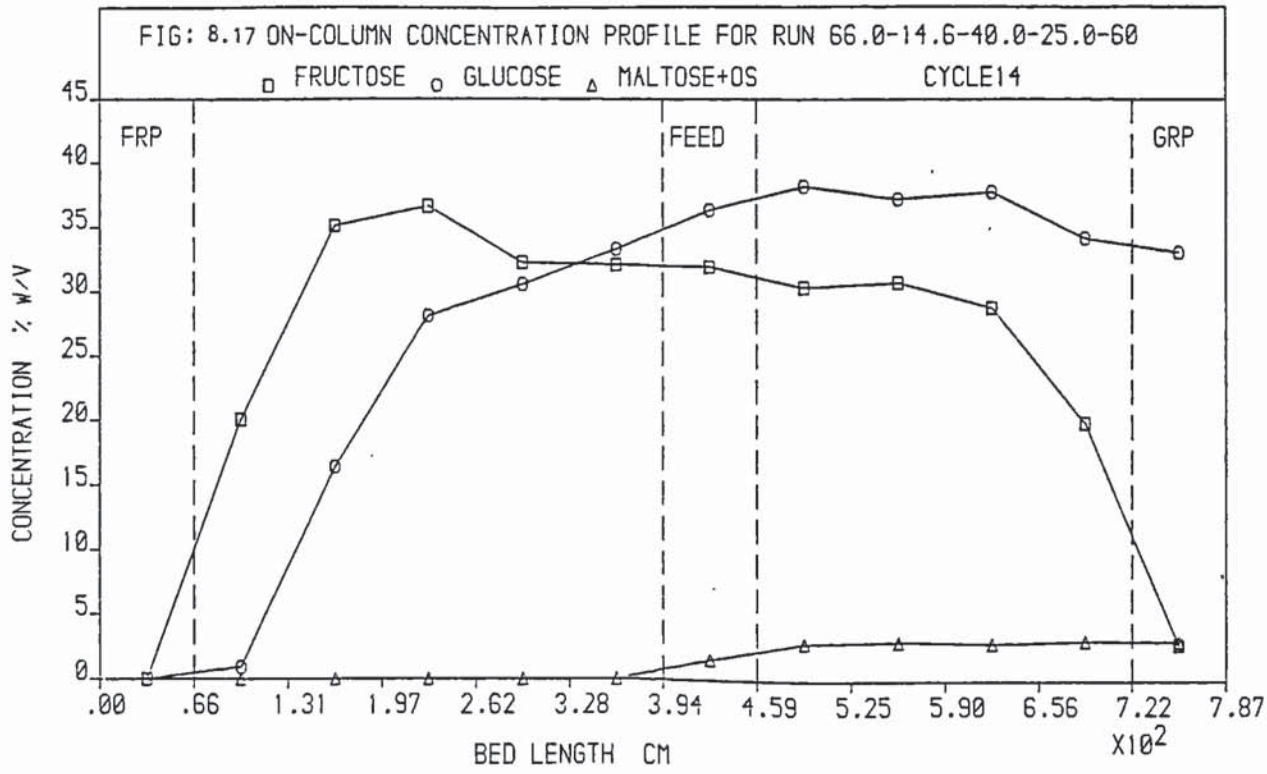


FIG: 8.17 ON-COLUMN CONCENTRATION PROFILE FOR RUN 66.0-14.6-40.0-25.0-60
□ FRUCTOSE ○ GLUCOSE △ MALTOSE+OS CYCLE14
FRP
FEED
GRP
CONCENTRATION % W/V
BED LENGTH CM
X10²
0
5
10
15
20
25
30
35
40
45
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87

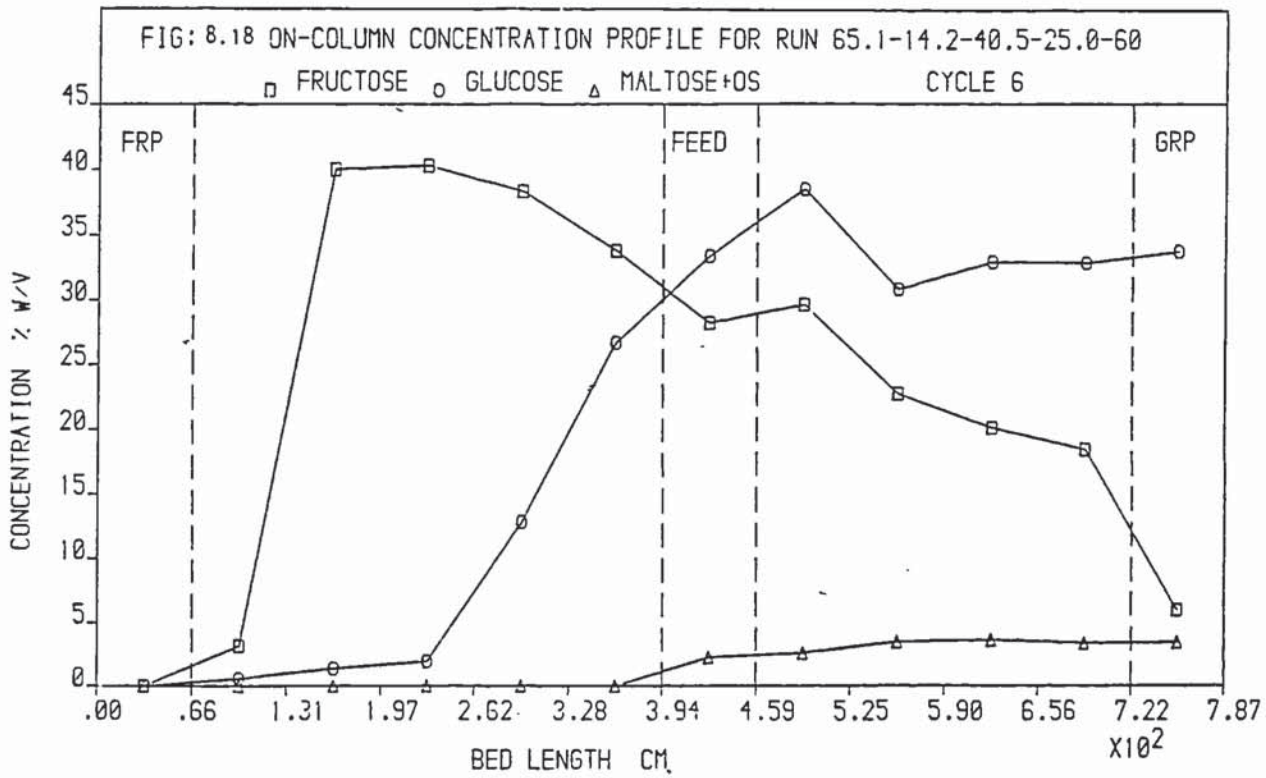


FIG: 8.18 ON-COLUMN CONCENTRATION PROFILE FOR RUN 65.1-14.2-40.5-25.0-60
□ FRUCTOSE ○ GLUCOSE △ MALTOSE+OS CYCLE 6
FRP
FEED
GRP
CONCENTRATION % W/V
BED LENGTH CM
X10²
0
5
10
15
20
25
30
35
40
45
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87

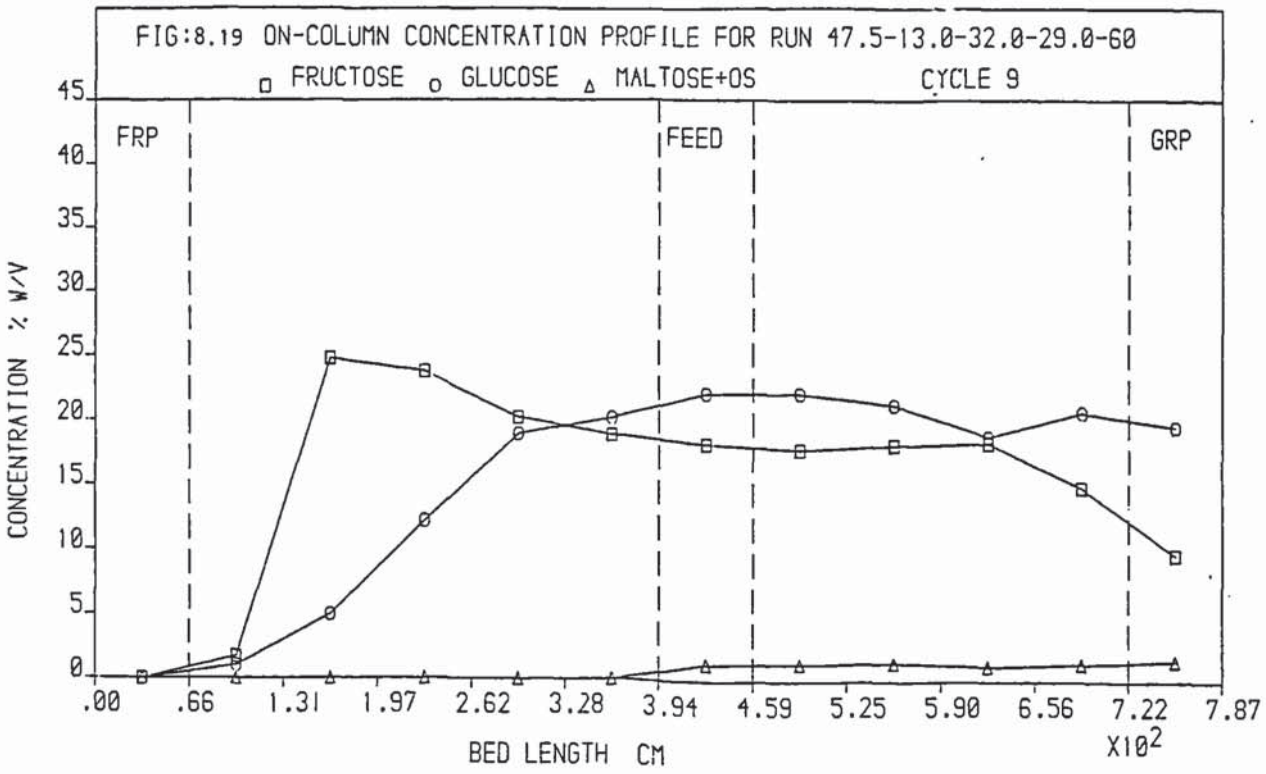


FIG:8.19 ON-COLUMN CONCENTRATION PROFILE FOR RUN 47.5-13.0-32.0-29.0-60
□ FRUCTOSE o GLUCOSE △ MALTOSE+OS CYCLE 9
FRP
FEED
GRP
CONCENTRATION % W/V
45
40
35
30
25
20
15
10
5
0
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87
BED LENGTH CM
X10²

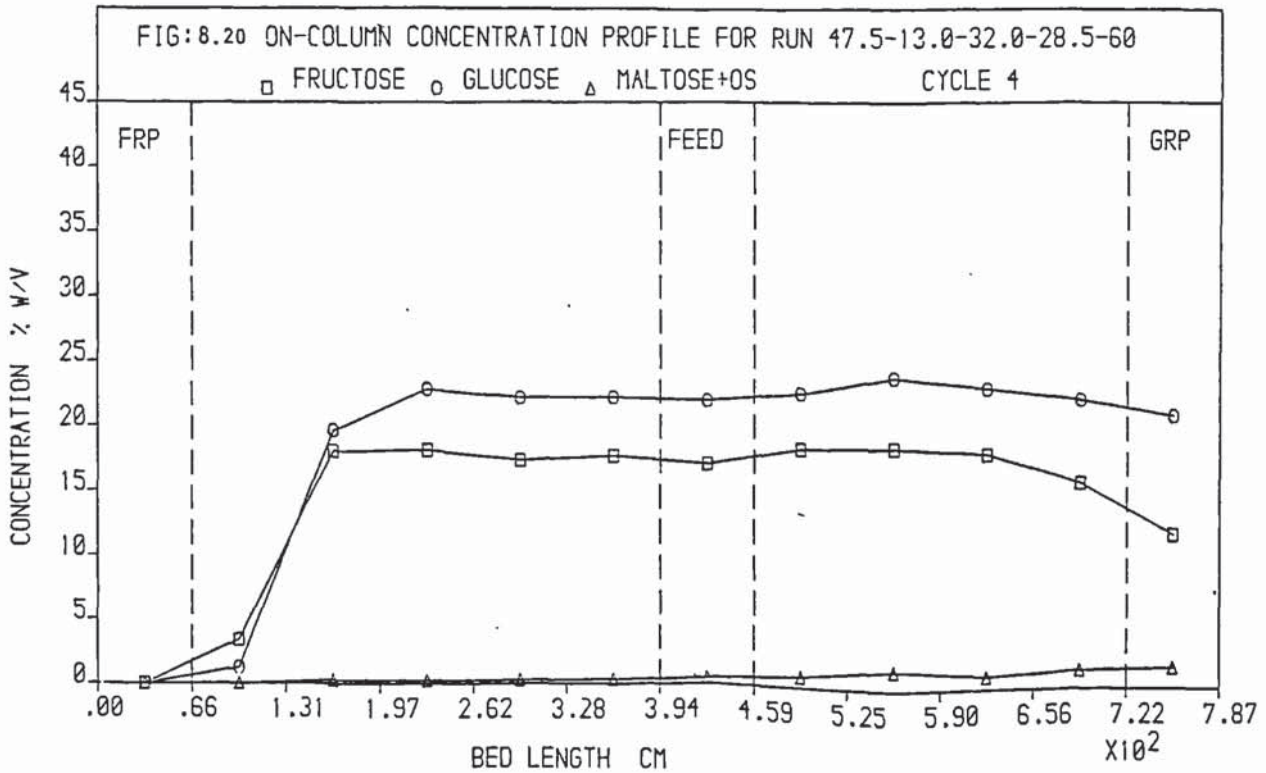


FIG:8.20 ON-COLUMN CONCENTRATION PROFILE FOR RUN 47.5-13.0-32.0-28.5-60
□ FRUCTOSE o GLUCOSE △ MALTOSE+OS CYCLE 4
FRP
FEED
GRP
CONCENTRATION % W/V
45
40
35
30
25
20
15
10
5
0
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87
BED LENGTH CM
X10²

Figure 8.21: GRP elution profile over a switch-run: 66-14.6-40-25-60

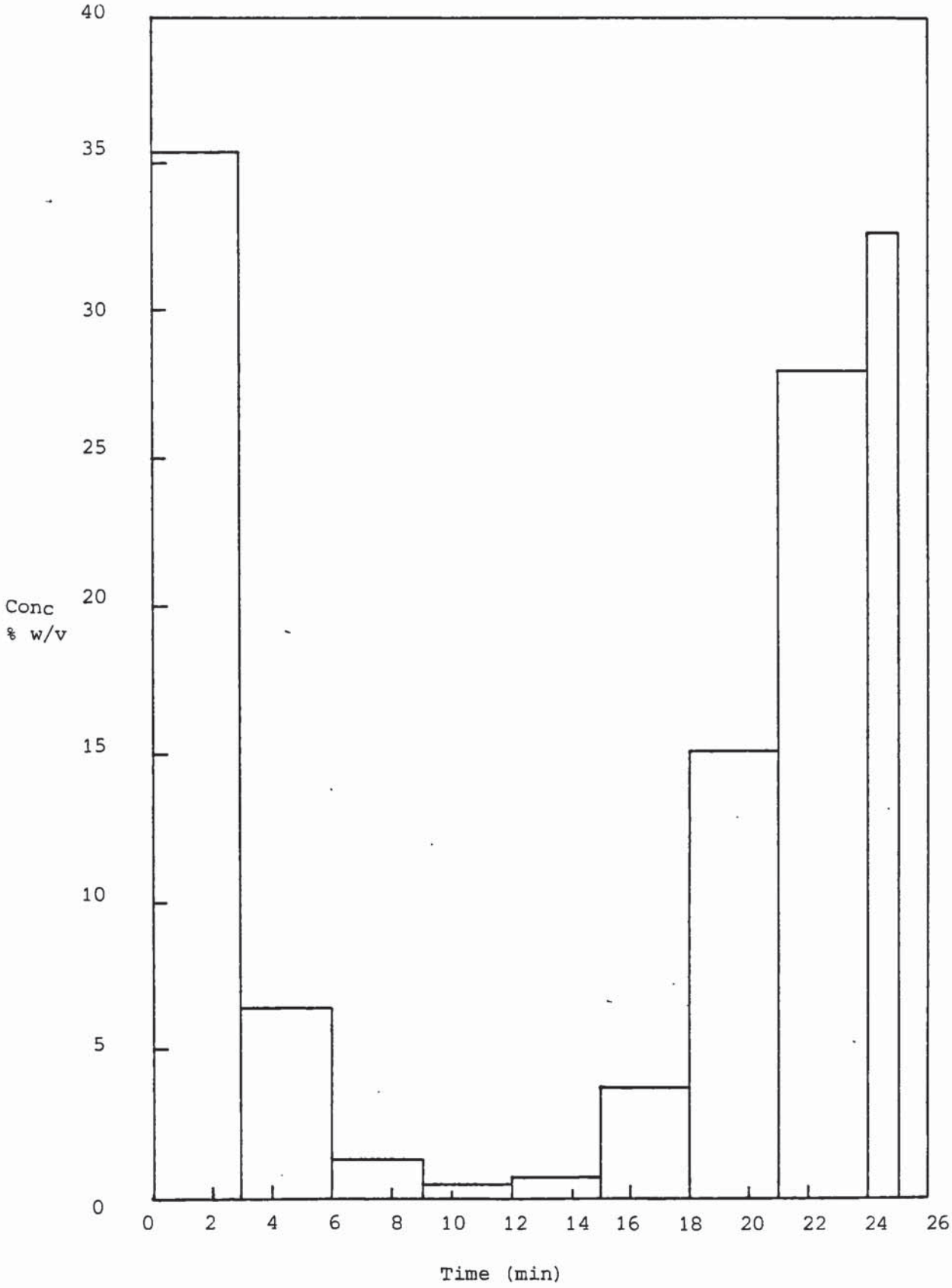
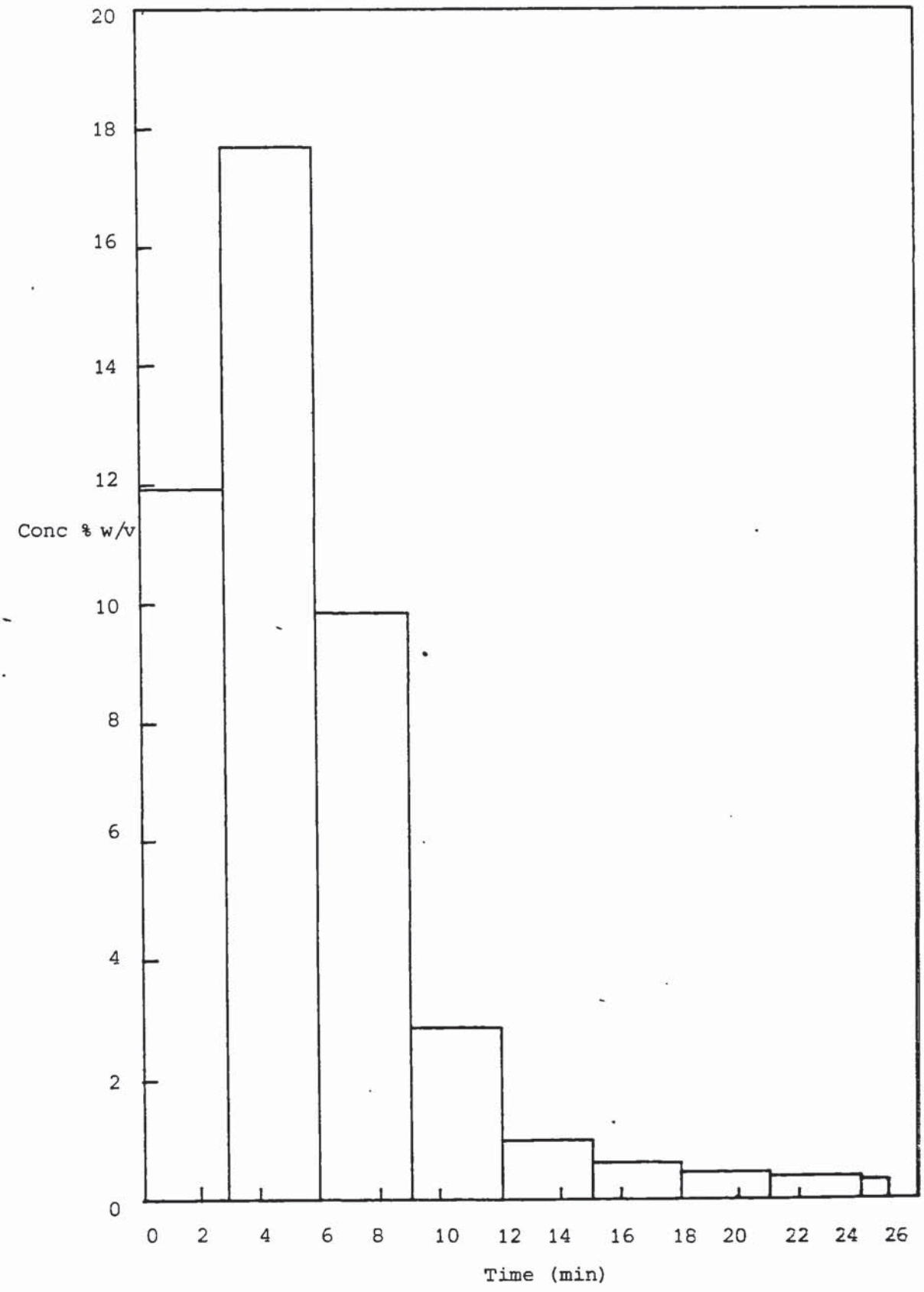


Figure 8.22: FRP elution profile over a switch-run+ 66-14.6-40-25-60



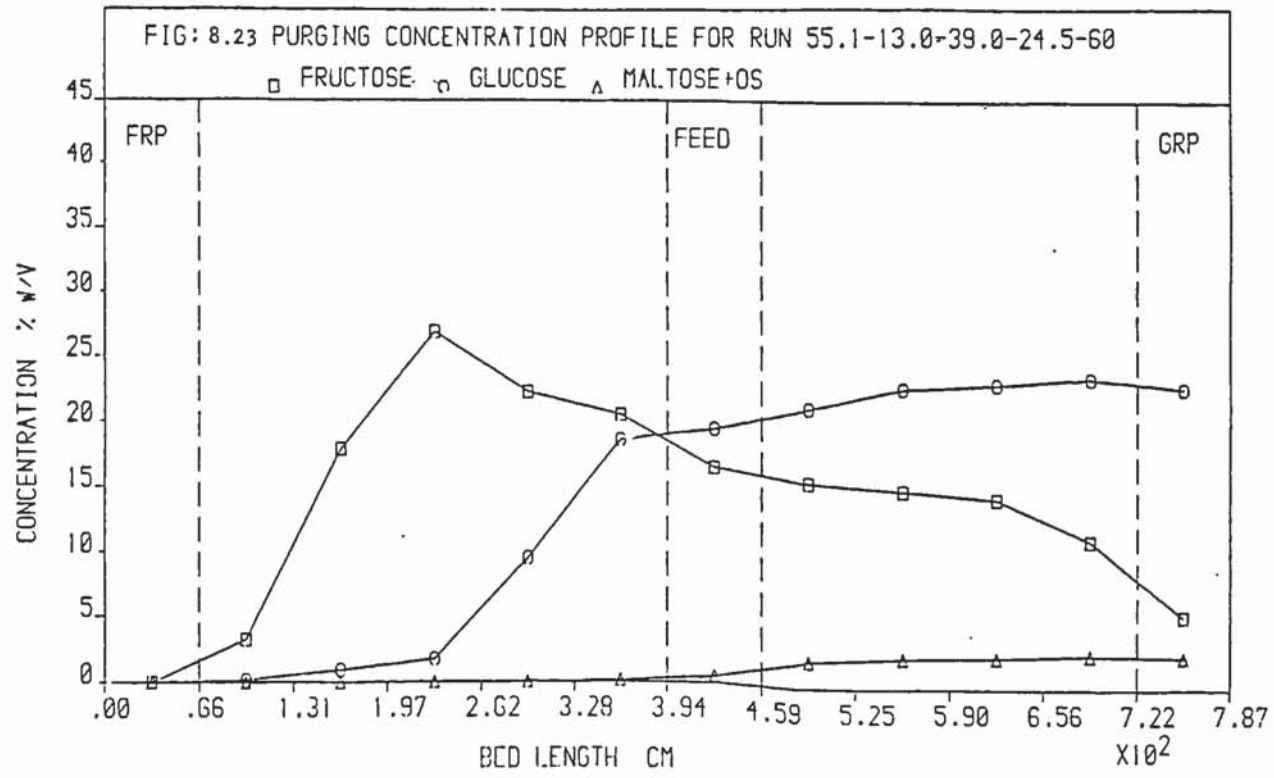


FIG: 8.23 PURGING CONCENTRATION PROFILE FOR RUN 55.1-13.0-39.0-24.5-60
□ FRUCTOSE ○ GLUCOSE △ MALTOSE+OS
FRP
FEED
GRP
CONCENTRATION % W/V
45
40
35
30
25
20
15
10
5
0
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87
BED LENGTH CM
X10²

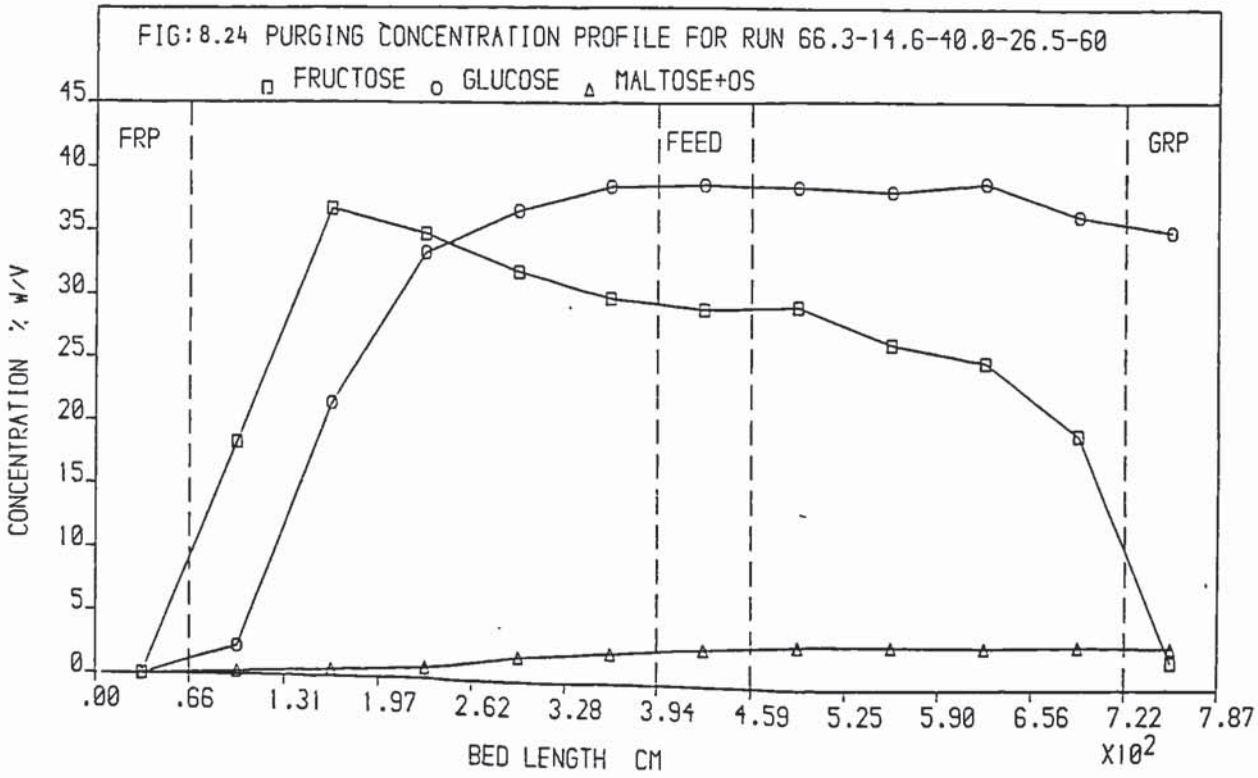


FIG: 8.24 PURGING CONCENTRATION PROFILE FOR RUN 66.3-14.6-40.0-26.5-60
□ FRUCTOSE ○ GLUCOSE △ MALTOSE+OS
FRP
FEED
GRP
CONCENTRATION % W/V
45
40
35
30
25
20
15
10
5
0
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87
BED LENGTH CM
X10²

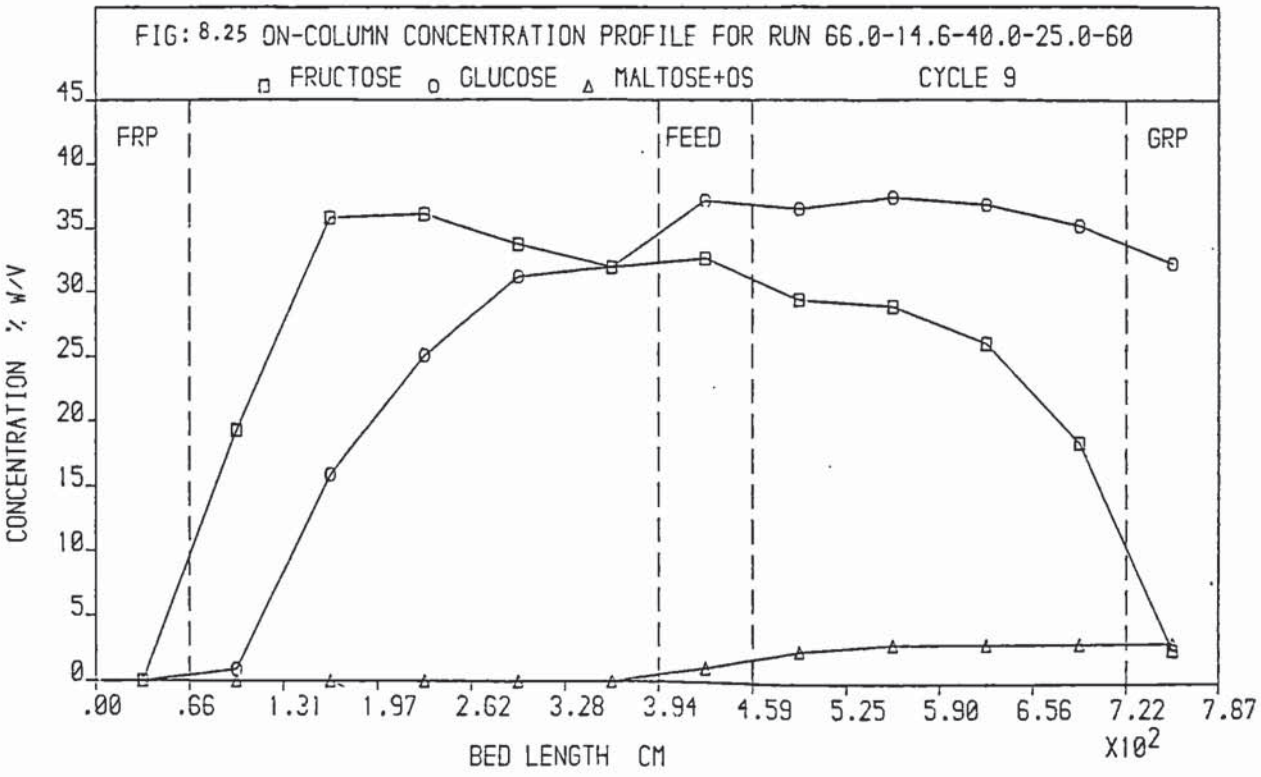


FIG: 8.25 ON-COLUMN CONCENTRATION PROFILE FOR RUN 66.0-14.6-40.0-25.0-60
□ FRUCTOSE o GLUCOSE △ MALTOSE+OS CYCLE 9
FRP
FEED
GRP
CONCENTRATION % W/V
45
40
35
30
25
20
15
10
5
0
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87
BED LENGTH CM
X10²

CHAPTER 9

9.0 THE SCALING UP OF THE SCCR CHROMATOGRAPHIC SYSTEMS

Existing theories and experimental results have been used in an attempt to develop a theoretical link between batch and semi-continuous chromatographic systems. The resulting data are compared and used to establish a method of calculating the required length to obtain 99.9% pure products in a semi-continuous system from batch data. A procedure for the selection of the correct operating conditions is also described.

9.1 Development of a Link Between Batch and Semi-continuous Liquid Chromatographic Systems

9.1.1 Introduction

A comparison of the results obtained by Thawait (202, 216) on the 10.8cm id SCCR6 system, operating in both batch and continuous modes, indicated that at high feed concentrations of binary mixtures the batch operation is slightly better in terms of product quality. The semi-continuous operation however has the additional advantages of being more flexible, requiring no recycling, allowing continuous unattended operation, and maintaining constant product quality. These inherent advantages of the semi-continuous operation favour its application in production scale plants, where continuous separation of multicomponent mixtures is usually carried out, and maximum throughputs with constant product quality are required. This highlights the need for a method of predicting the size and performance of the semicontinuous system from batch results. The evaluation of the system's length has been carried out in a way similar to the sizing of other separation processes, ie. through the determination of the height equivalent of a theoretical plate (HETP) and the number of theoretical plates (NTP) analogy. In this work a binary glucose-fructose feedstock is assumed and the calculation of both HETP and NTP values was based on the retarded component, fructose. The

calculations are carried out for both the 5.4cm id twelve column SCCR7 and 10.8cm id ten column SCCR6 equipments. The results of the later system (SCCR6) are shown in square brackets unless it is stated otherwise.

9.1.2 Calculation of HETPs

Ideally the HETP values can be predicted from the random walk model or the non-equilibrium theory (26) modified to allow for column diameter and larger sample sizes. Both theories, however, include a number of parameters with unknown or imprecise values. Because of these limitations the HETP values were calculated from the breakthrough curve using equation 6.6 for each column used in the batch mode and operating at infinite dilution conditions. A detailed description of the experimental procedure and the evaluation of the individual column parameters has been reported in Section 6 and reference 215 (for the SCCR6 system). Applying equation 6.6 to each one of the columns of both systems gave an average "apparent" number of theoretical plates per column of 32 [25] hence a total of 384 [250] plates. This resulted in an average fructose HETP value of 2.09 [2.58] cm and average infinite dilution distribution coefficients of K^{∞}_{df} = 0.472 [0.417] and K^{∞}_{dg} = 0.215 [0.168], giving a separation factor of 2.2 [2.48]. It will be recalled that the values above in turn refer to the 5.4cm dia SCCR7 (no brackets), and the 10.8cm dia SCCR6 column (square brackets).

9.1.3 Calculation of NTPs

The theoretical determination of the NTPs required to increase the fructose rich product (FRP) purity to 99.9% were carried out separately for the batch and semi-continuous operation. Thus, the corresponding theoretical lengths were obtained and compared.

9.1.3.1 Batch Operation

Glueckauf in his "theoretical plate concept" (31) expressed his findings graphically (Figure 9.1). Using this graph the NTP required for systems with a wide range of separation factors can be predicted provided that the band spreading is not dominated by lack of equilibrium between the sorbent and eluent phase. Also, although an ion exchange principle is applied for the separation, this is believed not to have any significant effect since the column characterisation indicated that over 250 plates were available (Chapter 6). The theoretical number of plates was found for each column based on the infinite dilution conditions, ie. $5cm^3$ injections of 10% w/v solutions. Figure 9.1 was used and the NTP value was the one corresponding to $\alpha = 2.2$ [2.48] and $x_g = 0.001$ (ie. 99.9% pure FRP) and assuming equimolar feed. This indicated that approximately 48 [42] theoretical plates were required per column instead of the "apparent" 32 [25]. Therefore in order to get 99.9% pure FRP without any recycle, 576 theoretical plates are needed for the SCCR7 and 420 for the SCCR6, instead of the 384 and 250 "apparent" plates respectively, found experimentally. This means a total theoretical system length of 1204cm for the SCCR7 and 1084cm for the SCCR6.

9.1.3.2 Semi-continuous Operation

Sciance and Crosser used the "probabilistic model" (227) to develop a continuous chromatographic model. The resulting equation accounts for feed location, system's length, degree of separation and flow rates. The semi-continuous operation of the SCCR units is an intermediate operation, but by assuming continuous operation and designing for 99.9% pure FRP the following form of the model was used (227), since the FRP was the desired product.

$$\ln(x_g) = \frac{Z.k'_g}{u_m}\left(K^{\infty}_{dg} - \frac{u_m}{u_p}\right) \qquad \text{..... 9.1}$$

The only unknown physical quantity is the k'_g term, and it was evaluated by using the results from a 10 column 2.54cm id system (SCCR4). Run 02-2.4-6.1-30-29 from Ching's thesis (203) was used, since due to the low feed concentration (2.15% w/v) it can be assumed that it approached "infinite dilution" conditions, hence the "infinite dilution" distribution coefficient (K^{∞}_{dg}) value could be used. The procedure followed was the direct scaling up from the 2.54cm id to the 10.8cm id semi-continuous chromatographic systems, and the calculations are shown in Appendix B. The x_g value in equation 9.1 is the mass of glucose in FRP over the mass of glucose in feed. In the actual calculations however the x_g value used was approximated to (% glucose in FRP/100). The accuracy of the mass balance was limited by the experimental errors associated with the scale of operation (ie. in weighting and analysing the products). Therefore the resulting inaccuracies of the above approximations were lower, and for feedstocks containing less fructose than glucose it would give slightly longer lengths than needed. It was found theoretically that a 1154cm long SCCR7 or a 1046cm long SCCR6 system was needed to produce 99.9% pure products; ie. 552 or 405 theoretical plates respectively. This theoretical length estimation of both semi-continuous systems was carried out using operating parameters identical to the ones used in the experimental runs 18.6-9-30-30-20 and 20-35-105-30-20 (202) for the SCCR7 and SCCR6 respectively. The experiments gave 99.9% pure products (Table 7.12 and Table 6 from reference 216), and the experimental concentration profiles (Figure 7.17) indicated that approximately nine columns were sufficient for complete separation; that means experimental lengths of 590cm for the SCCR7 and 585cm for the SCCR6 or 282 and 227 theoretical plates respectively. To account for this disagreement between the theoretical and experimental lengths of the semi-continuous systems, an Experimental-Theoretical-Correlation-Factor (ETCF) was introduced, where:

- For the SCCR7 system:

$$ETCF = \frac{\text{Experimental length}}{\text{Theoretical length}} = \frac{590}{1154} = 0.511 \qquad \text{..... 9.2}$$

- and for the SCCR6 system:

$$ETCF = \frac{585}{1046} = 0.559 \qquad \text{..... 9.3}$$

The above results indicate a similar experimental-theoretical relationship between the two preparative SCCR systems of different size, accounting for the different resins used and different feed composition. Therefore an average ETCF of 0.535 can be used for any SCCR system, based on the "apparent number" of theroetical plates obtained from the batch characterisation of the columns and at infinite dilution conditions. Although the actual operating conditions were used in the length estimation of the continuous systems, the effect of feed band width was assumed to be negligible.

9.1.4 Comparison between Batch and Continuous Systems at Infinite Dilution

Glueckauf demonstrated quantitatively the difference between continuous and batch chromatographic columns (228). The number of theoretical plates needed in the active part of a continuous system was given by:

$$N_t = \frac{6}{(\alpha - 1)} \qquad \text{..... 9.4}$$

Where N_t is the number of theoretical plates needed to achieve a shift between the peaks of two solutes equivalent to half the mean width of the peak (228). A shift which is sufficient to get pure products at both ends. For batch columns the number of theoretical

plates, N_b, needed is given by:

$$N_b = \frac{2}{(\alpha - 1)^2} \qquad \text{..... 9.5}$$

Therefore:

$$\frac{N_t}{N_b} = \frac{6/(\alpha - 1)}{2/(\alpha - 1)^2} = 3(\alpha - 1) \qquad \text{..... 9.6}$$

Now, generalising this equation for the two modes of operation:

$$\frac{NTP_{sc}}{NTP_b} = \lambda(\alpha - 1) \qquad \text{..... 9.7}$$

Substituting the NTP values evaluated above and the corresponding separation factors, α, then:

- For the SCCR7 system:

$$\frac{552}{576} = \lambda(2.2 - 1) \qquad \text{..... 9.8}$$

Therefore $\lambda = 0.799$ 9.9

- and, for the SCCR6 system:

$$\frac{405}{420} = \lambda(2.48 - 1) \qquad \text{..... 9.10}$$

Therefore $\lambda = 0.652$ 9.11

Therefore, the average λ value for carbohydrate systems was found to be 0.723. This is four times smaller than the value in equation 9.6, which was derived for batch and continuous circular gas chromatographic systems. This can also be attributed to the validity of the infinite dilution assumption, and to the stepwise operation of the semi-continuous system while the Sciance and Crosser model applies to "truly continuous" sytems. It must also be noted that the accuracy in obtaining graphically the number of theoretical plates for the batch system was low.

9.1.5 Discussion

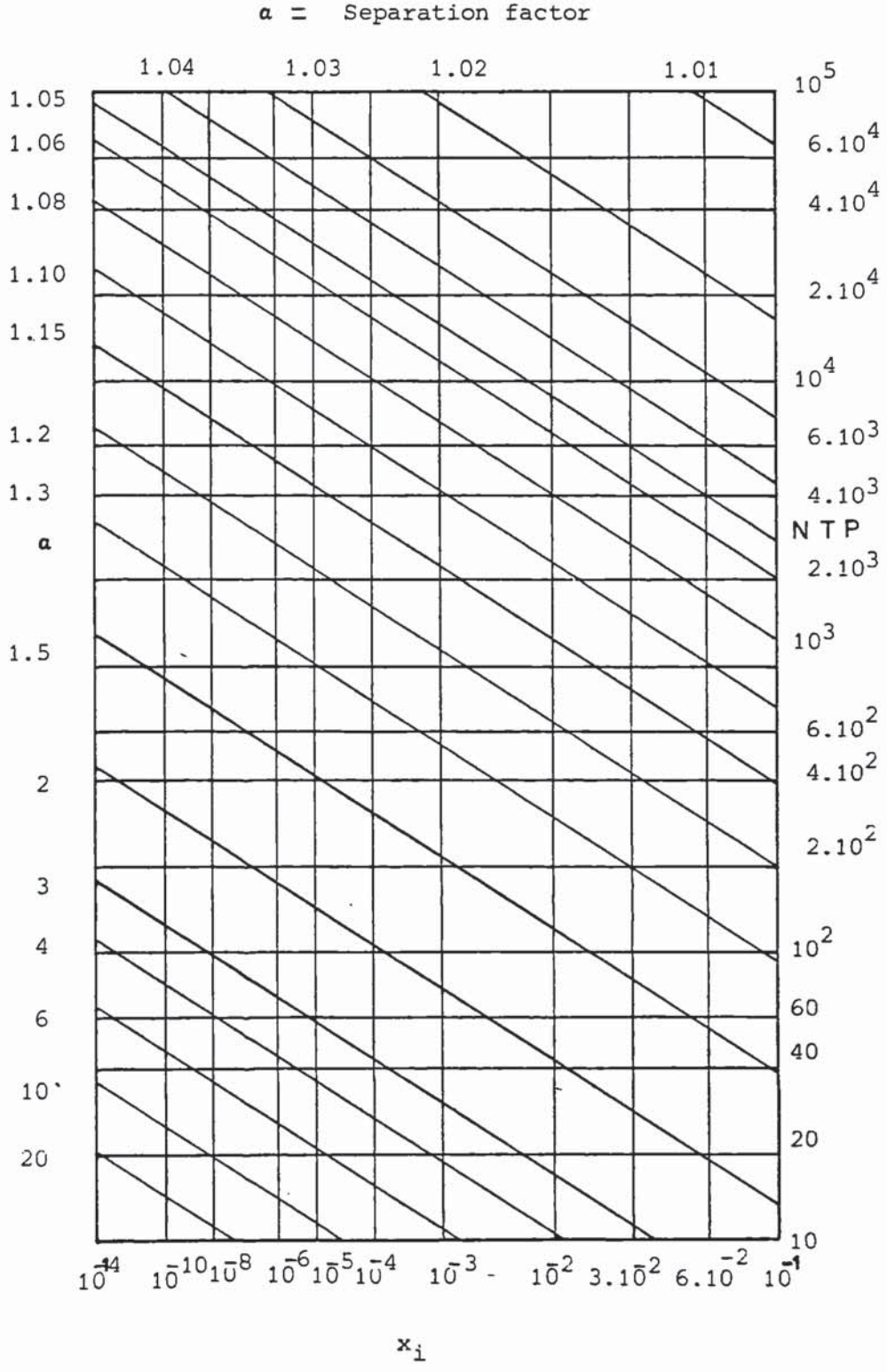
In the length estimation of the batch systems the infinite dilution separation factors, NTPs and HETP values were used. Since the column characterisation was carried out at infinite dilution the effects of concentration, temperature and flow rates are negligible and the "apparent" number of theoretical plates is accurate enough. The semi-continuous lengths however were obtained for the actual experimental conditions, and although the corresponding concentrations were low, they were higher than the infinite dilution ones. Therefore, the validity of this infinite dilution assumption is questionable, and its applicability is examined in the following section.

9.2 Length Calculations at Actual Operating Conditions

9.2.1 General Consideration

The above calculations were repeated at the actual operating conditions. The results of the continuous experimental runs 20-35-105-30 (SCCR6) and 18.6-9-30-30-20 (SCCR7) were used. The actual results of the batch run carried out on the SCCR6 at 20% w/v feed concentration were also used. Since the SCCR7 system was not used in the batch mode it was assumed it would exhibit similar performance to

Figure 9.1: Number of theoretical plates (NTP) as a function of separation factor (α), and mass fraction of component corrected according to the feed composition (x_i), in product stream (Ref (31) Fig 3)



the SCCR6 system operating in the batch mode. This assumption is supported by the similar separating performance of the two systems in the continuous mode and also by the relative low feed concentrations. Therefore, it was assumed that the SCCR7 would also produce 99.9% pure products operating in batch mode and using a 18.6% w/v feed of similar composition to the one used in the continuous run. All experiments were carried out at ambient temperature, and the results of the SCCR6 system are reported in square brackets. To incorporate the effects of temperature, flow rates and concentration the respective infinite dilution coefficients were modified as described in Chapter 7, using equations 7.3, 7.4, 7.5 and 7.19 to 7.24. The resulting fructose and glucose distribution coefficients were 0.54 [0.472] and 0.353 [0.267] respectively and the separation factors 1.53 (SCCR7) and 1.77 (SCCR6).

9.2.2 Batch Systems

The graphical evaluation of the number of theoretical plates (Figure 9.1) incorporates indirectly the effects of temperature, flow rates and concentration through the modified separation factors, but it does not account for throughput and feed bandwidth. The graphical reading represents the total theoretical plates required to achieve a specified separation. In the previous section the number of theoretical plates for each column was found graphically at infinite dilution conditions, giving a total of 250 (ten column/SCCR6) or 384 (twelve column SCCR7), at the corresponding total injection charges of 50cm^3 (SCCR6) and 60cm^3 (SCCR7). This approach requires a minimum amount of experimental data but is oversimplified, and corresponds to very low concentrations and throughputs. For a more accurate length estimation the actual experimental results were used (Table 9.3) and the whole batch system was considered as one long column. Therefore, from Figure 9.1, approximately 205 and 140 theoretical plates were needed to achieve 99.9% pure products on the SCCR7 and SCCR6 batch systems respectively.

An alternative way of evaluating the "true" number of theoretical plates is through the feed band broadening approach (Section 6.6). The "true" number of theoretical plates found from this alternative method was 710 and 464 for the batch SCCR7 and SCCR6 respectively, (Appendix A). The experimental results (Table 9.3) showed that the existing length was sufficient to obtain 99.9% purities. Therefore the resulting HETP values (based on fructose) were:

- From the graphical approach:

batch SCCR7, HETP = 3.84cm

batch SCCR6, HETP = 4.59cm

- From the feed band broadening approach:

batch SCCR7, HETP = 1.11cm

batch SCCR6, HETP = 1.39cm

9.2.3 Semicontinuous Systems

In Section 9.1.3.2 the semicontinuous length estimation was carried out using the experimental conditions and results of runs 18.6-9-30-30-20 and 20-35-105-30-20 and the respective infinite dilution coefficients. To account for the actual operating conditions the length evaluation was repeated using the modified distribution coefficients and separation factors, (Appendix B). Therefore, at the actual operating conditions of runs 18.6-9-30-30-20 (SCCR7) and 20-35-105-30-20 (SCCR6) the respective lengths for 99.9% purities were 1042cm and 1201cm instead of 1154 and 1046cm found when the infinite dilution distribution coefficients were used. The Sciance and Crosser paper (227) implies the use of the infinite dilution coefficients although it is not clear. The above results however show that it is essential to use the actual distribution coefficients found at the particular operating conditions. The true number of theoretical plates,

obtained from the modified Glueckauf (42) theory were 632 (SCCR7) and 360 (SCCR6), (Appendix A). From the experimental profiles (Figure 7.17) approximately nine columns were sufficient to give 99.9% purities, therefore the experimental lengths of the SCCR7 and SCCR6 systems were 590cm and 585cm respectively.

The new Experimental-Theoretical-Correlation-Factors applicable to the actual operating conditions were:

- For the SCCR7:

$$ETCF = \frac{590}{1402} = 0.421$$

- And for the SCCR6:

$$ETCF = \frac{585}{1201} = 0.487$$

The new λ values, for the batch-semicontinuous length comparison at the actual conditions were obtained from equation 9.7 using the respective NTP values found from the feed-broadening approach, thus maintaining a similar basis for comparison.

- For the SCCR7:

$$\frac{632}{710} = \lambda\ (1.53 - 1), \text{ therefore, } \lambda = 1.680$$

- And for the SCCR6:

$$\frac{360}{464} = \lambda\ (1.77 - 1), \text{ therefore, } \lambda = 1.008$$

Therefore, the average ETCF and λ values for the SCCR systems, obtained at the actual operating conditions using carbohydrate mixtures of approximately 20% w/v concentrations, were 0.454 and 1.344 respectively.

9.2.4 Discussion

In Section 9.1 the comparison between the relative lengths of the two modes of operation was oversimplified since it was carried out using the infinite dilution separation parameters, ie. K^{∞}_{d} and α values. The results however, obtained in this section are more representative since they correspond to the actual operating conditions and account for the effects of temperature, flow rates and concentration. The difference in the respective estimated lengths, NTP, ETCF and λ values is significant, and the results of the later approach should be used; that is ETCF and λ values of approximately 0.454 and 1.344 respectively. The new λ value is double the infinite dilution one, but is still lower than the original one used in equation 9.7 for the same reasons discussed in Section 9.1.4. In Section 9.1 the estimation of the number of theoretical plates for the batch mode was carried out for each column, and the resulting total number was too high considering the very low feed charge volumes and concentrations involved. In this section a similar estimation was carried out for the total batch system as a whole and at the actual operating conditions. The graphical evaluation of the number of theoretical plates for the batch operation (31) incorporates the effects of temperature, flow rates and concentration, but it does not account for increased throughputs and thus wide feed bands, and also is not accurate enough due to the difficulty in obtaining the exact value from the graph. On the other hand, the feed band broadening approach accounts for increased throughputs and feed volumes but it does not incorporate temperature and concentration effects. None of the approaches allows for repetitive injections; in the calculations a once through batch operation was considered. The number of theroetical

Table 9.1: Theoretical Link between Batch and Continuous Operation - Semicontinuous Operating Conditions

System	Experimental Run	Average Flow Rates $cm^3 min^{-1}$			Feed Concentration % w/v				Switch Time (min)	Throughput (sugar solids ($kg h^{-1}$)
		Feed	Eluent	Purge	G	F	M+OS	Total		
SCCR4	02-2.4-6-30-29 (Reference 203)	2.4	6.1	289	1.2	0.95	-	2.15	30	0.003
SCCR6	20-35-105-30-20 (Reference 202)	35	105	550	10	10	-	20	30	0.42
SCCR7	18.6-9-30-30-20	9	30	75	10.78	7.82	-	18.6	30	0.100
SCCR7	66-14.6-40-25-60	14.6	40	70	34.3	27.8	3.9	66	25	0.578

G: glucose; F: fructose; M+OS: maltose and oligosaccharides.

Table 9.2: Theoretical Link between Batch and Continuous Operation - Results of Semicontinuous Operation

Experimental Run	Glucose Rich Product		Fructose Rich Product	
	Glucose Purity %	Total Product concentration % w/v	Fructose Purity %	Total product Concentration % w/v
02-2.4-6-30-29 (Reference 203)	91.5	0.372	90.2	0.007
20-35-105-30-20 (Reference 202)	99.9	2.37	99.9	0.64
18.6-9-30-30-20	99.9	2.12	99.9	0.93
66-14.6-40-25-60	82.9	11.6	90.07	5.84

Table 9.3: Theoretical Link between Batch and Continuous Operation - Batch Operation

System	Feed Concentration			Average Flow Rate $cm^3\ min^{-1}$	Injection volume cm^3	Throughput (sugar solids) $kg\ h^{-1}$	GRP glucose purity %	FRP fructose purity %
	G	F	Total					
Batch SCCR6 (Ref 202, 216)	10.1	10.2	20.3	105	15000	0.3	99.9	99.9
Batch SCCR7*	10.78	7.82	18.6	35	3750	0.075	99.9	99.9

* Operating conditions and results assumed to be analogous to the ones obtained on the batch SCCR6

GRP: glucose rich product; FRP: fructose rich product.
G: glucose
F: fructose

plates obtained from the two approaches is different, due to the limitations of the respective theories. The theoretical plates obtained from the feed band broadening approach was greater, and these values were used in the calculations for both modes of operation, to maintain a similar basis of comparison.

In the semicontinuous length estimation the k'_g value was assumed to be constant; additional experiments however are needed to be carried out to investigate the effects, if any, of temperature, concentration, throughput and system geometry on its value. The difference in the K_d, α, NTP, λ, ETCF and theoretical lengths obtained on the SCCR6 and SCCR7 systems, was a result of the different sizes, packing material and packing density of the two systems, and the different feed fructose contents and concentrations used. Since the exact contribution of the above factors on the λ and ETCF values was not known the average of the respective values was used.

The above work has indicated the applicability and limitations of existing length estimation theories, showed the usefulness of a theoretical link between batch and semicontinuous operation, and high-lighted the necessity of improving the existing models or developing new ones especially for the batch operation, to account for throughput and column dimensions.

9.3 The Effect of the Actual Operating Conditions on the Length of the SCCR7

9.3.1 Introduction

To investigate the accuracy of the Sciance and Crosser model (equation 9.1) in predicting the length of the semicontinuous system, the results and actual experimental conditions of the runs carried out on the SCCR7 at increasing feed concentrations were used, (ie. runs 18.6-9-30-30-20, 36-13-40-23-60, 46-13-39-24.17-60, 54-13-39-24.5-60 and 66-14.6-40-25-60). The effects of increased concentrations,

Table 9.4: Length Estimation for the Continuous SCCR7 at Various Feed Concentrations

Run	Actual K_{dg}	Results for 99.9% pure FRP			Results for the actual FRP purities			
		NTP	Theoretical length (cm)	HETP (cm)	FRP purity	Equipment Lengths (cm)		ETCF
						Experi-mental	Theore-tical	
A	0.353	632	1402	2.22	99.9	590	1402	0.421
B	0.341	689	1591	2.31	92.4	787	592	1.329
C	0.361	710	1700	2.39	91.78	787	615	1.280
D	0.377	724	1710	2.36	91.35	787	606	1.299
E	0.401	803	1674	2.09	90.1	787	560	1.410

Where:

ETCF: Experimental-Theoretical-Correlation-Factor

A: experimental run 18.6-9-30-30-20
B: experimental run 36-13-40-23-60
C: experimental run 46-13-39-24.17-60
D: experimental run 54-13-39-24.5-60
E: experimental run 66-14.6-40-25-60

flow rates, temperature, reduced fructose content and multicomponent nature of the feed were incorporated using the modified distribution coefficients, as described in Chapters 7 and 8 (also Tables 8.3, 8.4 and 8.9). The FRP purities used in the model (x_g term in equation 9.1) were the actual purities obtained from the respective experimental run. A reverse approach was followed whereby, the theoretically obtained length was compared with the actual length which was found experimentally to be sufficient to give the particular FRP purity. Finally, a scaling up procedure is outlined and a method of selecting the correct operating conditions is described.

9.3.2 Discussion of the Theoretical and Experimental Length Estimation Results

The theoretical length estimation of the semicontinuous SCCR7 was carried out using the Sciance and Crosser model (equation 9.1), as described in the previous sections, (a more detailed description is shown in Appendix B), at feed concentrations of 18.6, 36, 46, 54 and 66% w/v. The actual operating conditions were used (Table 8.7), and the respective FRP purities used in the calculations were the same as the ones achieved experimentally (Table 8.8). The aim was to test the accuracy of the model in determining the required length. It was found experimentally that 590cm were required to give 99.9% pure FRP at 18.6% w/v feed concentrations. For the other feed concentrations the total length of the equipment, 787cm, was sufficient to give the FRP purities shown on Table 9.4. The theoretical lengths at these purities were 592, 615, 606 and 560cm for feed concentrations 36, 46, 54 and 66% w/v respectively. Because of the low feed concentration and flow rates (in run 18.6-9-30-30-20) the on-column sugar conentrations were very low resulting in an easier separation, thus requiring a shorter system. At those low concentrations the disagreement between the experimental and theoretical lengths is too high, thus questioning the model's applicability. At higher feed concentrations, when the effects of concentration and flow rates are more prominent, the experimental and theoretical lengths show similar variations, and the

model underdesigns by approximately 33%. Since in most industrial applications high feed concentrations (ie. over 35% w/v) are used, then on averge ETCF value of 1.33 can be used in the length calculations for the semicontinuous sytem. To estimate the length required to achieve 99.9% pure FRP, at various feed concentrations used in the experimental programme, the calculations were repeated using the actual operating conditions and an x_g of 0.001, and the results are shown in Table 9.4. As the feed concentration increases the required theoretical lengths increse sharply up to feed concentrations of about 46% w/v, and then seem to stabilise. As it was found in Section 8.4.2, this also indicates that at feed concentrations of approximately 45% w/v and at these operating conditions, the systems become overloaded. Assuming that the theoretical and experimental lengths vary to the extent found above, the actual SCCR7 lengths required to obtain 99.9% pure FRP would be approximately 1.33 times greater than the theoretical lengths shown in Table 9.4. That means, when operating at maximum throughput (as in run 66-14.6-40-25-60) a two order of magnitude increases in FRP purity, from 90.1% to 99.9%, requires a 2.83 times increase of the existing SCCR7 length, from 787 to 2227cm. In all these calculations the theoretical lengths were obtained using the same operating conditions with the respective experimental runs. This means that although the purities would be increased, the throughputs (kg dry solids/m^3 resin/hr) would decrease due to the additional volume of resin. The calculations also assume that the operating switch time remains unchanged. This and the accuracy in the length prediction calculations, need to be verified by using a number of SCCR equipment of the same column diameter and different length, and operating at the same conditions.

The number of theoretical plates (NTP) required for complete separation were calculated using the feed band broadening approach (Section 6.6 and Appendix A), since although its limitations (Section 9.2.4) have been found experimentally (Appendix A), to

be accurate enough and applies specifically to the particular system. The resulting average HETP value was 2.27cm. This compares well with the infinite dilution average HETP of 2.09cm (Table 6.2).

Although this modified length estimation approach, for the semicontinuous systems, is slightly more complicated than the "infinite dilution" one, it is more accurate because it includes the effects at temperature, flow rates and concentration apparent in the actual operating conditions, and it requires the same number of experimental parameters. That is, the k'_g value, and the infinite dilution number of theoretical plates and distribution coefficients.

9.3.3 A Stepwise Approach to the Scaling-up of a Semicontinuous Chromatographic Refiner and a Means of Selecting the Proper Operating Parameters

The above evaluation of the applicability of some of the existing design theories and their modifications derived from the experimental findings, led to the development of the following approach for the sizing of a scaled-up semicontinuous chromatographic refiner.

- The column diameter is derived from the required annual throughput, and is based on a similar linear feed flow velocity rate as in the small scale SCCR. For carbohydrate separations a linear feed flow velocity of 0.64cm min^{-1} (ie. the equivalent of 14.6cm^3 min^{-1} feed flow rate on the SCCR7) is proposed. If higher throughputs are desired the linear feed flow can be increased to 0.77cm min^{-1} (ie. corresponding to a feed flow rate of 17.6cm^3 min^{-1} on the SCCR7).

- To increase the accuracy of the length estimation the values of the distribution coefficients must be determined preferably at 25°C and linear flow rates of

1.09cm min^{-1}. It is therefore recommended that a column is constructed of the diameter found above and a characterisation procedure as discussed in Chapter 6 performed in the batch mode. This would give the number of theoretical plates and distribution coefficients at infinite dilution. A preliminary length estimation using the results of the small scale SCCR unit (ie. SCCR7) should give an approximate indication of the required length; this in conjunction with the desired number of columns in the large scale SCCR would determine the length of the individual columns. The incresed weight of the resin in larger columns results in compression and possible damage of the resin in the lower part of the column leading to high pressure drops and blockages. Also, the use of an increased number of shorter columns would make it easier to achieve a more uniform packing, and also the semicontinuous process would be nearer to true continuous counter-current operation. It is therefore proposed that the length of each column should not exceed 120cm and preferably be around 80cm. Using shorter columns would increase the dead volume in the interconnecting pipework and valves, but the overall dead volume is reduced in large scale units due to the larger volumes of stationary phase. Larger column diameters require careful liquid distribution designs to prevent zone spreading.

- The distribution coefficients derived above at infinite dilution conditions are modified to account for the effects of temperature, flow rates and concentration according to the nature of the feed using equations 7.3, 7.4, 7.5 and 7.19 to 7.24, as shown in Chapter 7.

- The required length of the semi-continuous sytem to give the specified product purities is evaluated using equation 9.1 and the Experimental-Theoretical-Correlation-Factor of 1.33.

- The separating efficiency of any chromatographic system is traditionally expressed in terms of the number of theoretical plates, NTP, and/or the height equivalent to a theoretical plate, HETP. Therefore from the characterisation results of the large column and the total number of columns found above, the total "apparent" number of theoretical plates at infinite dilution, N*, is found. Then, using various feed and eluent flow rates, the actual total number of theoretical plates vs the number of theoretical plates occupied by the feed relationships and graphs are developed as described in Section 6.6 and Appendix A. The "true" number of theoretical plates, NTP, for the whole SCCR system is obtained from these relationships or the graph at the particular operational feed flow rate, and the HETP value is derived from the total sytem's length and NTP.

Once the system design has been completed, the selection of the main operating conditions is carried out using the conclusions derived from the experimental program on carbohydrate feedstocks. Summarising:

- the eluent to feed flow rate ratio should be not less than 2.75:1 and preferably approximately 3:1;
- for maximum throughput operating conditions analogous to the ones in run 66-14.6-40-25-60 should be used;
- a reference switch time should be obtained by operating at intermediate feed concentrations (ie. 30-40% w/v) and standard operating conditions, (ie. corresponding to 13cm^3 min^{-1} feed flow rate on the SCCR7, a 3:1 eluent to feed flow rate ratio and at a representative feed fructose content). Then the proper switch time for any other condition can be obtained using the relationships 8.1 to 8.4.

This design approach requires a minimum amount of experimental data and offers increased accuracy since these data have been obtained from the actual system.

CHAPTER 10

10.0 MATHEMATICAL MODELLING AND COMPUTER SIMULATION OF THE SCCR7

10.1 Introduction

A number of mathematical models have been developed to simulate the batch chromatographic process and to predict the elution profiles (229, 230). Most of these models however involve statistical approaches which are simplified and applicable to special cases. Jonsson (231) using the plate theory developed a computer program which simulated the actual processes taking place in the chromatographic column, ie. diffusion and adsorption. Sciance and Crosser (227) proposed a probabilistic modelling approach for continuous chromatographic systems which related the degree of separation, system length and operating conditions for a binary feedstock and mid-point feed location, (Chapter 2). Al-Madfai (228) developed an alternative model to predict the plate height for moving bed continuous counter-current chromatographic gas-liquid systems based on the random walk approach (26) as follows:

$$H = d_p + \frac{2D_m}{u} + \frac{-2\gamma_1\gamma_2}{(\gamma_1+\gamma_2)^2} \cdot \frac{(u+u_L)^2}{u\gamma_2 - u_L\gamma_1} \qquad \text{..... 10.1}$$

Where:

H = plate height
d_p = average particle diameter
D_m = mobile phase diffusivity
u = mobile phase velocity
u_L = stationary phase velocity
γ_1 = rate of transfer of molecules from gas to liquid
γ_2 = rate of transfer of molecules from liquid to gas

A transfer unit concept was employed by Barker and Lloyd (232) in their modelling of counter-current gas-liquid chromatographic systems. The resulting equations predicting the number of overall gas phase transfer units (N_G) were:

- For the rectifying section

$$(N_G)_R = \frac{1}{V_G/(K_o V_L - 1)} \ln \left[\frac{(E_1/K_o V_L) - C_1 (V_G/K_o V_L - 1)}{(E_1/K_o V_L) - C_2 (V_G/K_o V_L - 1)} \right] \quad \text{..... 10.2}$$

- And for the stripping section:

$$(N_G)_S = \frac{1}{(1 - V_G/K_o V_L)} \ln \left[\frac{(E_2/K_o V_L) - C_1 (1 - V_G/K_o V_L)}{(E_2/K_o V_L) - C_2 (1 - V_G/K_o V_L)} \right] \quad \text{..... 10.3}$$

Where:

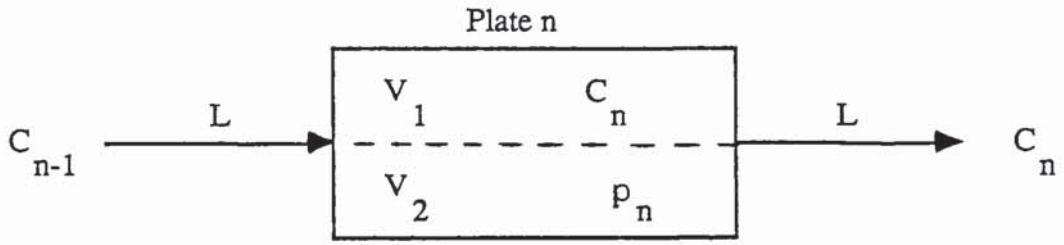
E_1 and E_2 = mass flow rates of solute leaving in products 1 and 2 respectively
C_1 and C_2 = gas phase solute concentrations at points 1 and 2 in the column
K_o = the partition coefficient
V_G and V_L = gas and liquid volumetric flow rates

These results indicated that the main resistance to mass transfer was in the gas phase.

10.2 Approach Employed for Simulating the Semicontinuous Operation of the SCCR7

Sunal (233) developed a simulation program for gas-liquid chromatography based on plate to plate calculations to describe the operation of a circular counter-current chromatographic equipment. This approach was adopted by Deeble (234) to simulate the operation of the SCCR1 gas-liquid chromatographic systems and developed further by Ching (203) in his liquid-liquid chromatographic studies on the SCCR4 system. The program uses the equilibrium stage or plate concept, where the separating length of the system is considered to consist of a number of theoretical plates, each containing a volume of mobile phase and a volume of stationary phase. The mobile phase leaving each stage is at equilibrium with the stationary phase in the stage. Therefore,

considering a mobile phase flow rate, L, passing through a plate "n" having an initial solute concentrationof C_{n-1} and an exit solute concentration of C_n, the conditions around the plate "n" may be represented by:



A mass balance over the plate "n" for the solute gives:

$$LC_{n-1} = LC_n + V_1 \frac{dC_n}{dt} + V_2 \frac{dp_n}{dt} \qquad \text{..... 10.4}$$

where:

C	= solute concentration in the mobile phase
p	= solute concentration in the stationary phase
V_1, V_2	= plate volumes of mobile and stationary phases respectively
L	= mobile phase flow rate

Equilibrium on the plate is represented by the distribution coefficient K_d, where by definition:

$$K_d = \frac{p_n}{C_n} \qquad \text{..... 10.5}$$

Substitution of equation 10.5 into equation 10.4 yields:

$$LC_{n-1} = LC_n + (V_1 + V_2 K_d) \frac{dC_n}{dt} \qquad \text{..... 10.6}$$

This equation can be integrated provided Δt is sufficiently small to allow C_{n-1} to be considered constant, to give:

$$C_n = C_{n-1}\left[1 - \exp\left(\frac{-L\,\Delta t}{V_1 + V_2 K_d}\right)\right] + C_n^{o} \exp\left(\frac{-L\,\Delta t}{V_1 + V_2 K_d}\right) \qquad \text{..... 10.7}$$

Where C_n^{o} is the initial concentration of the solute in the plate.

For multicomponent feedstocks similar concentration profile equations can be derived for each component by assuming no interaction between the components. The first term on the right hand side of equation 10.7 represents the material-transferred from plate (n-1) and the second term represents the material already present in the n^{th} plate. When this equation is applied for the post-feed section it is modified to account for the component feed concentration, C_f, and feed flow rate F, hence the term C_{n-1} is replaced by the ratio:

$$\frac{L\,C_{n-1} + FC_f}{L + F} \qquad \text{..... 10.8}$$

The model predicts the solute concentration in the mobile phase leaving each theoretical plate over a small time increment Δt, and the calculations are repeated over the total number of increments. When this predetermined total number of increments, which is equal to the switch period, has been reached, the sequencing counter-current action is simulated by stepping the concentration calculations by one column.

10.3 Model Developments

Ching (203) in his simulation work assumed that the glucose was not retained by the resin and used a glucose distribution coefficient equal to zero. Chuah (204) and

Gould (214) improved the Ching model by accounting for the glucose retention due to the size exclusion principle and used a finite value for the glucose distribution coefficient.

Abusabah (22) used the model to predict the concentration profiles in his anion exchange studies. In all of the above studies the distribution coefficients were assumed to be constant and the actual values used were obtained by trial and error to give the best fit. Thawait (235) modified the Gould's model to account for the effect of the background concentration on the distribution coefficients. The concentration-distribution coefficient relationships used however, were applicable to the small 0.5cm id x 50cm long batch column they were derived on, and their contribution on the simulation of the ten column, 10.8cm id x 70cm long, SCCR6 was limited.

An alternative approach for the modelling of the SCCR units was employed by England (236), where instead of assuming that C_{n-1} remains constant over a small time increment, Δt, he obtained n differential equations for the n plates, similar in form to equation 10.6, ie.

$$FC_{fn} + LC_{n-1} = (L + F)\, C_n - (V_1 + K_d\, V_2)\, \frac{dC_n}{dt} \qquad \text{..... 10.9}$$

Rearranging and dividing by $(V_1 + K_d\, V_2)$,

$$\frac{dC_n}{dt} = X_n\, C_{n-1} - Y_n C_n + Z_n\, C_{fn} \qquad \text{..... 10.10}$$

where:

$$X_n = \frac{L}{V_1 + K_d\, V_2}$$

$$Y_n = \frac{L + F}{V_1 + K_d\, V_2}$$

$$Z_n = \frac{F}{V_1 + K_d V_2}$$

Assuming that a chromatographic system is divided into n stages the resulting set of equations is as follows:

$$\dot{C}_1 = XC_o - YC_1 + Z C_{f1}$$

$$\dot{C}_2 = XC_1 - YC_2 + Z C_{f2}$$

.....

$$\dot{C}_n = XC_{n-1} - YC_n + Z C_{fn}$$

or

$$\dot{C}(t) = A C(t) + B C_f(t) \qquad \text{..... 10.11}$$

Equation 10.11 is solved in reference (236) and the solution is:

$$C(t) = \exp (At) C_o + \int_0^t \exp [A (t - \tau)] B C_f (\tau) d\tau \qquad \text{..... 10.12}$$

If the feed input is not time dependent the solution is:

$$C(t) = \Phi(t) Co + \Delta(t) B C_f$$

where:

$\Phi(t) = \exp (At)$

$\Delta(t) = A^{-1} [\exp (At) - I] = A^{-1} [\Phi(t) - I]$

Although this approach is theoretically better it has been found that it required excessive computing time exceeding the maximum CPU time (10000 seconds) available on the Harris 800. Therefore the original approach was followed in the simulation work, based on equation 10.7.

10.4 Improvements of the Simulation Program and its Application for the SCCR7 System

The final version of the simulation program developed by Thawait (202) and applied to the ten column 10.8cm id SCCR6 system, was used as a basis. The program was rearranged in a more general form to improve its compatibility with different SCCR systems of various configurations. All the system related parameters in the main program, ie. dimensions, numbers of columns, liquid inlet locations, number of theoretical plates, voidage and stationary and mobile phase plate volumes; the operating parameters, such as the infinite dilution distribution coefficients, flow rates, feed composition, and purging period; and program related parameters such as reference flow rates, number of cycles, and time increments were replaced by "dummy" variables, which were defined at the beginning of the program.

Because of the long computing time required and to give priority to other University Harris 800 computer users, the program was run as a "control point job" (237), whereby it was executed stepwise whenever some free computing time was available. When the initial version was run on the Harris computer with settings similar to the ones used by previous workers (202, 214), the program failed to complete the calculations within the 10000 seconds maximum computing time allowed. By minimising the amount of calculations performed inside the nested "DO" loops, the required CPU times was reduced to around two hours.

The actual operating switch time was predicted using equations 8.1 or 8.2 at the particular feed concentration, then it was adjusted using equation 8.3 to account for the feed flow rate, and adjusted further using equation 8.4 to account for the fructose content of the feed.

The simulation program was developed to perform a dual mode of operation; in addition to the concentration profile prediction, a system design was also carried out. The "true" number of theoretical plates at the particular feed and eluent flow rates were obtained using equations 6.9 or 6.10. The SCCR7 system length required to obtain a given separation, which in this work was chosen to be 99.9% pure FRP, at the particular operating conditions was obtained using the Sciance and Crosser model (equation 9.1). From these results the actual height equivalent to a theoretical plate was also determined.

In the previous versions the on-column "point" concentration profiles were predicted at a predetermined stage of the simulation. In this work the average concentrations for each colunn were calculated after nine cycles corresponding to the purging concentration profile, since it offers a more accurate representation of the separation. The purging period (PERTIM) used in the simulation was similar to the experimental. If required, the program can be modified easily to give both the on-column and the purging concentration profiles.

The program is rearranged to account for up to three components; the retarded fructose due to calcium ion complexing, the lesser retarded glucose due to size exclusion, and the faster moving impurities (maltose and oligosaccharides as one component). Because of the relatively low concentrations of these impurities it was assumed that they were totally excluded and migrated along the system in the mobile phase as one component. The program can be modified to account for more than three components by including the required additional mass balance equations in the feed and separating sections. The importance of the distribution coefficients on the simulation was identified by previous workers (22, 203, 204, 214) who used arbitrary values to achieve the best possible agreement between the experimental and simulated profiles.

Thawait (202) made a further contribution by identifying the effects of background concentration and temperature on the distribution coefficients, and incorporated a set of concentration vs distribution coefficients relationships and temperature adjustments in the program. Their contribution however was limited since

they were applicable to the system the experimental data were obtained from and not to the SCCR6 system. Also the actual distribution coefficient used was the maximum of the values obtained from the concentration vs K_d relationships.

In this work the infinite dilution coefficients obtained from the column characterisation of the SCCR7 system (Chapter 6) were modified to account for the effects of temperature and liquid flow rates using the general relationships 7.3, 7.4 and 7.5 (Chapter 7). During the simulation the general concentration vs K_d relationships (equations 7.19 to 7.24) were used to modify the above distribution coefficients according to the background concentration on each plate. The actual glucose and fructose distribution coefficients in the plate concentration calculations were the weighted average of the values obtained using equation 7.19 to 7.24 adjusted according to the composition on each plate. The average glucose and fructose distribution coefficients for the particular separation were obtained in a similar way and were based on the feed composition.

In the plate to plate concentration calculations the total "apparent" number of theoretical plates based on fructose was used. The use of the "true" number of theoretical plates would have led to more accurate results but it was not employed because of the computing time limitations. From the initial applications of the program in the simulation of the SCCR7 operation it was noted that the simulated concentration profiles of the two main components were "delayed", ie. appeared to be "shifted" to the right, especially the fructose, with respect to the experimental. This indicated either that the effect of changing the operating switch time was less pronounced in the simulation than in practice, or that the distribution coefficients, modified for the effects of temperature, flow rates and background concentration using equations 7.3 to 7.5 and 7.19 to 7.24, were still in some disagreement with the actual ones. To obtain a better agreement with the experimental profiles, two slightly different switch time approximations were used in the simulation (SWTIME and SWTFR for the glucose and

fructose respectively), which in general were smaller than the actual switch time. This resulted in an artificial "shift" of the simulated concentration profiles to the left.

To investigate the cause of this disagreement and identify which of the above reasons contributed to the shift, it is recommended in future work to obtain a new set of experimental data at various temperatures, concentrations and flow rates, operating on a production scale column (ie. 5.4cm id) at similar operating conditions to the actual ones, and test the accuracy of the relationships 7.3 to 7.5 and 7.19 to 7.24. In the data section of the program the parameter ILK2 = 86413 appears which is not used anywhere in the main program. This parameter was the final value of a counter used during the initial stages of the program development to check if all the iterations were carried out properly, and this testing sequence was removed in the later stages of the work. Although this parameter is no longer used, if it is eliminated the computing program would fail to run properly. This peculiarity of the compiler was brought to the attention of the advisory group in the Computing Centre but no satisfactory answer was given.

In the previous versions the simulation was carried out using a fixed number of time increments (KKINK) per sequence and a fixed switch period. Because of the various switch times used in this work, to provide a similar basis the duration of each time increment (DELTAT) was kept constant and it was chosen to be 2.5 seconds. The number of time increments per sequence (KKINK) was obtained using the DELTAT value and the operating switch time for the particular conditions. Reducing the DELTAT value to 2 seconds increased the CPU time by approximately 20% without any significant improvement in the predicted concentration profiles.

A flow chart of the simulation program is shown in Figure 10.1 and a listing of the setting used in the simulation and the total CUP time taken for each run is presented in Table 10.1. A program listing a typical set of the results obtained after executing the program and a comprehensive list of the parameters used are shown in Appendix C.

10.5 Results and Discussion

To examine the accuracy of the simulation program the experimental conditions of runs 18.6-9-30-30-20, 36-13-40-23-60, 46-13-39-24.17-60, 54-13-39-24.5-60 and 66-14.6-40-25-60 were used. These runs were chosen because the product purities were found experimentally to be within specification. Both the experimental and simulated concentration profiles are plotted in Figures 10.2 to 10.6 respectively. The very good overall agreement between the simulated and experimental profiles highlighted the contribution of the above modifications.

Although the existence of instantaneous equilibrium is arguable, and the column related parameters were evaluated for each column, operating separately in a batch mode, and not for the continuous systems as a whole, the model was found to describe the multicomponent separation well.

A close inspection of the respective concentration profiles shows that the "cross-over" point locations are almost identical, and the individual experimental concentration profiles, especially the fructose, are reproduced. The only exemption is for run 36-13-40-23-60 (Figure 10.3), because the experimental "on-column" concentrations ("point" instead of "bulk" values) were used, since the system was not purged at the end of this experimental run. The simulation profiles of the maltose and oligosaccharides, were starting later and the concentrations were lower than the corresponding experimental ones, because it was assumed that these components were totally excluded.

The fructose experimental profiles had slightly higher (sharper) concentrations in the third column where as the simulated ones were smoother. This could be attributed to the nature of operation.

The structural improvements of the program resulted in reductions of over 15% in the required CPU time (Table 10.1). The predicted switch times were in good agreement with the actual (Tables 10.1 and 10.2), and using the predicted ones for any

set of new operating conditions would minimise the experimental work substantially. The predicted "true" number of theoretical plates also agreed well with the values obtained in Chapter 9 (Table 9.4); the small differences were because, in the simulation the predicted switch times were used instead of the actual ones which were used in Chapter 9.

Table 10.2 includes the theoretical SCCR7 length required to obtain 99.9% pure products at the particular operating conditions, and also the resulting HETP values. The actual distribution coefficients of glucose and fructose were obtained based on the feed composition and using an average linear velocity, ie. based on the average liquid flow rate in the pre and post feed sections. The slight disagreement with the corresponding distribution coefficients obtained in Section 8, (Tables 8.3, 8.4 and 8.9), was because they were determined for the more adverse conditions possible, ie. using the post-feed liquid flow rate in the liquid velocity calculations.

Figure 10.7 is a plot of the results of run 47.5-13-32-28.5-60. The good agreement of the simulated and experimental profiles highlighted the model's flexibility in accommodating changes in the operating flow rates.

Summarising, the simulation program is very useful in presenting a fairly accurate overall picture of the separation expected for any set of operating conditions, and its application can minimise the experimental work required and provide a better understanding of the separation.

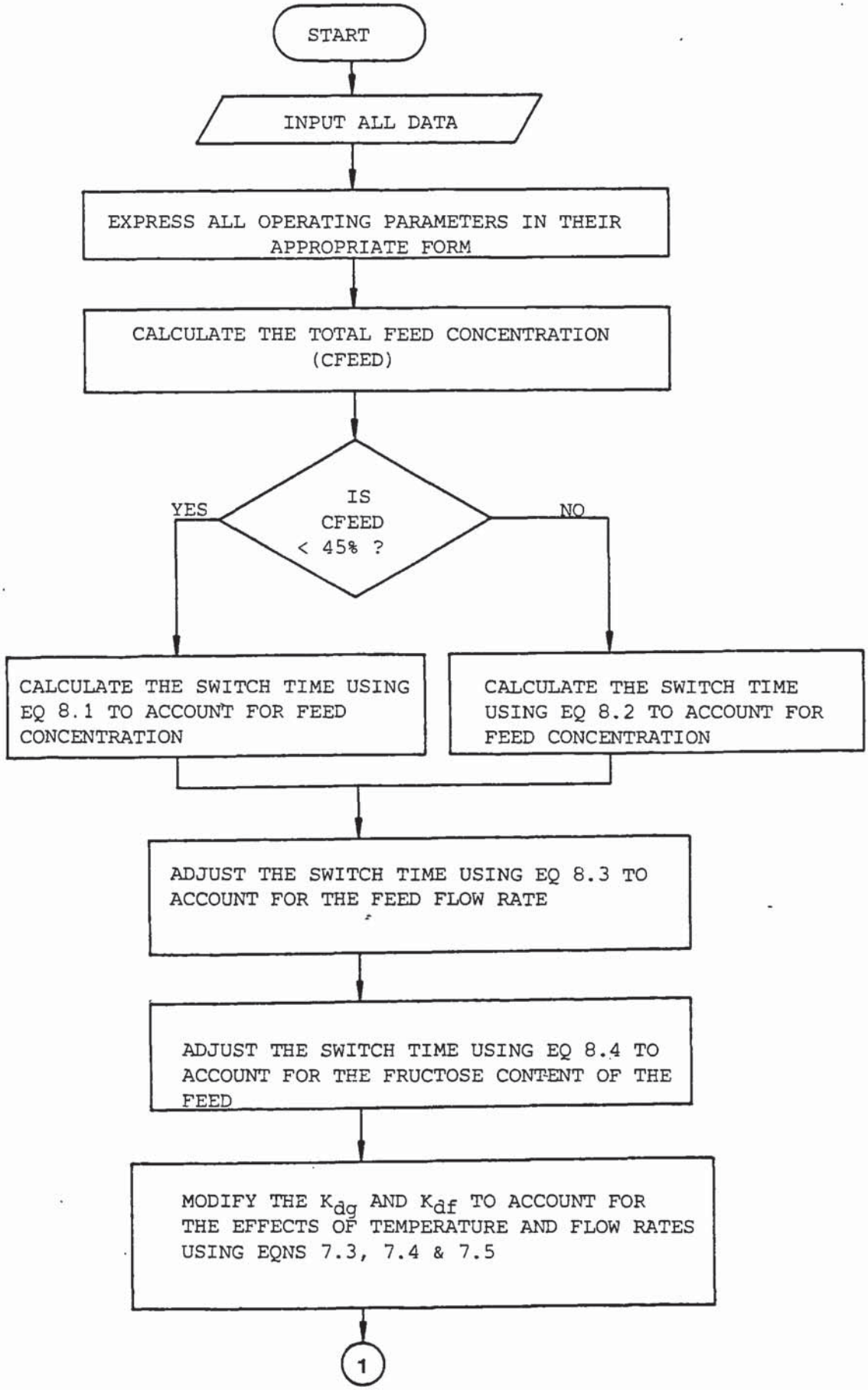
Table 10.1: Settings used in the Simulation of the SCCR7 Unit and Total Computing time taken to Carry out the Simulation

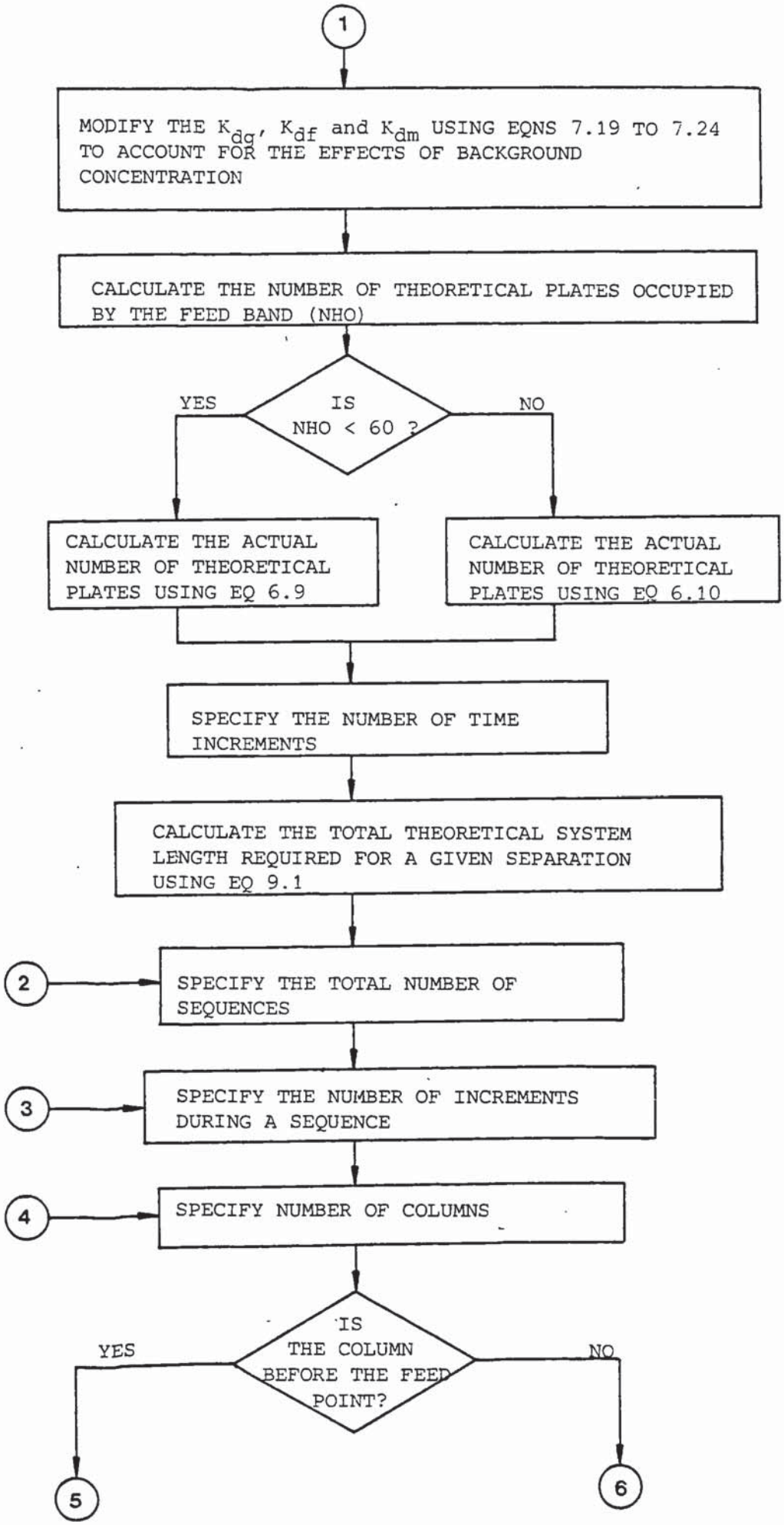
Feed Conc % w/v	Actual Switch Time (min)	Switch time approximations (min)		Purging period (min)	CPU Time (s)
		wrt glucose	wrt fructose		
18.6	30.0	30.4	23.9	13	8514
36	23.0	22.5	19.2	13	6312
46	24.17	23.3	20.1	13	6794
54	24.5	23.9	20.3	13	6850
66	25.0	23.3	19.7	13	6247
47.5	28.5	28.2	23.9	24	6845

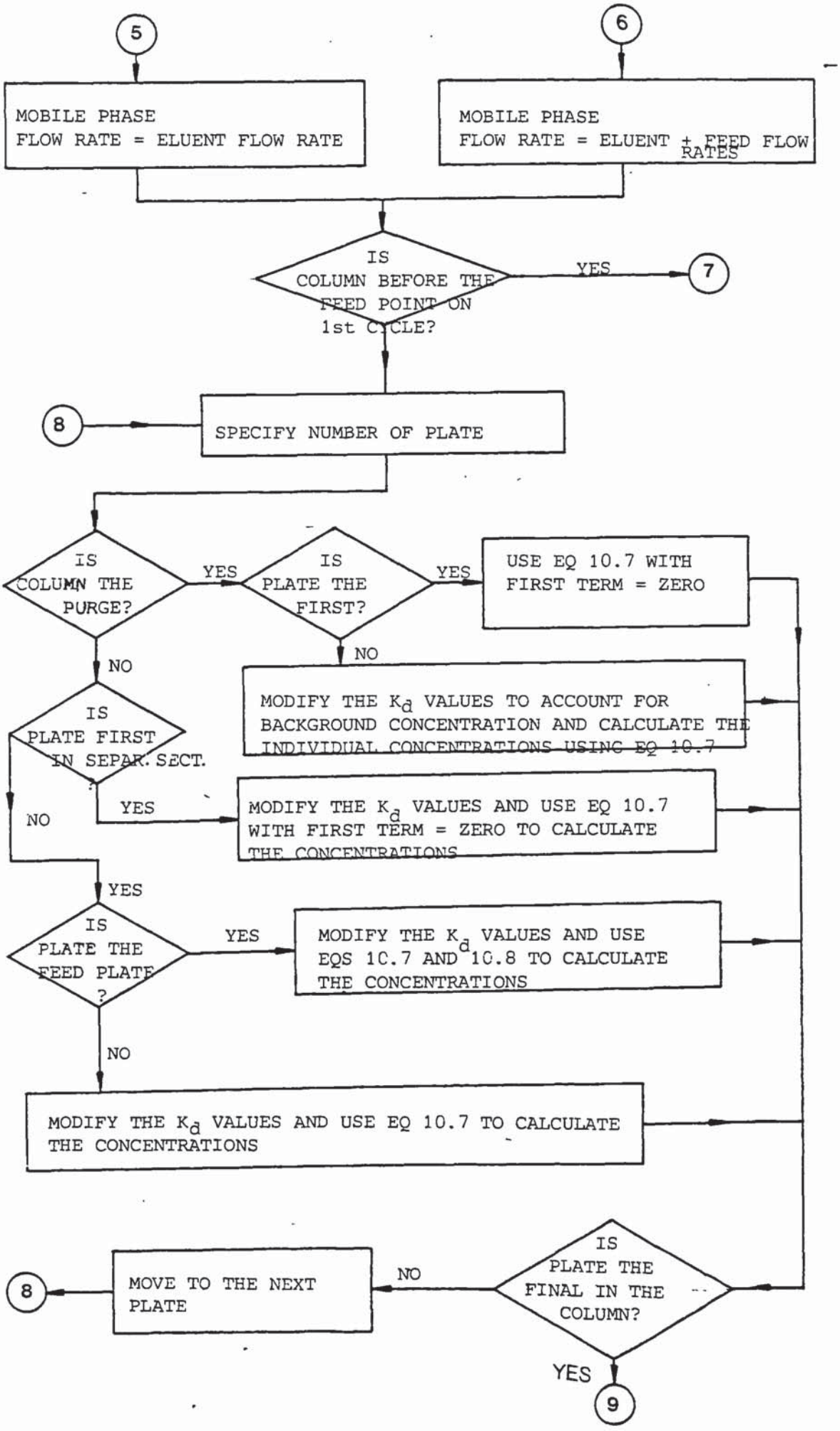
Table 10.2: Simulated Results for the SCCR7 System

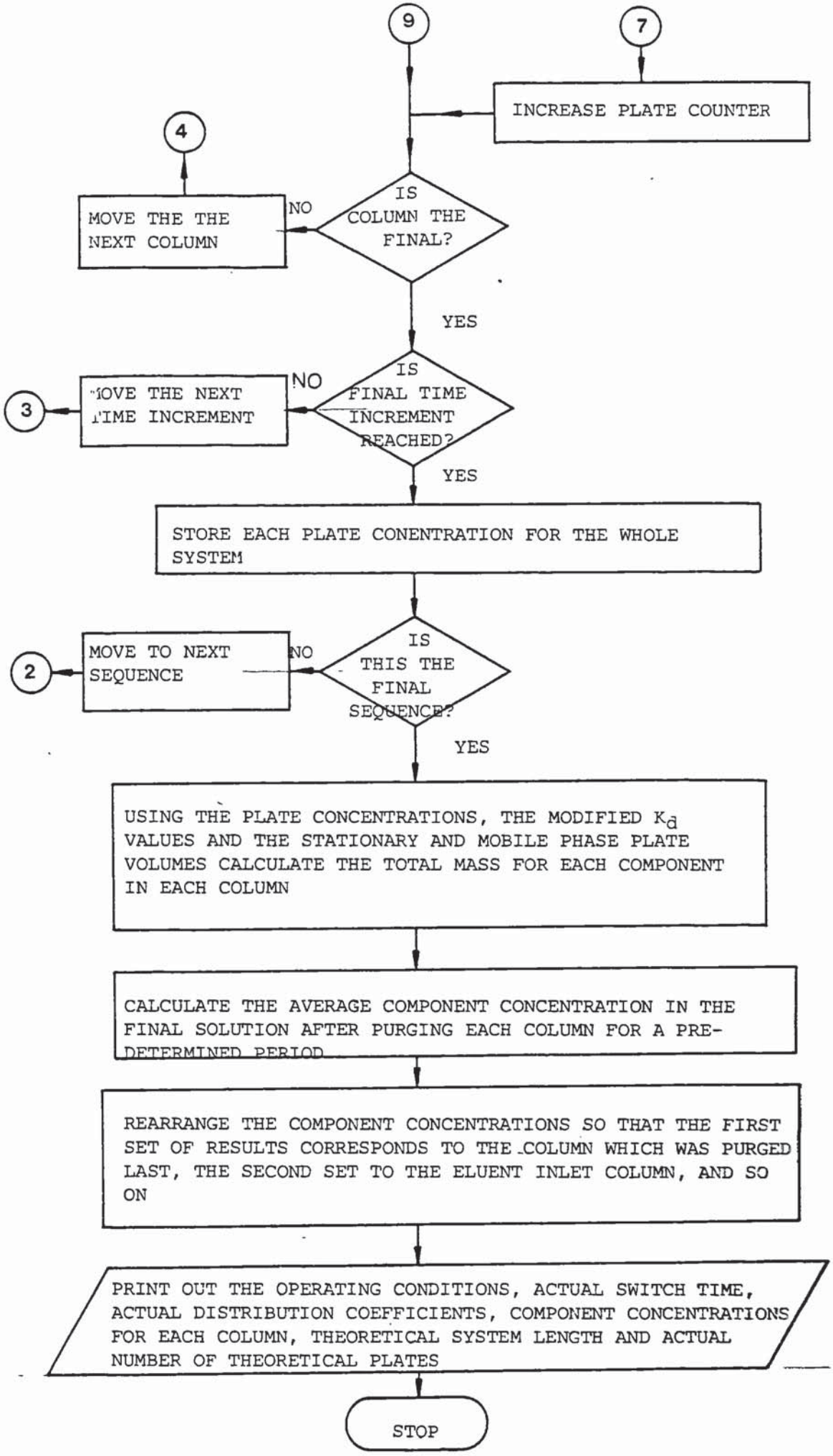
Feed Conc % w/v	Predicted Switch Time (min)	Actual distribution coefficients		"True" No of theoretical plates	SCCR length required to obtain 99.9% FRP (cm)	HETP (cm)
		K_{dg}	K_{df}			
18.6	30.12	0.335	0.509	633.7	1355.3	2.1
36	22.96	0.339	0.400	684.5	1766	2.6
46	24.30	0.361	0.420	716.1	1692.6	2.4
54	24.52	0.377	0.434	721.6	1707.2	2.4
66	22.32	0.398	0.450	732.8	1998.6	2.7
47.5	24.45	0.380	0.441	719.8	1912.2	2.7

Figure 10.1: Computer flow chart for the simulation of the continuous operation of the SCCR7 system




1
MODIFY THE K_{dg}, K_{df} and K_{dm} USING EQNS 7.19 TO 7.24 TO ACCOUNT FOR THE EFFECTS OF BACKGROUND CONCENTRATION
CALCULATE THE NUMBER OF THEORETICAL PLATES OCCUPIED BY THE FEED BAND (NHO)
IS NHO < 60 ?
YES
NO
CALCULATE THE ACTUAL NUMBER OF THEORETICAL PLATES USING EQ 6.9
CALCULATE THE ACTUAL NUMBER OF THEORETICAL PLATES USING EQ 6.10
SPECIFY THE NUMBER OF TIME INCREMENTS
CALCULATE THE TOTAL THEORETICAL SYSTEM LENGTH REQUIRED FOR A GIVEN SEPARATION USING EQ 9.1
2
SPECIFY THE TOTAL NUMBER OF SEQUENCES
3
SPECIFY THE NUMBER OF INCREMENTS DURING A SEQUENCE
4
SPECIFY NUMBER OF COLUMNS
IS THE COLUMN BEFORE THE FEED POINT?
YES
NO
5
6


5
6
MOBILE PHASE
FLOW RATE = ELUENT FLOW RATE
MOBILE PHASE
FLOW RATE = ELUENT + FEED FLOW RATES
IS COLUMN BEFORE THE FEED POINT ON 1st CYCLE?
YES
7
8
SPECIFY NUMBER OF PLATE
IS COLUMN THE PURGE?
YES
IS PLATE THE FIRST?
YES
USE EQ 10.7 WITH FIRST TERM = ZERO
NO
MODIFY THE K_d VALUES TO ACCOUNT FOR BACKGROUND CONCENTRATION AND CALCULATE THE INDIVIDUAL CONCENTRATIONS USING EQ 10.7
NO
IS PLATE FIRST IN SEPAR. SECT. ?
YES
MODIFY THE K_d VALUES AND USE EQ 10.7 WITH FIRST TERM = ZERO TO CALCULATE THE CONCENTRATIONS
NO
YES
IS PLATE THE FEED PLATE ?
YES
MODIFY THE K_d VALUES AND USE EQS 10.7 AND 10.8 TO CALCULATE THE CONCENTRATIONS
NO
MODIFY THE K_d VALUES AND USE EQ 10.7 TO CALCULATE THE CONCENTRATIONS
IS PLATE THE FINAL IN THE COLUMN?
NO
MOVE TO THE NEXT PLATE
8
YES
9


9
7
INCREASE PLATE COUNTER
4
MOVE THE THE NEXT COLUMN
NO
IS COLUMN THE FINAL?
YES
3
MOVE THE NEXT TIME INCREMENT
NO
IS FINAL TIME INCREMENT REACHED?
YES
STORE EACH PLATE CONENTRATION FOR THE WHOLE SYSTEM
2
MOVE TO NEXT SEQUENCE
NO
IS THIS THE FINAL SEQUENCE?
YES
USING THE PLATE CONCENTRATIONS, THE MODIFIED K_d VALUES AND THE STATIONARY AND MOBILE PHASE PLATE VOLUMES CALCULATE THE TOTAL MASS FOR EACH COMPONENT IN EACH COLUMN
CALCULATE THE AVERAGE COMPONENT CONCENTRATION IN THE FINAL SOLUTION AFTER PURGING EACH COLUMN FOR A PRE-DETERMINED PERIOD
REARRANGE THE COMPONENT CONCENTRATIONS SO THAT THE FIRST SET OF RESULTS CORRESPONDS TO THE COLUMN WHICH WAS PURGED LAST, THE SECOND SET TO THE ELUENT INLET COLUMN, AND SO ON
PRINT OUT THE OPERATING CONDITIONS, ACTUAL SWITCH TIME, ACTUAL DISTRIBUTION COEFFICIENTS, COMPONENT CONCENTRATIONS FOR EACH COLUMN, THEORETICAL SYSTEM LENGTH AND ACTUAL NUMBER OF THEORETICAL PLATES
STOP

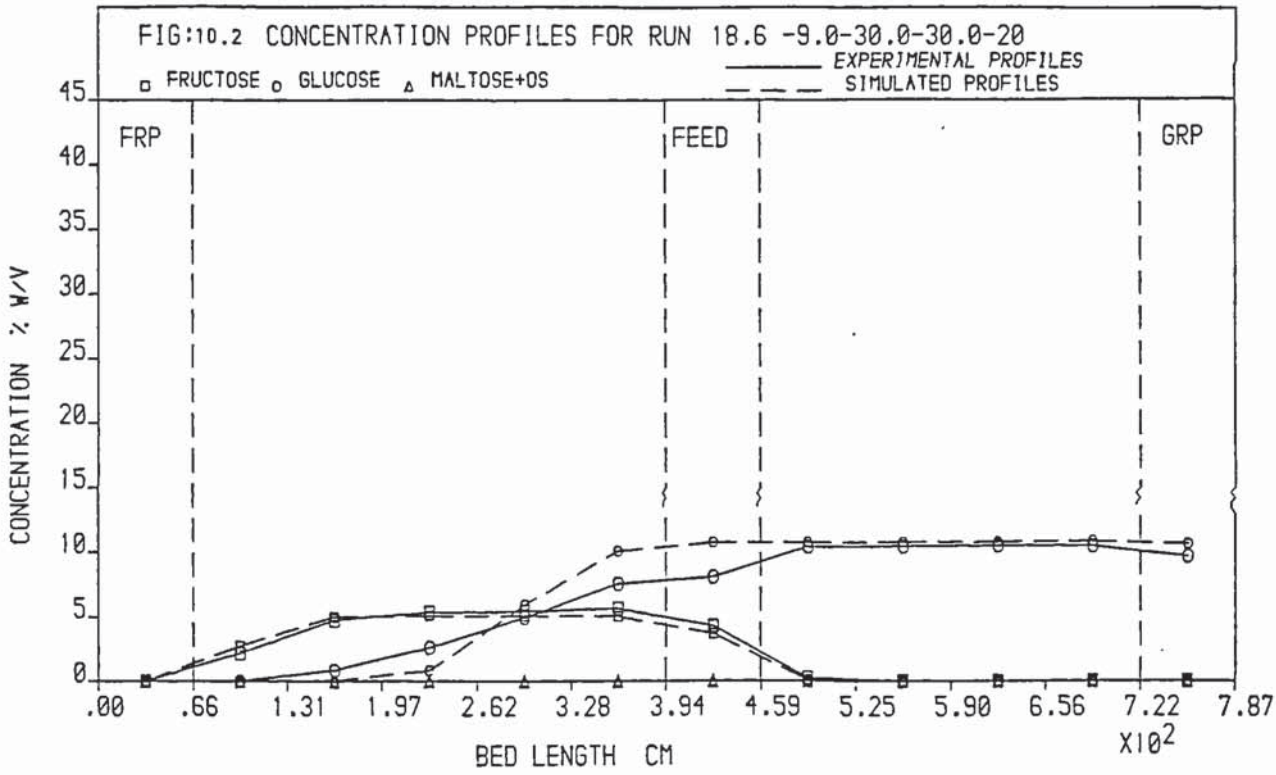


FIG:10.2 CONCENTRATION PROFILES FOR RUN 18.6 -9.0-30.0-30.0-20
□ FRUCTOSE ○ GLUCOSE △ MALTOSE+OS
EXPERIMENTAL PROFILES
SIMULATED PROFILES
FRP
FEED
GRP
CONCENTRATION % W/V
BED LENGTH CM
X10²
0
5
10
15
20
25
30
35
40
45
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87

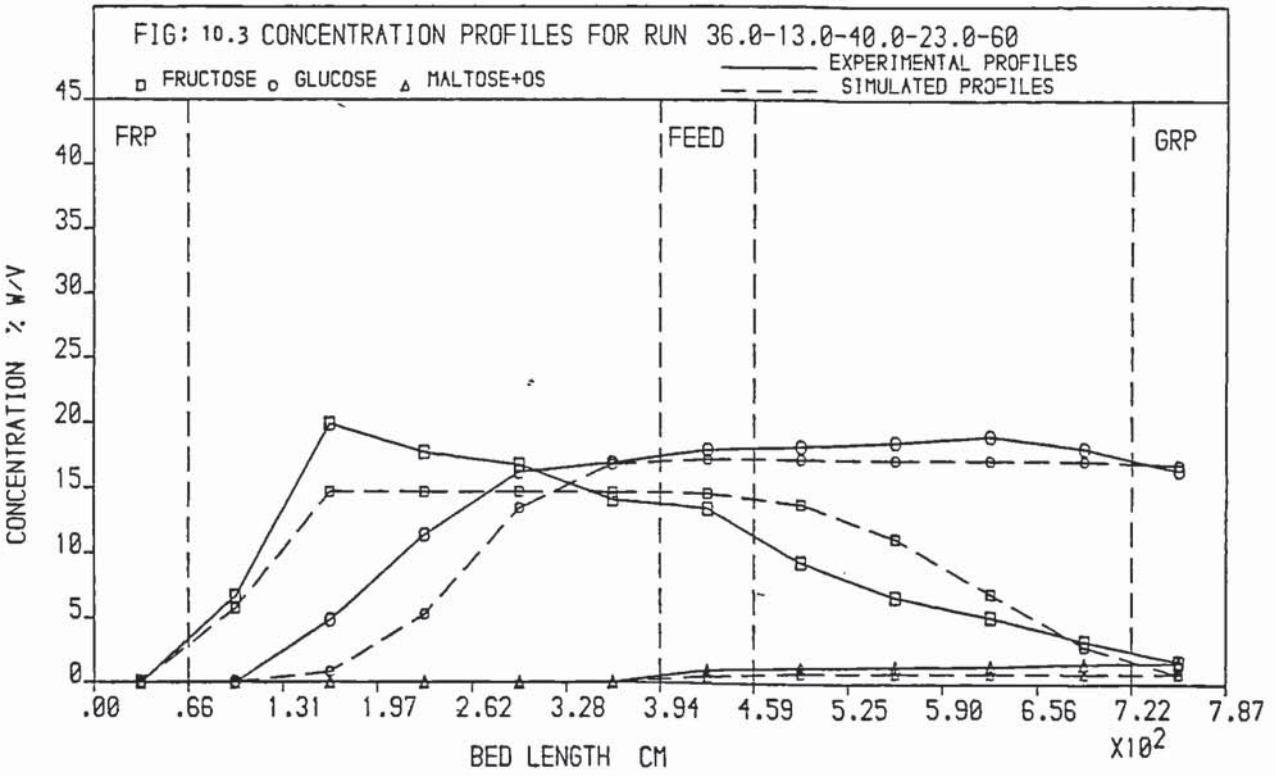


FIG: 10.3 CONCENTRATION PROFILES FOR RUN 36.0-13.0-40.0-23.0-60
□ FRUCTOSE ○ GLUCOSE △ MALTOSE+OS
EXPERIMENTAL PROFILES
SIMULATED PROFILES
FRP
FEED
GRP
CONCENTRATION % W/V
BED LENGTH CM
X10²
0
5
10
15
20
25
30
35
40
45
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87

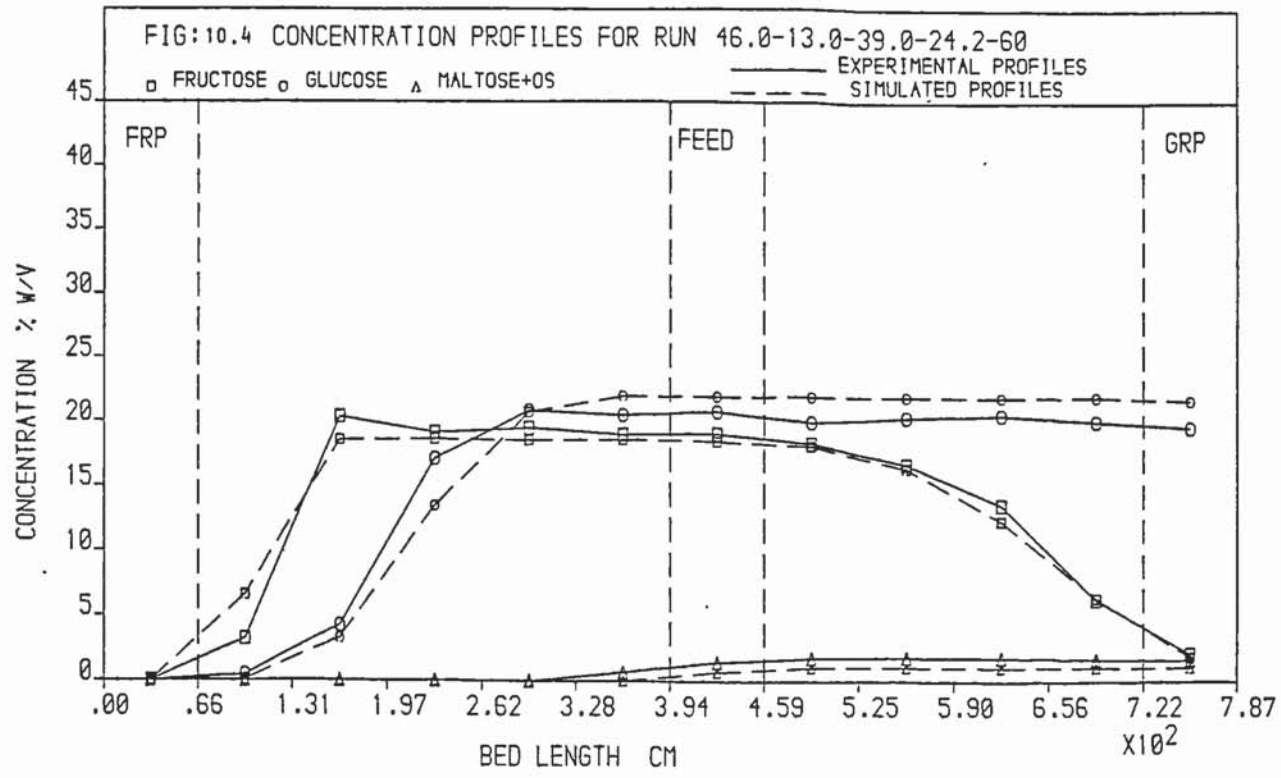


FIG:10.4 CONCENTRATION PROFILES FOR RUN 46.0-13.0-39.0-24.2-60
FRUCTOSE GLUCOSE MALTOSE+OS
EXPERIMENTAL PROFILES
SIMULATED PROFILES
FRP
FEED
GRP
CONCENTRATION % W/V
BED LENGTH CM
X10²
0
5
10
15
20
25
30
35
40
45
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87

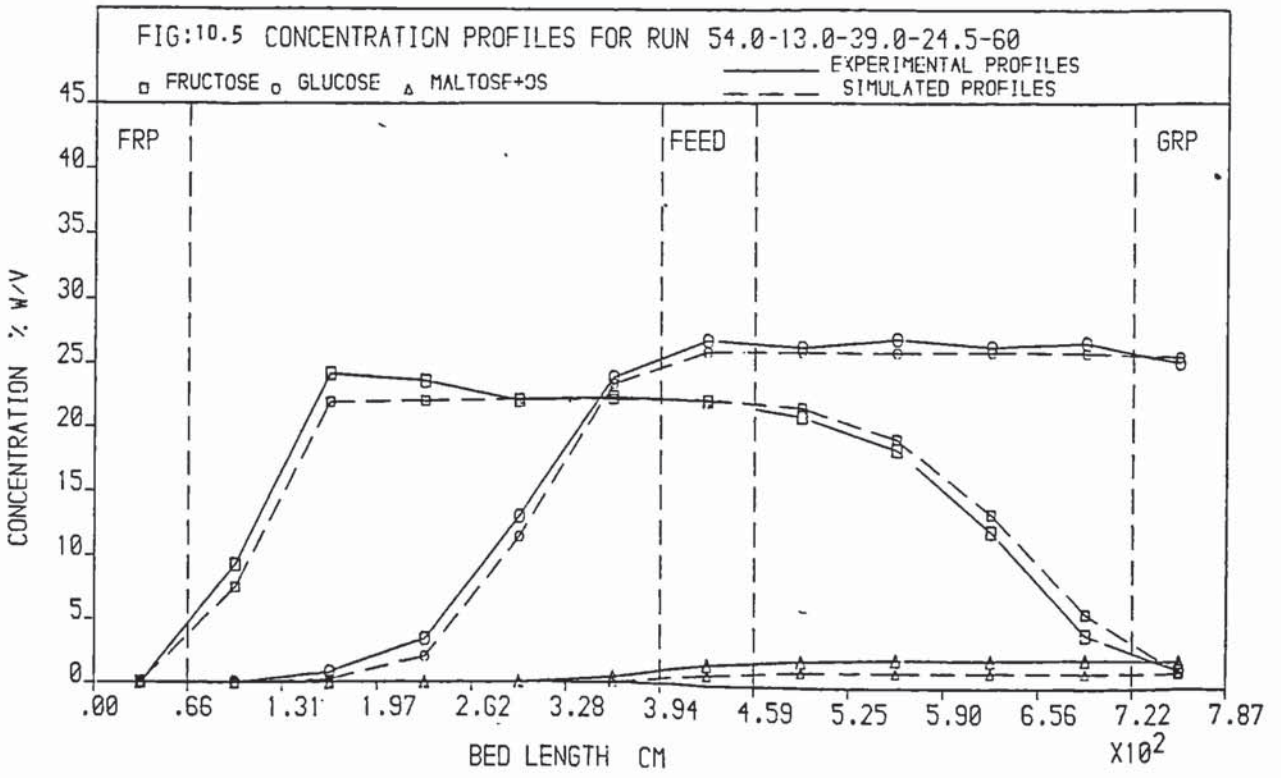


FIG:10.5 CONCENTRATION PROFILES FOR RUN 54.0-13.0-39.0-24.5-60
FRUCTOSE GLUCOSE MALTOSE+OS
EXPERIMENTAL PROFILES
SIMULATED PROFILES
FRP
FEED
GRP
CONCENTRATION % W/V
BED LENGTH CM
X10²
0
5
10
15
20
25
30
35
40
45
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87

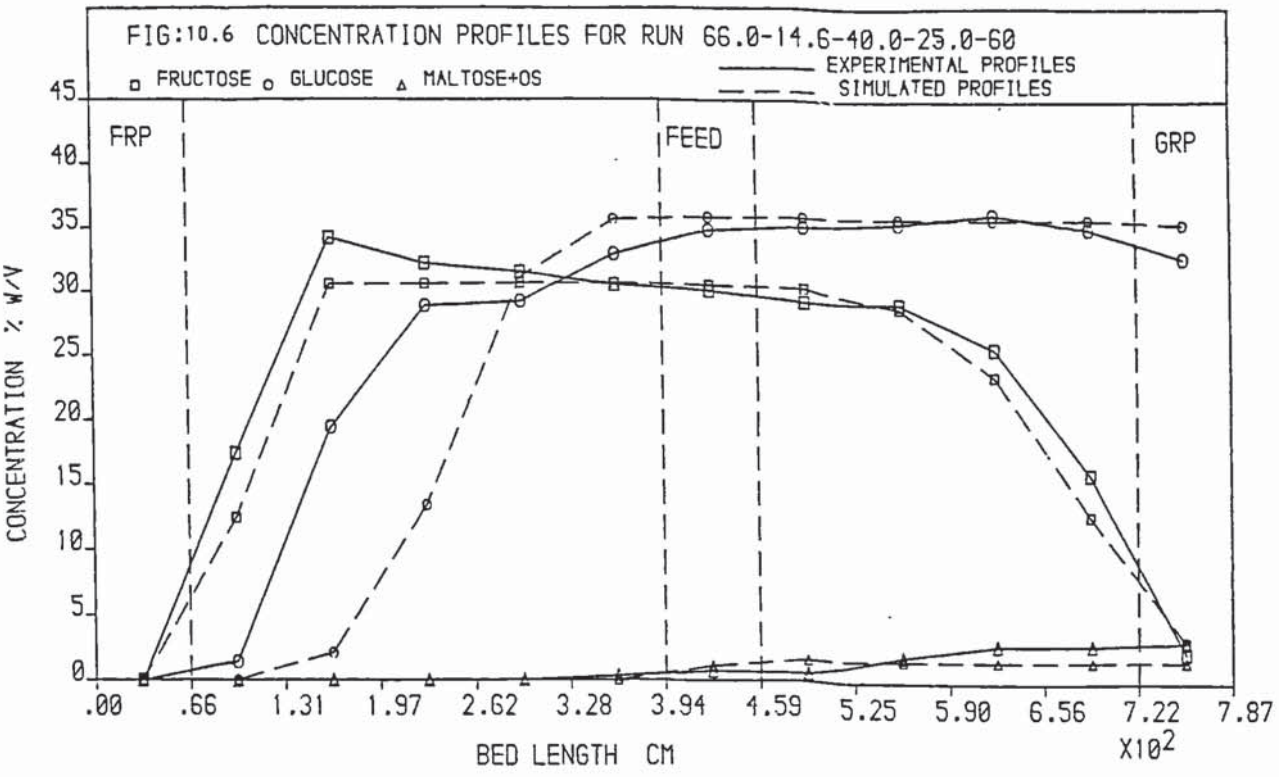


FIG:10.6 CONCENTRATION PROFILES FOR RUN 66.0-14.6-40.0-25.0-60
□ FRUCTOSE ○ GLUCOSE △ MALTOSE+OS
EXPERIMENTAL PROFILES
SIMULATED PROFILES
FRP
FEED
GRP
CONCENTRATION % W/V
BED LENGTH CM
X10²
0
5
10
15
20
25
30
35
40
45
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87

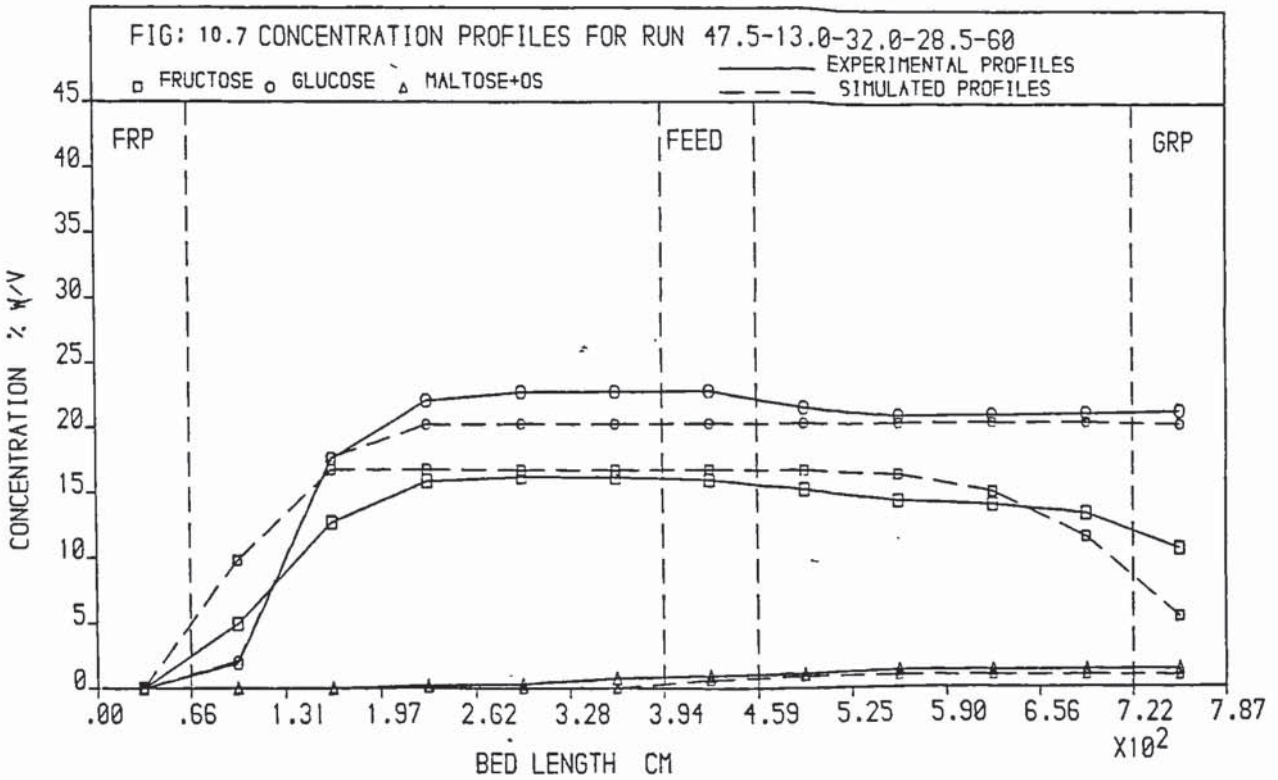


FIG: 10.7 CONCENTRATION PROFILES FOR RUN 47.5-13.0-32.0-28.5-60
□ FRUCTOSE ○ GLUCOSE △ MALTOSE+OS
EXPERIMENTAL PROFILES
SIMULATED PROFILES
FRP
FEED
GRP
CONCENTRATION % W/V
BED LENGTH CM
X10²
0
5
10
15
20
25
30
35
40
45
.00
.66
1.31
1.97
2.62
3.28
3.94
4.59
5.25
5.90
6.56
7.22
7.87

CHAPTER 11

11.0 BIOCHEMICAL REACTION - SEPARATION STUDIES

11.1 Introduction

In the previous chapters the successful application of the semicontinuous chromatographic refiner (SCCR7) as a separator has been described. The encouraging separation results and the potential of such a chromatographic system (SCCR) as a reactor-separator has led us in researching the biochemical synthesis of dextran from sucrose in the presence of dextransucrase enzyme and the simultaneous separation of the formed dextran and the valuable byproduct fructose. This combined reaction-separation operation would reduce the capital and operating costs, provide better product reproducibility and control and is expected to improve the dextran product yield by removing the acceptor fructose from the reaction mixture, immediately as it is formed, by the ion-exchange chromatographic principle. A provisional patent has been registered for this new process (3).

The aim of this work was to investigate the effect of scaling up the batch chromatographic reactor-separators on the extent of the sucrose conversion to dextran, and to derive a set of recommendations which would assist in the successful future application of the SCCR systems as semicontinuous chromatographic reactors separators (SCCRS). These increased scales of operation require larger amounts of purified dextransucrase and it was therefore necessary to investigate and select the appropriate processes that would purify the fermentation broth successfully, cause minimum enzyme denaturation, be economically viable, and provide successful integration between the fermenter and the reactor-separator.

11.2 Dextransucrase Purification

11.2.1 Introduction

The crude dextransucrase (from the fermentation broth), contains a number of impurities, such as dead cells, dextran, fructose, leucrose, mannitol, proteins and other oligosaccharides; small quantities of levansucrase and intervase are also present. The cells present result in mechanical problems and fouling of the stationary phase and the other impurities interfere in the HPLC analysis. The mannitol and fructose present prevent accurate quantification of the fructose formed, and similarly, the leucrose present overlaps the unreacted sucrose. The dextran and other proteins prevent the successful evaluation of the molecular weight distribution of the newly formed dextran. It was therefore considered necessary to purify the crude enzyme. A number of methods have been published (Chapter 2) for the purification of dextransucrase, and the selection of the proper ones was based on:

- the scaling up potential to obtain high purification throughputs;
- the principle of operation, process time and number of stages, to minimise enzyme denaturation due to large shear forces and heat generation;
- the economics and extent of purification, to select the required number of purification stages that give the necessary enzyme purity; any additional purification would result in further enzyme activity losses and additional capital and operating costs.

The separation processes judged to meet most of the above requirements were ultrafiltration, cross-flow microfiltration and centrifugation. A more detailed investigation of the above processes is described in the following sections.

Because of the large scale of operation, the enzyme requirements were high (typically 3600 DSU.hr^{-1} for a 5.4cm id x 230cm long batch reactor-separator), and a number of crude dextransucrase batches had to be bought from outside suppliers. For reference purposes these batches are identified as follows:

- FISONS batch; this was the original batch used. It was supplied by FISONS Pharmaceuticals (Holmes Chapel, Cheshire) and was produced by ABM (Wales). Its initial activity was 270 DSU.cm^{-3} and had a milky white colour;
- STURGE batch; also supplied by FISONS but it was produced under contract by STURGE (Selby, Yorkshire), having an initial activity of 170 DSU.cm^{-3} and a brown colouration.
- ASTON batch; produced in a 10 l New Brunswick fermenter at the Department of Chemical Engineering, Aston University. The fermenter was commissioned at the end of this research program and therefore this batch was used only in some of the purification studies. Its initial activity was 130 DSU cm^{-3}, and was also white.

11.2.2 Cell Removal by Centrifugation

To provide a basis for measuring the extent of cell removal the results obtained from a MSE SS50 Ultracentrifuge (FISONS UK) were used as a reference, having a capacity of 240cm^3 and capable of centrifugal forces up to 77000g. It has been found that at high centrifugal forces and/or long processing periods there are increasingly large activity losses in the supernatant, partly due to enzyme denaturing from the large shear forces and partly due to enzyme removal with the cells. The reference operating conditions were therefore chosen to be 15 minutes and 10000g. At these settings there was an activity loss of up to 6%.

During the initial cell removal experiments a batch centrifuge (CU5000 KONTRON UK) was used, having a maximum capacity of 480cm^3 per batch and a maximum centrifugal force of around 2000g. At a process time of 15 minutes up to 75% of the solids were removed and the resulting activity losses were between 4 and 5%.

Because of the low centrifugal forces and capacity of the above centrifuge a continuous centrifuge was evaluated and used to purify the large quantities of enzyme required for the experimental program. The centrifuge was a New Brunswick Scientific LE model, capable of centrifugal forces up to 50000g. Over 90% of the cells were removed from the enzyme by passing the fermentation broth once through at 30000g and a flow rate of 5000cm^3 hr^{-1}. The low processing time resulted in a reduced activity loss of only 3%. The only limitation of this type of continuous centrifuge is the need for dismantling approximately every 2 hours to clean the cylinder. This would present additional problems in maintaining sterile conditions and it could limit its applications in the cell removal of intra-cellular enzymes. A centrifuge with continuous flow of the two layers would be preferred.

There was no temperature control in the centrifuge but because of the short residence time it is believed not to be necessary providing the initial broth is at about 5^{o}C. Although the very small amount (< 10%) of cells still present could have some effect on the life expectancy of the membranes used in the subsequent purification stages, any additional centrifugation would result in higher activity losses.

11.2.3 Removal of Non-solid Matter by Membranes

11.2.3.1 Introduction

Because of their specific selectivity, negligible heat generation and low energy costs, membranes are used increasingly in the concentration and purification of proteineous solutions. Although the actual effect of proteins on the mechanism around

and inside the membrane layer is not well known and still under dispute various workers have made considerable progress in the use of membranes (238). The aim of the work was to remove the low molecular weight impurities such as leucrose, mannitol and fructose at sufficient throughputs and with minimum activity losses.

Jackson and Stewart (239) reported a molecular weight of 171000 daltons for the dextran sucrose, while Ebert and Schenk (157) claim that the dextransucrase in association with dextran has a molecular weight of 280000 daltons. According to the literature membranes have only been used to concentrate the dextransucrase and not for its purification. Kaboli (170) used an Amicon 402 ultrafilter fitted with an XM100A (100000 daltons cut-off) flat disc membrane to obtain a 30 fold concentration. Monsan and Lopez (174) used a Sartorious SM 12134 filter unit to concentrate the enzyme from 24.5 to 93 DSU.cm^{-3}.

Robyt and Walseth (169) used an Amicon ultrafiltration cell having a PM-10 (10000 daltons cut-off) membrane to get a 25 fold concentration increase. Zafar (240) in his experimental work proposed the use of an Amicon ultrafiltration membrane with a 30000 dalton normal molecular weight cut-off.

In this work during the ultrafiltration operation the centrifuged fermentation broth was used and the low molecular weight (< 500) impurities were removed in the permeate and the dextransucrase was kept in the retentate. For comparison purposes and in view of a better process selection the more novel cross-flow microfiltration process was also evaluated; theoretically the large pore sizes (0.2 μm) of these membranes should allow all the non-solid matter, including the enzyme, through in the permeate.

The optimum operating conditions were obtained experimentally and the manufacturers figures were only used as initial guesses because of the varying solution concentrations, pressure drops and particularly the proteineous nature of the mixture. A general equipment layout for both the ultrafiltration and cross-flow microfiltration operation is shown in Figure 11.1.

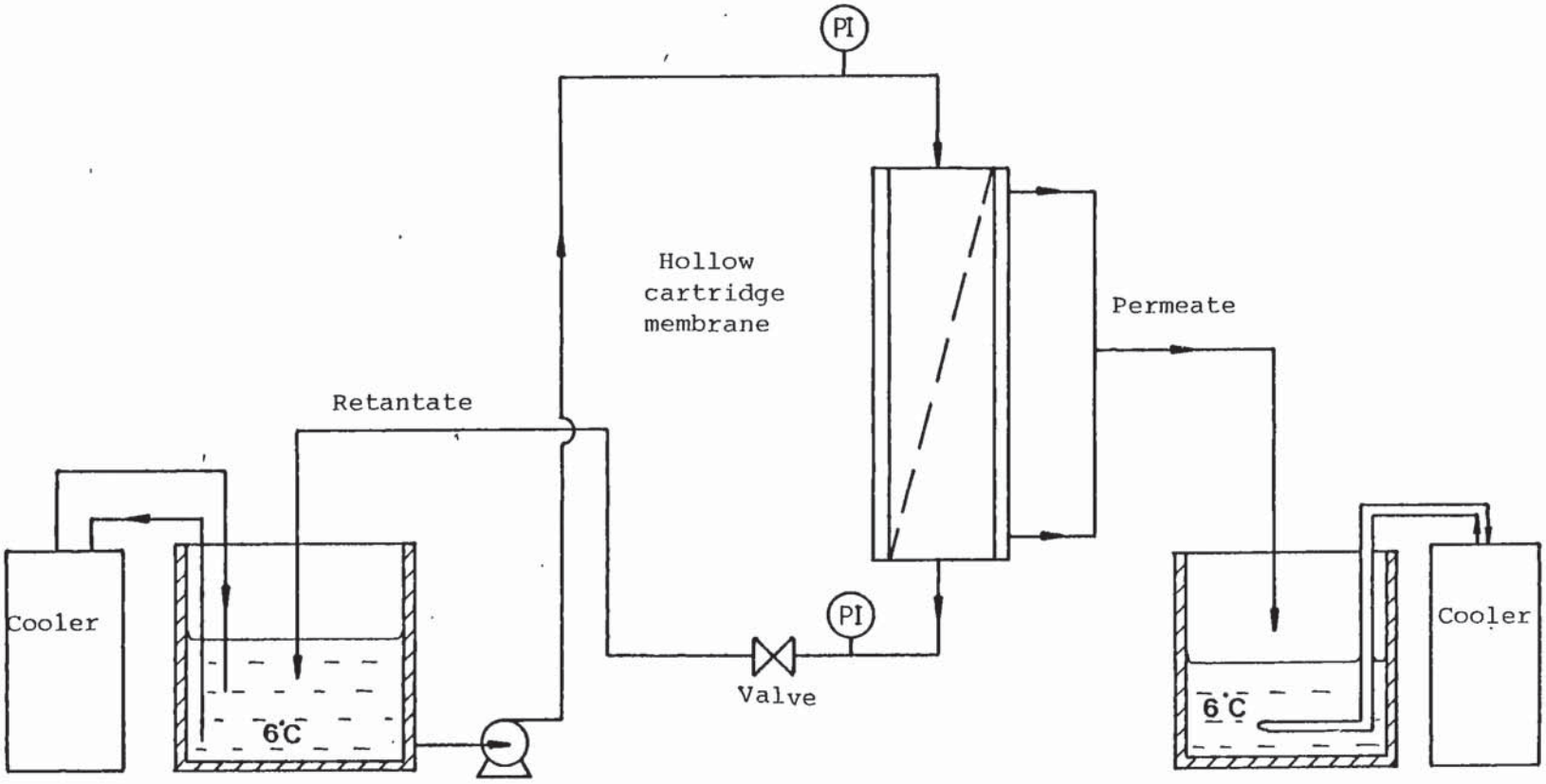
The application of membranes in enzyme purification has been found to result in substantial activity losses, sometimes in excess of 50%. These losses could be due to adsorption and denaturation or due to enzyme permeation through the pores and entrainment. Adsorption losses occur due to the formation of finite non-covalent bonds with the membrane, and are a direct function of membrane surface area. Its effect is increased in dilute solutions. Denaturation can occur as a result of shear fields created during the enzyme processing, the presence of air-liquid interfaces due to the unfolding of the enzyme molecule, and also due to plating out in stainless-steel surfaces (134). Retention of a complicated solute like an enzyme is not only a function of molecular size and shape but of the enzyme nature, ie. enzyme molecules tend to aggregate and thus increasing their effective molecular size. Membrane manufacturing is still a developing area, the available membrane range is limited, and the various manufacturers tend to follow different membrane characterisation procedures. Therefore the selection of the right membrane type is very difficult and usually requires experimenting.

11.2.3.2 Ultrafiltration Studies

Because of the increased enzyme requirements the Amicon (Stonehouse, Glos) HP10P30-20 hollow fibre cartridge was used. The cartridge had a total membrane surface area of 0.9m^2 and a 30000 daltons cut-off.

It was connected onto an existing rig in a similar arrangement to the one shown in Figure 11.1. The rig consisted of two stainless-steel tanks which were lagged, a Stuart Turner centrifugal pump and a Filterite inline filter to remove any fine solids present. The system was operated at 7 kNm^{-2} back pressure and an overall pressure drop of 62 to 70 kNm^{-2}. CHURCHILL (Middlesex) and GRANT (Cambridge) cooler units were used to maintain the permeate and retentate at 6^{o}C to prevent any activity losses due to high temperatures. The diafiltration mode of operation

Figure 11.1: Membrane purification equipment layout



was followed where deionised water was added at three times the volume of the initial enzyme volume and the operation was carried out until the solution volume reached the initial enzyme volume which was 8 litres for all the purification runs. The diafiltration operation was repeated until the required purification levels were reached. Throughout the operation the pH was kept around 5.3. When only deionised water was used to rinse the membrane out during the initial period, the permeate rate was around 500 cm^3 min^{-1}. Over the purification period the permeate flow rate was dropped to an average of 105 cm^3 min^{-1}. The proteins present are believed to form a gel layer on the membrane surface and/or partly block the pores, thus reducing the selectivity and permeate rate. This membrane fouling from the proteins is a common phenomenon in biotechnological applications and presents a major area for further development. At the end of each run the membrane was washed with 0.1 M NaOH for 20 minutes from each side in turn, and then flushed through with deionised water for 15 minutes. This washing procedure was sufficient to get the initial permeate rates of 500 cm^3 min^{-1} when only deionised water was used. The membrane cartridge was stored in deionised water containing 0.02 $g.l^{-1}$ of sodium azide. From the purification results (Table 11.1) it can be seen that on average over 90% of the non-solid impurities can be removed. The membrane fouling is believed to cause the large reduction in % impurity removal. For the first three batches four diafiltrations were sufficient to remove over 93% of all the major impurities, although there was a progressive reduction in the purification achieved from the first batch to the third. For the fourth batch (STURGE 2) after five diafiltrations the impurity removal was still below 90%. In the fifth batch (STURGE 3) it was decided to carry out six diafiltrations, and although the purification levels were better than the previous batch they only just managed to exceed the 90% level. These results indicate clearly that although the ultrafiltration cartridge can be used to separate successfully the non-solid matter of the fermentation broth, it is very prone to fouling; some of the proteins present are trapped within the membrane layer and thus alter the membrane selectivity. The

results also indicated excessively high activity losses. These increased from around 43% to 49.7% when the number of diafiltrations increased from 4 to 6. Since the temperature was kept below 6°C and the pH at 5.3, this enzyme denaturation is believed to be mainly due to the shear fields present. The ultrafiltration operation involves a continuous recycling of the retentate through the narrow hollow membrane tubes, which form the ultrafiltration module, thus generating considerable shear forces. The main source of shear formation is attributed to the centrifugal pump used. The centrifugal pump also resulted in foam formation which caused additional activity losses due to the uncoiling of the enzyme molecules at the liquid-gas interfaces (134). As expected the activity losses increased with increasing process time. It is therefore proposed to use a peristaltic pump, preferably with two heads operating out of phase, to reduce these activity losses and also minimise the pressure fluctuations due to the pulsations. In addition to that the pH must be kept within 4.6 and 5.4, the operating temperature as close to 1°C as possible, and the process time must be reduced, ie. using a larger ultrafiltration cartridge such as the AMICON H53P30-20, having a total membrane area of $5m^2$.

11.2.3.3 Cross-flow Microfiltration Studies

The recent increased reported (241, 242) interest in the application of cross-flow microfiltration for the removal of particles and emulsions led us to evaluate such a system for the dextransucrase purification. An ENKA LM2N06 module (marketed by APV Membrane Processes Ltd, Crawley) was used, having a total membrane area of $0.04m^2$. The module had a total of 85 capillaries, each of 0.06cm id and 35cm long made of hydrophilic membrane having 0.2 micron pores and it was connected in a similar arrangement to that shown in Figure 11.1.

Table 11.1: Ultrafiltration Results Obtained from the Amicon HIOP30-20 Cartridge

Batch	Total Number of Diafiltrations	Total Volume of water added (l)	Total Process time (min)	Total Activity loss (%)	% impurity removal		
					Leucrose	Fructose	Mannitol
FISONS 1	4	96	970	42.7	97.7	98.4	99.0
FISONS 2	4	96	980	42.9	95.1	94.6	98.2
STURGE 1	4	96	990	43.2	93.5	95.3	95.9
STURGE 2	5	120	1230	47.0	86.5	89.7	90.3
STURGE 3	6	144	1480	49.7	89.3	90.1	91.5

Table 11.2: Cross Flow Microfiltration Results

Batch	Volumes added (l)		Total process time (min)	Enzyme recovery as % activity in		Total non-solid impurities as a % in	
	Water	Enzyme		Permeate	Retentate	Permeate	Retentate
ASTON	18	6	1200	9	75	71	29

Initially an MPL L150 pump, fitted with a single L-PC50 head, was used capable of operating within the required flow rates which were 1 to 5 l min^{-1}. Operating at these flow rates however resulted in high pressure drops without any sufficient increase in the permeate rate, and prohibited the use of any back pressure. Also the single head arrangement gave pressure fluctuations of around 33 kN m^{-2}. It was therefore decided to use an MPL PG136X pump fitted with two heads (operating out of phase), and operating at 800 cm^3 min^{-1}.

A 35 kNm^{-2} back pressure was used giving an overall pressure drop of 138 kNm^{-2} + 14 kNm^{-2} due to the pulsations, which was just below the maximum operating pressure drop of 160 kNm^{-2}. Eighteen litres of deionised water were added to six litres of enzyme and the operation continued until the final concentrated retentate had a volume of around six litres. The operating temperature was 15°C and the retentate pH was kept at 5.3. The enzyme used was the ASTON batch as obtained from the fermenter and without any pretreatment, ie. containing all solid matter and non-solid impurities.

Initially the membrane was prewetted for 5 minutes with a mixture of 70% isopropyl alcohol and 30% water, followed by a water wash. The initial permeate flow rate (water only) was 110 cm^3 min^{-1}, and during the enzyme purification it dropped to an average of 15.5 cm^3 min^{-1}. When water only was used the permeate flow rate increased to 107 cm^3 min^{-1}, ie. almost to its original value. This indicated that only a very small number of enzyme molecules or cells are trapped within the membrane due to the larger pore sizes, and also that the drop in permeate flux during purification was mainly due to the gel layer formation by the proteins and also possibly due to increased diffusion difficulties of the small molecules through the concentrated cell and protein suspension to the membrane surface.

At the end of the run a washing procedure similar to the one used for the Amicon H10P30-20 module (Section 11.1.3.2) was followed.

In hollow fiber cartridges, operating either as ultrafilters or cross-flow microfilters, the high fluid velocities inside the membrane tubes sweep particles tangentially across the membrane surface. This high velocity theoretically minimizes the solids layer build up and maintains high permeate fluxes compared to static filtration. The ultrafiltration and cross-flow microfiltration results however indicated that this continuous "cleaning" effect was not sufficient. The Enka microfiltration unit offers an additional facility where the permeate is at certain intervals back flashed through the membrane, for a few seconds in the opposite direction to the permeate without interrupting recirculation, removing most of the gel layer from the membrane surface (244). The reduced permeate fluxes observed during the purification indicated that this continuous gel layer removal technique does not overcome the problem completely. Although the cross-flow principle removes the deposited enzyme ("static" gel layer) the incoming enzyme still tends to be deposited due to the increased concentration gradient. Therefore a continuous deposition and removal takes place which effectively leads to a "dynamic" moving gel layer. This reduction in flux through a simple cross-flow filter due to the so called "particle polarisation" has also been identified by Wakeman (137), and a possible way of minimising this effect is by applying a pulsating electric field.

Originally it was decided to use microfiltration as an alternative to centrifugation ie. for cell removal only, followed by ultrafiltration to remove the other non-solid impurities from the enzyme and concentrate the final enzyme mixture. The experimental results (Table 11.2) however showed that only a very small amount of the enzyme is removed in the permeate, approximately 9% in activity terms, and the remaining is in the retentate. This could be attributed to:

- the globular nature of the enzymes which tend to aggregate so that their effective molecular size is much larger than the native molecule;
- the increased effective size due to the associated dextran;

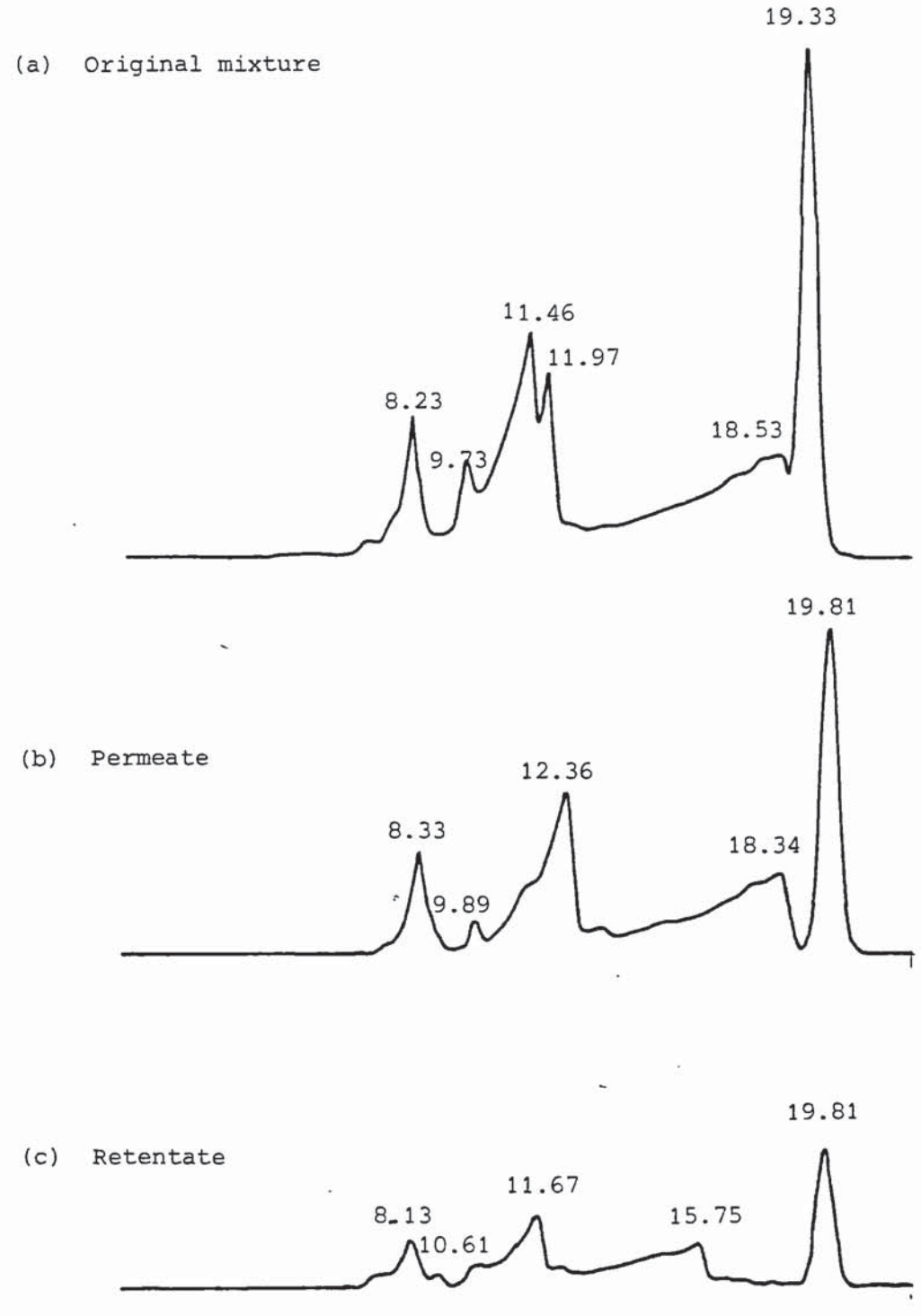
- the ionic changes in the enzyme mixture and the electrokinetic properties of the membrane;
- and finally, although the dextransucrase is an extracellular enzyme it is possible that a greater proportion of it is still attached to the surface of the cells and moves with them.

Because of the low permeate flux the operation took 1200 minutes and only one concentration was carried out. An HPLC analysis, using the BIO-RAD Aminex HPX87C calcium changed column, showed that over 70% of the non-solid impurities were removed in the permeate and also indicated (Figures 11.2 a,b,c) that the Enka membrane was not selective to any of these impurities.

Centrifugation tests, using the reference MSE SS50 ultracentrifuge, showed that the permeate was free of cells. The purification was carried out over two and a half working days and to compare the activity losses a sample of the initial unpurified enzyme (pH 5.2) was kept in a flask for the same period of time at 15^{o}C. At the end of the purification an activity test showed that this reference sample retained 87% of its activity, while a total of 84% of the activity was recovered in the processed enzyme; (9% in the permeate and 75% in the retentate). A number of conclusions can be derived from these activity results and can be summarised as follows:

- approximately 13% of the activity losses were due to the enzyme denaturation resulting from keeping the enzyme solution at relatively high temperature (15^{o}C) for over 60 hours and can be reduced further by operating at lower temperatures (between 1 and 5^{o}C) and maintaining the pH within 4.5 and 5.4;
- the application of the double head positive displacement pump minimised the enzyme denaturation (only 3% activity losses) by offering low shear and foam free operation;

Figure 11.2: HPLC analysis of the original mixture and products of the microfiltration enzyme purification



- by purifying first the impurities from the fermentation broth containing the cells and enzyme, the enzyme remains stable for long periods, possibly because most of it is still attached to the surface of the cells, or due to extra enzyme stability in concentrated solutions and/or due to the presence of the protective dextran (166);
- the low activity losses and the fact that at the end of the run the permeate flux almost returned to its original value showed that the membrane fouling was kept to a minimum.

By carrying out a second or third concentration well over 90% of the impurities are expected to be removed and the activity losses are not expected to exceed 15%. This however would require increased permeate fluxes and can be achieved either by using a larger microfiltration cartridge or using eight or more of these LM2N06 cartridges in parallel.

11.2.4 Downstream Process Integration for the Purification of Dextransucrase

The criteria for the selection of the purification processes were minimum enzyme denaturation, extent of purification, low capital and operating costs, scaling up potential, ease of operation and compatability for successful integration with the fermentation and reaction-separation (dextran production) stages.

Enzymes are generally sensitive to pH and temperature changes, shear forces and air-liquid interfaces, ie. in the presence of foams or bubbles. The various enzyme solutions used were kept frozen and were left at room temperature to thaw before using them. During thawing, activity losses between 5 and 15% occurred. Although enzymes are expected to be stable at sub-zero temperatures it has been reported (166) that dextransucrase lost over half of its activity when stored at -15^{o}C for two days. When

Table 11.3: Enzyme Stability during Purification - Aston Batch

(The results correspond to similar solution concentrations)

Enzyme Solution	Activity (DSU.cm^{-3})	Loss in activity (%)
Original enzyme	112	0 (basis)
Centrifuged	109	2.7
Microfiltered	107	4.4
Microfiltered & centrifuged	101	9.8

Table 11.4: Enzyme Sensitivity to Storage Temperature - Aston Batch (the results represent the activity testing of the above samples carried out 24 hours later. The activities correspond to similar solution concentrations)

Enzyme Solution	Storage Temperature ^{o}C	Activity DSU.cm^{-3}	Loss in activity (%)
Original	5	108	3.6
Centrifuged	5	89	20.5
Microfiltered	5	103	8.0
Microfiltered & centrifuged	5	80	28.6
Original	20	96	14.3
Centrifuged	20	77	31.2
Microfiltered	20	90	19.6
Microfiltered & centrifuged	20	69	38.4

the unpurified FISONS and STURGE batches were left for 24 hours at 5°C in the refrigerator activity losses of up to 8 and 12% occurred respectively. The FISONS and STURGE batches were purified by removing the cells first using centrifugation and then diafiltration; activity tests on the purified batches showed that for every 24 hours they were kept at 5°C, the activity was reduced by up to 20% and 43% respectively. This presented an additional problem in the reaction-separation studies since it was very difficult to maintain constant enzyme strength and thus obtain reproducable results. The STURGE batch was also more sensitive and unstable during both storage and handling. The above results indicate that after removing the cells the enzyme solution becomes more unstable even at low temperatures. To verify this a comprehensive activity analysis was carried out on unpurified, centrifuged, ultrafiltered, ultrafiltered and centrifuged samples of the Aston enzyme, both immediately after purification (Table 11.3) and after storing the samples over 24 hours at 5°C and at room temperature (Table 11.4). The CU 5000 KONTRON batch centrifuge was used for the cell removal. An analysis of the results led to the following findings and conclusions:

- cell removal using centrifugation resulted in just under 3% of activity loss;
- after cross-flow microfiltration (one concentration only) the activity losses were 4.5%;
- a complete purification, where the microfiltration was followed by centrifugation, resulted almost in a 10% activity loss;
- a 24 hour storage of the fermentation broth at room temperature resulted in an over 14% activity loss; this loss can be reduced to under 4% by storing the unpurified enzyme at 5°C. All solutions lost 12 to 14% more of their activity when they were stored at 20°C instead of 5°C;
- the microfiltered enzyme mixture (containing the cells) retained over 96% of its activity when stored at 5°C, and over 84% when stored at room temperature for 24 hours;

- after 24 hours the centrifuged enzyme lost over 18% of its activity when stored at 5°C and over 29% when stored at 20°C. The high centrifugal forces and the associated shear remove any enzyme attached to the cells and also cause denaturation. The results of the microfiltered and centrifuged samples show similarly high enzyme losses, ie. over 20% and 31% respectively;
- a comparison of the results immediately after centrifugation and 24 hours later indicated that the enzyme becomes very unstable when the cells are removed. The cell removal alters slightly the enviroment in which the enzyme was grown and this possibly leads to this instability;
- this cell removal effect is supported by the corresponding results of the ultrafiltered samples, where the presence of the cell in the enzyme medium resulted in a lot lower activity losses.

The application of membranes and centrifugation for the enzyme purification offers a relatively simple operation which can be easily automated, involves no heat generation and relatively low shear forces, giving reproducable and highly pure enzyme solutions (whose purities can be increased further after carrying out an optimisation between the activity losses and purification levels); the scaling up is simple and presents no variations in the purification characteristics and the capital and operating costs are low. The disadvantages are the membrane fouling by proteins, cells or silicones (present as antifoams), and the sterilisation and containment difficulties. The centrifuge bowl must be sterilised every time after cleaning and microfiltration cartridges made of ceramic materials would be preferred to allow steam sterilisation. The application of centrifugation and ultrafiltration or microfiltration would remove the cells and the other non-solid impurities but not the enzyme associated dextran. To provide accurate characterisation of the dextran produced from the chromatographic reactor separator this associated dextran and any remaining proteins can be purified using polyethylene glycol

(PEG) precipitation (177). Although this technique has been carried out on a small scale (240), it is difficult to scale up, time consuming and relatively expensive, and its overall efficiency and reproducibility is questionable. Concentration gradients usually exist between the two layers and the final product contains some PEG which introduces a new problem in the dextran characterisation. The activity losses are typically around 50% (240) and from the production point of view it was therefore considered not to be necessary. Summarising, two alternative techniques have therefore been investigated for the purification of the dextransucrase:

- the CUF process, where the cells were removed first using the continuous New Brunswick LE clarifying centrifuge, and the non-solid impurities were separated using the Amicon H10P30-20 ultrafiltration cartridge;
- and the MFC process, where the non-solid impurities were removed first using the ENKA LM2NO6 cross-flow microfiltration cartridge, and the cells were then separated using the continuous New Brunswick LE clarifying centrifuge.

Both techniques provide satisfactory separation efficiency and involve similar capital and operating costs. In the CUF process the activity losses were very high due to the centrifugal pump used and due to the initial cell removal, and the ultrafiltration cartridge was particularly prone to fouling. In the MFC process, although some of the enzyme is lost in the permeate, the enzyme denaturation is lower and therefore it offers higher enzyme recovery levels than the CUF. The Enka microfiltration module is also a lot less susceptible to fouling. The purified enzyme is denatured during storage and is best used soon after purification. Although the enzyme denaturation during centrifugation is small due to the low process time, its long term stability is reduced substantially. This can be contributed to two reasons; the deformation of the enzyme's

original structure due to the high shear fields and its removal of the outer surface of the cells. To minimise the activity losses it is therefore proposed to remove the non-solid impurities first using microfiltration and then store the partially purified enzyme. The very low centrifuging process time enables the cell removal to be carried out just before using the enzyme. In view of the above merits the MFC dextran purification process is therefore recommended.

11.3 The Scaling-up of Chromatographic Biochemical Reactor-Separators

11.3.1 Introduction

In the existing industrial process (FISONS Pharmaceuticals, Holmes Chapel), dextran is formed in a conventional enzyme batch reactor, where sucrose solutions are innoculated with a Leuconostoc Mesenteroides culture and the dextran synthesis is carried out without any process control of the dextran chain length. At the end of the fermentation, ethanol precipitation removes the dextran formed from the reaction products and an acid hydrolysis brakes the larger dextran molecules down. For a more efficient and economical operation dextran with a high (over 150000 daltons) and narrow molecular weight range is preferred as starting material for the hydrolysis. The final clinical dextran is produced after a series of ethanol precipitation stages which reduces its molecular weight within the specified limits. Using a chromatographic reactor-separator sucrose can be converted to dextran and fructose and at the same time the reaction products can be separated simultaneously. This would minimise the need for additional separation steps, the acceptor fructose can be removed immediately from the reaction mixture thus giving more high molecular weight dextran and can be marketed separately, and it would provide better control of the reaction. This alternative dextran synthesis has been carried out successfully on glass 1 cm id by 200cm long and 2 cm id by 175 cm long batch chromatographic columns (240) where high sucrose convertions have been

achieved. A characterisation (3) of the dextran produced by the conventional batch reactor and the above chromatograph reactor-separators (Table 11.5) showed that the conversion of high concentration (over 15% w/v) sucrose feedstocks on the chromatographic reactor-separators gives a lot more high molecular weight dextran (over 156980 daltons) than the conventional batch process due to the simultaneous removal of fructose from the reaction mixture. These results led us into pursuing this novel process further and to investigate the effects of scaling up on the extent of sucrose conversion.

11.3.2 Equipment Description

The experimental programme was concentrated on the scaling up of batch chromatographic reactor-separator columns, with special emphasis on the effects of the materials of construction, packing density, and the nature of the enzyme batches on the sucrose conversion.

Dextransucrase was added in the eluent stream (deionised water) at various strengths since it has not been immobilised successfully yet. A number of alternative batch chromatographic systems, all packed with the calcium charged KORELA VO7C resin (Chapter 6) were used. These were:

- the 5.4cm id x 230cm long stainless steel system. This system was used during the initial part of the experimental work and it consisted of three 5.4cm id x 30cm long stainless steel columns and three of the SCCR7 columns (5.4cm id x 64.5 cm effective height) directly linked together (bypassing the valves) to minimise the dead volume. The shorter column also acted as a guard column. The overall voidage was 0.31.
- An MPL Series II metering pump with a maximum flow rate of 20 cm^3 min^{-1} was used for the eluent delivery and the enzyme was delivered to the column

Table 11.5: Dextran Characterisation Results (3)

Reactor	Sucrose concentration % w/v	% dextran over 156980 daltons
Conventional batch	2	97.62
Reactor-separator	2	89.04
Conventional batch	15	68.98
Reactor-separator	15	80.7
Conventional batch	20	43.65
Reactor-separator	20	77.35

inlet using a second short stroke Series II metering MPL pump having a maximum flow rate of 3.8 cm^3 min^{-1}. The eluent was kept in a 20 l glass aspirator and the enzyme in a 5 l plastic container placed in an ice bath to minimise the activity losses. The columns were located inside a heated enclosure and the operating temperature was controlled using an air heater (Chapter 5). The product streams were collected in a 20 l glass aspirator placed in a hot bath (60°C) to quench the enzyme present and stop further conversion of any unreacted sucrose;

- the 5.4cm id x 30cm long stainless steel columns. The first column had a voidage of 0.41 and the second 0.56 and they were used separately to evaluate the effect of voidage on conversion. Their mechanical design was similar to the design of the twelve SCCR7 columns (Chapter 5);
- the 5.4cm id x 30cm long glass column, having a voidage of 0.46. Glass wool was fitted at the column's inlet and outlet to support the packing. The operating temperature of this column and of the two 30cm long stainless steel columns was controlled by placing them inside the heating enclosure;
- the 5.4cm id x 175cm long jacketed glass column. Glass wool was also used as packing support and the voidage was 0.38. The column design was similar to the 1cm id x 175cm long glass columns (240), and the column was packed to within 1cm from the top. The inlet and outlet liquid flow rates were controlled so that the packing was always immersed in water to prevent drying, and having this open top arrangement any air in the inlet stream was removed minimising air bubbles from being formed inside the packing.

The smaller batch systems used for the comparison (1cm id x 200cm and 2cm id x 175cm) were also jacketed and were packed with the LEWATIT TSW 40 calcium charged resin having a similar size range to the KORELA resin used in this work.

A general equipment layout (not applicable to the 5.4cm id x 230 long stainless steel system), is shown in Figure 11.3. A ten litre glass aspirator was used to contain the eluent-enzyme mixture and it was kept in an insulated stainless steel tank. The tank was filled with ice to maintain the temperature below 5°C thus preserving the enzyme activity. For minimum shear forces and accurate flowrate control a COLE-PARMER peristaltic pump (BDH Chemicals Ltd, Atherstone) was used, fitted with two pump heads controllable between 0.21 and 21 cm^3 min^{-1}. The pump heads were fitted with the shear and thermal resistant Masterflex Norprene (BDH Chemicals Ltd) tubing which operated efficiently for over 600 hours. One of the pump heads was used to deliver the eluent to the column's inlet and the other at the column's outlet delivering the product stream to the collection reservoir. This arrangement was chosen to provide uniform flow through the column. A Churchill (Churchill Instrument Co, Perivale, Middx) heater-cooler was used to circulate the water in the jacketed outer section of the column (applicable to the 5.4cm id x 175cm long glass column only). When the system was not in operation the circulator was switched to the cooler mode maintaining the column temperature at 2°C thus minimising the denaturation of the enzyme present in the column.

A fraction of the product stream was pumped, using a TECHNICON (London) peristaltic pump, through a Jobling Refracto Monitor, to detect the elution of the reactants, and the results were plotted on a Servoscribe 1S Venture RE (Phase Sep, Queensferry, Flint), chart recorder. The product stream was collected in a ten litre glass aspirator which was kept in a water bath inside a stainless steel tank. The bath temperature was maintained at 60°C to quench the reaction products thus deactivating the enzyme present and preventing further conversion of any unreacted sucrose.

To obtain the desired operating temperature the circulator was set to the heating mode and the required temperature was chosen. The eluent and enzyme delivering tubing was wrapped around the column to heat the solution up to the operating temperature. A photograph of the equipment is shown in Figure 11.4.

Figure 11.3: Batch chromatographic reactor-separator equipment layout

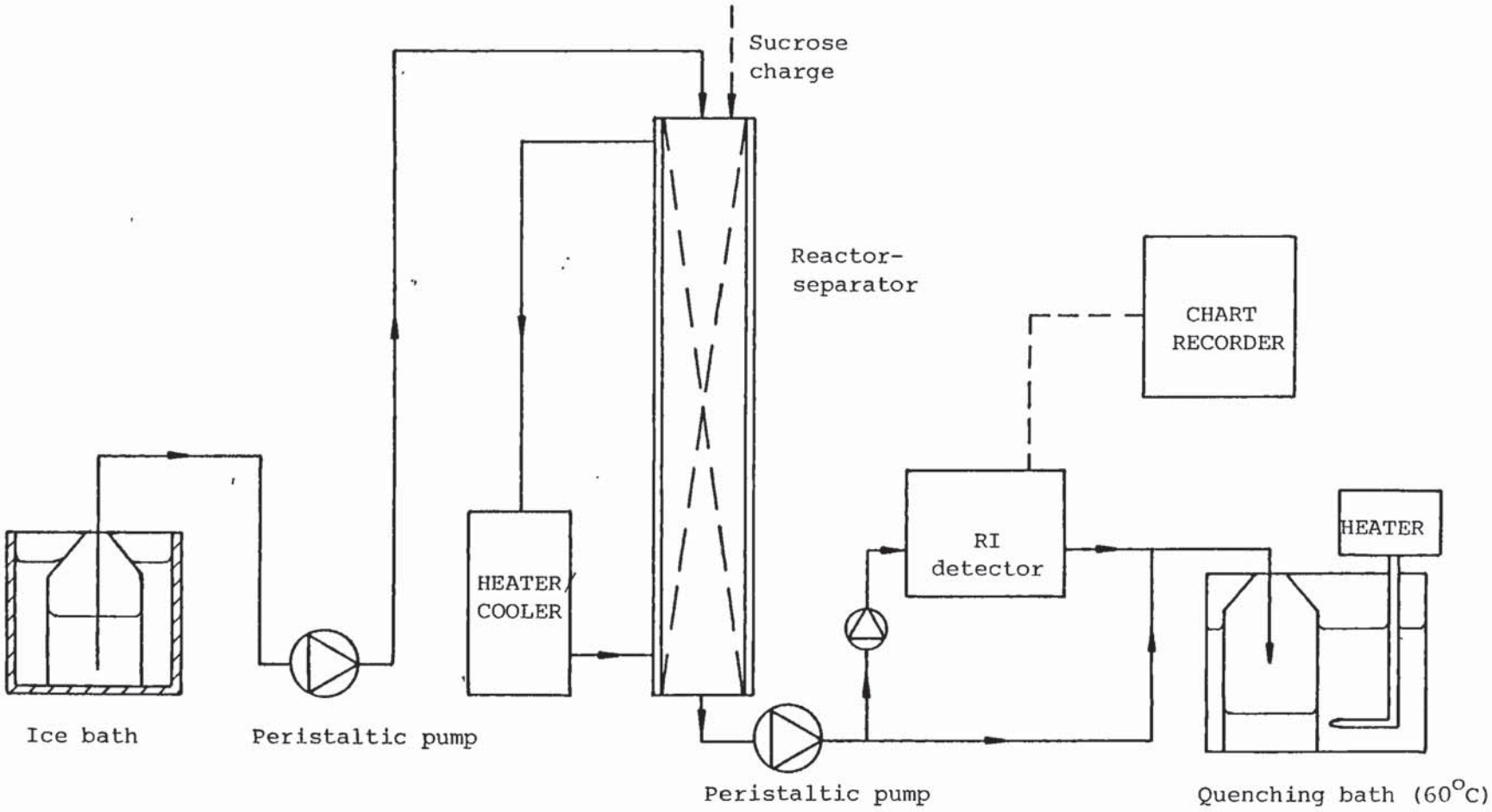


Figure 11.4: Photograph of a Chromatographic Reactor-Separator

Key:

1. Chart recorder
2. Eluent + enzyme tank and ice bath
3. product collector and quenching unit
4. Refractive index detector
5. Pump
6. Heater/cooler circulator
7. Chromatographic reactor-separator column (glass 5.4cm id x 175cm long)

7

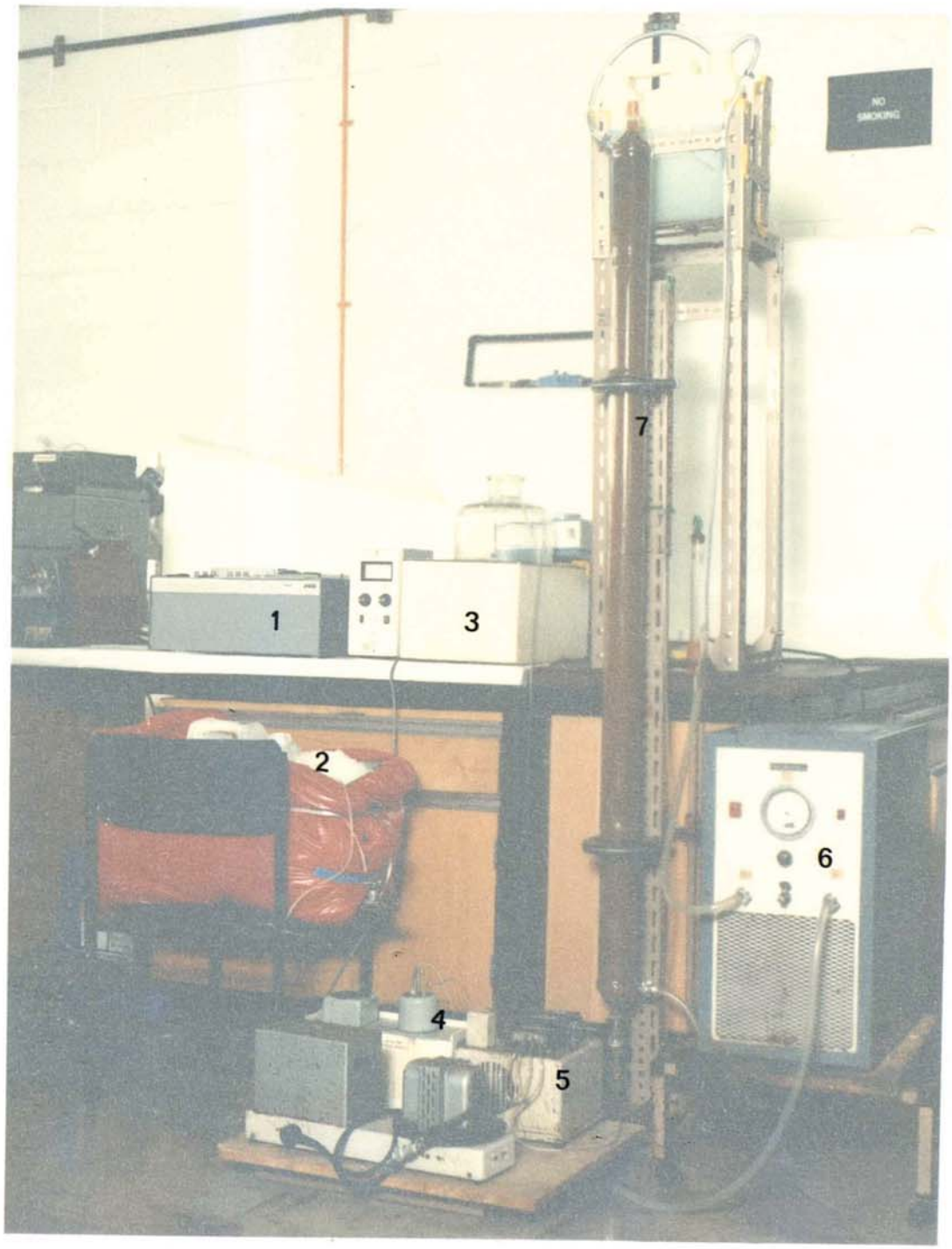
1 3

2

6

4

5


NO
SMOKING
1
2
3
4
5
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7

11.3.3 Experimental Technique

After packing each column a voidage determination was carried out following the procedure described in Chapter 6. In the 230 x 5.4cm id stainless steel system the desired eluent-enzyme composition was achieved by adjusting the stroke lengths of the corresponding metering pumps. For the other systems the required enzyme content of the eluent was obtained by adding the required enzyme volume in the eluent storage tank. This was performed at the beginning of each run and usually after carrying out an enzyme activity test.

Although the dextransurcase cannot be immobilised yet, it has been established (240) that after prolong enzyme passage through the packed column some enzyme is adsorbed on the resin. This "partial" immobilisation is believed to be possibly due to the physical entrapment of the relatively large enzyme agglomerates and their adsorption on the packing. An activity analysis of the resin obtained at the top, middle and bottom parts of the column showed that there was an activity gradient along the column indicating that more enzyme was adsorbed towards the column inlet.

Before passing any enzyme through the column, dextran, surcose and fructose charges were introduced at various eluent flow rates to obtain their relative retention times. To maintain a similar basis with the experiments on the 1cm id and 2cm id systems (240) an eluent flow rate was chosen which gave a sucrose retention time of approximately 4 hours. Then, the newly packed column was charged for approximately 40 hours by passing through only the diluted enzyme-eluent mixture. When the column was not used for a short period (ie. weekends), it was recharged for 3 to 5 hours. Before each run the required enzyme-eluent flow rate was chosen, and was pumped through the column for 1 hour, and the column heating procedure was switched on (ie. heated enclosure or heater circulator). When the operating temperature of 25^{o}C (3) was reached the enzyme-eluent delivery was stopped and the required volume of the sucrose

feed charge was introduced containing enzyme at a similar concentration to that used in the eluent stream. When the feed slug was completely immersed inside the packing the enzyme-eluent delivery was switched on again. During these switch overs extra care was taken to ensure that the packing was always covered by liquid. During operation the pH was checked frequently and since it was between 5 and 5.4 it needed no adjustment.

During operation the once through injection approach was followed, ie. only one feed charge was injected per run. At the end of the operation the volume collected was measured accurately, and by using the operation time, the exact eluent flow rate was determined. Because of the low product concentrations a sample of the products was concentrated 5 to 10 times by boiling and its composition was obtained using the HPLC analytical system. Using the HPLC results and the total product volume a mass balance gave the total fructose formed and the total unreacted sucrose. Knowing the total sucrose in the feed charge and also the total expected fructose for 100% conversion, the actual extent of sucrose conversion was obtained. A complete sucrose conversion theoretically gives 52.6% fructose (154), therefore for a 210cm^3 feed charge containing 2% w/v sucrose:

Total sucrose in feed = 4.2g

Fructose expected for 100% conversion = 4.2 x 0.526 = 2.21g

$$\% \text{ conversion based on surcrose disappearance} = \frac{4.2 - \text{sucrose in product}}{4.2} \times 100$$

$$\% \text{ conversion based on fructose formation} = \frac{\text{fructose in product}}{2.21} \times 100$$

The dextran results were not used in the calculations because the dextran associated with the enzyme and the presence of other protein impurities were interferring with the dextran peak. The feed charge volume was expressed as a percentage of the

total empty column volume and for comparison purposes (240) it was chosen to be around 4%. Each experimental run was defined with a set of four figures - for example, the set of 2-210-5-12 corresponds to sucrose content (% w/v), feed charge (cm^3), enzyme activity in eluent stream ($DSU.cm^{-3}$), and eluent flow rate ($cm^3\ min^{-1}$).

11.3.4 Experimental Results and Discussion

During the investigtion of the scaling up of the batch reactor-separators special emphasis was given to the system's reproducibility. Over sixty experimental runs have been carried out and a selection of the results of the more representative runs are listed in Tables 11.6 to 11.8, and used for the comparison. Most of the conversion results are expressed by tracing both the unreacted sucrose and the fructose formed. Both sets of values should be identical; analytical inaccuracies however, due to the presence of some impurities, resulted in some disagreement. Runs 1 to 10 were carried out within three months from the time the 5.4cm id x 230cm system was charged, using the purified FISONS enzyme batch. The eluent flow rate was about 12 $cm^3\ min^{-1}$ (corresponding to a 4 hour sucrose retention), and had an enzyme concentration of 5 DSU cm^{-3}. A $210cm^3$ feed charge was used, ie. 4% of the total empty system volume, having a 2% w/v sucrose concentration, ie. a total of 4.2g of sucrose. For the first four runs the conversion was obtained using the fructose formed and was on average 77.5%. In the following four runs the conversion was approximately 61% and was based on the unreacted sucrose. During the three months of continuous operation a progressive increase in the operating pressure was observed. In runs 7 and 8 the eluent flow rate was reduced to 9.8 and 8.6 $cm^3\ min^{-1}$ respectively to maintain the sucrose retention at around 4 hours. Theoretically, a reduction in the carrier flow rate should have increased the retention time. Because of its relatively large size the enzyme was expected to travel with the dextran through the void volume and be eluted completely. However, this

Table 11.6: Extent of Conversion using the 5.4cm id x 230cm long Stainless steel Reactor Separator

Run No	Experimental Code No	Enzyme used	% conversion	
			Based on sucrose disappearance	Based on fructose formation
1	2-210-5-12	FISONS	-	71
2	2-210-5-12	FISONS	-	77
3	2-210-5-12	FISONS	-	83
4	2-210-5-12	FISONS	-	79
5	2-210-5-12.6	FISONS	60	-
6	2-210-5-12.3	FISONS	62	-
7	2-210-5-9.8	FISONS	64.3	-
8	2-210-5-8.6	FISONS	57.9	-
9	2-210-5-12	FISONS	-	79
10	2-843-5-12	FISONS	-	47
11	2-210-10-11.9	FISONS	96.8	89
12	2-210-5-12	FISONS	87.6	68
13	2-210-2-12.7	FISONS	73	45
14	2-210-5-12.0	FISONS	87.6	68
15	2-210-5-12.6	FISONS	70	68
16	2-210-5-11.4	FISONS	71	69
17	2-210-5-9.2	FISONS	99	62
18	8-210-5-9.2	FISONS	52	21
19	20-210-5-8.3	FISONS	32	11.4

progressive increase in pressure drop and reduction in sucrose retention indicated that the enzyme was agglomerating and was gradually trapped on the resin blocking some of its pores. Usually, the relatively smaller sucrose molecules diffuse in and out of the resin pores and are retarded. Since however, some of these pores are gradually blocked by the enzyme agglomerates the sucrose molecules are retained less and travel faster through the column.

Zafar (240) in his experimental work reduced the enzyme concentration in the eluent stream to minimise the enzyme usage and claimed that a concentration within 0.8 and 1.6 DSU cm^{-3} was sufficient. A comparison of the above results with the ones obtained on the 1cm id x 200cm (run 2-6-1.2-0.21, Table 11.7) and 2cm id x 175cm (run 2-20-0.8-1-38) systems, operating at similar conditions, and bearing in mind the different resins used, shows similar conversion percentages (based on fructose) indicating that a scaling up of 1 to 4 to 29 times (based on cross-sectional area) has no significant effect on the extent of reaction. These 1 and 2cm id columns were packed with LEWATIT resin, but it is expected to behave similarly since it is of similar size range and in the calcium form. Although these results are very encouraging, in the 1cm id x 2cm id systems the enzyme concentration was over 5 times lower; the effect of the enzyme concentration on conversion will be considered later on. Runs 9 and 10, carried out on consecutive days, were performed to investigate the effect of increased feed charge size. When the feed volume was increased from $210cm^3$ (4% empty column volume and a total of 4.2g of sucrose) to $843cm^3$ (16% empty column volume and a total of 16.86g of sucrose), the conversion dropped from 79 to 47%. This reduction was in agreement with the results obtained by Matsen in his gas chromatography studies (186). An increase in feed charge from 4% (run 2-6-1.2-0.21, Table 11.7) to 10.7 (run 2-16-1.2-0.21) on the 1cm id x 200cm system reduced the conversion from 71.5 to 39.5% (based on fructose). The first impression of these results is that the large scale system (5.4cm id) is more efficient than the smaller 1cm id at high feed charge volumes.

Table 11.7: Results Obtained on the 1cm id x 200cm long and 2cm id x 175cm long Glass Reactor-Separators (240), for a Four Hour Sucrose Retention. The FISONS Enzyme was used and the Columns were packed with the Calcium Charged LEWATIT Resin

Rig	Experimental Code Number	Feed charge as % empty column volume	% conversion	
			Based on Sucrose disappearance	Based on fructose formation
1cm id x 200cm	2-6-1.2-0.21	4	94.8	71.5
1cm id x 200cm	2-16-1.2-0.21	10.7	45.2	39.5
1cm id x 200cm	2-6-0.6-0.22	4	60.9	42.7
2cm id x 175cm	2-20-0.8-1.38	3.7	79.3	

This however is arguable and is probably due to the very low enzyme strengths in the eluent stream of the 1cm id unit. In fact a comparison of runs 2-6-1.2-0.21 and 2-6-0.6-0.22 (Table 11.7) shows that a reduction in the eluent enzyme concentration from 1.2 to 0.6 DSU cm^{-3} reduces the conversion by almost 40%.

Runs 11 to 19 were carried out after repacking the first (5.4cm id x 30cm long) column of the 5.4cm id x 230cm system, and recharging it. The first three runs (11 to 13) were carried out consequently at decreasing eluent enzyme concentrations, (10, 5 and 2 DSU cm^{-3} respectively). This decrease in eluent enzyme concentration reduced the conversion from 96.8 to 87.6 to 73% (based on sucrose) or 89 to 68 to 45% (based on fructose) proving that the enzyme content of the eluent is important and an optimisation should determine its exact value. In this experimental programme it was chosen to be 5 DSU cm^{-3}. The following three runs (14, 15, 16) were carried out within three weeks during which time the system was used continuously. The results showed that over 70% conversions can be achieved, and also exhibited similar pressure increases and variations in the sucrose retention times to those of the earlier runs. This supported the claim made above, ie. increasing resin pore blockaging due to the progressive adsorption of enzyme. The fact that the equipment's performance was reproduced after repacking the first small column indicates that the early part of the packed bed acts as a depth filter for the enzyme agglomerates and any cells present. It is therefore recommended to use such a "guard column" arrangement in all reactor separators to maintain a stable operation. Finally, in the last three runs (17 to 19), the same feed volumes were injected but at increasing concentrations, ie. 2%, 8% and 20% respectively. This led to reductions in conversion from 62% to 21% to 11.4% (based on fructose formation) respectively. By keeping the charge volume constant ($210cm^3$) and increasing its concentration, the sucrose population per unit column volume is increased, therefore there are more sucrose molecules available competing for the same enzyme active sites, hence the chances for them to be reacted are reduced.

This can be verified by comparing the results of runs 10 and 18, in which the total amount of sucrose injected was the same, ie. 16.8g. Because of the broader feedband in run 10 ($843cm^3$) there were four times more enzyme sites available to catalyse the same sucrose molecules thus doubling the conversion, ie. from 21% for run 18 to 47% for run 10 (based on the fructose formed). Although larger feedbands of low concentration give higher conversions they have an adverse effect on the dextran yield. The reactor-separator results of Table 11.5 (3, 240) were obtained using feed charges of increased concentration and reduced volumes so that the total amount of sucrose injected was constant.

At low feed concentrations and high feed volumes the fructose formed in the front part of the feedband is interferring with the reaction mixture in the back end of the band terminating the dextran synthesis. This is supported by the results (Table 11.5) at 2% w/v which produced even less high molecular weight dextran than the corresponding batch process. As the feed concentrations increase and the feed band is reduced this interference is also reduced giving more high molecular weight dextran. Therefore, for the best product yield, small feed volumes of high concentrations should be used, and a repetitive injection technique can be employed provided that the extent of overlapping is carefully controlled. This would also result in higher throughputs and increased product concentrations. In addition to the above the feedband spreading along the system should be kept to a minimum.

To investigate the effect of materials of construction and voidage on the conversion the 5.4cm id x 30cm glass and two stainless steel colums were used. All columns were charged for approximately 15 hours using the same FISONS enzyme batch. The eluent flow rate was also chosen to give a 4 hour sucrose retention, and 4% (empty column volume) feed charges were used. The results are shown in Table 11.8. Because of the short charging period sufficient enzyme had not been adsorbed on the resin, resulting in lower than expected conversions. Since however, all columns were

Table 11.8: Effects of Materials of Construction and Voidage on Conversion

The FISONS enzyme was used

Experimental Code Number	Rig	Voidage	% conversion based on sucrose disappearance
2-28-5-1.95	5.4cm id x 30cm long Glass	0.46	42
2-26-5-2.3	5.4cm id x 30cm long Stainless steel	0.56	46
2-26-5-2.4	5.4cm id x 30cm long Stainless steel	0.41	35

Table 11.9: Reaction-Separation Results Obtained on the 5.4cm id x 175cm Glass Column

Experimental Code Number	Enzyme	% Conversion	
		Based on Sucrose disappearance	Based on Fructose Formation
2-80-5-8.6	STURGE	69	74
2-80-5-8.6	STURGE	70	67
2-400-5-7.8	STURGE	21	22

charged for the same time length using identical enzyme batches and concentrations, the relative conversion changes are used for the comparison. The results obtained on the two stainless steel columns show that the less dense packed (higher voidage) column gave substantially higher conversions (46% instead of 35%). At increased void volumes more enzyme is available in the column and better mixing of the sucrose molecules takes place. In chromatographic separations however the increased voidages have an adverse effect on the degree of separation, therefore a compromise has to be made between the separation efficiency and the extent of conversion of the chromatographic reactor-separator.

Robyt and Walseth (169) have reported that certain metal ions such as zinc, cadmium, lead, copper etc are inhibitory during enzyme production to various degrees. Stainless steel is an iron-nickel-chromium alloy that can oxidize to form soluble metal ions under aqueous conditions, and it was suspected that some of these soluble ions might poison the enzyme and inhibit the sucrose conversion. The results obtained on the glass column however were still not very clear. The conversion obtained (42%) was better than the one obtained on the low voidage stainless steel (35%), but lower than the one of the high voidage stainless steel (46%). Since the voidage of the glass column was between the voidages of the two stainless steel columns, this difference in the percentage conversion is probably due to the different void volumes and not due to the different materials.

In all the above systems the operation temperature was maintained using the heated enclosure, and none of the systems were fitted with a device to cool them down (below 5^{o}C) during shut-off, thus preserving the activity of the adsorbed enzyme. To investigate further the effect of materials of construction and enable visual observation the glass 5.4cm id x 175cm column was used. The column was jacketed and the heater-cooler circulator maintained the water in the outer annulus at 25^{o}C during operation and at 2^{o}C when not in use. The operating conditions were similar to the ones

used in the other systems, ie. 4 hour sucrose retention, 5 DSU cm^{-3} eluent enzyme concentration. In these experiments the STURGE enzyme was used and the feed charge was 2% empty column volume. The conversions obtained were almost identical to the ones on the 5.4cm id x 230cm stainless steel system (about 70%), using similar operating conditions. This showed that the different materials of construction did not have any significant effect on the conversion, bearing in mind the different enzyme batches used and system voidages. A five times increase in the feed charge (to 20% empty column volume) reduced the conversion down to 22%. The careful temperature control and the overnight cooling did not appear to have any positive effect on conversion, possibly due to the less stable STURGE enzyme. The system also exhibited pressure increases and retention time fluctuations, and there was a bacterial growth in the open column inlet. Since the use of sodium azide as a bacteriostat or high temperatures would denaturate the enzyme on the column, special care must be exercised to maintain sterile conditions.

11.3.5 Conclusion

The experimental results have shown that the fermentation broth can be purified successfully to give cell free and over 90% pure dextransucrase, using continuous centrifugation and cross-flow microfiltration. The enzyme stability is reduced after removing the cells. To minimise these activity losses it is therefore proposed to purify the enzyme first from the non-solid impurities using cross-flow microfiltration and remove the cells immediately before usage using continuous centrifugation at 30000g.

The reactor-separator can be used efficiently for approximately two months before becoming less efficient due to the fouling of the resin. It is recommended to use a short column of the same id and packed with the same material as a guard column, which should be accessible and easy to dismantle. After approximately two months, or as soon

as a drop in conversion efficiency is observed, the guard column should be replaced and the system should be backflashed and recharged.

The reaction separation results have shown that the batch chromatographic reactor-separators can be scaled-up successfully without any significant reduction in conversion. For a four hour sucrose retention over 80% conversions were obtained, and the usage of stainless steel did not appear to have any significant effect. It is esential however to maintain sterile conditions and employ good management practice. The relatively low conversions (70 to 80%) could be improved further by using longer retention times. It has been reported (243) that a three times increase in eluent flow rate resulted in activity losses of 20%, 13% and 5% at the top, middle and bottom parts of a reactor-separator column respectively. Therefore by prolonging the sucrose retention time, the eluent flow rate would be reduced, thus preserving the activity of the adsorbed enzyme. This would reduce the enzyme usage and increase the overall conversion.

12.0 CONCLUSIONS AND RECOMMENDATIONS

12.1 CONCLUSIONS

The aims of this work have been to optimise the separation performance of the production scale semi-continuous chromatographic refiner (SCCR7), by identifying and quantifying the effects of various operating parameters, and to investigate the application of chromatographic systems as biochemical reactor-separators. The overall findings and conclusions can be summarised and classified as follows:

12.1.1 Separation work using the SCCR7

1 The switch time was found to have a major effect on the separation performance of the SCCR7 system, and was the controlling parameter during the operation. Small switch time changes "shifted" the relative positions of the concentration profiles and were enough to determine whether the final products were going to be within the specified purities or not. The system was found to be very sensitive to switch time changes as small as 10 seconds. It has been suggested by previous workers that at certain difficult operating conditions the respective product purities would be improved by altering the feed point location so that extra system length would be available for the separation. This is no longer required since the same effect can be achieved by making the appropriate alteration in the switch time.

2 During operation "pseudo-equilibrium" was achieved approximately 6 cycles after starting from a completely purged system. If however, one or more of the operating conditions are changed during operation, the new "pseudo-equilibrium" will be reached after a further 3 to 4 cycles, providing the system has not been purged.

3 By increasing the feed concentration the throughput is also increased but at the expense of the separation efficiency. At about 19% w/v feed concentration 99.9% purities are obtained, and as the feed concentration increases to 66% w/v the product purities are still within specification but are reduced to just over 90% (FRP at 66% w/v feed concentration). At approximately 45% w/v the system becomes overloaded and the rate with which the switch time increases with concentration is reduced. General switch-time concentration relationships have been developed to predict the correct switch time at the particular operating conditions.

4 The reduced fructose content in the feed has an adverse effect on the separation, ie. becomes more difficult. This was counteracted by increasing the operating switch time. Feedstocks with feed fructose contents as low as 36.8% have been separated efficiently to give products within specification.

5 As a rule of thumb a satisfactory separation can be expected when the "cross-over" point is situated in or very near column 6, the column before the feed inlet column on the FRP side.

6 By reducing the eluent to feed flow rates ratio the product concentration increase. The minimum ratio, at which the product purities were still within specification, was found to be 2.74 to 1; a further reduction however would affect the separation and thus the product purities. To overcome the increased difficulty due to the reduced eluent to feed flow rate ratios the switch time needs to be increased. When a 66% w/v feed concentration was used, a 9.5% decrease in the above ratio (from 3:1 to 2.74:1) required a 13% increase in switch time (from 22.15 to 25 minutes).

7 To increase the economical viability of the chromatographic equipments in general the throughputs and product concentrations need to be increased. When a 66%

w/v feed concentration was introduced into the SCCR7 at 14.6 cm^3 min^{-1}, and a 40 cm^3 min^{-1} eluent flow rate was used, a throughput of 0.578 kg sugar solids per hour, or 32.1 kg sugar solids/m^3 resin/hr was achieved and the product purities were still within specification, (ie. 90.1% pure FRP and a GRP containing only 6.4% fructose). To increase the product concentrations, the purge flow rate was reduced and the product splitting technique was employed. Only the "concentrated" splits were collected and the dilute splits were discarded. To obtain complete purging the minimum purge flow rate must be 60 cm^3 min^{-1} (or 1.5 times the eluent flow rate). When a 70 cm^3 min^{-1} purge rate was used and the product splitting technique was employed the product concentration increased from 5.8 to 11.3% w/v for the FRP, and from 11.6 to 22.56% w/v for the GRP. Only 2% of the glucose and 2.8% of the fructose in the feed was lost in the dilute GRP and FRP splits respectively. The product purities were also increased, ie. the FRP purity increased from 90.1 to over 95% and the fructose content of GRP reduced from about 6.7 to 4.5%. Over 94% of the glucose in the feed was recovered in the GRP, and 95.78% of the fructose in the feed was recovered in the FRP.

8 By reducing the purge rate to 60cm^3 min^{-1} and recycling the dilute product splits as eluent and purge water, the FRP concentration increased to almost 13% w/v and the GRP to 25.4% w/v, recovering over 84 and 87.8% of the glucose and fructose in the feed respectively. The product purities were still within specification, ie. 6.69% fructose in GRP and 90.2% pure FRP.

12.1.2 <u>The effects of flow rates, temperature and concentration on the separation</u>

9 By operating at higher temperatures (60°C) the pressure drop is reduced and the microbial growth is suppressed, but at these temperatures the amount of the β-D-fructopyranose, the fructose form that forms the complex with the calcium ions of

the resin and also the sweetest form, is reduced and therefore the separation efficiency is also slightly affected. This effect is indirectly incorporated in the K_{df} vs temperature general relationship.

10 Temperature, flow rates and background concentration were found to affect the distribution coefficients. General relationships were obtained, relating their effects on the distribution coefficients for any chromatographic system used in the separation of carbohydrate mixtures.

12.1.3 A theoretical link between batch and continuous systems, and the computer simulation of the SCCR7

11 A theoretical link between batch and semicontinuous chromatographic systems has been developed which provided a new and simpler system length estimation procedure requiring the minimum of experimental data. These data can be easily obtained on a batch column operating at infinite dilution conditions or preferably at the actual operating conditions.

12 The computer simulation of the semi-continuous operation has been carried out successfully. The "plate" model approach was employed and the general relationships correlating the effects of temperature, flow rates and background concentration on the distribution coefficients were incorporated. The simulated concentration profiles corresponded to the actual purging concentration profiles, thus giving a more accurate representation since the average sugar solids concentrations in each column were predicted. The simulated profiles showed a very good agreement with the experimental values. The model was capable of predicting the profiles at different flow rates, feed compositions and concentrations, and giving an estimation of the correct switch time for the particular operating conditions. By carrying out a number of modifications in the structure of the computing program a reduction of over 15% in the required CPU time

was achieved. The simulation program also carried out a design procedure, predicting the required system length, "true" number of theoretical plates and HETP values, to obtain a given separation at the particular experimental operating conditions.

12.1.4 Biochemical reaction-separation work

The successful operation of the SCCR7 as a continuous separator led us into investigating ways that would enable its application as a reactor-separator. Before making any recommendations and indeed using the SCCR7 in the reaction-separation mode it was decided to investigate the scaling up of batch chromatographic reactor-separators and to identify the factors that affect their scaling up. Because of specific industrial interest and the very valuable products, the enzymatic conversion of sucrose into dextran and fructose in the presence of the dextransucrase enzyme was chosen. It has been found in this department that the dextran yield is improved by separating the acceptor fructose from the reaction mixture immediately as it is formed. The increased scale of operation required relatively high enzyme volumes that needed purification before using the enzyme.

12.1.4.1 Dextransucrase purification

13 Two different approaches were followed for the purification of the crude dextransucrase enzyme. First a continuous centrifuge was used, operating at 30000g and a throughput of 5000 $cm^3\ hr^{-1}$, to remove over 90% of the cells present. The activity losses during the operation were 3%.

Then an Amicon H10P30-20 ultrafiltration membrane with a 30000 "cut-off" was used to remove the non-solid impurities. On average over 95% of the impurities were removed but the activity losses approached 50%. The enzyme was denaturated

rapidly when large shear forces were present and at moderately high temperatures (over 6°C).

Using the above purification sequence, ie. centrifugation and ultrafiltration the activity losses during processing were around 50% and the enzyme became more unstable. For every 24 hours, over 20% of activity was lost even when it was stored in a refrigerator (4-6°C). This was believed to be due to the removal of cells and the protective dextran from the enzyme mixture.

14 In the second approach a cross-flow microfiltration cartridge was used first to remove the non-solid impurities from the crude enzyme mixture. Both the enzymes and the cells were retained in the retentate. During processing there was only a 4.4% activity loss, and the enzyme was found to be more stable during storage.

15 When the microfiltered mixture was centrifuged the enzyme stability reduced and for every 24 hours of storage in a refrigerator (4-6°C) about 20% of the activity was lost. By removing the cells the enzyme environment changes. Therefore, to minimise the activity losses, it is recommended that the non-solid impurities are removed first by cross-flow microfiltration and then the enzyme is either stored or is centrifuged to remove the cells and used immediately. For minimum activity losses it is essential that the cells are removed by continuous centrifugation immediately before using the enzyme.

16 During both ultrafiltration and cross-flow microfiltration the permeate flux was reduced and a "gel" layer was formed altering the characteristics of the membranes.

12.1.4.2 Chromatographic reaction-separation studies

17 During the reaction-separation work on batch chromatographic sytems some "immobilisation" was achieved probably due to the physical entrapment of the enzyme

molecules and agglomerates on the packing. The continuous usage however of enzyme in the eluent stream was found necessary.

18 The use of a stainless-steel column instead of glass column did not have any affect on the sucrose conversion to dextran and fructose.

19 The voidage did have an affect on the conversion, with higher voidages giving better conversions. However, as the void volume increases the separation efficiency decreases and a compromise is necessary.

20 The scaling up of batch chromatographic reactor-separators, from 1 to 2 to 5.4 cm id columns showed that at comparable conditions (4 hr sucrose retention time) similar conversions were obtained, ie. between 70 and 80% (based on fructose formed). To obtain higher conversions and better product yield (more high molecular weight dextran) it is believed that longer sucrose residence times should be used. When operating at maximum throughput, it is preferable to employ repetitive injection techniques and to use small sucrose feed charges (about 2% empty column volume) at high concentrations (20% w/v).

12.1.5 Overall comments

The semicontinuous chromatographic refiner has been shown capable of handling industrial feedstocks and producing HFCS and a glucose rich product meeting the strictest industrial specifications, without any recycling. It provided excellent reproducibility, allowed continuous unattended operation and showed great scaling up potential.

The reactor-separation results looked promising and it is anticipated that the SCCR equipments could be used successfully as continuous biochemical reactor-separators. Unfortunately lack of time did not permit this study to be

performed as it was initially intended.

12.2 RECOMMENDATIONS

The inherent advantages, the separation capabilities of the SCCR systems and their potential as reactor-separators provide the basis for a very attractive commercial proposition. From the work caried out in this research the following suggestions are recommended:

1 Carry out more separation experiments, operating at intermediate conditions to the ones used in this work to provide more data and improve the accuracy of the relationships derived to predict the effect of the various operating conditions on the switch time.

2 Carry out a set of experiments where only the very concentrated part of the FRP is collected and the rest, containing increasing amounts of fructose, is recycled in the eluent stream. Employing this technique the fructose content in the column, which is to be purged next, would be increased "artificially", and the FRP concentration would also be increased. A similar approach can be followed for the GRP, but to a lesser extent, and the "dilute" split can be used to dilute the feed.

3 Obtain a new theoretical approach that would characterise the system's separation potential in the continuous operation, instead of the traditional approach that employs individual column characterisation on a batchwise basis.

4 If the SCCR system is to be used for the desugarisation of molasses or other feedstocks, where ions are present that displace the calcium ions on the resin, continuous resin regeneration is advantageous. This can be achieved by isolating a second column

in the same way as with the purge column (Figure 12.1a). This second column preferably should be the column just purged. The regeneration flow is recommended to be in the opposite direction to the mobile phase flow so that some back flashing occurs. The column can be regenerated for the whole or part of the switch time. This modification will also increase the versatility of the equipment by allowing three separate products to be collected. For example, when a feedstock contains a component or components that are eluted with the mobile phase (GRP), a retarded component (FRP) and a totally adsorbed component (RO), the first two components will be separated in the usual fashion and the last by "purging" with an alternative solvent or using one with a different pH.

The above modification would require one inlet and one outlet port and extra corresponding valves per column, an additional inlet and outlet liquid network, and a simple reprogramming of the pneumatic controller.

Another alternative is illustrated in Figure 12.1(b) and can be employed on its own or in addition to the above. By using one inlet and one outlet port and two extra valves per column, an additional inlet and outlet liquid network, and one extra column connected to the new networks (preferably identical to the other columns), any column in the system could be isolated and by-passed. This would enable the isolated column to be regenerated or to be repacked without stopping the operation. Alternatively, this extra column can be used as a reactor. For example, the glucose in the post feed section can be isomerised continuously to fructose by redirecting the mobile phase, at any desired position, through the reactor.

The above modifications should enhance the versatility of the SCCR systems and increase their flexibility. These suggestions can be achieved either by reprogramming the existing pneumatic controllers or preferably by interfacing the equipment to a computer, which would allow automatic reprogramming while in operation.

6 In fact, it is recommended to use on line monitoring (concentration, flow rates, temperature etc) interfaced with a computer which would be programmed, using the correlations mentioned above, to make the required changes automatically.

7 In the reaction-separation work, it is recommended that different ultrafiltration and/or microfiltration units are tested from other manufacturers to establish the ones that provide equally good or better separation efficiencies, at a reduced process time and which would allow sterilisation (ie. ceramic units).

8 Examine the effect of repetitive injections on the sucrose conversion and dextran yield using the batch chromatographic reactor-separator.

9 And, carry out the sucrose conversion to dextran and fructose on the SCCR unit. Figure 12.2 illustrates the expected concentration profiles. The prime objective should be to establish the correct operating conditions that permit immediate separation of the fructose from the reaction mixture thus minimising the acceptor reactions and improving the dextran yield. At the same time the relevant publications should be reviewed to establish if and how the successful immobilisation of the dextransucrase enzyme has been achieved. The SCCR system can also be used as a reactor separator to study the sucrose inversion to glucose and fructose and their separation.

10 Finally, carry out a feasibility study and an economic evaluation to estimate the system's (SCCR) competitiveness as a separator and/or as a reactor separator in current and future industrial applications.

Figure 12.1: Proposed alternative operating techniques

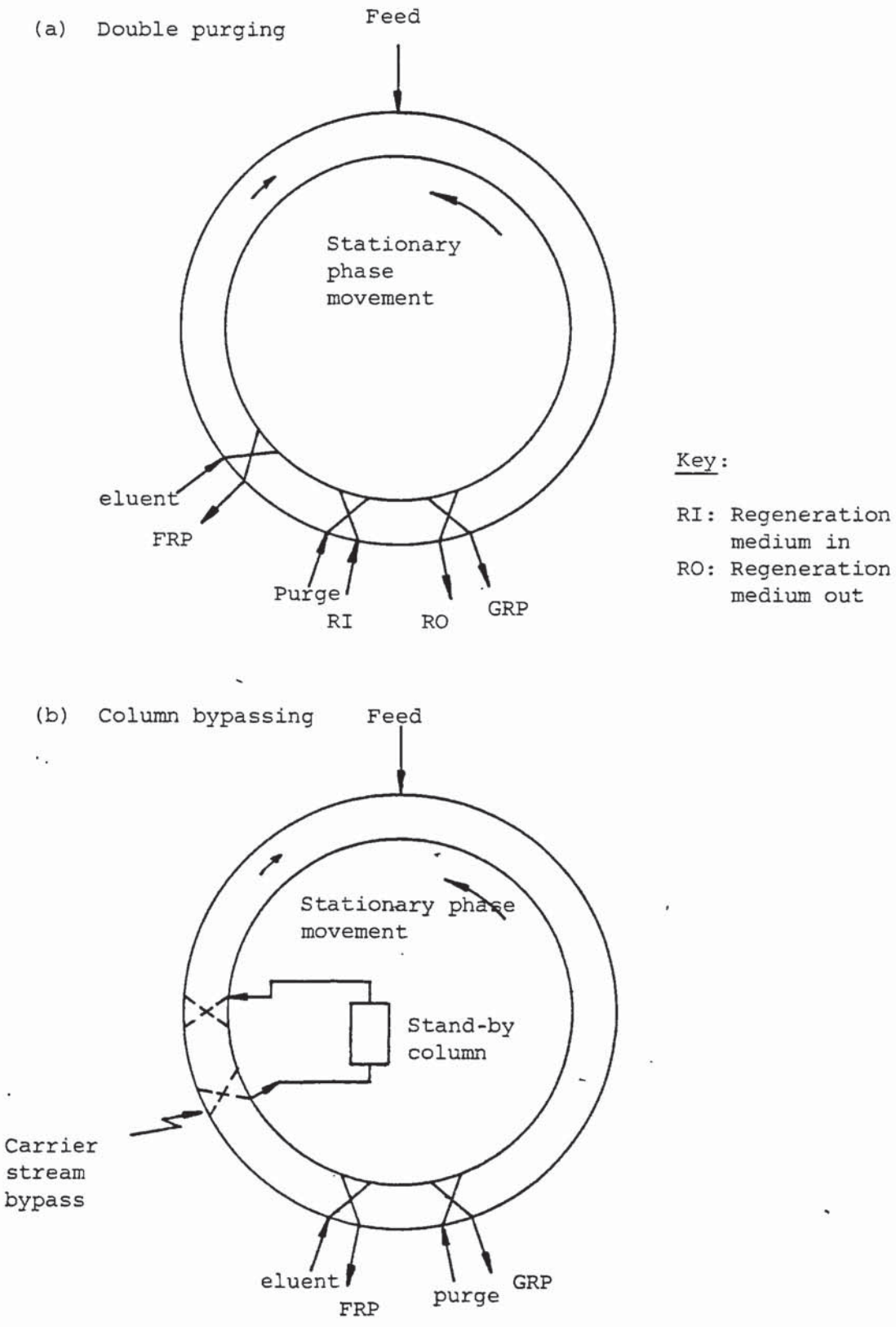
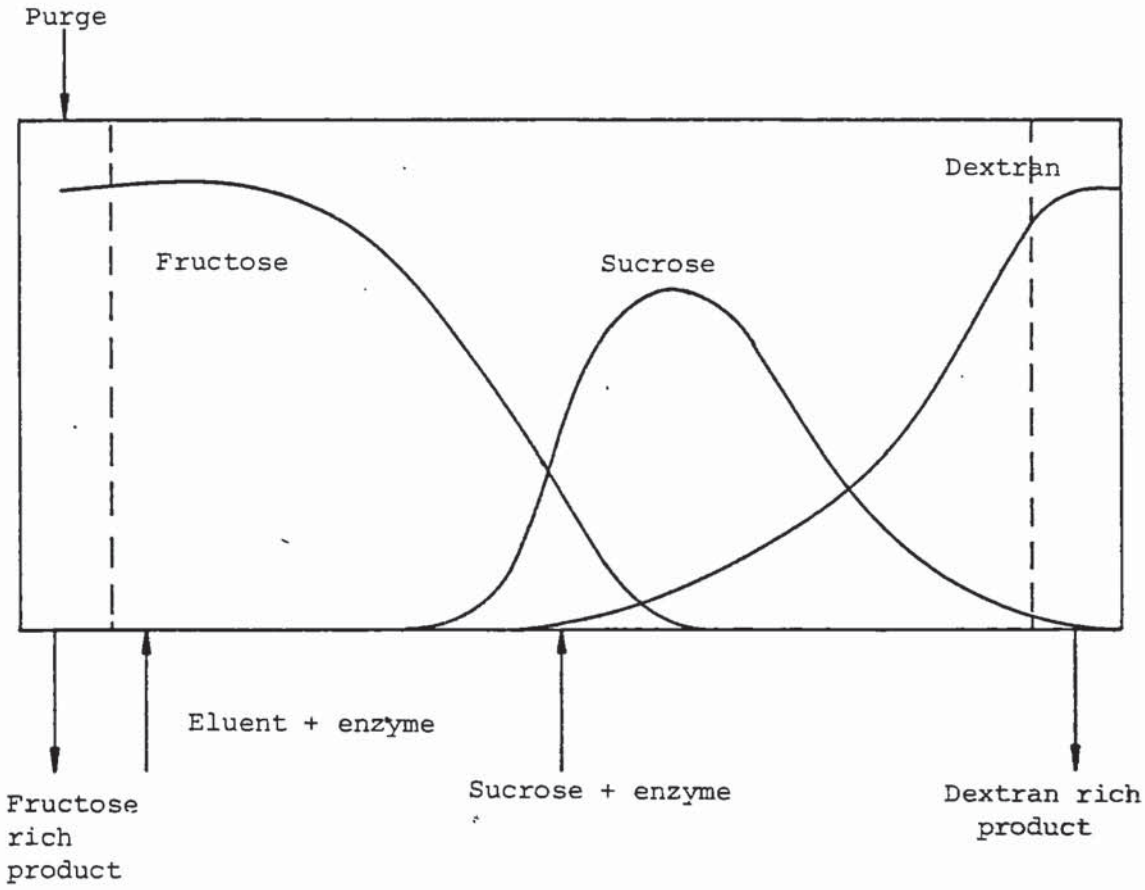


Figure 12.2: Diagramatic representation of the operation of the SCCR system as a continuous reactor-separator



APPENDIX A

FEED BAND WIDTH EVALUATION

The modified Glueckauf equation (2.17) was rearranged as follows:

$$B1 = \frac{N^*}{N}\left[1 + \frac{P}{3} + \frac{7P^2}{45} + \frac{0.0765\,P^3}{1 - 0.565\,P}\right] \qquad \text{..... A1}$$

Where P: a systems loading related parameter given by:

$$P = \left(\frac{N_o}{N}\right)^2 \frac{N^*}{4} \qquad \text{..... A2}$$

N^* = "apparent" number of theoretical plates obtained by equation 2.11 for the whole system

N_o = "apparent"~number of theoretical plates occupied by the feed band.

The solution to this equation is the N value for which the B1 is equal to 1, and it was solved by trial and error using a simple interactive FORTRAN 77 program for a wide range of N_o values.

The calculation procedure was generalised to make it applicable to any system and has been included in the computing simulation program (Chapter 10) to give the required true number of theoretical plates for the particular conditions.

Calculation of N_o

The value of No represents the number of "apparent" theoretical plates occupied by the feed band and its approximate value was found as follows:

For a batch system:

$$N_o = \frac{\text{Feed volume}}{\text{Total empty system volume}} \times N^* \qquad \text{..... A3}$$

For a semicontinuous system the calculation was carried out over a cycle by visualising the whole system as a "static" column, ie:

$$No = \frac{\text{Feed flow rate x Switch time x No of columns}}{\text{Total empty system volume}} \times N^* \qquad \text{..... A4}$$

These N_o values are the minimum ones since they do not account for any additional longitudinal diffusion during the feed loading. The validity of this assumption was tested by injecting blue Dextran into a packed glass column and measuring the relative lengths. The resulted error was relatively low (± 5%).

A plot of the true number of theoretical plates (NTP), and system loading related parameter (P), against the number of theoretical plates occupied by the feed band (N_o), for the SCCR6 system, is shown in Figure A1. The NTP vs N_o profile is approximated to two straight lines as follows:

- For N_o less than 40:

$$NTP = 229 + 2.7 \times N_o \qquad \text{..... A5}$$

- And for No values equal or greater than 40:

$$NTP = 113.3 + 5.48 \times N_o \qquad \text{..... A6}$$

<u>Calculation of "True" Number of Theoretical Plates at the Actual Conditions</u>

<u>Batch SCCR6</u>

For a 20% w/v feed concentration (Table 9.3):

$$N_o = \frac{15000}{10 \times 5889} \times 250 = 64 \text{ plates}$$

Therefore, from relationship A6:

NTP = 464 plates

Batch SCCR7

For an 18.6% w/v feed concentration (Table 9.3):

$$N_o = \frac{3750}{12 \times 1500} \times 384 = 80 \text{ plates,}$$

Therefore, from relationship 6.10,

NTP = 710 plates

SCCR6 Semi-continuous Mode

Using the operating conditions of run 20-35-105-30-20 (Table 9.1), then:

$$N_o = \frac{35 \times 30 \times 10}{10 \times 5889} \times 250 = 45 \text{ plates}$$

and from relationship A6:

NTP = 360 plates

SCCR7 Semi-continuous Mode

Using the operating conditions of run 18.6-9-30-30-20 (Table 9.1), then:

$$N_o = \frac{9 \times 30 \times 12}{12 \times 1500} \times 384 = 69 \text{ plates}$$

and for relationship 6.10:

NTP = 632 plates

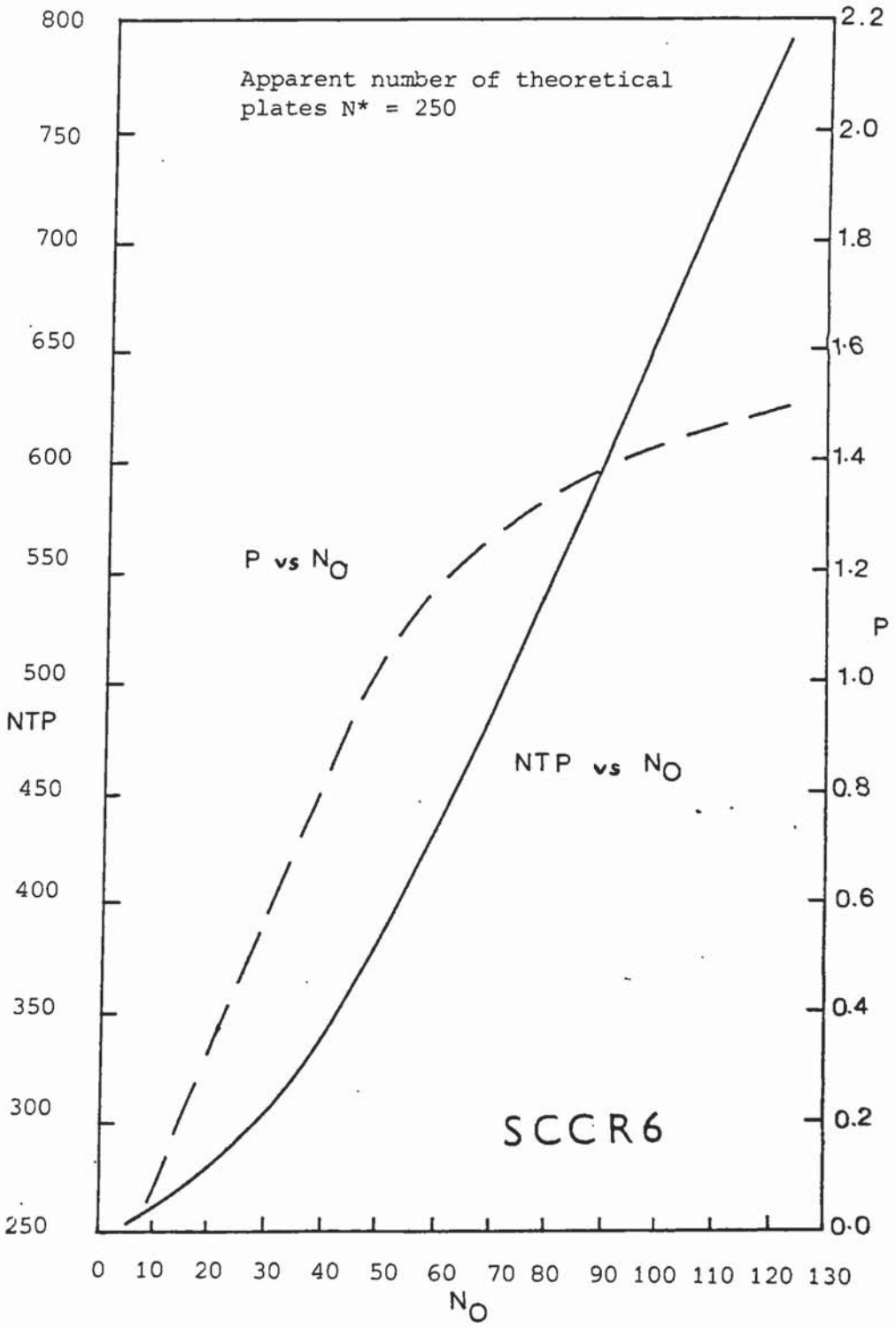
And at the maximum throughput, run 66-14.6-40-25-60 (Table 9.1)

$$N_o = \frac{14.6 \times 25 \times 12}{12 \times 1500} \times 384 = 93 \text{ plates}$$

Therefore, from relationship 6.10:

NTP = 803 plates.

Figure A1: Plot of number of theoretical plates (NTP) and system loading related parameter (P), against the number of theoretical plates occupied by the feed band (No)



APPENDIX B

k'_g DETERMINATION

Determination of k'_g for 10 column x 2.54cm id SCCR4 Unit

The operating conditions and results of run 02-2.4-6.1-29 (203) were used since this run approached the "infinite dilution" conditions, ie:

$x_g = 0.098$, $Z = 638/2 = 316$cm, $K^{\infty}_{dg} = 0.23$, and switch time = 30 min

Also,

$$u_m = \frac{V_g}{XSA} = \frac{\text{Average mobile phase flow rate}}{\text{Cross sectional area}}$$

$$= \frac{(6.1 + 8.5)/2}{\pi\,(2.54)^2/4} = 1.44\text{cm min}^{-1}$$

$$u_p = \frac{V_p}{XSA} = \frac{\text{Average volume of packing per column/switch time}}{\text{Cross sectional area}}$$

$$= \frac{320/30}{\pi\,(2.54)^2/4} = 2.11\text{cm min}^{-1}$$

Therefore,

$$\ln(0.098) = \frac{316.k'_g}{1.44}\left(0.23 - \frac{1.44}{2.11}\right)$$

Therefore: $k'_g = 0.023$

Prediction of Total Length of the 12 column x 5.4cm id SCCR7 Unit

Using similar operating conditions to the 2.54cm id x SCCR4 unit, the same switch time of 30 min and the following data:

Feed flow rate = 9 $cm^3\ min^{-1}$

Eluent flow rate = 30 $cm^3\ min^{-1}$

and $K^{\infty}dg = 0.215$ (run 18.6-9-30-30-20)

And designing for 99.9% pure FRP (ie. $x_g = 0.001$) then,

$$u_m = \frac{(30 + 39)/2}{\pi (5.4)^2/4} = 1.51 cm\ min^{-1}$$

$$u_p = \frac{1037/30}{\pi (5.4)^2/4} = 1.51 cm\ min^{-1}$$

Hence,

$$\ln (0.001) = \frac{0.023 Z}{1.51}\left(0.215 - \frac{1.51}{1.51}\right)$$

Therefore, Z = 577cm

With a central feed location on a continuous unit, the overall theoretical SCCR7 system length is 1154cm.

Prediction of Total Length of the 10 column x 10.8cm id SCCR6 Unit

Using a 30 min switch time as in the 2.54 id SCCR4 unit, similar operating conditions, ie.

Feed flow rate = $35cm^3\ min^{-1}$

Eluent flow rate = $105cm^3\ min^{-1}$

and $K^{\infty}_{dg} = 0.168$

and designing for 99.9% pure FRP, then

$$u_m = \frac{(105 + 140)^2}{\pi\,(10.8)^2/4} = 1.34cm\ min^{-1}$$

$$u_p = \frac{3940/30}{\pi\,(10.8)^2/4} = 1.43cm\ min^{-1}$$

Therefore,

$$\ln(0.001) = \frac{0.023.Z}{1.34}\left(0.168 - \frac{1.34}{1.43}\right)$$

Therefore Z = 523cm

With a central feed location the overall theoretical length for the SCCR6 is 1046cm.

Length Calculation at Actual Experimental Conditions

In the theoretical estimation of the continuous length it was assumed that the k'_g value remains constant irrespective of flow rate, temperature and concentration changes.

SCCR6 System

Using the conditions of run 20-35-105-30-20 (Table 9.1) and the modified glucose distribution coefficient to account for the actual flow rates and concentrations (K_{dg} = 0.267), then:

$$u_m = \frac{(105 + 140)/2}{\pi (10.8)^2/4} = 1.34\text{cm min}^{-1}$$

$$u_p = \frac{3940/30}{\pi (10.8)^2/4} = 1.43\text{cm min}^{-1}$$

And for a 99.9% pure FRP:

$$\ln (0.001) = \frac{0.023.Z}{1.34} \left(0.267 - \frac{1.34}{1.43} \right)$$

Therefore, Z = 600.5cm or a total SCCR6 length of 1201cm.

SCCR7 Systems

Using the conditions of run 18.6-9-30-30-20 (Table 9.1) and the modified glucose distribution coefficient, K_{dg} = 0.353, then:

$$u_m = \frac{(30 + 39)/2}{\pi (5.4)^2/4} = 1.51\text{cm min}^{-1}$$

$$u_p = \frac{1037/30}{\pi (5.4)^2/4} = 1.51\text{cm min}^{-1}$$

For 99.9% pure FRP:

$$\ln(0.001) = \frac{0.023.Z}{1.51}\left(0.353 - \frac{1.51}{1.51}\right)$$

Therefore, Z = 701cm, or a total length of 1402cm.

Using the conditions of run 66-14.6-40-25-60 (Table 9.1) and designing for 90.1% pure FRP, then:

Modified $K_{dg} = 0.401$

$$u_m = \frac{(40 + 54.6)/2}{\pi (5.4)^2/4} = 2.07\text{cm min}^{-1}$$

$$u_p = \frac{1037/25}{\pi (5.4)^2/4} = 1.81\text{cm min}^{-1}$$

$$\ln(0.099) = \frac{0.023.Z}{2.07}\left(0.401 - \frac{2.07}{1.81}\right)$$

Therefore, for 90.1% pure FRP, Z = 280cm or a total length of 560cm. And for a 99.9% pure FRP at a similar switch time,

$$\ln(0.001) = \frac{0.023.Z}{2.07}\left(0.401 - \frac{2.07}{1.81}\right)$$

Therefore, Z = 837cm, or a total SCCR7 length of 1674cm.

APPENDIX C

LISTING OF COMPUTER PROGRAM, RESULTS AND SYMBOLS USED FOR THE SIMULATION OF THE SCCR7 SYSTEM

FIG: C1 – LISTING OF THE SIMULATION PROGRAM.

```
$JOB GG4 2299GGS ppn OUT=RES4
$FORTRAN.S
      DIMENSIONG(500),F(500),AG(500),AF(500),
     1FMASS(500),FCUM(500) ,GCONC(500),FCONC(500)
      DIMENSION GMASS(500),GCUM(500)
      REAL KDM,M(500),AM(500),MMASS(500),MCUM(500)
      REAL KD1,KD2,KD11,KD12,KD13,KD21,KD22,KD23
      REAL MCONC(500),KDG,KDF,HETP,KKK,VFCN(500)
      REAL MFEED,VG1(20),VF1(20),VM1(20),NTTP,NHO
      REAL LVT,LVSP,KDGMD,KDFMD,VGCN(500),VMCN(500)
      INTEGER X,KNL(20),NTCLS,PRFD
C     INPUT ALL DATA
      DIA=5.4
      TLENGT=787.0
      CFLOW=0.65
      FFLOW=0.217
      SFLOW=1.333
      GFEED=0.2808
      FFEED=0.2273
      MFEED=0.0319
      PERTIM=13.0
      KKK=0.023
      REFST=23.0
      PURITY=99.9
      VOPKPC=1037
      NTCLS=12
      KCYC=9
      KCYLST=8
      TEMP=60
      NFEED=7
      NNBED=32
      V1NV=700.0
      V2NV=800.0
      KDM=0.19
      KDG=0.215
      KDF=0.472
      DELTAT=2.5
      SWTIME=23.9
      SWTFR=20.3
      PURC=(100.0-PURITY)/100.0
      REFLOW=13.0
      ILK2=86413
      CFLOWM=CFLOW*60.0
      SFLOWM=SFLOW*60.0
      FFLOWM=FFLOW*60.0
      KTOTAL=KCYC*NTCLS
      VRT1=SFLOWM*PERTIM
      PRFD=NFEED-1
      XSA=(3.1416*(DIA**2))/4
```

```
      TESV=XSA*TLENGT
C     ESTIMATION OF ACTUAL SWITCH TIME
      CFEED=(GFEED+FFEED+MFEED)*100.0
      IF (CFEED.LT.45.) THEN
       OPSWP=(0.542*CFEED+80.29)*REFST/100.
      ELSE
       OPSWP=(0.175*CFEED+97.05)*REFST/100.
      ENDIF
      IF (FFLOWM.NE.REFLOW) THEN
       OPSWP=(REFLOW/FFLOWM)*OPSWP
      ENDIF
      CFF=((FFEED*100.)/CFEED)*100.
      OPSWP=(152.89-1.25*CFF)*OPSWP/100.
      LVT=((CFLOWM*2)+FFLOWM)/(XSA*2)
C     THE INFINITE DILUTION DISTRIBUTION
C     COEFFICIENTS ARE MODIFIED TO ACCOUNT
C     FOR THE EFFECTS OF TEMPERATURE,
C     FLOW RATES AND CONCENTRATION.
      KDF=(114.55-0.563*TEMP)*KDF/100
      KDF=(112.68-11.593*LVT)*KDF/100
      KDG=(111.71-10.631*LVT)*KDG/100
      E1=492.5*KDG/(2*100)
      C1=164.83*KDG/100
      E2=173.1*KDF/100
      C2=95.71*KDF/100
      E3=198.3*KDG/100
      C3=107.43*KDG/100
      E4=38.9*KDF/(2*100)
      C4=101.62*KDF/100
      E5=627.4*KDG/100
      C5=111.6*KDG/100
      E6=289.1*KDF/100
      C6=112.2*KDF/100
      KD11=E1*GFEED+C1
      KD12=E3*FFEED+C3
      KD13=E5*MFEED+C5
      KD21=E2*GFEED+C2
      KD22=E4*FFEED+C4
      KD23=E6*MFEED+C6
      TFCS=CFEED/100.0
      KDFMD=(GFEED/TFCS)*KD21+(FFEED/TFCS)*KD22+(MFEED/TFCS)*KD23
      KDGMD=(GFEED/TFCS)*KD11+(FFEED/TFCS)*KD12+(MFEED/TFCS)*KD13
C     CALCULATION OF THE ACTUAL NUMBER OF
C     THEORETICAL PLATES USING THE FEED
C     BAND BROADENING APPROACH.
      FVPC=FFLOWM*OPSWP*NTCLS
      NHO=FVPC*NNBED*NTCLS/TESV
      IF (NHO.LT.60.0) THEN
       NTTP=NHO*3.85+339.4
      ELSE
       NTTP=NHO*7.13+139.53
      ENDIF
      KKINK=OPSWP*60.0/DELTAT
      DT=SWTIME*60/KKINK
```

```
      DTF=SWTFR*60/KKINK
      SWPF=KKINK*DTF/60
      SWP=KKINK*DT/60.0
C     THEORETICAL LENGTH ESTIMATION USING
C     THE SCIANCE AND CROSSER MODEL.
      LVSP=(VOPKPC/OPSWP)/XSA
      SLTH=2*ALOG(PURC)*LVT/(KKK*(KDGMD-(LVT/LVSP)))
      HETP=SLTH/NTTP
      V1=V1NV/NNBED
      V2=V2NV/NNBED
C     CALCULATION OF THE CONCENTRATION PROFILES
      DO 99 I=1,500
      G(I)=0.0
      F(I)=0.0
      M(I)=0.
      AM(I)=0.0
      AG(I)=0.0
      AF(I)=0.0
   99 CONTINUE
      NNTOT=NNBED*NTCLS
      NNNINE=NNBED*(NTCLS-1)+1
      NNFEED=(NFEED-1)*NNBED+1
C     CALCULATION OVER TOTAL NUMBER OF SEQUENCES
      DO 100 K=1,KTOTAL
      ISTKK=KKINK*(K-1)+1
      LSTKK=KKINK*K
C     TOTAL NUMBER OF TIME INCREMENTS DURING A SEQUENCE
      DO 200 KK=ISTKK,LSTKK
C     COLUMN FUNCTIONING MODE
      DO 300 N=1,NTCLS
      IF(N.LE.PRFD)CFLOWC=CFLOW
      IF(N.GE.NFEED)CFLOWC=CFLOW+FFLOW
      IF(N.LE.(NFEED-K))GO TO 300
      NNFST=NNBED*(N-1)+1
      NNLST=NNBED*N
C     CONCENTRATION CALCULATION FOR THE
C     PARTICULAR PLATE.
      DO 400 NN=NNFST,NNLST
      IF(N.EQ.1)GO TO 80
      IF((N.EQ.2).AND.(NN.EQ.NNFST))GO TO 40
      IF(NN.EQ.NNFEED) GO TO 50
      GO TO 60
   40 G(NN-1)=G(NNBED)
      F(NN-1)=F(NNBED)
      M(NN-1)=M(NNBED)
      GO TO 70
   50 A=CFLOWC*DT
      AAF=CFLOWC*DTF
C     CALCULATION OF THE DISTRIBUTION COEFFICIENTS FOR
C     THE PARTICULAR BACKGROUND CONCENTRATION.
      KD11=E1*G(NN)+C1
      KD12=E3*F(NN)+C3
      KD13=E5*M(NN)+C5
      KD21=E2*G(NN)+C2
```

```
      KD22=E4*F(NN)+C4
      KD23=E6*M(NN)+C6
      CTL=G(NN)+F(NN)+M(NN)
       IF (CTL.LT.0.01) THEN
        KD1=AMAX1(KD11,KD12,KD13)
        KD2=AMAX1(KD21,KD22,KD23)
       ELSE
       KD1=G(NN)*KD11/CTL+F(NN)*KD12/CTL+M(NN)*KD13/CTL
       KD2=G(NN)*KD21/CTL+F(NN)*KD22/CTL+M(NN)*KD23/CTL
       ENDIF
      IF(G(NN-1).LT.0.1E-10)G(NN-1)=0.0
      IF(F(NN-1).LT.0.1E-10)F(NN-1)=0.0
      IF(M(NN-1).LT.0.1E-10)M(NN-1)=0.0
      RR=EXP(-A/(V1+V2*KD1))
      SS=EXP(-AAF/(V1+V2*KD2))
      RM=EXP(-A/V1)
      M(NN)=(1.-RM)*((CFLOW*M(NN-1)+FFLOW*MFEED)/CFLOWC)+RM*M(NN)
      G(NN)=(1.-RR)*((CFLOW*G(NN-1)+FFLOW*GFEED)/CFLOWC)+RR*G(NN)
      F(NN)=(1.-SS)*((CFLOW*F(NN-1)+FFLOW*FFEED)/CFLOWC)+SS*F(NN)
      GO TO 400
   60 IF(G(NN-1).LT.0.1E-10)G(NN-1)=0.0
      IF(F(NN-1).LT.0.1E-10)F(NN-1)=0.0
      IF(M(NN-1).LT.0.1E-10)M(NN-1)=0.0
   70 A=CFLOWC*DT
      AAF=CFLOWC*DTF
C     THE DISTRIBUTION COEFFICIENTS ARE MODIFIED FOR
C     THE PARTICULAR BACKGROUND CONCENTRATION.
      KD11=E1*G(NN)+C1
      KD12=E3*F(NN)+C3
      KD13=E5*M(NN)+C5
      KD21=E2*G(NN)+C2
      KD22=E4*F(NN)+C4
      KD23=E6*M(NN)+C6
      CTL=G(NN)+F(NN)+M(NN)
       IF (CTL.LT.0.01) THEN
        KD1=AMAX1(KD11,KD12,KD13)
        KD2=AMAX1(KD21,KD22,KD23)
       ELSE
       KD1=G(NN)*KD11/CTL+F(NN)*KD12/CTL+M(NN)*KD13/CTL
       KD2=G(NN)*KD21/CTL+F(NN)*KD22/CTL+M(NN)*KD23/CTL
       ENDIF
      RR=EXP(-A/(V1+V2*KD1))
      SS=EXP(-AAF/(V1+V2*KD2))
      RM=EXP(-A/V1)
      M(NN)=(1.0-RM)*M(NN-1)+RM*M(NN)
      G(NN)=(1.0-RR)*G(NN-1)+RR*G(NN)
      F(NN)=(1.0-SS)*F(NN-1)+SS*F(NN)
      GO TO 400
   80 IF(NN.EQ.NNFST)GO TO 90
      IF(G(NN-1).LT.0.1E-10)G(NN-1)=0.0
      IF(F(NN-1).LT.0.1E-10)F(NN-1)=0.0
      IF(M(NN-1).LT.0.1E-10)M(NN-1)=0.0
      GO TO 95
   90 G(NN-1)=0.0
```

```
      F(NN-1)=0.0
      M(NN-1)=0.0
   95 A=SFLOW*DT
      AAF=SFLOW*DTF
C     THE DISTRIBUTION COEFFICIENTS ARE MODIFIED FOR
C     THE PARTICULAR BACKGROUND CONCENTRATION.
      KD11=E1*G(NN)+C1
      KD12=E3*F(NN)+C3
      KD13=E5*M(NN)+C5
      KD21=E2*G(NN)+C2
      KD22=E4*F(NN)+C4
      KD23=E6*M(NN)+C6
      CTL=G(NN)+F(NN)+M(NN)
       IF (CTL.LT.0.01) THEN
        KD1=AMAX1(KD11,KD12,KD13)
        KD2=AMAX1(KD21,KD22,KD23)
       ELSE
       KD1=G(NN)*KD11/CTL+F(NN)*KD12/CTL+M(NN)*KD13/CTL
       KD2=G(NN)*KD21/CTL+F(NN)*KD22/CTL+M(NN)*KD23/CTL
       ENDIF
      RR=EXP(-A/(V1+V2*KD1))
      SS=EXP(-AAF/(V1+V2*KD2))
      RM=EXP(-A/V1)
      M(NN)=(1.0-RM)*M(NN-1)+RM*M(NN)
      G(NN)=(1.0-RR)*((G(NN-1)))+RR*G(NN)
      F(NN)=(1.0-SS)*F(NN-1)+SS*F(NN)
  400 CONTINUE
  300 CONTINUE
  200 CONTINUE
      DO 500 NN=1,NNBED
      AG(NN)=G(NN)
      AF(NN)=F(NN)
      AM(NN)=M(NN)
  500 CONTINUE
      DO 600 NN=1,NNTOT
      IF(NN.GE.NNNINE)GO TO 2010
      NNADJ=NN+NNBED
      G(NN)=G(NNADJ)
      F(NN)=F(NNADJ)
      M(NN)=M(NNADJ)
      GO TO 600
 2010 NNADJ=NN+1-NNNINE
      G(NN)=AG(NNADJ)
      F(NN)=AF(NNADJ)
      M(NN)=AM(NNADJ)
  600 CONTINUE
  100 CONTINUE
      WRITE(3,5)
      WRITE(3,6)
      WRITE(3,7)
      WRITE(3,8)
      WRITE(3,51)CFEED,CFLOWM,FFLOWM,SFLOWM,OPSWP,NNBED,TEMP
      WRITE(3,91)
      WRITE(3,12)KCYC
```

```
      WRITE(3,10)
      WRITE(3,34)
      GCUM(1)=0.0
      FCUM(1)=0.0
      MCUM(1)=0.0
      DO 11 I=1,NNTOT
C     THE DISTRIBUTION COEFFICIENTS ARE MODIFIED FOR
C     THE PARTICULAR BACKGROUND CONCENTRATION.
      KD11=E1*G(NN)+C1
      KD12=E3*F(NN)+C3
      KD13=E5*M(NN)+C5
      KD21=E2*G(NN)+C2
      KD22=E4*F(NN)+C4
      KD23=E6*M(NN)+C6
      CTL=G(NN)+F(NN)+M(NN)
       IF (CTL.LT.0.01) THEN
        KD1=AMAX1(KD11,KD12,KD13)
        KD2=AMAX1(KD21,KD22,KD23)
       ELSE
       KD1=G(NN)*KD11/CTL+F(NN)*KD12/CTL+M(NN)*KD13/CTL
       KD2=G(NN)*KD21/CTL+F(NN)*KD22/CTL+M(NN)*KD23/CTL
       ENDIF
      GMASS(I)=G(I)*V1+G(I)*KD1*V2
      FMASS(I)=F(I)*V1+F(I)*KD2*V2
      MMASS(I)=M(I)*V1
   11 CONTINUE
      L=NNBED
   27 DO 14 I=1,NNTOT+1
      IF(I.EQ.(L+1))GCUM(I)=GMASS(I)
      IF(I.EQ.(L+1))FCUM(I)=FMASS(I)
      IF(I.EQ.(L+1))MCUM(I)=MMASS(I)
   14 CONTINUE
      L=L+NNBED
      IF(L.EQ.(NNTOT+NNBED))GO TO 15
      GO TO 27
   15 L=NNBED
C     CALCULATION OF THE AVERAGE INDIVIDUAL
C     COMPONENT CONCENTRATION IN EACH COLUMN
C     DURING THE FINAL CYCLE.
      I=2
      Z=0
      GCUM(1)=0.0
      FCUM(1)=0.0
      MCUM(1)=0.0
   13 DO 16 X=I,L
      GCUM(X)=GMASS(X)+GCUM(X-1)
      FCUM(X)=FMASS(X)+FCUM(X-1)
      MCUM(X)=MMASS(X)+MCUM(X-1)
      MCONC(X)=MCUM(X)/VRT1
      GCONC(X)=GCUM(X)/VRT1
      FCONC(X)=FCUM(X)/VRT1
       VMCN(X)=100*MCONC(X)
       VGCN(X)=100*GCONC(X)
       VFCN(X)=100*FCONC(X)
```

```
      KOLNO=X/NNBED
      IF(X.EQ.L) GO TO 18
      GO TO 16
   18   Z=Z+1
        KNL(Z)=KOLNO
        VG1(Z)=VGCN(X)
        VF1(Z)=VFCN(X)
        VM1(Z)=VMCN(X)
   16 CONTINUE
      I=L+2
      L=L+NNBED
      IF(L.EQ.(NNTOT+NNBED))GO TO 355
      GO TO 13
  355   WRITE(3,4)KNL(1),VG1(NTCLS),VF1(NTCLS),VM1(NTCLS)
C     PRINT OUT THE RESULTS
C     COLUMN 1 REPRESENTS THE PURGED COLUMN
       FINLST=NTCLS-1
       DO 886 Z=1,FINLST
        TST=Z+1
        WRITE(3,4)KNL(TST),VG1(Z),VF1(Z),VM1(Z)
  886   CONTINUE
      WRITE(3,156)
      WRITE(3,158)
      WRITE(3,157)KDGMD,KDFMD
      WRITE(3,162)PURITY
      WRITE(3,163)SLTH
      WRITE(3,164)NTTP
      WRITE(3,165)HETP
      WRITE(3,159)
  162 FORMAT(//,10X,'FOR A ',F4.1,'% PURE FRUCTOSE RICH PRODUCT',/)
  163 FORMAT(10X,'THE REQUIRED TOTAL SCCR LENGTH IS :',F6.1,' CM')
  164 FORMAT(10X,'THE TRUE NUMBER OF THEORETICAL PLATES IS :',F6.1)
  165 FORMAT(10X,'RESULTING IN A  HETP  OF :',F4.1,' CM')
  156 FORMAT(//,10X,'THE ACTUAL DISTRIBUTION COEFFICIENTS BASED')
  159 FORMAT(/////////////////)
  158 FORMAT(10X,'ON THE FEED COMPOSITION WERE : ')
  157 FORMAT(12X,'KDG =',F5.3,10X,'KDF =',F5.3)
    4 FORMAT(11X,I2,10X,F5.2,15X,F5.2,12X,F5.2)
    5 FORMAT(1H1,////////////////,12X,'FEED',1X,'ELUENT',
     11X,'FEED',3X,
     1'PURGE',3X,'PREDICTED ',1X,'APPARENT NO',3X,'TEMP')
    6 FORMAT(12X,'CONC',1X,'FLOW',3X,'FLOW',3X,'FLOW',3X,
     1'SWITCH TIME'1X,' OF PLATES ')
    7 FORMAT(11X,'%W/V',2X,'RATE',3X,'RATE',3X,'RATE',5X,
     1'MINUTES',3X,'PER COLUMN',6X,'C')
    8 FORMAT(17X,'ML/MIN',1X,'ML/MIN',1X,'ML/MIN',8X,/)
   91 FORMAT(//,17X,'AVERAGE CONCENTRATIONS OF SUGARS ON EACH')
   12 FORMAT(25X,'COLUMN AFTER',I3,' CYCLES')
   10 FORMAT(/,10X,'COL NO',2X,'AV GLUCOSE CONC',2X,'AV',
     11X,'FRUCTOSE CONC',2X,'AV MALTOSE CONC'/)
   34  FORMAT(23X,'% W/V',15X,'% W/V',12X,'% W/V'/)
   51 FORMAT(12X,F4.1,1X,F5.1,2X,F4.1,3X,F5.1,5X,F5.2,8X,
     1I2,7X,F5.1)
        STOP
```

```
      END
$VU.S
$RUN
$EOJ
```

FIG: C 2 – RESULTS OBTAINED USING THE SIMULATION PROGRAM.

FEED CONC %W/V	ELUENT FLOW RATE ML/MIN	FEED FLOW RATE ML/MIN	PURGE FLOW RATE ML/MIN	PREDICTED SWITCH TIME MINUTES	APPARENT NO OF PLATES PER COLUMN	TEMP C
54.0	39.0	13.0	80.0	24.52	32	60.0

AVERAGE CONCENTRATIONS OF SUGARS ON EACH COLUMN AFTER 9 CYCLES

COL NO	AV GLUCOSE CONC % W/V	AV FRUCTOSE CONC % W/V	AV MALTOSE CONC % W/V
1	0.00	0.00	0.00
2	0.00	7.49	0.00
3	0.21	21.90	0.00
4	2.00	21.98	0.00
5	11.30	21.98	0.00
6	23.19	21.98	0.00
7	25.72	21.96	0.77
8	25.79	21.43	1.15
9	25.78	19.08	1.17
10	25.78	13.34	1.17
11	25.77	5.75	1.17
12	25.43	1.26	1.17

THE ACTUAL DISTRIBUTION COEFFICIENTS BASED
ON THE FEED COMPOSITION WERE :
KDG =0.377 KDF =0.434

FOR A 99.9% PURE FRUCTOSE RICH PRODUCT

THE REQUIRED TOTAL SOCR LENGTH IS :1707.2 CM
THE TRUE NUMBER OF THEORETICAL PLATES IS : 721.6
RESULTING IN A HETP OF : 2.4 CM

List of Symbols used for the Simulation of the Continuous Operation of the SCCR7 System

Ai	Array containing concentrations of component i
CFLOW	Mobile phase flow rate ($cm^3.s^{-1}$)
CTL	Total sugar concentration at any plate in the column ($g.cm^{-3}$)
DELTAT	Time increment (s)
DIA	Column inside diameter (cm)
DT, DTF	Time increments based on the initial switch time approximations (s)
F	Array containing the fructose concentration in each plate ($g\ cm^{-3}$)
FFLOW	Feed flow rate ($cm^3.s^{-1}$)
G	Array containing the glucose concentration in each plate ($g.cm^{-3}$)
HETP	Height equivalent to a theoretical plate (cm)
iCONC	Average concentration of component i in each column ($g\ cm^{-3}$)
iCUM	Cumulative mass of component i (g)
iFEED	Feed concentration of component i ($g.cm^{-3}$)
iMASS	Arrays containing the mass of component i for each plate (g)
ISTKK	First time increment in a sequence
KCYC	Total number of cycles
KCYLST	Penultimate cycle number
KD1	Modified glucose distribution coefficient
KD11	Glucose distribution coefficient in glucose background
KD12	Glucose distribution coefficient in fructose background
KD13	Glucose distribution coefficient in maltose background
KD2	Modified fructose distribution coefficient
KD21	Fructose distribution coefficient in glucose background
KD22	Fructose distribution coefficient in fructose background
KD23	Fructose distribution coefficient in maltose background
KDi	Infinite dilution coefficient of component i
KDiMD	Actual distribution coefficient of component i based on the feed composition
KKINK	Number of time increments in a sequence

KKK	Dissociation constant used in equation 9.1
KNL	Array containing the column numbers
KTOTAL	Total number of sequences
LSTKK	Last time increment in a sequence
LSVP	Linear velocity of stationary phase ($cm.min^{-1}$)
LVT	Average linear velocity of the mobile phase ($cm.min^{-1}$)
M	Array containing the maltose concentration in each plate ($g\ cm^{-3}$)
N	Column number
NFEED	Number of feed column
NHO	Number of "apparent" plates occupied by the feed band
NN	Plate counter
NNBED	Number of "apparent" plates in each column (based on fructose)
NNFEED	Number of feed plate
NNFST	First plate in a column
NNLST	Last plate in a column.
NNNINE	Last plate in the previous column
NNTOT	Total number of "apparent" plates on the system
NTCLS	Total number of columns
NTTP	Total number of theoretical plates found using equations 6.9 or 6.10
OPSWP	Actual switch time (min)
PERTIM	Purping period (min)
ppn	User's personal computer number
PRFD	Column before the feed column
PURITY	Predetermined FRP purity for which the theoretical length estimation has been carried out (%)
REFLOW	Reference feed flow rate ($cm^3\ min^{-1}$)
REFST	Reference switch time (min)
SFLOW	Purge flow rate ($cm^3\ s^{-1}$)
SLTH	Theoretical system length found using equation 9.1 (cm)

SWTFR, SWTIME	Initial switch time approximations (min)
TEMP	Operating temperature (^{o}C)
TESV	Total empty system volume (cm^3)
TFCS	Total feed concentration ($g.cm^{-3}$)
TLENGT	Total system length (cm)
ViCN	Average concentration of component i in each column (% w/v)
VOPKPC	Volume of stationary phase per column (cm^3)
VRT1	Total purge volume (cm^3)
V1NV	Mobile phase plate volume (cm^3)
V2NV	Stationary phase plate volume (cm^3)
XSA	Column cross-sectional area (cm^2)

The subscript i represents glucose, fructose and maltose

NOMENCLATURE

A	Eddy diffusion term in the Van Deemter equation
A'	Cross-sectional area
B	Longitudinal diffusion term in the Van Deemter equation
C	Mass transfer term in the Van Deemter equation
c_i	Concentration of component i in the mobile phase
C_m	Contribution of the extraparticle effect on the C term in the Van Deemter equation
C_s	Contribution of the stationary phase mass transfer effects on the C term in the Van Deemter equation
C_{sm}	Contribution of the stagnant mobile phase effects on the C term in the Van Deemter equation
C_o	Solute concentration at the elution peak
D	Dextran
d	Column diameter
d_p	Particle diameter
d_f	film thickness
D_m,D_{sm}, D_s	Solute diffusion coefficients corresponding to extra-particle, stagnant mobile phase and stationary phase respectively
D_r	Solute radial diffusivity
e	Natural logarithm
ETCF	Experimental-Theoretical-Correlation-factor
F	Fructose
FFRN	Actual operating feed flow rate
FFRR	Reference feed flow rate
f_i	Feed concentration of component i
FRP	Fructose rich product
G	Glucose
GRP	Glucose rich product

H	Plate height
h	Peak height
H_c	Height contribution due to uneven velocity profiles
HETP	Height equivalent to a theoretical plate
HFCS	High Fructose Corn Syrup
K''	Kozeny's constant
K_{di}	Distribution coefficient of component i
K^*_{di}	Reference distribution coefficient of component i calculated at infinite dilution, 25°C and at 1.09 cm min^{-1} linear velocity
K^{∞}_{di}	Infinite dilution distribution coefficient of component i
K^J_{di}	Distribution coefficient of component i in background of component J
$^{25}K_{di}$	Distribution coefficient of component i evaluated at 25°C
k_i	Capacity factor
k'_g	Probability per unit time that a molecule will dissociate from the surface bed (for component i)
k_1,k_2,k_3,k_{-1}	Reaction constants
L	System's length
Le	Pre-feed mobile phase flow rate
Li	Mobile phase flow rate
Lm	Average mobile phase flow rate
L_1	Eluent phase flow rate
L_2	Feed flow rate ,(L_f)
L_3	Purge flow rate
M	Maltose
N	"True" number of theoretical plates
N*	"Apparent" number of theoretical plates
N_b	Number of theoretical plates needed in a batch system

N_G	Number of overall gas phase transfer units
N_i	Number of theoretical plates for component i
N_o	Number of plates occupied by the feed band
N_t	Number of theoretical plates needed in a continuous system
NTP_b	Number of theoretical plates for the batch system
NTP_{sc}	Number of theoretical plates for the semicontinuous system
OS	Oligosaccharides
P	Stationary phase effective flow rate
p_i	Concentration of component i in the stationary phase
r	Column radius
r_{max}	Maximum rate of product formation
r_o	Initial rate of product formation
R_s	Resolution
s	Switch period
S	Packing specific surface area
S'	Predicted switch time
$[S]_o$	Initial substrate concentration
ST	Actual switch time
STR	Reference switch time for the particular SCCR system
TEMP	Temperature
t_{Ri}	Retention time of component i
u_m	Mobile phase velocity
u_p	Effective stationary phase velocity
V_i	Retention volume of component i
V_o	Total void volume in the chromatographic column
V_s	Volume of stationary phase = $V_T - V_o$

V_T	Total empty column volume
W_i	Peak width at the base of the elution curve of component i (in time units)
$W_{h/e}$	Band width measured at a height equal to h/e
X_A, X_B	Concentrations of ions A and B in the solute
x_i	Stationary phase concentration of component i
XSA	Cross-sectional area
Y_A, Y_B	Concentrations of ions A and B in the resin
y_i	Mobile phase concentration of component i
Z	System's length/2

Greek Letters

α	Separation factor
β	Affinity coefficient
γ	Radial Labyrinth factor
γ_1	Rate of transfer of molecules from gas to liquid
γ_2	Rate of transfer of molecules from liquid to gas
δ_{1i}	Fractional changes due to the effect of concentration on the distribution coefficient of component i
δ_{2i}	Fractional changes due to the effect of system characteristics on the distribution coefficient of component i
ε	Column voidage
λ	Parameter relating the relative lengths of batch and continuous systems required to perform the same separation
μ	Fluid viscosity
σ^2	Peak variance in units of volume

Subscripts

b	Batch operation
f	Fructose
g	Glucose
m	Maltose
sc	Semi-continuous operation

Superscripts

D	Dextran
F	Fructose
G	Glucose
M	Maltose

REFERENCES

1 Martin AJP and Synge RLM, Biochem J (London), 35, (1941), 1358

2 Foster FH, "Dextran Manufacture and Use", Parts 1 and 2, Process Biochem, Feb 1968

3 Barker PE, Zafar I, Alsop RM, "Reactor Separator for Dextrans", Brit Pat application 86/05975

4 "Liquid Column Chromatography", A survey of modern techniques and applications, J of Chromatography Library, Vol 3, edited by Deyl Z, Macek K and Janak J, Elsevier Scientific Publishing Co (1975)

5 Williams RJP, Analyst, 77, (1952), 915

6 Tiselius A, Mineral Geol, 14B, No 22, (1940), 5

7 Tiselius A, Mineral Geol, 16A, No 18, (1943), 11

8 Claesson S, Mineral Geol, 23A, No 1 (1946), 133

9 Claesson S, Discussions Faraday Soc, 34, No 7 (1949)

10 Griffiths JH and Phillips CSG, J Chem Soc, (1954), 3446

11 Lindqvist B and Storgards T, Nature (London), 175, (1955), 511

12 Synge RLM, Inst Int Chim Solvay, Cons Chim (Rapp Discuss), 9, (1953), 163

13 Porath J and Foldin P, Nature (London), 183, (1959), 1657

14 Dorfner K, Ionenaustauschchromatographie, Akademie-Verlag, Berlin, (1963b)

15 Helfferich FG, "Ion Exchange", McGraw-Hill, NY, (1962)

16 Paterson R, "An introduction to Ion Exchange", Heyden and Sons, Philadelphia, (1970)

17 Samuelson O, "Ion Exchange Separation in Analytical Chemistry", Wiley, NY, (1963)

18 Genge JAR, "Chromatography", Browning DR (Ed), McGraw-Hill, London, (1969)

19 Fowkes FM, "Encyclopedia of Science and Technology", Vol 1, 5th Edition, McGraw-Hill, NY, (1982)

20 Reiner RH and Walch A, Chromatographia, 4, (1971), 578

21 Dorfner K, "Ion Exchangers, Properties and Applications", Ann Arbor Science, Michigan, (1972)

22 Abusabah EKE, PhD Thesis, University of Aston, Birmingham, (1983)

23 Kirkland JJ, "Modern Practice of Liquid Chromatography", J Willey, NY, (1971)

24 Purnell JH, J Chem Soc, (1960), 1268

25 Rodrigues AE, and Tondeur DT, "Percolation Processes: Theory and Application", Synthoff and Noorhoff (1981), 264

26 Giddings JC, "Dynamics of Chromatography, Part 1, Principles and Theory", Edward Arnold, London, (1965)

27 Van Deemter JJ, Zuiderweg FJ and Klinkenberg A, Chem Eng Sci, 5, (1956), 271

28 Wilson JN, J Am Chem Soc, 62, (1940), 1583

29 De Vault DJ, J Am Chem Soc, 65, (1943), 532

30 Mayer SW, Tompkins ER, J Am Chem Soc, 69, (1947), 2866

31 Glueckauf E, Trans Faraday Soc, 51, (1955), 34

32 Lapidus L, Amundson NR, J Phys Chem, 56, (1952), 984

33 Tunitskii NN, Doklady Akad Nauk SSCR, (Compt rend acad sci, URSS), 99 (1954), 577

34 Giddings JC, Euring H, J Phys Chem, 59, (1955), 416

35 Klinkenberg A, Sjenitzer F, Chem Eng Sci, 5, (1956), 258

36 Littlewood AB, "Gas Chromatography" 2nd ed, Academic Press, NY, (1970)

37 Dal Nogare S, Juvet RS, "Gas-liquid Chromatography", Wiley, NY, (1962)

38 Phillips CSG, "Gas Chromatography", Academic Press, NY (1956), 95

39 Yau WW, Kirkland JJ, Bly DD, "Modern Size-exclusion Liquid Chromatography", Wiley, NY (1979)

40 Keulemans AIM, "Gas Chromatography", Verner CG (Ed), Reinhold Publishing Corporation, NY, (1959)

41 Hamilton RJ, Sewell PA, "Introduction to High Performance Liquid Chromatography", Wiley, NY (1977), Chapter 2.

42 Glueckauf E, Trans Faraday Soc, 60, (1964), 729

43 Giddings JC, J Gas Chrom, 1, (4), (1963), 38

44 Bayer E, Hupe K, Mack H, Anal Chem, 35, (1963), 492

45 Giddings JC, Jensen GE, J Gas Chrom, 2, (9), (1964), 290

46 Huyten FH, Van Beersan W, Rijnders GWA, "Gas Chromatography", Scot RPW (Ed), Butterworth, London, (1960)

47 Pretorius V, De Clark K, "Prep Gas Chromatography", Wiley, Interscience, London, (1971)

48 Helfferich FJ, Chem Educ, 41, (1964), 410

49 Vermulen T, Ind Eng Chem, 45, (1953), 1658

50 Conder JR, Shingari MK, J Chrom Sci, 73, 525

51 Janson Jan-Christer, Hedman Per, "Large Scale Chromatography of Proteins", paper presented in the Biochemical Engineering Course at the University College in London, (1985)

52 Sachs DH, Painter E, Science, 175, (1972), 781

53 Janson JC, Dunnill P, "Industrial Aspects of Biochemistry", Spencer B (Ed), Amsterdam, (1974)

54 Boeing J, Muller HA, US Pat 4259186, (1978)

55 Janson JC, Agr Food Chem, 19, (1971), 581

56 Musser WN, Sparks J, J Chron Sci, 9, (1971), 116

57 Timmins RS, Mir L, Ryan JM, Chem Eng, 76, (1969), 170

58 Baddour RF, US Pat: 3250058, (1966)

59 Higgins GMC, Smith JE, Gas Chromatography, Goldup A (Ed), Institution of Petroleum, London (1965)

60 Martin AJP, Dis Faraday Soc, (London), 7, (1949), 332

61 Svensson H, Swedish Pat: 133951

62 Fox JB, J Chromatogr, 43, (1969), 55

63 Nicholas RA, Fox JB, J Chromatogr, 43, (1969), 61

64 Taramasso M, Dinelli D, J Gas Chromatogr, 2, (1964), 150

65 Turina S, Krajovan V, Kostomaj, Anal Chem, 34, (1962), 100

66 Barker PE, Critcher D, Chem Eng Sci, 13, (1960), 82

67 Berg C, TAIChE, 42, (1946), 665

68 Kehde H, Fairfield RG, Frank IC, Zahenstecher LW, Chem Eng Prog, 44, (1948), 575

69 Pichler H, Schultz H, Brennst-Chem, 39, (1958), 48

70 Gulf Research and Development, US Pat: 2893955

71 Luft L, Mine Safety Appliances, US Pat: 3016107

72 Barker PE, Huntington DH, Gas Chromatogr, Littlewood AB (Ed), Inst of Petroleum, London, (1966)

73 Wankat PC, Oritz PM, Int Eng Chem Process, Res Dev, 21, (1982), 416

74 Broughton DB, Chem Eng Progr, 64, (8), (1968), 60

75 Thornton DP, Hydrocarbon Process, 49, (11), (1970), 151

76 Broughton DB, Neuzil RW, Pharis JM, Brearley CS, Chem Eng Progr, 66, (9), (1970), 70

77 Barker PE, Deeble, RE, Anal Chem, 45, (1973), 1121

78 Barker, PE, Deeble RE, Brit Pat: 1418503, US Pat: 4001112

79 Szepesy L, Sebestyen Zs, Feher I, Nagy Z, J of Chromatog, 108, (1975), 285

80 Ryan JM, Timmins RS, O'Donnell JF, Chem Eng Prog, 64, (8), (Aug, 1968), 53

81 "Plant Scale Gas Chromatography Envisioned", C&EN, June 28 1965, 46

82 "Liquid Chromatography Looms as Full-scale Separation Method for Production Uses", C&EN, March 25 1968, 62

83 Chem Show '79, Chem Processing, mid-November 1979, 86

84 "Liquid Chromatography: Ready for Bigger Rate", Chem Eng, Aug 28 1978, 58

85 Bernard JR, Gourlia JP, Guttierrez MJ, Chem Eng, May 18 1981, 92

86 "Gas Chromatography Tackles Production Jobs", Chem Eng, Nov 30 1981, 70

87 Chem Eng (Int Ed), 89, (24), 29 Nov 1982, 11

88 Ferrier RJ, Collins PM, "Monosaccharides Chemistry", Penguin Library, London (1972)

89 Guthrie RD, Honeyman J, "An Introduction to the Chemistry of Carbohydrates", 3rd Edition, Clarendon Press, Oxford (1968)

90 Hyvonen L, Pertti V, Kovistoinen P, J of Food Science, 42, (3), (1977), 652

91 Hyvoven L, Pertti V, Kovistoinen P, J of Food Science, 42, (3), (1977), 657

92 Hyvonen L, Pertti V, Kovistoinen P, J of Food Science, 42, (3), (1977), 654

93 Shallenbeiger et al, "Sugar Chemistry", AVI Publishing Co Inc, (1971), 116

94 Barker SA, Proc Biochem, 10, (1975), 39

95 Birch GG, Green LF, Coulson CB, "Sweetness and Sweeteners", Applied Science Publishers, London, (1971)

96 Birch GG, Parker KJ, "Sugars: Science and Technology", Applied Science Publishers, London, (1979), Chapter 14

97 Bruyn L, Van Eckenstein A, Rec Fran Chim, 14, (1895), 203

98 Dale JK, Langlosis DP, US Pat: 2201609

99 Marshall RO, Kooi ER, Science, 125, (1957), 648

100 Marshall RO, US Patent: 2950228, (1960)

101 Tsumara N, Sato T, Agri Biol Chem, 25, (1961), 616

102 Natake M, Yoshimura S, Agri Biol Chem, 27, (1963), 342

103 Sato T, Tsumara N, Agri Biol Chem, 29, (1965), 1129

104 Takasaki Y, Tanable O, US Pat: 3616221

105 Takasaki Y, Kosugi Y, Kanbayashi A, "Fermentation Advances", Academic Press, NY (1969), 561

106 Newton A, Wardip G, In Symp: Sweeteners, "High Fructose Corn Syrup", Inglett GE (Ed), Ch 8, AVI, NY, (1974)

107 Charley PJ, Abstract from Rendleman JA, Adv in Carbohydr Chem and Biochem, 24, (1966), 209

108 Angyal SJ, J Chem, 25, (1972), 1957

109 Angyal SJ, J Chem, 27, (1974), 1447

110 Angyal SJ, J Chem, 28, (1975), 1279

111 Angyal SJ, J Chem, 28, (1975), 1541

112 Angyal SJ, Olis J, James VJ, Pojer PM, Carbohydr Res, 60, (1978), 219

113 Angyal SJ, Evans ME, Beveridge RJ, Methods in Carbohydrate Chemistry, 8, (1980), 233

114 Lindberg B, Carbohyd Res, 5, (1967), 286

115 Schnyder BJ, Starke, 26, (1974), 409

116 Zittan L, Poulsen PB, Hemmingsen SH, Starke, 27, (1975), 236

117 Lauer K, Weber M, Stoek G, US Pat: 3483031 (1969)

118 Lauer K, Springler H, Wallach KE, Stoeck G, US Pat: 3694158, (1972)

119 Lauer K, Budka HG, Stoeck G, US Pat: 3785864, (1974)

120 "And Rice to Fructose is the Goal of a New Process being used in Pakistan", Chem Eng, Sept 20 1982, 19

121 Hollo J, Laszlo E, Hoschke A, Bende P, Bolgar P, Wieg A, Process Biochemistry, June 1985, 79

122 Hashimoto K, Adachi S, Noujima H, Ueda Y, Biotech and Bioeng, Vol XXV, (1983), 2371

123 Hongisto HJ, Int Sug J, 79, (1977), 100

124 Heikkila H, Chem Eng, Jan 24 1983, 50

125 Munir M, Int Sugar J, 78, (1976), 100

126 The Colonial Sugar Refining Co, Brit Pat: 1083500 (1967)

127 Boehringer CF, Haftung SGMB, of Maunheim-Waldhof, Brit Pat: 1085696 (1967)

128 Broughton DB, "Industrial Applications of Preparative Chromatography", book on "Percolation Process Theory and Applications", Rodrigues AG and Tondeur D (Ed), Applied Science Publishers, 249

129 Bieser HJ, de Rosset AJ, Die Starke, 29, (1977), 392

130 Odawara H, Ohno M, Yamazaki T, Kanaoka M, US Pat: 4157267, (1979)

131 Spinks A, BIOTECHNOLOGY, Report of a joint Working Party, HMSO, London, March 1980

132 Vlachogiannis G, PhD Thesis, University of Aston, Birmingham, 1982

133 Ganetsos G, Final Year Design project, BSc Chem Eng, University of Aston, Birmingham, 1983

134 Dunnill P, "Protein Processing", Course in Biochemical Engineering, University College, London, Sept 1985

135 Thorley JF, "The Fermentation of Recombinant Organisms", paper presented in the "Engineering Developments in the Pharmaceutical and Biological Industries" Symposium, IChemE, 21 May 1986

136 Datar R, Process Biochemistry, Feb 1986, 19

137 Wakeman R, The Chemical Engineer, June 1986, 65

138 Meares P, The Chemical Engineer, Feb 1986, 38

139 Le MS, Billinghelmer PJ, The Chemial Engineer, July/August 1985, 48

140 Dillman WJ, Miller IF, J Coll Int Sci, 41, (1973), 221

141 Morrissey BW, Hau CC, J Coll Int Sci, 65, (1978), 423

142 Sternberg M, Hershberger D, Biochim Biophys Acta, 34, (1974), 195

143 Bergmeyer HV, Naher G, Weimann G, Thum W, US Pat: 3794562, (1974)

144 Dextran Fractions, Dextran Sulphate, DEAE-Dextran, Publication material by Pharmacia Fine Chemicals (Sweden)

145 Lindquist LO, Williams KW, Dairy Ind Int, 38, (1973), 459

146 Cohn EJ et al, J Am Chem Soc, 68, (1946), 459

147 Notarianni AF, Ghielmetti G, US Pat: 3586607, (1971)

148 Chase HA, Chem Eng Sci, 39, (7/8), (1984), 1099

149 Janson JC, Trends in Biotechnology, 2, (2), (1984), 31

150 Scheibler C, Rudenzucker Ver, Ind, 24, (1874), 309

151 Nilsson K, WHO/IABS Symposium, Geneva 1980, Develop Biol Standards, 48

152 Murphy PT, Whistler RL, "Dextrans" in Industrial Gums: Polysaccharides and their derivatives, 2nd ed, Academic Press, NY, (1973), 513

153 Baker PJ, Industrial Gums, Chapter XXIII, publishers Whistler, 531

154 Alsop RM, "Industrial production of Dextrans" Progress in Industrial Microbiology, Bushell ME (Ed), Elsevier, (1983)

155 Jeanes A, Encyclopedia of Polymer Science and Technology, John Wiley and Sons Inc, Volume 4 (1966), 805

156 Neely WB, Adv Carbohydr Chem, 15, (1960), 341

157 Ebert KH, Schenk G, Adv Enzymol, 30, (1968), 179

158 Sidobotham RL, Adv Carbohydr Chem Biochem, 30, (1974), 371

159 Robyt JF, Kimble BK, Walseth TF, Arch Biochem Biophys, 165, (1974), 634

160 Robyt JF, Corrigan AJ, Arch Biochem Biophys, 183, (1977), 726

161 Robyt JF, Tanigvchi H, Arch Biochem Biophys, 174, (1976), 129

162 Robyt JF, Walseth RF, Carbohyd Res, 61, (1978), 433

163 Robyt JF, Eklund SH, Bioorganic chemistry, 11, (1982), 115

164 Stodola FH, Sharpe ES, Koepsell HJ, J Amer Chem Soc, 78, (1956), 2541

165 Bhatnagar R, Bhatnagar N, Prabhu KA, IRCS Med Sci, 13, (1985), 129

166 Miller AW, Robyt JF, Biochimica et Biophysica Act, 785, (1984), 89

167 Koepsell HJ, Tsuchiya HM, J Bacteriol, 63, (1952), 293

168 Lawford CR, Kligerman A, Williams T, Biotech and Bioeng, 21, (1979), 1121

169 Robyt JF, Walseth TF, Carbohydrate Research, 68, (1979), 95

170 Kaboli H, Reilly PJ, Biotech and Bioeng, 22, (1980), 1055

171 Kobayashi M, Matsuda K, Biochimica et Biophysica Acta, 397, (1975), 69

172 Kobayashi M, Matsuda K, J Biochem, 79, (1976), 1301

173 Kobayashi M, Matsuda K, Biochimica et Biophysica Acta, 614, (1980), 46

174 Lopez A, Monsan P, Biochimie, 62, (1980), 323

175 Chen YE, Kaboli H, Proc Ann Bioch Eng Symp, 6 April 1976, 41

176 Paul F, Auriol D, Oriol E, Monsan P, Annals NY Academy of Sciences, 434, 267

177 Paul F, Monsan P, Auriol D, "Procede de Purification de La Dextrane-saccharase", European Pat: 125981-A (1984)

178 Schneider M, Guillot C, Ayerbe A, UK Pat: 2079290 B (1982)

179 Ogino S, Agri Biol Chem, 34, (1970), 1268

180 Edwards CR, Drew SW, Abstr Ann Meet Am Soc Microbiol, 77, (1977), 251

181 Roginskii SZ, Yanovskii MI, Gazier GA, Doklady Akademii Nauk SSSR, Vol 140, No 5, (1961), 1125

182 Roginskii SZ, Semenenko EI, Yanovskii, Doklady Akademii Nauk SSSR, Vol 153, No 2 (1963), 383

183 Roginskii SZ, Rozental AL, Doklady Akademii Nauk SSSR, Vol 162, No 3 (1965), 621

184 Yanovskii MI, Berman AD, Russian Chemical Reviews, 42, (1), (1973), 72

185 Magee EM, I&EC Fundamentals, Vol 2, No 1, Feb 1963, 32

186 Matsen JM, Harding JW, Magee EM, The J of Phys Chem, 69, No 2, Feb 1965, 522

187 Villermaux J, Schweich D, Chem Eng J, 24, (1982), 99

188 Villermaux J, Nato Aso Ser E 33, (Percolation Processes: theory and applications), (1985), 539

189 Langer SH, Bohne MW, The J of Phys Chem, 87, No 18, 1983, 3363

190 Langer SH, Chu AHT, Anal Chem, 57, (1985), 2197

191 Mile B, Morton L, Sewell PA, J of Chromatography, 204, (1981), 35

192 Unger BD, Rinker RG, Ind Eng Chem Fundam, 15, (3), (1976), 225

193 Takeuchi K, Uraguchi Y, J of Chem Eng of Japan, 10, (6), (1977), 455

194 Takeuchi K, Miyauchi T, Uraguchi Y, J of Chem Eng of Japan, 11, (3), (1978), 216

195 Petroulas T, Aris R, Carr RW, Chem Eng Sci, 40, (12), (1985), 2233

196 Viswanathan S, Aris R, Adv Chem Ser 197, 133, (1970), 191

197 Cho BK, Carr RW, Aris R, Chem Eng Sci, 35, (1980), 74

198 Cho BK, Carr RW, Aris R, Separation Sci and Technol, 15, (3), (1980), 679

199 Hashimoto K, Adachi S, Hiromitsu N, Ueda Y, Biotechnology and Bioengineering, Vol XXV, (1983), 2371

200 Ishimatsu Y, Chem Economy and Engin Review, No 185, Vol 17, (1-2), (1985), 36

201 Deeble RE, PhD thesis, University of Aston, Birmingham, (1974)

202 Thawait S, PhD thesis, University of Aston, Birmingham, (1983)

203 Ching CB, PhD thesis, University of Aston, Birmingham, (1978)

204 Chuah CH, PhD thesis, University of Aston, Birmingham, (1980)

205 Ellison JF, PhD thesis, University of Aston, Birmingham, (1976)

206 England K, PhD thesis, University of Aston, Birmingham, (1980)

207 Coulson JM, Richardson JF, Chemical Engineering, Vol 2, 3rd Ed, Pergamon Press, 1978

208 Barker PE, Barker SA, Hatt BW, Somers PJ, Chem Proc Eng, 52, (1971), 64

209 Barker PE, Al-Madfai S, J Chromatog Sci, 7, (1969), 425

210 Barker PE, Deeble RE, Chromatographia, Vol 8, No 2, Feb 1975

211 Barker PE, Ellison FJ, Hatt BW, Ind Eng Chem Process Des Dev, Vol 17, No 3, (1978), 302

212 Barker PE, Alsop RM, Vlachogiannis G, Chromatographia, Vol 17, No 3, March 1983, 149

213 Barker PE, Chuah CH, The Chemical Engineer, Aug/Sept 1981, 389

214 Gould JC, PhD thesis, University of Aston, Birmingham, (1980)

215 Barker PE, Thawait S, "The Separation of Fructose from Carbohydrate Mixtures by Batch and Semi-continuous Operation", paper accepted for publication in Chem Eng Res and Design (London)

216 Barker PE; Ganetsos G, Thawait S, "Development of a Link between Batch and Semi-continuous Liquid Chromatographic systems", paper accepted for publication in Chem Eng Science

217 Barker PE, Abusabah EKE, Chromatographia, Vol 20, No 1, Jan 1985

218 Barker PE, Abusabah EKE, Irlam GA, Chromatographia, Vol 18, No 10, Oct 1984, 567

219 Barker PE, Thawait S, J of Chromatography, 295, (1984), 479

220 Ruthven DM, Department of Chemical Engineering, University of New Brunswick, Canada, communication in 1985

221 Thawait S, Barker PE, Chemistry and Industry, 7 Nov 1983

222 "Guidelines for Use and Care of Aminex Resin based Columns", BIO-RAD (Watford, UK), product information

223 SUGAR-PAK 1 use guidelines, WATERS ASSOCIATES, product information

224 Zafar I, Department of Chemical Engineering, University of Aston, Birmingham, private communication

225 Joshi K, Department of Chemical Engineering, University of Aston, Birmingham, private communication

226 "Handbook of Chemistry and Physics", Weast RC, Astle MJ, 62nd Edition, CRC Press Inc, 1981-82

227 Sciance CT, Crosser OK, AIChE Journal, Jan 1966, 100

228 Al-Madfai S, PhD thesis, University of Aston, Birmingham, (1969)

229 Huang S, Wilson JW, Overholser KA, J Chromatography, 89, (1974), 119

230 Zhitomirskii BM, Agafonow AV, Berman AD, Yanowskii MI, J Chromatography, 94, (1974), 1

231 Jonsson JA, Chromatographia, 13, 5, (1980), 273

232 Barker PE, Lloyd D, Symposium on the Less Common Means of Separation, Inst Chem Eng, (1963)

233 Sunal AB, PhD thesis, University of Aston, Birmingham, (1973)

234 Deeble RE, PhD thesis, University of Aston, Birmingham, (1974)

235 Barker PE, Thawait S, Chem and Ind, 7 Nov 1983, 817

236 Barker PE, England K, Vlachogiannis G, Chem Eng ResDes, Vol 61, July 1983

237 Control Point Jobs, Harris Handbook, Section H2-09, Jan 1982

238 Le MS, Atkinson T, Process Biochem, Feb 1985, 26

239 Jackson C, Steward G, Fisons UK, Loughborough, personal communication

240 Zafar I, Interim reports, Department of Chemical Engineering, University of Aston, Birmingham, 1984-85

241 Le MS, Spark LB, Ward PS, J of Membrane Sci, 21, (1984), 219

242 Le MS, Spark LB, Ward PS, Ladwa N, J of Membrane Sci, 21, (1984), 307

243 Lilly MD, "Immobilised Enzyme/Cell Reactor Operation", paper presented in the Biochemical Engineering course, University College, London, 1985

244 Enka Cross-flow microfiltration modules, product information, (UK distributors APV Membrane Process Ltd, Crawley).

APPENDIX 1

Determination of K_g' for 10 column x 2.54cm dia SCCR4 Unit

The operating conditions and results of run 02-2.4-6.1-29 ... reference (12) were used since this run approached the "infinite dilution" conditions, ie.

$$\chi_g = 0.098,\ Z = 638/2 = 316\text{cm},\quad K_{dg}^{\infty} = 0.23$$

Switch time = 30 min

$$u_m = \frac{Vg}{XSA} = \frac{\text{Average mobile phase flow rate}}{XSA} = \frac{(6.1+8.5)/2}{\pi\,(2.54)^2/4} = 1.44\text{cm.min}^{-1}$$

$$u_p = \frac{Vp}{XSA} = \frac{\text{Average volume of packing per column/switch time}}{XSA}$$

$$= \frac{320/30}{\pi\,(2.54)^2/4} = 2.11\text{cm.min}^{-1}$$

Therefore

$$\ln(0.098) = \frac{316k_g'}{1.44}\left(0.23 - \frac{1.44}{2.11}\right) \qquad \therefore\ k_g' = 0.023$$

Prediction of total length of the 10 column x 10.8cm dia SCCR6 Unit

Using similar operating conditions to the 2.54cm dia SCCR4 Unit, the same switch time of 30 min and the following data

$$\text{feed flow rate} = 35\text{cm}^3\,\text{min}^{-1}$$

$$\text{eluent flow rate} = 105\text{cm}^3\,\text{min}^{-1} \quad \text{and} \quad \overset{\infty}{K}_{dg} = 0.168$$

and designing for 99.9% pure FRP (ie. $\chi_g = 0.001$) then,

$$u_m = \frac{(105 + 140)/2}{\pi\,(10.8)^2/4} = 1.34\ \text{cm.min}^{-1}$$

$$u_p = \frac{3940/30}{\pi\,(10.8)^2/4} = 1.43\ \text{cm.min}^{-1}$$

Hence,

$$\ln\,(0.001) = \frac{0.023Z}{1.337}\left(0.168 - \frac{1.34}{1.43}\right) \qquad \therefore\ Z = 522\text{cm}$$

With a central feed location on a continuous unit, the overall theoretical SCCR6 system length is 1044cm.

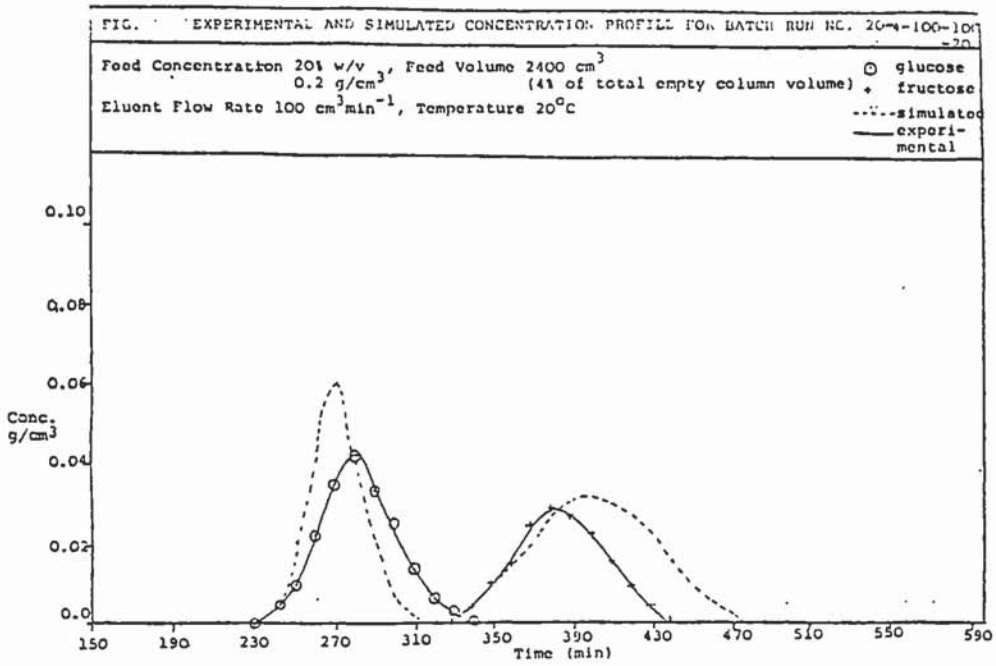


FIG. EXPERIMENTAL AND SIMULATED CONCENTRATION PROFILE FOR BATCH RUN NO. 20-4-100-100-20
Feed Concentration 20% w/v, Feed Volume 2400 cm3
0.2 g/cm3 (4% of total empty column volume)
Eluent Flow Rate 100 cm3min-1, Temperature 20°C
glucose
fructose
simulated
experimental
Conc. g/cm3
0.10
0.08
0.06
0.04
0.02
0.0
150 190 230 270 310 350 390 430 470 510 550 590
Time (min)

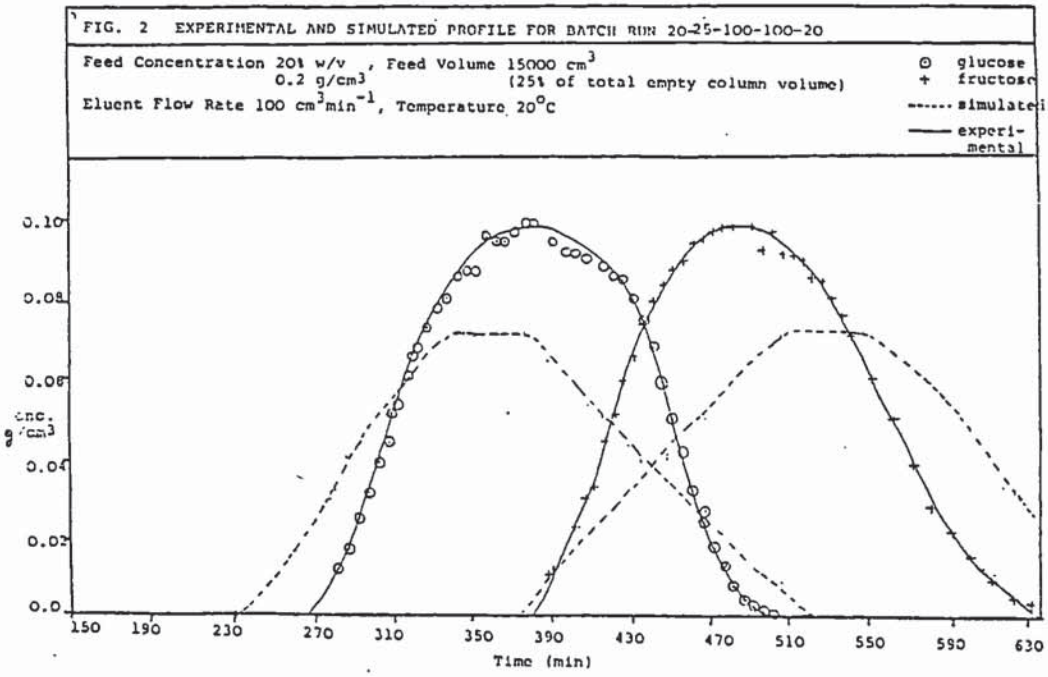


FIG. 2 EXPERIMENTAL AND SIMULATED PROFILE FOR BATCH RUN 20-25-100-100-20
Feed Concentration 20% w/v, Feed Volume 15000 cm3
0.2 g/cm3 (25% of total empty column volume)
Eluent Flow Rate 100 cm3min-1, Temperature 20°C
glucose
fructose
simulated
experimental
Conc. g/cm3
0.10
0.08
0.06
0.04
0.02
0.0
150 190 230 270 310 350 390 430 470 510 550 590 630
Time (min)

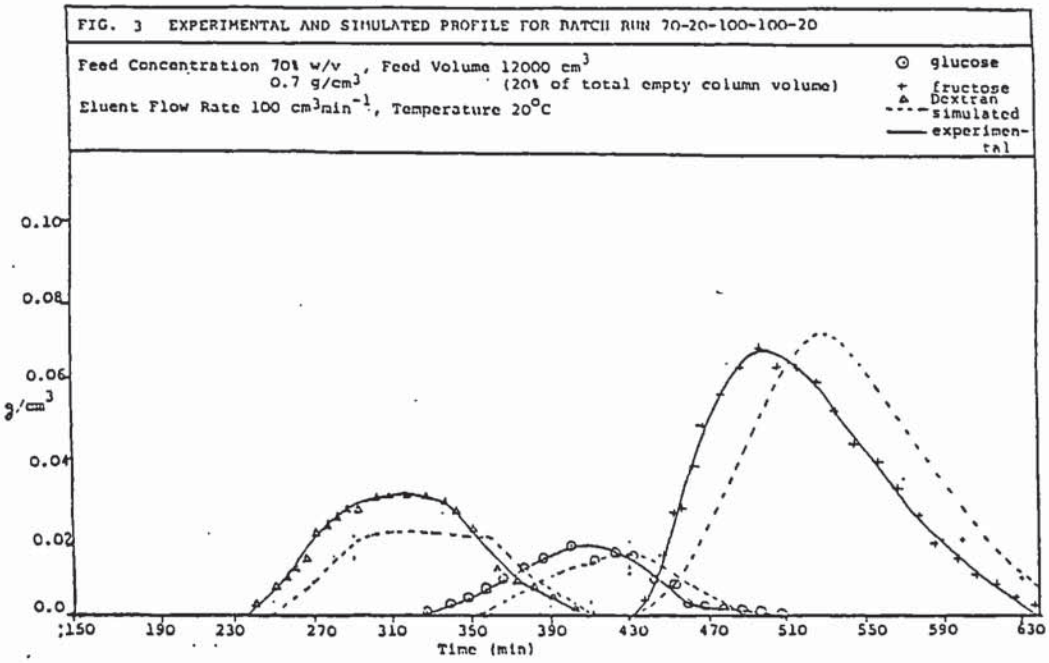


FIG. 3 EXPERIMENTAL AND SIMULATED PROFILE FOR BATCH RUN 70-20-100-100-20
Feed Concentration 70% w/v, Feed Volume 12000 cm3
0.7 g/cm3 (20% of total empty column volume)
Eluent Flow Rate 100 cm3min-1, Temperature 20°C
glucose
fructose
Dextran
simulated
experimental
g/cm3
0.10
0.08
0.06
0.04
0.02
0.0
150 190 230 270 310 350 390 430 470 510 550 590 630
Time (min)

Figure 4: Plot of number of theoretical plates (NTP) and system loading related parameter (P), against the number of theoretical plates occupied by the feed band (No)

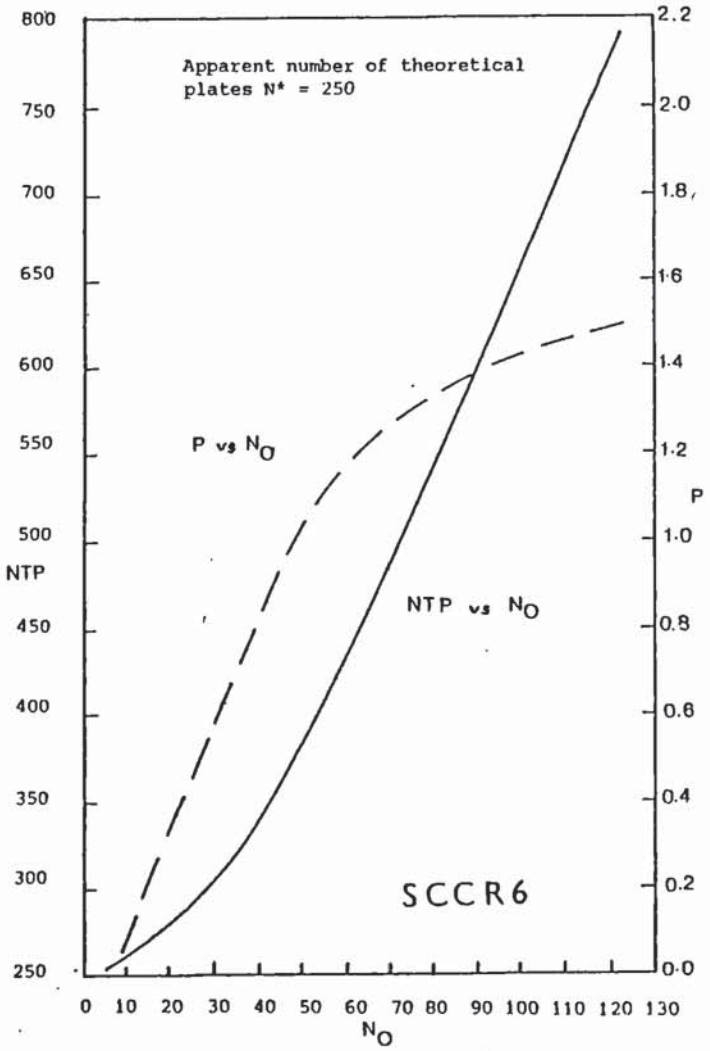


Figure 5 : Number of theoretical plates (NTP) as a function of separation factor (α), and mass fraction of component corrected according to the feed composition (x_i), in product stream (Ref (31) Fig 3)

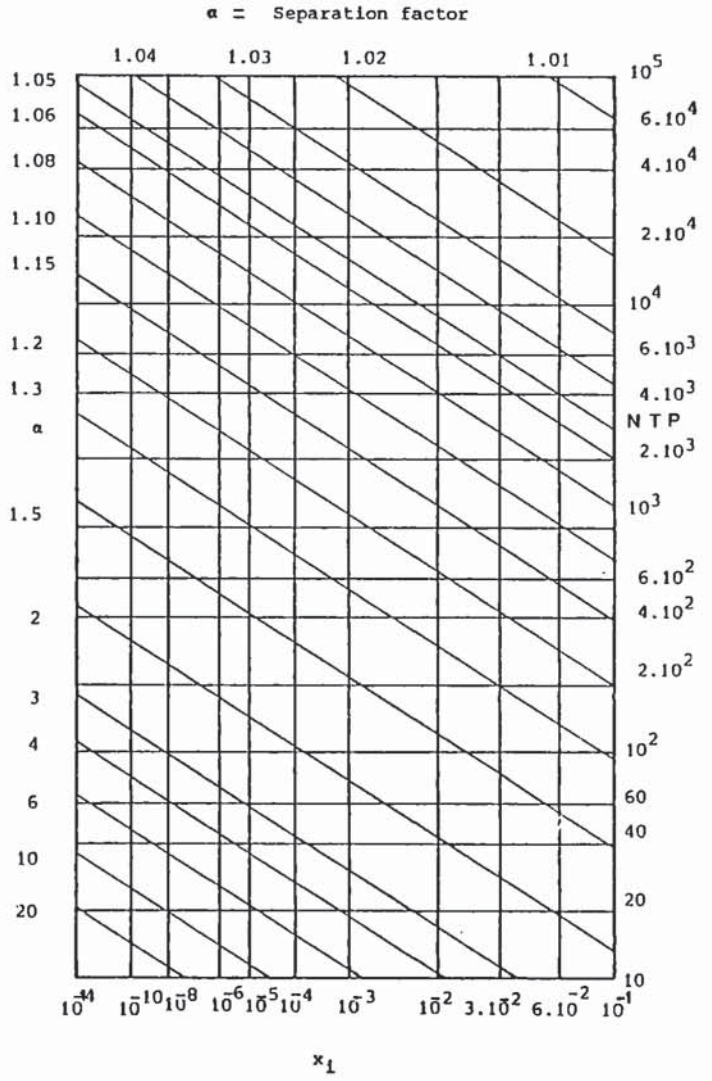


TABLE 1.. SIMULATION RESULTS FOR THE COMPARISON OF BATCH AND SEMI-CONTINUOUS OPERATION FOR GLUCOSE FRUCTOSE FEED AT AMBIENT TEMPERATURE

FEED CONC. %w/v		BATCH								SEMI CONTINUOUS									THROUGH-PUT RATIO
		SAMPLE VOLUME INJECTED (1)	FRUCTOSE PRODUCT			GLUCOSE PRODUCT			THROUGH-PUT kg/h	AVERAGE FLOW RATES		SWITCH PERIOD min	FRUCTOSE PRODUCT			GLUCOSE PRODUCT			THROUGH-PUT kg/h	
G	F		PURITY %	MASS BAL. %	PROD CONC %w/v	PURITY %	MASS BAL. %	PROD CONC. %w/v		FEED $cm^3 min^{-1}$	ELUENT $cm^3 min^{-1}$		PURITY %	MASS BAL. %	PROD CONC. %w/v*	PURITY %	MASS BAL. %	PROD CONC %w/v		SEMI CONT / BATCH
10.0	10.0	15.0	99.9	100	2.6	99.9	100	5.7	0.25	70.2	210	15	96.1	100	1.05	99.9	100	0.9	0.84	3.36
20.0	20.0	15.0	84.0	100	9.1	99.9	100	12.1	1.11	70.2	210	15	80.0	100	2.97	99.9	100	1.4	1.68	1.51
25.0	25.0	15.0	82.0	100	12.9	99.9	100	14.6	1.21	70.2	210	15	75.0	100	3.4	99.9	100	1.8	2.1	1.73

* fructose concentration in bulk fructose product over the switch period of 15 minutes

TABLE 2. SIMULATION RESULTS FOR THE COMPARISON OF BATCH AND SEMI-CONTINUOUS OPERATION FOR GLUCOSE, FRUCTOSE, DEXTRAN FEED AT AMBIENT TEMPERATURE

FEED CONCENTRATION % w/v			BATCH									SEMI-CONTINUOUS										THROUGH-PUT
			SAMPLE INJECTED (1)	FRUCTOSE PRODUCT			DEXTRAN PRODUCT				THROUGH-PUT kg/h	AVERAGE FLOW RATES		SWITCH PERIOD (min)	FRUCTOSE PRODUCT			DEXTRAN PRODUCT				THROUGH-PUT kg/h	
G	F	D		PUR. %	MASS BAL. %	FRUC. CONC. %w/v	PUR. %	MASS BAL. %		D CONC %		FEED $cm^3 min^{-1}$	ELUENT $cm^3 min^{-1}$		PUR. %	MASS BAL. %	FRUC. CONC. %w/v*	PUR. %	MASS BAL. %		D CONC %		SEMI CONT / BATCH
								D	G										D	G		
0.07	0.49	0.15	12	99.9	100	10.3	68	100	100	3.0	0.6	70.2	210	15	99.9	100	6.2	99.2	100	100	3.7	2.94	4.26

* fructose concentration in bulk fructose product over the switch period of 15 minutes

TABLE 3 RESULTS FOR THE COMPARISON OF BATCH AND CONTINUOUS OPERATION OF GLUCOSE FRUCTOSE MIXTURE

Feed Conc % w/v		Batch							Semi-Continuous							Throughput Ratio Semi/Batch
		F.R.P.			G.R.P.			Throughput kg h^{-1}	F.R.P.			G.R.P.			Throughput kg h^{-1}	
G	F	Purity %	Mass Bal.	Fruct. Conc. % w/v	Purity %	Mass Bal.	Gluc. Conc. % w/v		Purity %	Mass Bal %	Fruct. Conc. % w/v	Purity %	Mass Bal. %	Gluc. Conc. % w/v		
10.1	10.2	99.9	102	5.44	99.9	103	7.65	0.3	85.0	105	1.22	99.9	69	1.4	0.84	2.8
20.0	20.0	84.0	105	12.01	99.9	101	15.52	0.98	76.0	102	3.2	99.9	98	2.98	1.68	1.72
25.0	25.1	82.0	103	13.1	99.9	106	21.85	1.21	70.0	111	7.6	99.9	100	2.7	2.1	1.74

TABLE 4 RESULTS FOR THE COMPARISON OF BATCH/CONTINUOUS OPERATION FOR FISONS SYNTHETIC FEED

Feed Conc.			Batch							Semi-Continuous							Throughput Ratio Semi/Batch
			F.R.P.			D.R.P.			Throughput kgh^{-1}	F.R.P.			D.R.P.			Throughput kgh^{-1}	
G	F	D	Purity %	Mass Bal. %	Fruct. Conc. % w/v	Purity %	Mass Bal. % D	Mass Bal. % G	Dext. Conc. % w/v	Purity %	Mass Bal. %	Fruct Conc. % w/v	Purity %	Mass Bal. % D	Mass Bal. % G	Dext. Conc. % w/v		
6.3	18.3	14.9	99.9	114	8.64	66	110	108	3.84	0.56	99.9	114	16.3	72	101	102	4.2	2.94	5.25

TABLE 5: Experimental Operating Conditions for the Evaluation of the SCCR6 Total System Length

Run Number	Average Flow Rates cm^3 min^{-1}			Switch Time Min	Feed Concentration % w/v	Temperature °C
	Eluent	Feed	Purge			
20-35-105-30-20	105	35	550	30	20	20

TABLE 6: Results Obtained at the Above Operating Conditions

Feed Throughput kg/hr	Fructose Rich Product			Glucose Rich Product		
	Purity %	Product Concentration % w/v	Total Fructose Recovered %	Purity %	Product Concentration % w/v	Total Glucose Recovered %
0.42	99.9	0.64	98	99.9	2.37	101