THE PRODUCTION OF FRUCTOSE FROM CARBOHYDRATE FEEDSTOCKS

A thesis submitted by

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PhD Mr Ketan Joshi April 1988

SUMMARY

A review of the general chromatographic theory and of continuous chromatographic techniques has been carried out.

Three methods of inversion of sucrose to glucose and fructose in beet molasses were explored. These methods were the inversion of sucrose using the enzyme invertase, by the use of hydrochloric acid and the use of the resin Amberlite IR118 in the H^+ form. The preferred method on economic and purity considerations was by the use of the enzyme invertase.

The continuous chromatographic separation of inverted beet molasses resulting in a fructose rich product and a product containing glucose and other non-sugars was carried out using a semi-continuous counter-current chromatographic refiner (SCCR6), consisting of ten 10.8cm x 75cm long stainless steel columns packed with a calcium charged 8% cross-linked polystyrene resin Zerolit SRC 14. Based on the literature this is the first time such a continuous separation has been attempted.

It was found that the cations present in beet molasses displaced the calcium ions from the resin resulting in poor separation of the glucose and fructose. Three methods of maintaining the calcium form of the resin during the continuous operation of the equipment were established. Passing a solution of calcium nitrate through the purge column for half a switch period was found to be most effective as there was no contamination of the main fructose rich product and the product concentrations were increased by 50%.

When a 53% total solids (53 Brix) molasses feedstock was used, the throughput was 34.13kg sugar solids per m^3 of resin per hour. Product purities of 97% fructose in fructose rich (FRP) and 96% glucose in the glucose rich (GRP) products were obtained with product concentrations of 10.93 %w/w for the FRP and 10.07 %w/w for the GRP.

The effects of flowrates, temperature and background sugar concentration on the distribution coefficients of fructose, glucose, betaine and an ionic component of beet molasses were evaluated and general relationships derived.

The computer simulation of inverted beet molasses separations on an SCCR system has been carried out successfully.

KEY WORDS: Chromatography, fructose, glucose, molasses, sucrose inversion

Dedicated to my family

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CHAPTER ONE

INTRODUCTION

The production of sugar worldwide depends on the success of the harvest of the sugar producing crops. These crops vary with location - sugar beet in the temperate zones and sugar cane in the tropical zones of the world. The most common sugar produced in large quantities is sucrose, but since the late 1970's considerable interest has been shown in the production of monosaccharides from starch sources such as maize, corn or rice by enzymatic inversion.

British Sugar plc controls sugar production from sugar beet in Britain whereas Tate and Lyle Ltd is the main producer of sugar from imported sugar cane.

The production of sugar in the European Economic Community member countries is controlled by the commission to protect the sugar beet farmers in the community. There are three different quotas set for the quantity of sucrose produced by British sugar from sugar beet:

Quota A - a high price is guaranteed by the EEC for the sucrose produced in this quota.

Quota B - a lower price is guaranteed for sucrose produced within this quota. The B quota is 10% of the A quota in tonnage.

Quota C - any C quota sugar produced must be

exported outside the EEC, and hence it is quoted at the world market price. This price paid is much lower than the two other prices which currently makes it uneconomical to produce.

But there is an interest in the recovery of sucrose from beet molasses using other cheaper processes which could be sold under quota C.

Presently there are no quotas for sucrose produced from imported sugarcane molasses.

The annual output of sugar in Britain is 2.4 million tons in 1987; approximately 1.2 million tons from British Sugar PLC and 1.0 million tons from Tate and Lyle Ltd, the rest produced by smaller independent companies.

The 1.2 million tons of sucrose is obtained from 8 million tons of sugar beet and this yields 0.3 million tons of molasses. British sugar sells approximately 25% of the molasses to fermentation companies and about 60% as animal feedstock.

Fructose is a monosaccharide and is the sweetest of all natural sugars. In cold solution it is 1.8 times as sweet as an equivalent amount of sucrose. Due to this high sweetness of fructose per unit weight, high fructose corn syrups (HFCS) are useful to the food industry for the production of low calorie drinks and foods. Currently 40% of the sweetener market in the States is supplied by HFCS.

A similar trend has appeared in Japan and the Scandinavian countries. The HFCS market specification is for syrups containing either 55, 70 or 90% fructose (the balance being glucose, maltose and other oligosaccharides).

There are quotas on isoglucose syrups ie. syrups containing >10% fructose, but these are only for syrups derived from corn. No quotas exist for fructose made from any other raw material.

The bulk prices for molasses, sucrose, glucose and fructose are:

Molasses	£75/Tonne
Sucrose	£350/Tonne - Quota A price (1)
Glucose	£600/Tonne
Fructose	£1000/Tonne

Thus the recovery of fructose from beet molasses is very much favoured.

Chromatography can be defined as the unit operation where the separation of solutes is brought about due to the differential migration of the solutes through a system of two phases, the stationary (column packing) and the mobile phase (a liquid in the case of liquid chromatography). Chromatography has been proved to be one of the most useful separation techniques of recent times. It is particularly useful if the compounds involved have similar physical or chemical properties. The application of chromatography to industrial processes was hampered for many years due to

several factors, the main problem being the preference for batch separations and the scale up to large diameter columns. This has, to a large extent been overcome so that now one can find columns of up to 4 meters diameter in commercial use. Application of chromatography for industrial processes are found in the sugar industry in the separation of the two isomers, glucose and fructose by Boehringer-Mannheim (2), Finnsugar (3) and Suddeutsche zucker (4).

The development of the Semi-Continuous Chromatographic Refiner (SCCR) systems has been carried out successfully in this department since 1974 by Barker and co-workers (5)-(10) and one of these systems (SCCR6) was used during the separation studies described in this work. It consists of ten stainless steel columns, each 10.8cm inner diameter by 65cm long, and was packed with an 8% cross-linked polystyrene resin in the calcium form (ZEROLIT SRC14).

The aims of this research were:

(1) To run the SCCR6 equipment using synthetic glucose/fructose mixtures as feedstock under similar conditions as previous workers (8) and (9) to test the operational condition of the equipment as it had not been used for approximately 1.5 years.

(2) To run the SCCR6 using inverted sucrose as feedstock. The inversion of sucrose to be

performed by:

(a) Enzymatic inversion (using INVERTASE) or

(b) Acid inversion (using HCl) or

(c) H^+ resin inversion (using AMBERLITE IR118)

(3) To compare the three inversion techniques mentioned in terms of costs, ease of inversion, purity of products recovered etc.

(4) Based on the inversion technique selected from (3) above, to invert the sucrose in sugar beet molasses and pretreat (if necessary) the inverted molasses for use as a feedstock on the SCCR6.

(5) To run the SCCR6 using the inverted molasses as feedstock and optimise the equipments separating performance by evaluating various operating parameters such as feed concentration, temperature and switch time.

(6) To devise a method whereby the glucose recovered could be isomerised to fructose using immobilised GLUCOSE ISOMERASE enzyme, and since the inversion is incomplete, to study methods of recycling this material to enhance fructose recovery.

(7) To study the variation of the distribution coefficients of some of the key components of inverted beet molasses with different flowrates, temperatures, background and on-column

concentrations.

(8) To model and develop a computer simulation program to predict throughputs, yields and purities of products under any operating conditions.

CHAPTER TWO

THE THEORY OF CHROMATOGRAPHY

2.1 Introduction

The literature related to this research has been divided into two sections. This chapter discusses the basic concept of chromatography and the development and scaling up of liquid chromatographic processes. Chapter 3 reviews the operating techniques and equipment used for continuous and batch chromatography.

2.2 Types of Chromatographic Separation Techniques

2.2.1 Introduction

Chromatography is defined as a separation technique in which a mobile phase containing more than one component is passed through a stationary phase. Each component has a different affinity for the stationary phase and thus each component proceeds through the stationary phase at a different rate. Thus the separation in chromatography is due to the different migration velocities of the components resulting from the relative distribution of each component between the stationary and mobile phases. This is expressed for each component by the distribution coefficient K_{di} which is defined by:

$$K_{di} = \frac{S_i}{M_i} \qquad \ldots\ldots 2.1$$

where

S_i = concentration of component i in the stationary phase (in moles per litre),

M_i = concentration of component i in the mobile phase (in moles per litre).

Thus for component X; if it is mainly found in the stationary phase at equilibrium, the distribution coefficient K_{dx} is high. Similarly if component Y is found mainly in the mobile phase, K_{dy} is low and the migration rate for component Y is greater than that for component X. This also means that in an extreme case, if the distribution coefficient is equal to zero, then the component is present only in the mobile phase.

2.2.2 Classification of chromatographic operations

We have seen that in chromatography, the components of the separation mixture are distributed between the two phases - the stationary and mobile phases. Depending on the nature of these phases we have four main chromatographic systems as follows:

A - Solid stationary phase
- (a) Gaseous mobile phase "Gas - Solid chromatography" (GSC)
- (b) Liquid mobile phase "Liquid - Solid chromatography" (LSC)

B - Liquid stationary phase
- (c) Gaseous mobile phase "Gas - Liquid chromatography" (GLC)
- (d) Liquid mobile phase "Liquid - Liquid chromatography" (LLC)

In this work the Liquid - Solid chromatographic system (system (b)) was used.

2.2.3 Modes of chromatographic operation

There are three modes of chromatographic operations that each of the four methods mentioned above can be carried out by, namely:

(a) elution chromatography

(b) frontal chromatography

(c) displacement chromatography

(a) Elution chromatography

A small quantity of mixture is injected onto a column and component separation is achieved by its distribution between the two phases. As different components migrate through the bed at different rates, both qualitative and quantitative separation of the mixture occurs. This is the most commonly practiced mode of chromatography.

(b) Frontal chromatography

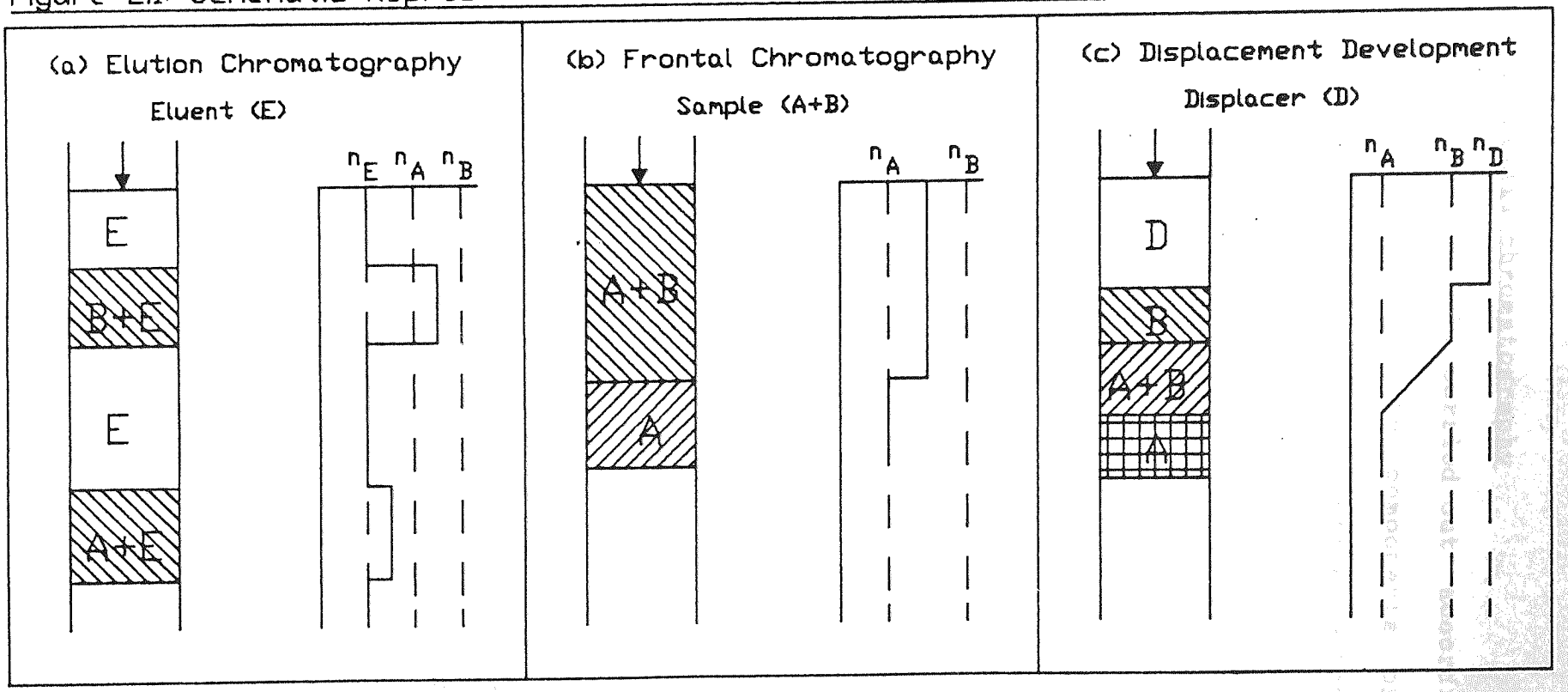
A sample is introduced into the system continuously with the mobile phase. The less strongly adsorbed component

migrates faster and is eluted pure until the accumulated strongly adsorbed component breaks through with the mobile phase, after which there is no separation. This system can be advantageous when the separation of a powerfully adsorbed minor component from a mixture is required.

(c) Displacement chromatography

In this mode, the mobile phase is more strongly retained by the stationary phase than the sample mixture. The sample is then pushed through the bed by the advancing mobile phase. This provides much poorer separation but increased sample loading can be applied to the bed.

Figure 2.1: Schematic Representation of the Three Chromatographic Techniques



2.2.4 Types of liquid chromatography

Liquid chromatography can be carried out according to the mechanism of retention of the components by the stationary phase. The six types of 'retention mechanisms' are:

(a) Adsorption chromatography - the retention of molecules by physical or chemical association between the solute and active sites on the stationary bed.

(b) Ion exchange chromatography - the retention of molecules by different affinities of the solute molecules in a multi-component mixture for the charged stationary bed. A continuous reversible exchange of ions between electrolytes and ion exchangers is involved.. This principle was employed for the separation studies carried out in this work.

(c) Exclusion chromatography - separation occurs due to the difference in size of the sample molecule. Larger molecules are less likely to penetrate the pores of the packing. The more frequently that a molecule can penetrate these pores, the more highly retained the molecule will be. Larger molecules are therefore eluted at a faster rate and smaller molecules are eluted more slowly.

(d) Ion exclusion chromatography - this involves the charged molecules on an ion exchange resin having the effect of repelling similar molecules in the mobile phase. These ions in the mobile phase are therefore not retained

and are eluted rapidly.

(e) Partition chromatography - this is dependent on the adsorption of solutes by an inert solid support coated with a liquid stationary phase.

(f) Ligand exchange chromatography - is a form of adsorption chromatography whose retention mechanism relies on the association of the solute with a suitably charged ion-exchanger on the stationary phase; no ion exchange actually occurs.

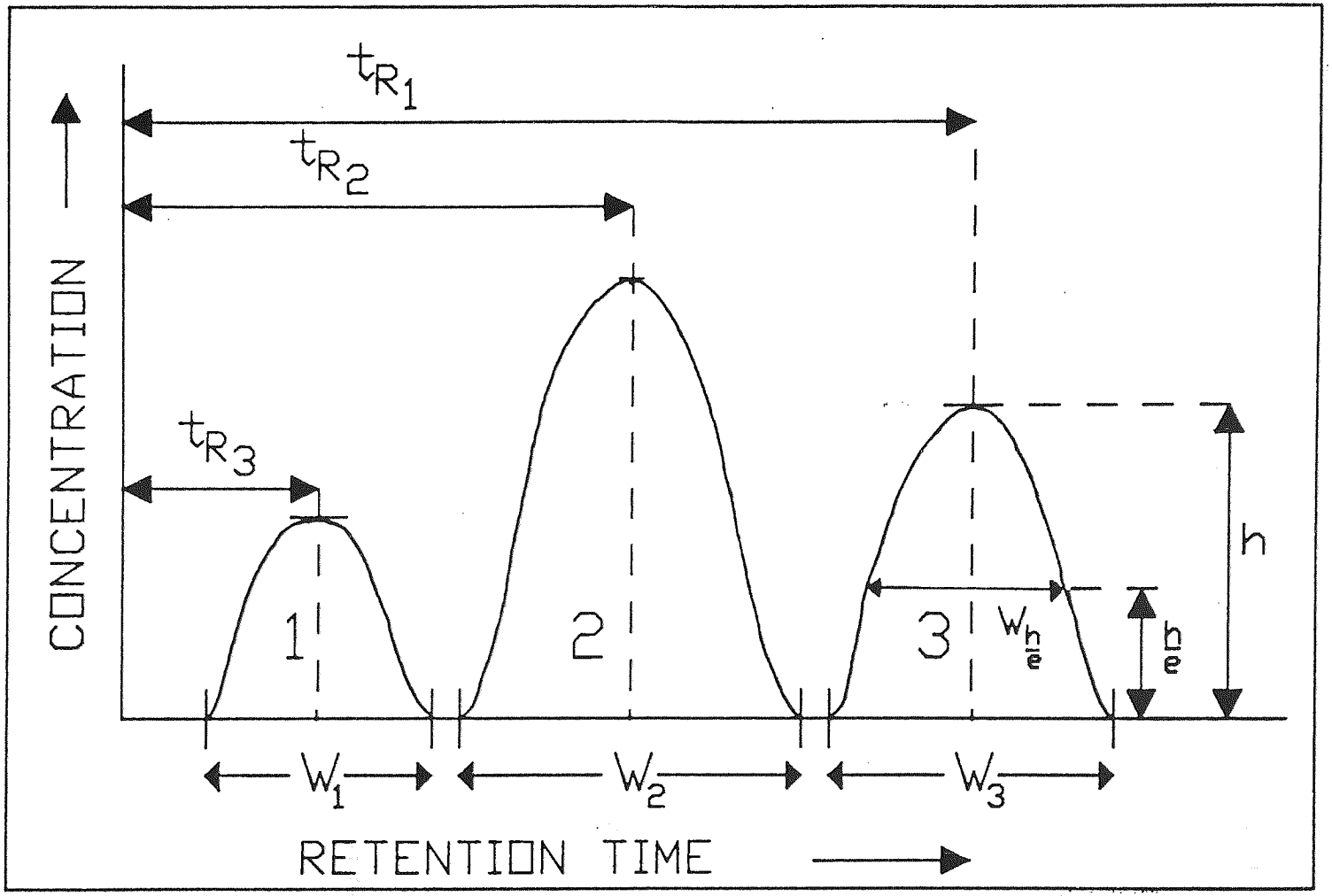
2.3 Chromatographic Terminology and Definitions

A profile as shown in Figure 2.2 is obtained when a three component mixture travels through a chromatographic column where separation occurs due to the relative affinities of the components for the packing. The concentration of the product stream is plotted with time and these concentration-time profiles are called 'chromatograms'.

The retention time :- (also known as elution time) is defined as the average time a molecule takes to travel along the column and is measured to the midpoint of the elution curve ie. t_{R1}, t_{R2}, t_{R3}.

The retention volume :- is defined as the volume of the mobile phase required for complete elution of a component, and is obtained by multiplying the retention time by the mobile phase flowrate.

Figure 2.2: A Typical Chromatogram From a Three Component Mixture



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

$$k_i = K_{di} \frac{V_s}{V_o} \qquad \ldots\ldots 2.2$$

where

K_{di} = distribution coefficient of component i

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

V_o = total void volume in column occupied by the mobile phase

V_T = total empty column volume

The resolution :- is a measure of the degree of separation and is defined (for components 1 and 2 in Figure 2.2) by:

$$R_s = \frac{2(t_{R2} - t_{R1})}{W_2 + W_1} \qquad \ldots\ldots 2.3$$

where

R_s = resolution

t_{Ri} = retention time for component i

W_i = peak width at the base of the elution curve of component i (in time units)

2.4 The Theory of Band Broadening

Various researchers have shown that many factors affect band broadening. Some concepts and theories have been used in this section to explain the mechanisms

involved.

2.4.1 The theoretical plate concept

This concept was first applied by Martin and Synge (11) who suggested that a chromatographic column consisted of a number of layers of packing, each equivalent to a theoretical plate (HETP). To simplify the mathematical model for this concept, it was assumed that the distribution coefficient K_d is constant throughout the column and is independent of concentration. It was also assumed that the diffusion along the column in any phase was negligible and that the mobile phase is discontinuous, consisting of stepwise additions of volumes of mobile phase equal to the mobile phase volume per plate.

One further assumption was made in that at equilibrium, the distribution ratio of one solute between the two phases must be independent of the absolute value of its concentration and of the presence of other solutes.

The plate height, H, is thus defined by:

$$H = \frac{d\sigma^2_z}{dz} = \frac{\sigma^2(L)}{L} \qquad \ldots\ldots 2.4$$

where

σ^2_z = variance of Gaussian curve (m^2)

z = distance along the column length (m)

This assumes that the single solute band has spread

into a Gaussian distribution curve.

A modification to this model was made by Gleukauf (12) who converted the discrete plate model into a continuous one by reducing the volume of the plate to an infinitely small value. The predicted concentration profile exhibited a Poisson distribution which became a Gaussian distribution when the total number of plates (NTP) exceeded 100 plates.

Thus the plate theory model can be summarised as follows:

- significant error arose from the assumption of plate wide equilibrium,
- a Gaussian peak shape,
- peak width increased linearly with retention volume,
- the number of theoretical plates increased with column length,
- the model fails to take into account the effect of molecular structure, sorption phenomena, temperature, molecular distribution and flow patterns towards zone broadening.

2.4.2 The 'Rate' theory

This theory was originally proposed by Lapidson and Amundson (13) who defined the column as a continuous medium where mass transfer and diffusion were accounted for. This was later modified by Deemter, Zuiderweg and Klinkenberg (14) who took into account the effects of flow behaviour on

a band in the column and the rate of adsorption or reaction.

The Van Deemter equation can be represented by:

$$H = A + \frac{B}{u} + Cu \qquad \ldots\ldots 2.5$$

where

H = height equivalent to a plate

A = eddy diffusion term

B = longitudinal diffusion term

C = mass transfer term

u = mobile phase velocity

Equation 2.5 can be represented as Figure 2.3. The solid line in the figure is the sum of all three dispersion processes and shows a minimum in plate height (H_{min}) which corresponds to the 'optimum' velocity (u_{opt}); at this velocity the column has a maximum separation efficiency. The C term in the equation is the sum of the contributions from three possible processes:

(i) extra particle effect (C_m)

(ii) stagnant mobile phase effect (C_{sm})

(iii) conventional liquid chromatography stationary phase mass transfer effect involving the basic sorption process (C_s)

Therefore the expanded Van Deemter equation is:

$$H = A + \frac{B}{u} + C_{mu} + C_{smu} + C_{su} \qquad \ldots\ldots 2.6$$

2.4.3 The Random Walk theory

Equation 2.6 shows a linear relationship of plate height with increasing mobile phase velocity. In practice the plate height begins to level off at high mobile phase velocities. Giddings (15) proposed the random walk theory or coupling theory, which takes the eddy and lateral-diffusion terms into account which have been ignored in classical plate height theory. This gives:

$$H = \frac{B}{u} + C_{smu} + C_{su} + \left(\frac{1}{A} + \frac{1}{C_{mu}} \right)^{-1} \qquad \ldots 2.7$$

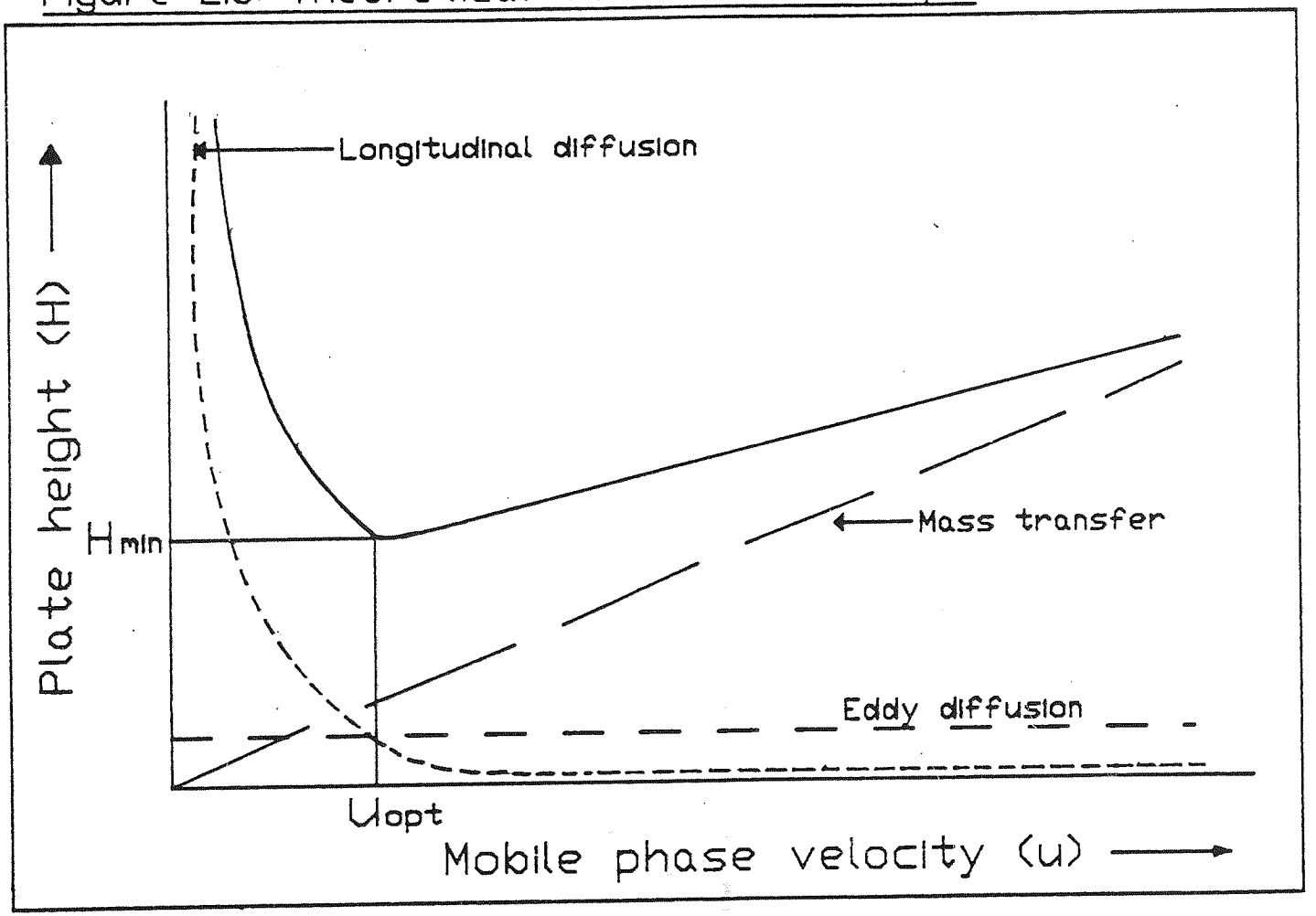
2.5 Column Packing Techniques

A number of packing techniques have been developed and are classified as dry packing or wet (slurry) packing, with the second being more popular. Higgins and Smith (16) provide a comprehensive description of various techniques employed in gas chromatography. Bayer (17) introduced mechanical tapping and vibration to improve packing efficiency.

Slurry packing techniques include pouring under vacuum, bulk pouring and bulk pouring with vibration. The columns used in this work were packed using slurry packing

under vacuum. The resin was introduced at the top as a 50:50 slurry and some column vibration was applied.

Figure 2.3: Theoretical Van Deemter Graph



CHAPTER THREE

CONTINUOUS AND BATCH CHROMATOGRAPHY

3.1 Introduction

The first part of this chapter outlines the operating techniques used for continuous chromatography. The rest of the chapter then describes production scale batch and continuous chromatographic processes.

3.2 Operating Techniques and Equipment used for Continuous Chromatography

The use of batch chromatographic process employs a repetitive feed injection technique which results in band overlapping and thus reduced purities. Therefore an interest has been directed in continuous chromatographic processes which offer uniform product quality and greater throughputs.

According to the relative movements of the mobile and stationary phases, the continuous processes fall into two broad categories:

(i) Counter-current flow processes

(ii) Cross-current flow processes

3.2.1 Counter-current flow processes

In counter-current processes, the stationary phase is moved in the opposite direction to that of the mobile phase

by a number of methods which include:

(i) Moving bed systems

(ii) Moving column systems

(iii) Moving feed point systems

(iv) Simulated moving bed systems

3.2.1.1 Moving bed systems

In moving bed systems, the column packing flows under gravity counter-currently to the upflowing mobile phase. The sample mixture is injected in the center of the column and the highly retained components move with the packing and are stripped off at the bottom. Components which are less strongly retained exit the column with the mobile phase. An example of this kind of system is the Hypersorption process developed by the Universal Oil Company, California, USA, (18,19). Barker and Critcher (20) also developed a Moving bed system in the 1960s.

3.2.1.2 Moving column systems

This type of system (21), (22), (23), consists of a circular arrangement of parallel columns which rotate past stationary ports which interconnect the columns. Problems associated with these systems are in achieving reliable mechanical seals between the static ports and moving columns.

3.2.1.3 Moving feed point systems

This process behaves as an intermediatory between conventional batch and simulated countercurrent systems. A continuous feed switching system introduces a feed pulse into each column for a predetermined time period. Wankat and Oritz (24) used such a system to separate dextran 2000 from cobalt chloride.

The switching period was controlled so that the velocity of the feed point advancement was between the migration velocities of the least and the more strongly adsorbed components. The problem with this system is that it only utilises a part of the total adsorbent packing in the system at any time.

3.2.1.4 Simulated moving bed systems

In these systems, a number of stationary interlinked columns are employed and the counter-current movement is achieved by sequentially moving the inlet and outlet ports in the direction of the mobile phase.

To eliminate flat faced moving seals, Barker and co-workers developed a technique to simulate the counter-current movement of both phases. They developed the 'moving port' multicolumn system (SCCR) where substantially all moving parts were eliminated by using valves of proven commercial reliability. The counter-current 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.

The largest of these systems, the SCCR6, was used for the research carried out in this work and a detailed description is outlined in chapter 5.

3.2.2 Cross-current flow processes

In cross-current systems, the 'stationary' phase moves perpendicular to the mobile phase. These systems can be divided into two categories:

(i) Moving column - open end systems

(ii) Moving annulus systems

3.2.2.1 Moving column - open end systems

In these systems, parallel tubes rotate through a fixed top inlet and stationary product receivers are at the open bottom ends. Although the unit rotates as a whole, they are effectively a series of batch columns where in each one the mobile phase flows through a fixed stationary phase. These systems have the same effect as for a large batch column and are therefore only of increased complexity with no likely practical significance.

3.2.2.2 Moving annulus systems

This process was first suggested by Martin (25) who proposed the use of an annulus packed with a

chromatographic resin, rotating through a fixed feed inlet. The mobile phase is introduced all the way around the top of the annulus and leaves from the bottom of the packed bed. The components travel in helical paths around the annulus at different angles according to the different affinities of the components for the packing and are eluted at different points, with the strongly retarded components travelling along longer helical paths. This arrangement can provide the successful separation of multicomponent mixtures.

One such system has been constructed in the Department of Chemical Engineering and Applied Chemistry at Aston. Thirkill (26) in this department has used this system to recover sucrose from molasses using sodium charged resin particles.

3.3 Production Scale Chromatographic Processes

A brief description of the batch and continuous commercial chromatographic processes used to separate carbohydrates are outlined in this section. A detailed literature search was carried out on these processes by Ganetsos (10), Thawait (9) and Gould (8) and is fully reported in their theses.

3.3.1 Batch systems

3.3.1.1 The Finn-Sugar Process

In the 1950s and 1960s, insulated columns of 2 to 12 metres high and 0.5 to 4 metres in diameter were constructed and used to separate sucrose from beet molasses. The separation took place semicontinuously by employing repetitive injection techniques. In 1975, Finn-Sugar installed a large plant consisting of 2.7 metres id columns by 3 to 6 metres high resin recovering up to 95% of the sugar in the molasses at purities of up to 92%, (27). They have also installed in 1983, a plant in Germany for processing 60000 tons/year of molasses using 3.6 metres id by 12 metres high columns, (28). Another plant in the USA for the production of 13000 tons/year of crystalline fructose from corn syrup has also been built by Finn-Sugar, (28). The syrup is isomerised to a 42% fructose solution and is separated in 17 chromatographic columns.

Finn-sugar company has also developed commercial scale systems for the production of mannitol, xylose and betaine. Pilot units for the separation of raffinose, arabinose, lactulose, galactose, mannose and some amino acids have also been used.

3.3.1.2 The Sudzucker process

This process was developed by Munir of Suddeutsche

Zucker AG (Germany) in the mid-seventies (4). The process used liquid distribution chromatography for the separation of molasses in columns of 18 metres length by 1 metre diameter packed with a 4% cross linked resin in the calcium form. 50% w/w molasses was injected at charges equal to 6% of the bed volume and product purities of 90% was obtained. 95% of the sugar was reported to have been recovered and the product contained 11% total solids on dry basis.

3.3.1.3 The Boehringer process

In 1969, Boehringer of Mannheim-Waldhof company (Germany) obtained a patent for a process for obtaining pure glucose and fructose from inverted sucrose (2). A separating length of 9 metres was used in six columns each of 15 cm. id and 2 metres high. The packing used was a 4% divinylbenzene crosslinkage resin in the calcium form with between 5 - 30% of its active sites in the hydrogen form (Dowex 50WX4). Thus simultaneous hydrolysis of the sucrose present and the separation of the glucose and fructose was achieved. The fructose molecules complex with the calcium ions on the resin but the glucose molecules do not. This difference was exploited to achieve a separation.

3.3.1.4 The Colonial sugar process

A patent was granted to the Colonial Sugar Company (Australia) in 1967 (29) for the separation of fructose and

glucose from inverted sugar feedstocks using a sulphonated polystyrene cation resin crosslinked with 4% divinylbenzene (Dowex 50W). This process looked promising but there are no further reports available about its industrial success.

3.3.2 Continuous Systems

3.3.2.1 The Sarex process

This is a continuous process for producing up to 90% pure fructose syrups (30). The feed is charged continuously to a single adsorption column via a rotary valve distribution system. At the same time, desorbent water is passed through different parts of the rotary valve. The extract (high purity fructose) and raffinate (fructose depleted glucose) streams are removed, each from a different section of the column on a continuous basis, (see Figure 3.1). The fructose rich product contains over 90% of the fructose in the feed and has a purity of 90-94%. The glucose stream contains approximately 80% glucose. The product concentrations are around 20%w/v.

3.3.2.2 The Odawara process

A continuous process using zeolite in the barium form to absorb the fructose was patented by Odawara et al of Toray Industries ltd (Japan) in the 1970s (31). The equipment consisted of eleven 2.5cm id by 1.5 metres long

columns packed with the zeolite. The liquid streams flow through three serially and circularly interconnected zones including a desorption zone (5 columns), rectification zone (2 columns) and a sorption zone (4 columns). The continuous operation was simulated using 66 valves and a timer by advancement of entry and exit ports around a closed loop.

Figure 3.2 shows the flow scheme of the process. Water was fed continuously as a desorbent at 2.9Kg.hr^{-1}. A 7% w/v feed containing 57.5% glucose and 42.5% fructose was introduced at 1.4Kg.hr^{-1} and another stream containing 1% w/v sugar solids was refluxed continuously at 8.5Kg.hr^{-1}. The desorped effluent was removed continuously at 12.7Kg.hr^{-1} and had a concentration of 1%w/v containing pure fructose. The raffinate effluent was removed at 0.2Kg.hr^{-1} and had a concentration of 45%w/v containing 3% fructose. The products were concentrated by evaporation and the desorbent was recycled.

Figure 3.1: Flowsheet of the Sarex Process

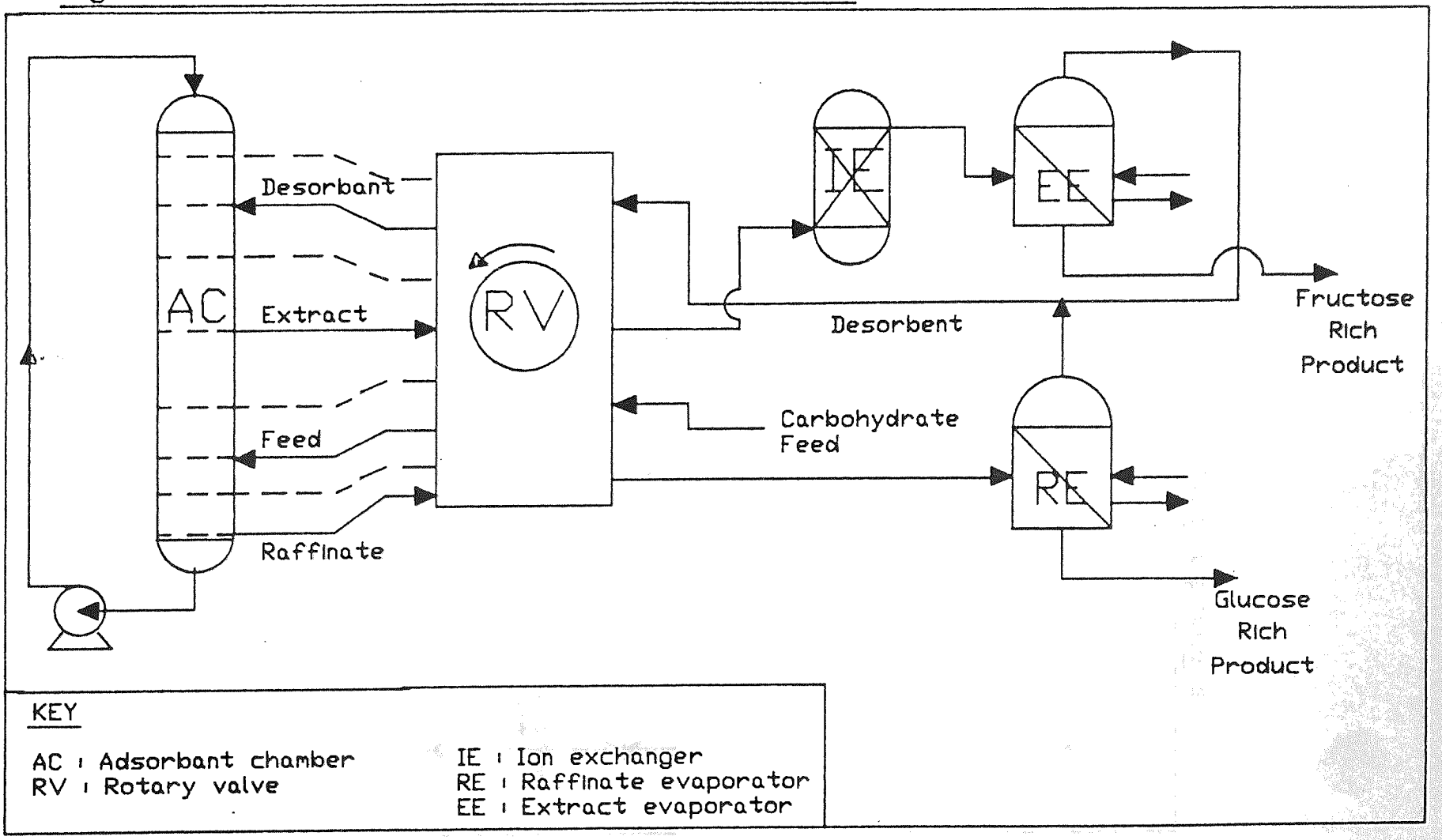
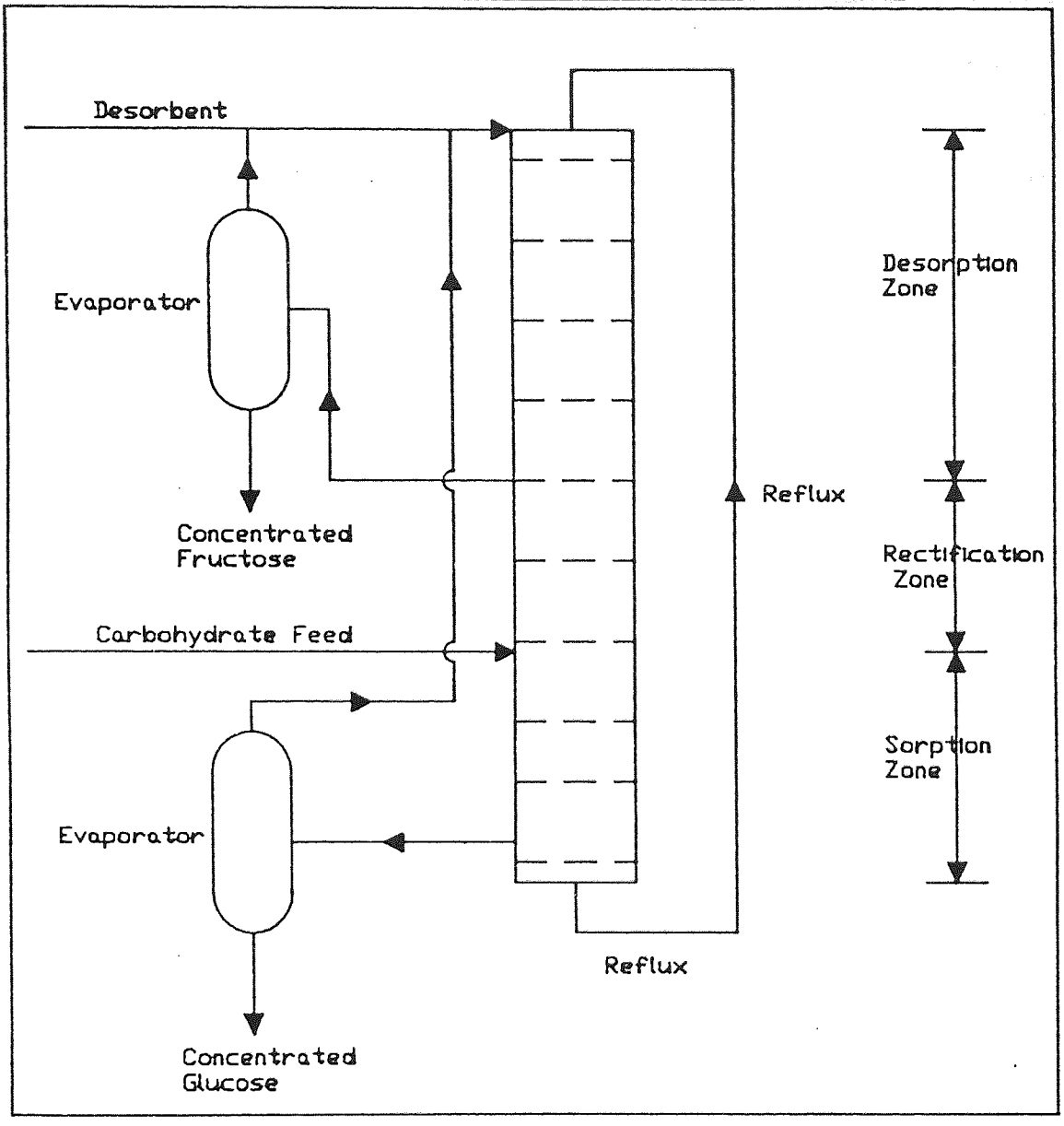


Figure 3.2: Flowsheet of the Odawara process



3.3.2.3 The Illinois Water Treatment process

The Illinois Water Treatment company (USA) in conjunction with Finn-Sugar company used a continuous system consisting of a number of vertical columns for the desugarisation of molasses. They developed a new method of switching of feed and eluent inlets and product outlet points to simulate the operation. Unfortunately not enough information is available about the system operation or commercial viability.

CHAPTER FOUR

PROPERTIES OF MOLASSES AND SUGARS

4.1 Introduction

This chapter is divided into two parts. The first part details the molasses properties and the latter part details the sucrose, fructose and glucose properties.

4.2 Molasses - Composition and Properties

Molasses is the final syrup spun off after repeated crystallisations in the extraction of sugar. It is so low in purity that further crystallisation from it is impractical. It is discarded from the sugar end, carrying with it all the non-sugars of the beet not eliminated by the juice purification process, the soluble impurities added in processing, and the degradation products formed during processing (32).

Sugar beet molasses is a complex mixture of inorganic and organic components, the largest single component being sucrose which accounts for approximately 52% of the molasses. Molasses also contains approximately 74-78% dry substance, organic non-sugars 12-17%, nitrogen free compounds 6-8% and ash 10-12%. Betaine is the most abundant nitrogenous compound found in molasses, approximately 3 to 4% in British molasses increasing somewhat towards the end of the campaigns.

Table 4.1 indicates the approximate percentages of each of the main types of compounds found in beet molasses. The main difference between cane molasses and beet molasses is the high percentage of nitrogen compounds found in the beet molasses. In terms of sugar content, beet sugar has a relatively lower percentage of reducing sugars present.

The sugar in the molasses is the largest single loss from the beet sugar refining process. This large loss prompted the study and effort since the beginning of the industry to reduce it. Obviously, more complete exhaustion of the final syrups can be achieved by boiling to high density, cooling to lower temperature and allowing longer periods of time for crystallisation. But, as a practical matter however, there are limitations to this approach. As the density of raw molasses increases, the viscosity rapidly increases, making separation of crystallised sugar slow and unsatisfactory. The viscosity of molasses approximately doubles with each increase of 2 in percentage dry substance (33). Similarly, lowering the temperature by 10°C doubles the viscosity.

Molasses, therefore, cannot be defined as the syrup from which no more sugar ultimately can be crystallised, but rather a syrup from which 'no more appreciable amounts of sugar can be recovered by crystallisation at economic cost'.

Table 4.1: Composition of Beet Molasses (34)

Dry Matter		74 - 78%
Sucrose		50 - 52%
Reducing Sugars	0.2 - 1.5%	
Raffinose	0.5 - 2.0%	
Organic non-sugars		12 - 17%
of which: nitrogen compounds	6 - 8%	
which include		
Betaine	3 - 4%	
Glutamic Acid	2 - 3%	
non nitrogen compounds ie. organic acids, lactic acid, malic acid, acetic acids, oxalic acids and hemicelluloses	6 - 8%	
Sulphated Ash		10 - 12%
of which: Sodium Na^+	0.3 - 0.7%	
Potassium K^+	2.0 - 7.0%	
Calcium Ca^{2+}	0.1 - 0.5%	
Chlorine Cl	0.5 - 1.5%	
Phosphorous P	0.02 - 0.07%	
Others	5.5%	

4.3 Chemistry of Glucose and Fructose

Carbohydrates have the general empirical formula $(C.H_2O)_n$, where $n \geq 3$. They are divided into three basic categories :- monosaccharides, oligosaccharides and polysaccharides, with the monosaccharides usually having between three to nine, usually five or six, carbon atoms and contain only one aldehyde or ketone functional group or are derivatives of molecules that do.

The oligosaccharides are generally two to ten monosaccharides linked together by the formation of glycosidic bonds. These are subdivided into disaccharides (eg. maltose and sucrose), trisaccharides (eg. raffinose) and so on, depending on the carbohydrate units they contain.

Carbohydrate polymers containing more than ten monomeric units are called polysaccharides and can have molecular weights of many million. Starch, glycogen and cellulose are examples of polysaccharides.

Glucose is the most common monosaccharide found in fruits, honey and other living material. Fructose is another common monosaccharide found frequently in fruits with glucose. Both these monomers are soluble in water with fructose also soluble in ethers and alcohols while glucose only slightly soluble in alcohol (35). Glucose is dextrorotatory with a specific rotation of $(\alpha)^{20}_D = 52.7°$ in water while fructose is strongly levorotatory with

$(\alpha)^{20}_D$ = -132.2° (36). Thus glucose is often referred to as dextrose and fructose as laevoluse.

Glucose in solution exists with the isomeric ring structures as shown in Figure 4.1. An equilibrium exists between these forms and is only slightly dependent on concentration and temperature as shown in Table 4.2. The average composition is 40% of α-D-glucopyranose and 60% of β-D-glucopyranose (37 - 39).

Fructose in solution is in the isomeric forms shown in Figure 4.2. The equilibrium between the α-D-fructofuranose, β-D-fructofuranose and β-D-fructopyranose is affected by temperature as shown in Table 4.3, but the effect of solution concentration is minimal (37, 38, 39).

Figure 4.1i Glucose Isomers in solution

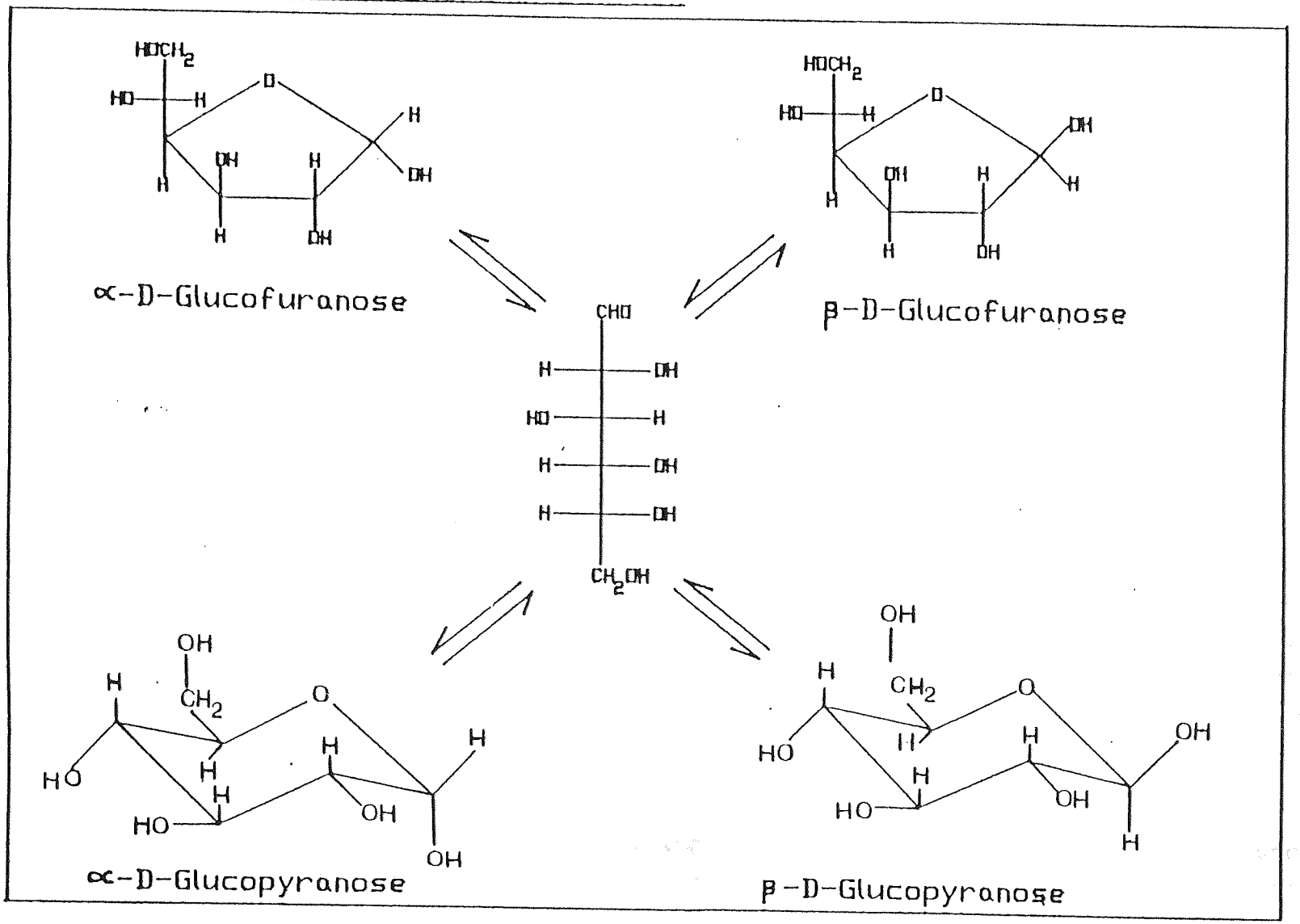


Figure 4.2: Fructose Isomers In Solution

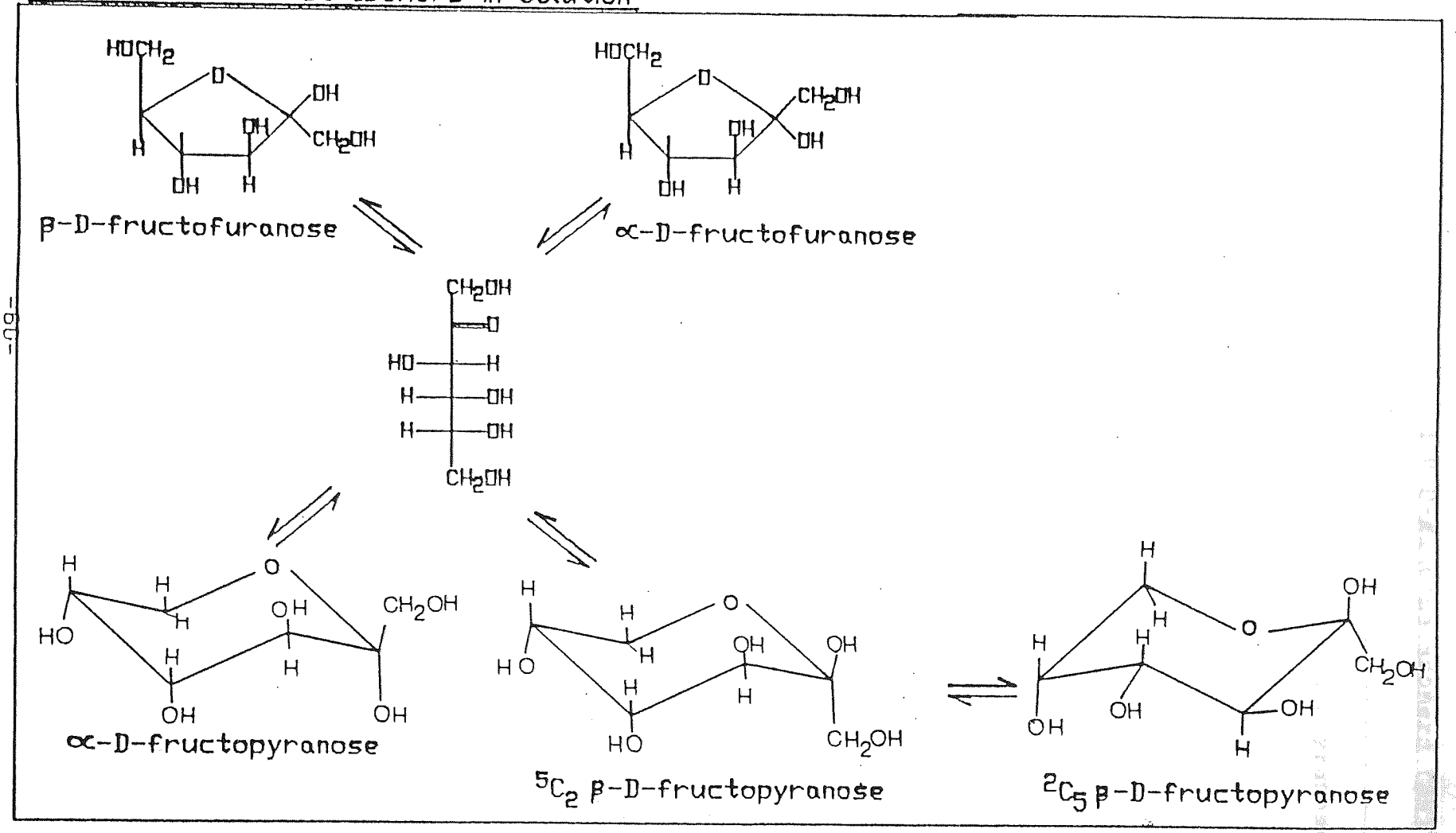


Table 4.2: Equilibria of α- and ß-D-glucose anomers (38)

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

Table 4.3: Tautomeric Equilibria of Fructose (38)

Fructose conc. (%)	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

4.4 Uses of Fructose

Fructose has been established as a substitute to sucrose in beverages as well as various diabetic food products. As part of a diabetic's diet, it offers metabolic and organoleptic advantages, and has minimal influence on the sugar and insulin levels in blood plasma. Fructose can also be used as a raw material in the industrial manufacturing of flavours and as a flavour enhancer in meat dishes.

Fructose is a natural sugar without any toxic properties, and has traditionally been used as an alternative to glucose in infusion therapy for patients with reduced tolerance to intravenous glucose. Fructose is 1.8 times sweeter than sucrose in cold solution and has a calorific value slightly lower than sucrose ($3.7 kcal.kg^{-1}$ compared to $4 kcal.kg^{-1}$ for sucrose). The solubility of fructose in water is 15% higher than that of sucrose and fructose causes less dental plaque formation than sucrose according to some medical statistics.

Synthetic sweeteners such as saccharin and cyclamate have been produced which are totally non calorific, but there is increased speculation about their possible health side effects, and saccharin provokes a bitter after taste. The use of sugar alcohols such as sorbitol has increased but they have a laxative effect and are of lower sweetness. Thus fructose is the best alternative to sucrose.

The β-D-fructopyranose is the sweetest fructose form and the solution sweetness depends on the equilibrium between the β-D-fructopyranose and the β-D-fructofuranose. As seen in the previous section, the equilibrium depends on the temperature, concentration, pH and time. 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.

The usage of fructose is expanding rapidly and though it is unlikely to substitute sucrose completely, it should become more favourable when new economical production methods have been developed and utilised. The separation efficiency and low energy intensiveness of chromatography favour its application to this field.

4.5 Commercial Production of Fructose

The production of high fructose syrups is classified into four main categories according to the raw materials used:

- Hydrolysis of sucrose
- Hydrolysis of inulin, a polyfructosan
- Enzymatic isomerisation of corn starch
- Dextran synthesis

The research program included the study of various techniques for the inversion of sucrose to glucose and fructose and this is described in detail in Chapter 8.

4.5.1 Hydrolysis of sucrose

Sucrose is a disaccharide consisting of one fructose and one glucose molecule. On hydrolysis, this breaks into the two monosaccharides. The hydrolysis (or inversion) can be carried out either by 'acid hydrolysis' where the sucrose solution is mixed with mineral acids directly, or by 'hydrogen ion resin treatment' where the sucrose solution is passed through a cation exchange resin in the hydrogen form, which allows continuous sucrose inversion, or by using 'enzyme hydrolysis'. Lauer et al (40 - 42) have been granted a patent for the inversion of sucrose and the separation of the glucose-fructose mixtures in the same column using calcium charged DOWEX WX4 resin.

The three inversion techniques mentioned were also used for this research and are described in detail in Chapter 8.

4.5.2 Hydrolysis of inulin

Inulin is a polysaccharide found in the roots of compositae like Jerusalem artichoke, dandelion and dahlia tubers. Its molecule consists of about 30 D-fructofuranose units and can be hydrolysed easily to produce fructose. This was the earliest method for fructose production but was not economically viable and was discontinued (43).

4.5.3 Enzymatic isomerisation of corn starch

This was also carried out in this research and is described in detail in Chapter 12.

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.

Bruyn and Van Eckenstein (44) isomerised glucose to fructose in 1895 using an alkaline catalyst at elevated temperatures. Saccharifying enzymes were also used in the 1930s and 1940s to hydrolyse starch into glucose and the glucose then partially isomerised in the presence of the enzyme isomerase (45).

In 1957, Marshall and Kooi (46) discovered the enzyme 'xylose isomerase' for isomerising glucose to fructose. The presence of toxic arsenate and fluoride in the enzyme and the unavailability of cheap xylose for the initial enzyme manufacture made this unsuitable for food production.

In 1975, Novo Industries of Denmark introduced an immobilised glucose isomerase enzyme under the trade name of Sweetzyme which was suitable for continuous on-column isomerisation (47). This enzyme is now also available in the non-immobilised form under the same trade name.

The typical composition of the product mixture is 42% fructose and 52% glucose, the balance being other oligosaccharides. This enzyme is very sensitive and is

poisoned by the presence of calcium ions. The presence of magnesium ions enhances the isomerisation (48).

4.5.4 Dextran synthesis

Sucrose can be converted to dextran and fructose using the dextransucrase enzyme. This technique has been developed by Barker, Zafar and Alsop (49, 50) and the process offers promising commercial viability.

4.5.5 Fructose Enrichment

The products obtained by hydrolysis or by isomerisation indicate that there is a further need for separation to increase the fructose purity. This can be achieved by 'chemical precipitation' or chromatography. The chemical precipitation technique is outdated and the application of the novel chromatographic technique is increasing fast worldwide.

4.6 Mechanism of Separation - Complex Formation

It was reported by Saltman and Charley (51) that Ca^{2+}, Ba^{2+}, Mg^{2+} and Sr^{2+} ions form soluble compounds in aqueous alkaline solutions with galactose, arabinose, maltose, lactose and fructose. Although their work showed the existing of such complexes, the chemical formulae and explanation for such complex formations is still hypothetical.

Angyal (52 - 57) 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 Angyal's hypothesis, only C^{2}_{5}-β-D-fructopyranose is expected to form a complex with cations since it has an axial-equitorial-axial hydroxide group arrangement (Figure 4.2). Such arrangements do not exist in any of the glucose forms (Figure 4.1) and is therefore not expected to form a complex.

The percentage of the β-D-fructopyranose form in equilibrium has been found to be most affected by temperature ie. as the temperature increases, the amount of this fructose form is reduced. Since this form is also the sweetest, then during the chromatographic separation of fructose, it is important to maintain the temperature low to have more of this form available for complexing. Alternatively it has been found (58) that the use of strong basic, ion exchange resins in the bisulphite form results in a complex forming between the glucose and the anions.

In this work a cationic ion exchange resin in the calcium form was used. The fructose was retarded due to the complex formation and the glucose was carried with the mobile phase and was eluted first.

CHAPTER FIVE

THE SEMICONTINUOUS CHROMATOGRAPHIC REFINER (SCCR6) SYSTEM

5.1 The Semicontinuous Principle of Operation

5.1.1 Introduction

If a multicomponent carbohydrate mixture of fructose, glucose and other polysaccharides is eluted through a chromatographic column packed with a cross-linked polystyrene resin charged in the calcium form, a separation occurs (2). Fructose forms a weak chemical complex with the calcium ions on the resin and is retarded, thus travelling through the column at a slower rate than glucose and other components of the mixture. Size exclusion also separates the glucose from the larger polysaccharides as the smaller glucose molecules tend to diffuse into and out of the pores in the resin and are delayed while the larger polysaccharides are excluded and are eluted faster. Although this results in an additional separation between the glucose and the polysaccharides, the chemical principle, ie. the fructose retention, is the more prominent one. A typical elution concentration profile of such a batch system is shown in Figure 5.1.

If however a continuous system is employed, where the mobile phase (eluent) and the stationary phase (resin) move countercurrently at suitable conditions, the elution

concentration profile is as shown in Figure 5.2. The advantages of continuous processes, such as increased throughputs, product reproducibility and continuous operation led Barker and Deeble (5) to construct and operate successfully a continuous gas-liquid chromatographic system. Since then, similar liquid-liquid continuous chromatographic systems have been developed at Aston and one such system (SCCR6) was used for this research.

Figure 5.1: Concentration Profile For a Batch Cocurrent System

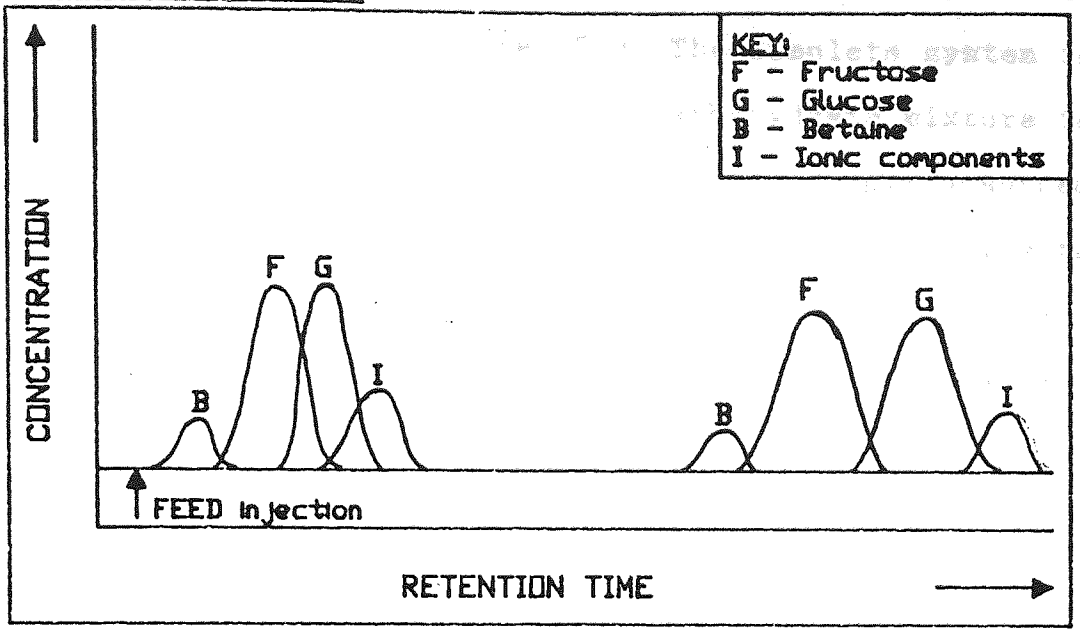
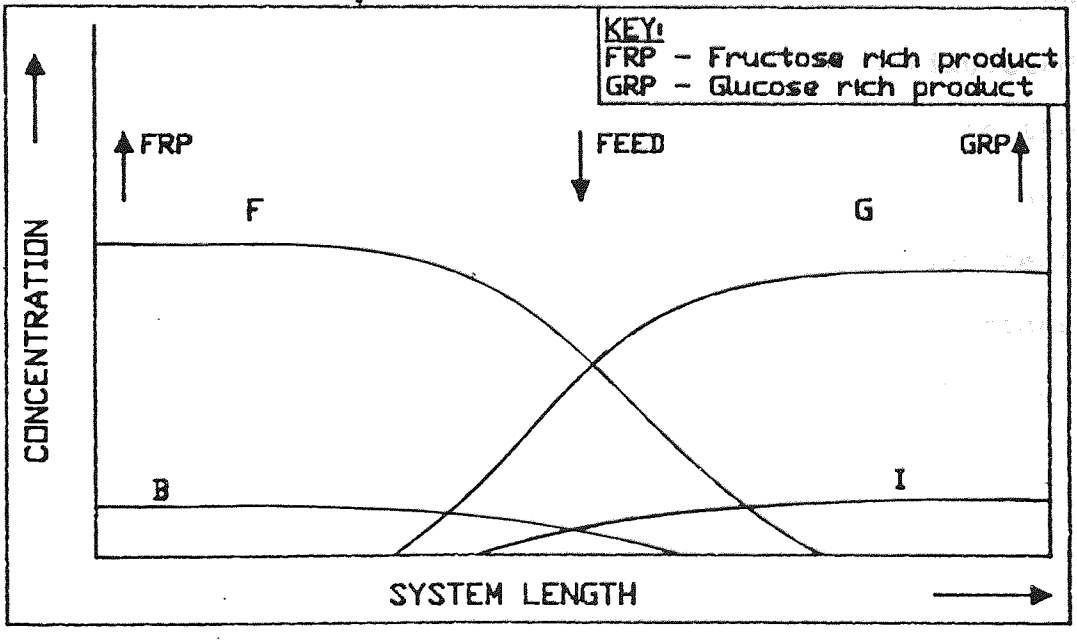


Figure 5.2: Concentration Profile of a Continuous Countercurrent system

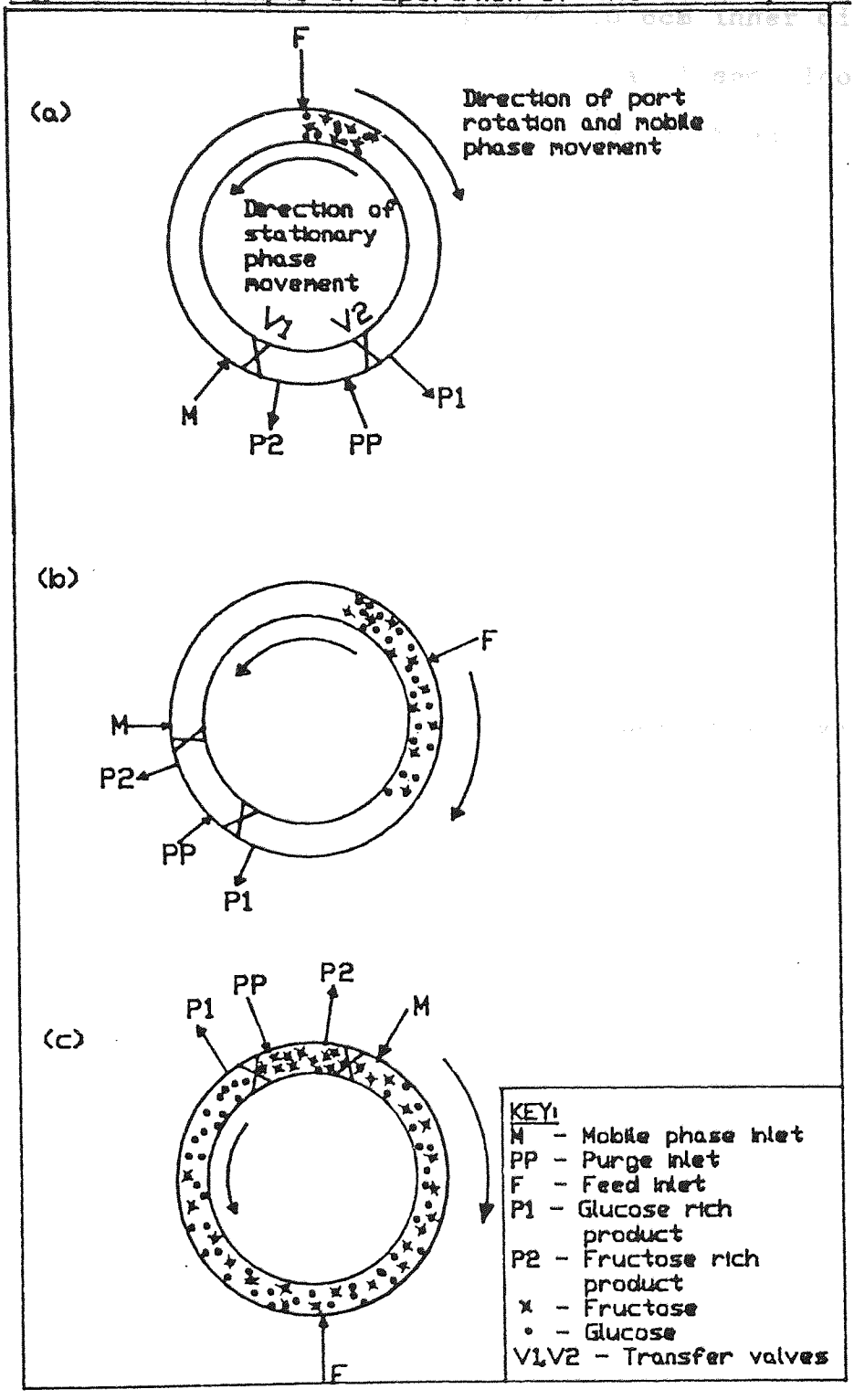


5.1.2 SCCR Principle Of Operation

The principle of operation of the SCCR system is schematically shown in Figure 5.3. The complete system is illustrated as a closed loop. The carbohydrate mixture is fed into the system at port F. The less strongly adsorbed glucose and other polysaccharides move preferentially with the mobile phase towards the glucose rich product (GRP) offtake port P1. A section of the loop is isolated at any time by two locks V1 and V2, and an independent purge fluid stream enters at point PP, removes the adsorbed fructose and exists from port P2 as the fructose rich product (FRP).

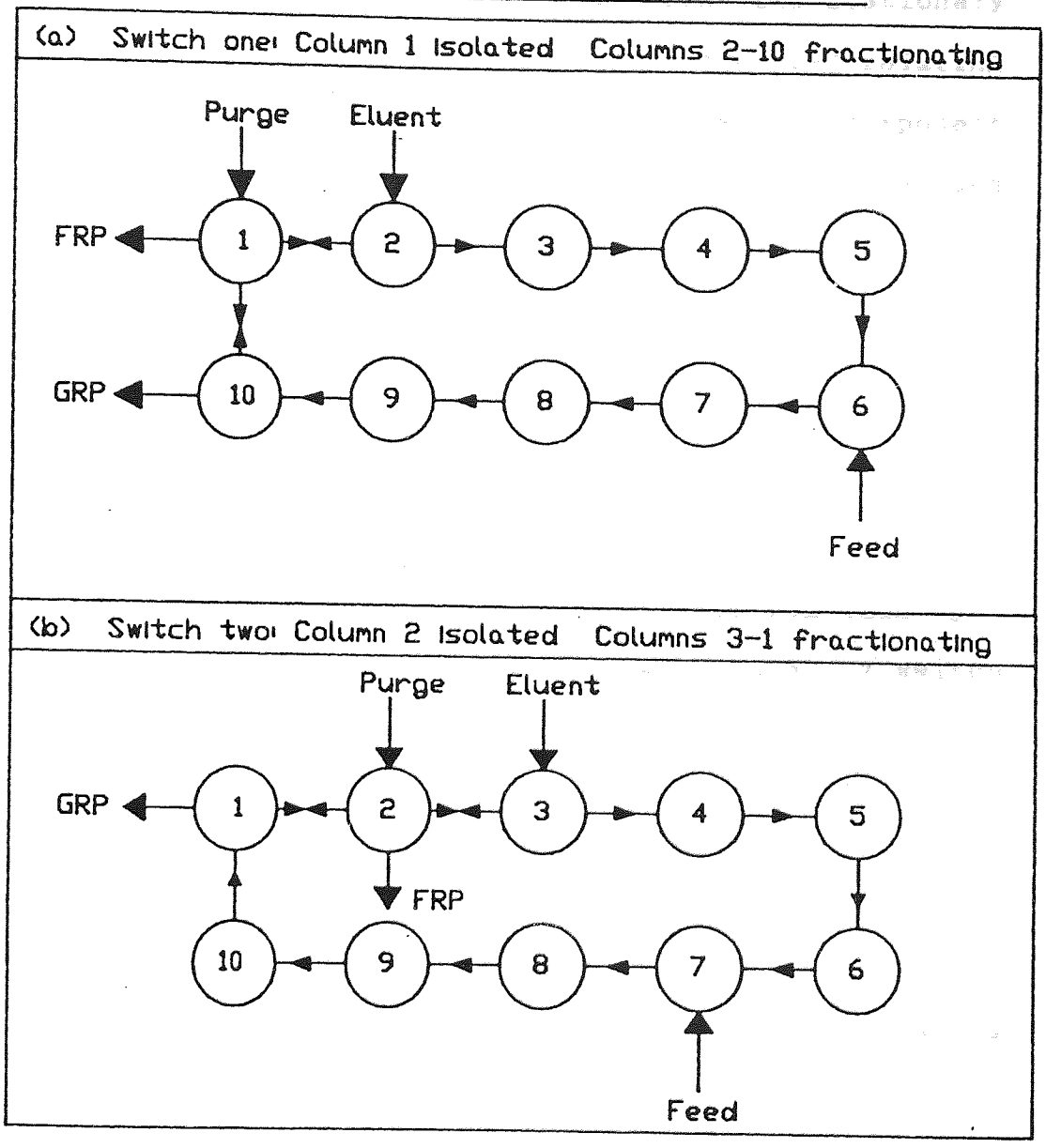
Figure 5.3(a) represents the component distribution within the system soon after 'start-up'. Figure 5.3(b) represents the situation where all the port functions have been advanced by one position in the direction of the mobile phase. This port advancement results in a simulated movement of the stationary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

Figure 5.3: Principle of Operation of the SCCR System



The countercurrent operation mode of the SCCR6 is shown in figure 5.4. The system consists of ten stainless steel columns each 75cm long and 10.8cm inner diameter linked at the top and bottom to form a closed loop. Six pneumatic poppet valves are associated with each column- feed,eluent and purge inlets and the glucose rich (GRP) and fructose rich (FRP) products outlets together with 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 6 and 2 respectively and the GRP is eluted from column 10. In the next switch period, Figure 5.4(b), all the ports are advanced by one position; column 2 is purged, feed and eluent enter columns 7 and 3 and the GRP is eluted from column 1. After 10 such advancements the 'cycle' is completed.

Figure 5.4: Sequential Operation of the SCCR6 system



5.1.3 Idealised Operating Conditions

In the separation of a glucose/fructose mixture on the countercurrent semicontinuous system, the glucose travels with the mobile phase and the fructose with the stationary phase. An idealised model can be constructed relating mobile and stationary phase flowrates as well as component separation. A material balance on glucose about the feed point (see Figure 5.5) gives:

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

where:

L_2 = feed flowrate ($cm^3.min^{-1}$)

L_e = mobile phase flowrate ($cm^3.min^{-1}$)

P = stationary phase effective flowrate ($cm^3.min^{-1}$)

≡ total system volume/(total columns x switch time).

f_g = glucose concentration in the feed ($g.cm^{-1}$)

y_g = glucose concentration in the mobile phase

x_g = glucose concentration in stationary phase

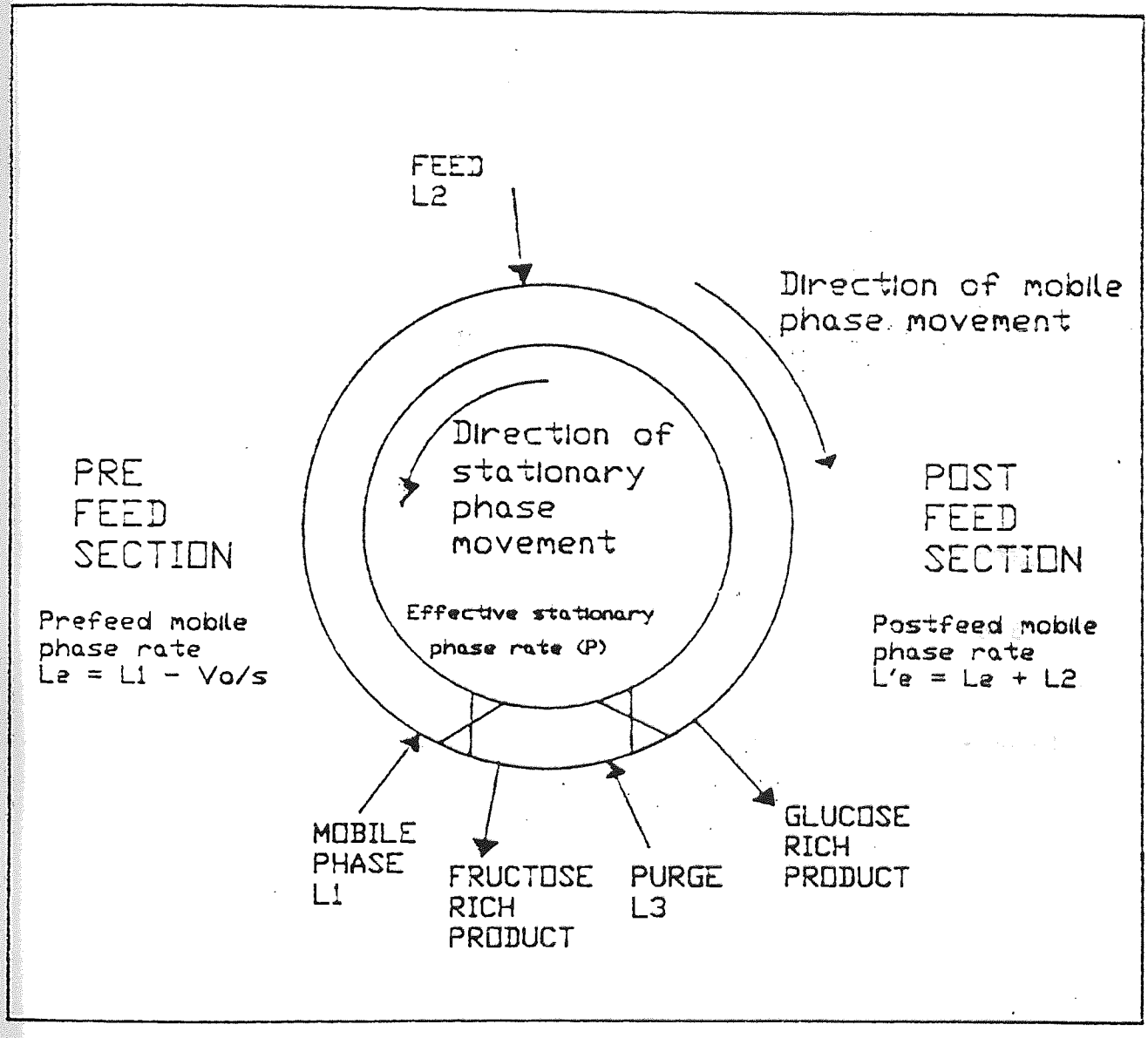
For a glucose molecule to move preferentially with the mobile phase,

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

Rearranging,

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

Figure 5.5: Schematic Representation of the SCCR Principle of Operation



and since by definition of the equilibrium distribution component (K),

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

Then,

$$\frac{L_e}{P} > K_{dg} \qquad \ldots\ldots 5.4$$

Similarly for fructose to move with the stationary phase,

$$\frac{L_e}{P} < K_{df} \qquad \ldots\ldots 5.5$$

Combining equations 5.4 and 5.5 gives the theoretical limits of mobile and stationary phase flowrates to achieve separation of glucose and fructose, ie.

$$K_{dg} < \frac{L_e}{P} < K_{df} \qquad \ldots\ldots 5.6$$

As each column contains mobile phase in the void volume, V_o, the effective mobile phase flowrate is reduced to:

$$L_e = L_1 - \frac{V_o}{s} \qquad \ldots\ldots 5.7$$

where:

L_1 = mobile phase inlet flowrate ($cm^3.min^{-1}$)

s = switch period (min)

Because of the feed flowrate L_2, the effective mobile phase flowrate is different before and after the feed point. This means that the effective mobile phase flowrate in the post feed section, L'_e, becomes:

$$L'_e = L_e + L_2 = (L_1 + L2) - (V_o/s) \qquad \ldots\ldots 5.8$$

Therefore equation 5.6 becomes:

$$K_{dg} < \frac{L_e}{P} < \frac{L'_e}{P} < K_{df} \qquad \ldots\ldots 5.9$$

and this equation now gives the true theoretical limits.

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

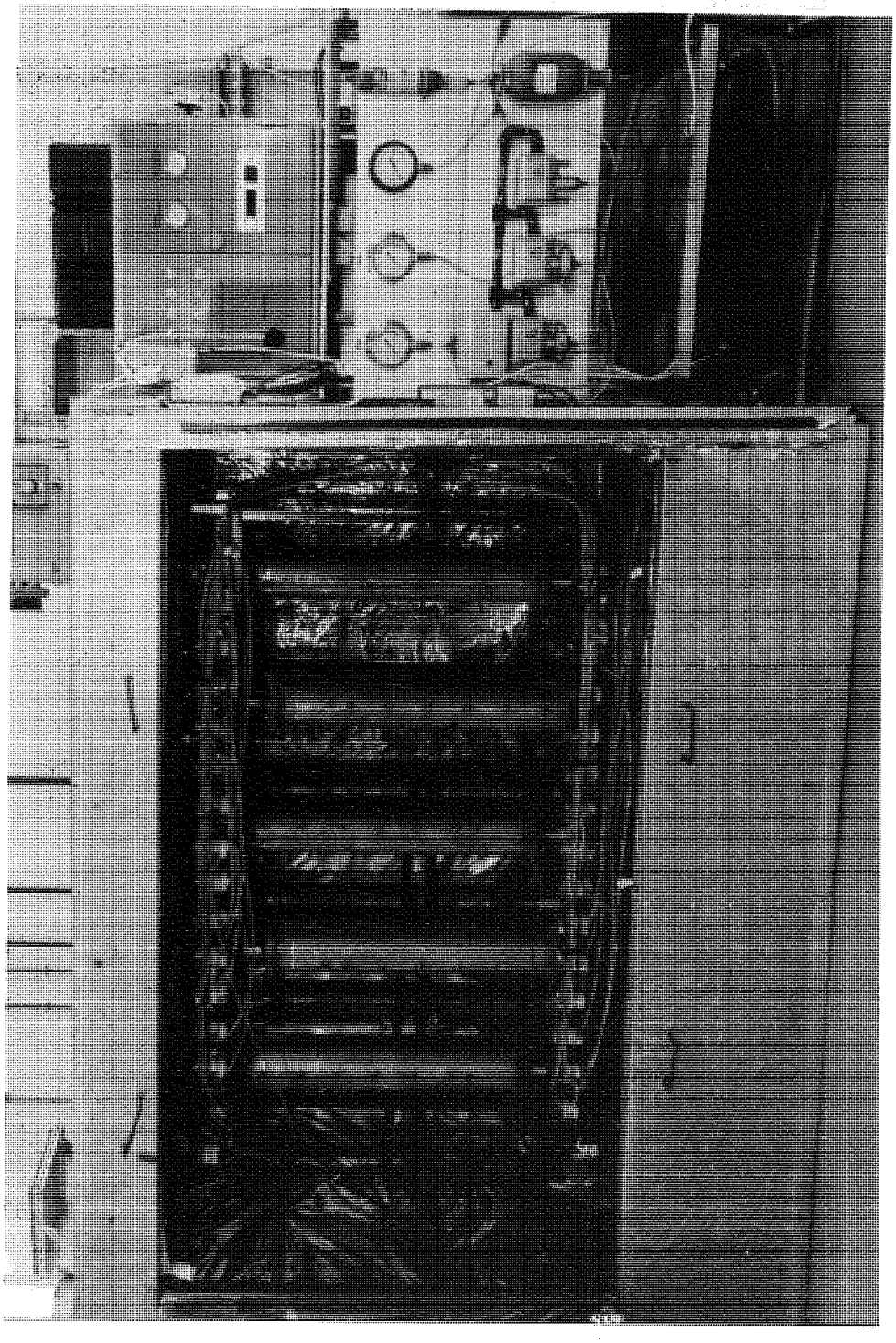
$$\frac{L_3}{P} >> k_{df} \qquad \ldots\ldots 5.10$$

5.2 Description of the SCCR6 System

5.2.1 Introduction

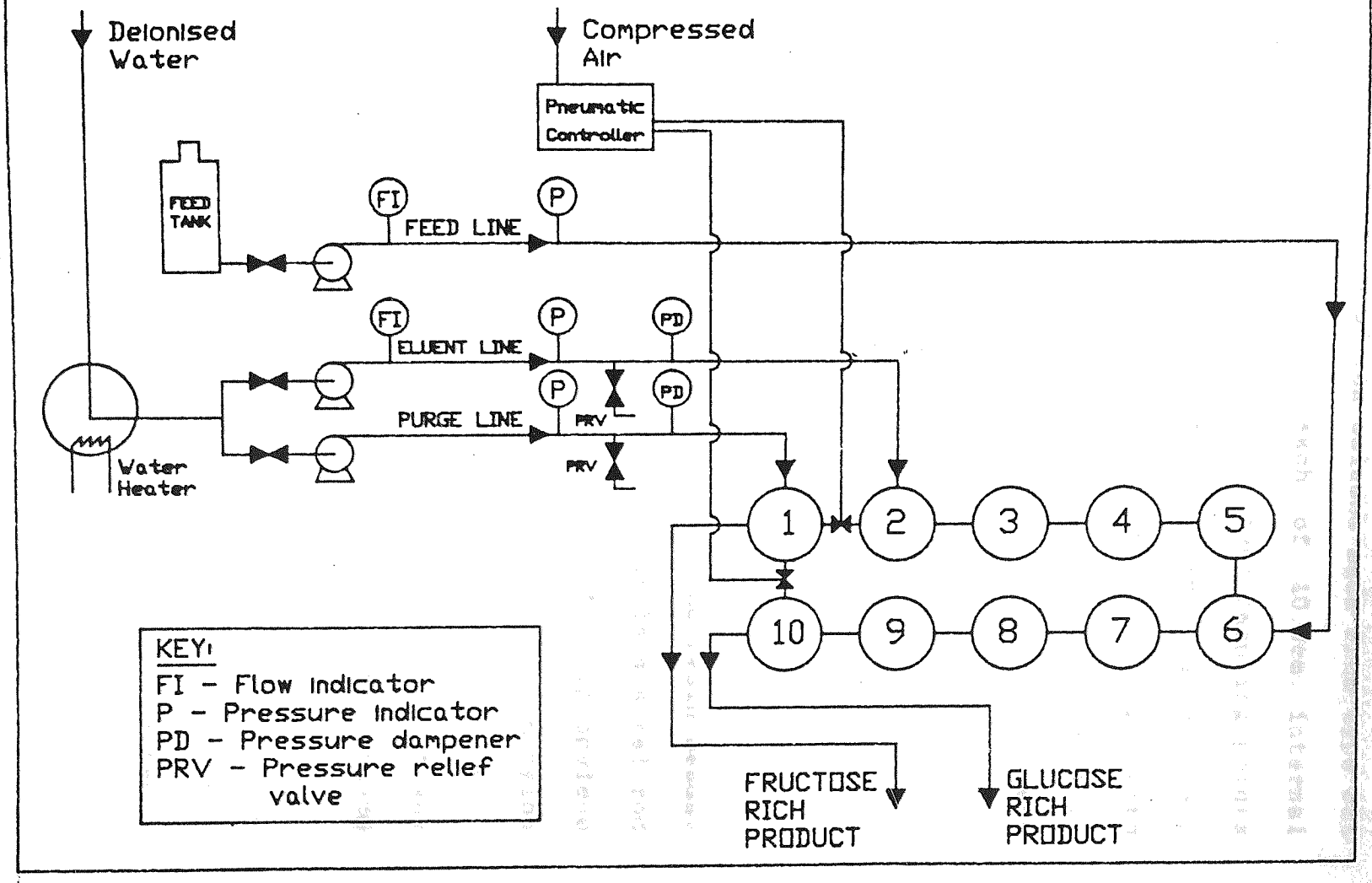
The separation section of the SCCR6 system consists of 10 stainless steel columns 75cm long and 10.8cm internal diameter. The columns were packed with an 8% cross-linked polystyrene resin charged in the calcium form. The resin was of type ZEROLIT SRC14 supplied by PUROLIT LTD, Hounslow Middlesex, and the resin bed length was 65cm. The equipment was constructed by Gould (8) and later modified by Thawait (9). Figure 5.6 is a photograph of the apparatus as used by Thawait and the earlier part of this research.

Figure 5.6: Picture of the SCCR6 System



The equipment layout is shown in Figure 5.7. The columns are mounted on a frame and are linked at the top and bottom to form a loop. The inlet and outlet lines from all columns are arranged in a ring distribution network connected to supply pumps and collection units outside the system. The poppet valves are activated by a pneumatic controller. The columns, valves and the ring distribution network are enclosed in a constant temperature enclosure. A description of the individual items of equipment is given in the following sections.

Figure 5.7: Equipment Layout for the SCCR6 System



5.2.2 The columns and fittings

The refiner consists of ten columns made from type 321 seamless stainless steel tube each of 10.8cm. internal diameter and 75cm. long. Stainless steel sampling points were constructed and welded to the columns radially and longitudinally. The sample point ends were plugged with silicone rubber septa held by simplifix nuts. Samples were taken by inserting hypodermic needles into the columns through the septa. Mild steel flanges were welded to each end of the column and a 2mm. thick neoprene gasket was used to seal the two flanges 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 beads. The plunger was made of a 130 x 19mm. stainless steel rod and a 108 x 55mm. polypropylene piston. A polypropylene ring was fitted to the piston base to retain a 100 μm nylon mesh and a distributor was used to ensure even distribution at the inlet. The first 20cm. of the column's inlet was accurately machined and two Dowty o-rings were fitted round the head to ensure perfect sealing.

Five ¼" BSP ports were tapped on the inlet head to accommodate CK-¼-PK4-KU type FESTO plastic fittings (FESTO PNEUMATICS LTD, West Bromwich, West Midlands) for the feed, eluent, purge and transfer lines and the piston rod. Liquid channels of 5mm. diameter were drilled through the head,

plunger and the piston.

The outlet assembly consisted of a stainless steel packing support and a T-shaped polypropylene head to form a bottom flange. Three ¼" BSP ports were tapped on the outer head and were fitted with the same festo connectors as the inlet head. These were connected to the fructose rich and glucose rich product lines and the transfer line.

5.2.3 Resin Compression

The resin was kept compressed by tightening the screw on the aluminium backing plate placed on top of the inlet head.This forced the piston into the column compressing the resin.

5.2.4 Pneumatic Poppet Valves and Controller

The countercurrent operation was achieved by the pneumatic operation of 60 double acting poppet valves. The valves were constructed by Aston Technical Services Ltd and the prime objective was to minimise the internal volume thus minimising the system dead volume. The sequence of the valve settings for every switch in a cycle is shown in Table 5.1. Figure 5.8 is a picture of a valve with its parts. Locking washers were added to prevent the nuts on the valve stems from working loose and preventing the valve from closing fully. Compressed air was supplied by the department's main compressor with a Broom and Wade

Compressor as a standby.

The supply was divided into bias and actuating streams whose pressures were regulated to 240 KN.m^{-1} and 550 KN.m^{-1} respectively. The bias supply was branched and led directly to the poppet valves while the actuating line was led to the controller. Normally the valves were closed due to the bias pressure 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.

The operation of the valves was governed by a pneumatic controller. The control mechanism consisted of a cam unit with ten programmable disks operating ten on-off roller valves. The gaps on consecutive disks were set at 36° out of phase and each gear wheel movement represented one sequence on the SCCR6 process. The cam was driven by a single speed motor. When a particular cam opened its roller valve, the actuating air flowed through the valve and was then split into four streams; one stream entering a closed ring main which opened five functional valves, namely the inlets for feed, eluent and purge and outlets for glucose and fructose rich products. The second and third of the four streams were each attached to a shuttle valve and each one of these in turn closed an isolating valve. The fourth stream travelled to an indicator to aid visual display of the eluent entry column. A further branch allowed air to flow to an electro-pneumatic switch which

Table 5.1: Valve Sequence

SWITCH NUMBER	VALVES ACTIVATED						
	TO OPEN					TO CLOSE	
	ELUENT	FEED	PURGE	FRP	GRP	TRANSFER	TRANSFER
1	1	5	10	10	9	10	1
2	2	6	1	1	10	1	2
3	3	7	2	2	1	2	3
4	4	8	3	3	2	3	4
5	5	9	4	4	3	4	5
6	6	10	5	5	4	5	6
7	7	1	6	6	5	6	7
8	8	2	7	7	6	7	8
9	9	3	8	8	7	8	9
10	10	4	9	9	8	9	10

Figure 5.8: Photograph of a poppet valve and its parts

stopped the voltage to the motor. This switch zeroed the manually adjustable digital timer which permitted the various sequencing intervals.

At the end of the sequence the timer activated the electro-pneumatic switch which rotated the camshaft another 36° and the sequence repeated itself.

5.2.5 Liquid Delivery and Pressure Control

All the deionised water used in the SCCR6 system was produced from an Elgastat B224 deioniser unit (Elga Products Ltd. High Wycombe). The water was stored in two stainless steel tanks of total capacity 750 litres, and were placed one floor higher in the building to provide a positive suction head to the SCCR6 supply pumps. The deionised water passed through a Gelman Acroflow II cartridge (Gelmen Sciences Ltd, Northampton) to remove any impurities in the water.

The synthetic and sucrose inverted feed mixtures were prepared in a 150 litres polypropylene tank and transferred to 20 litre glass aspirators approximately 2.5 metres above the pump to provide the positive head. The inverted molasses feed was prepared and mixed separately for every cycle in a 30 litre plastic container and transferred to the glass aspirators to feed the SCCR6.

Two sets of pumps were used during the research. For the initial non molasses feed runs on the SCCR6 two types

of pumps were used:

Purge pump

Supplier - Metering Pumps Ltd. Ealing, London

Pump Type - Series L

Stroking Speed - 150 per minute

Flow Rate - 0-6500 $cm^3.min^{-1}$

Maximum Operating Pressure - 1160 $KN.m^{-2}$

Eluent and Feed pump

Supplier - Metering Pumps Ltd. Ealing London

Pump Type - Series K Twin head

Stroking Speed - 96 per minute

Flow Rate - 0-360 $cm^3.min^{-1}$

Maximum Operating Pressure - 1360 $KN.m^{-2}$

These pumps proved unreliable for the runs using molasses as feedstock and one pump with three pumpheads was bought to replace the two pumps.

New Pump for the SCCR6

Supplier - Metering Pumps Ltd. Ealing London

Pump Type - Type E With three heads

Stroking Speed - 144 per minute

Purge Head:

Type - PG20L Glass plunger in polypropylene head

Flowrate - 0-20000 $cm^3.min^{-1}$

Maximum Operating Pressure - 1420 $KN.m^{-2}$

Eluent Head:

Type - PG10L Glass plunger in polypropylene head

Flowrate - 0-10000 $cm^3.min^{-1}$

Maximum Operating Pressure - 1420 $KN.m^{-2}$

Feed Head:

Type - SS6C Stainless steel plunger in steel head

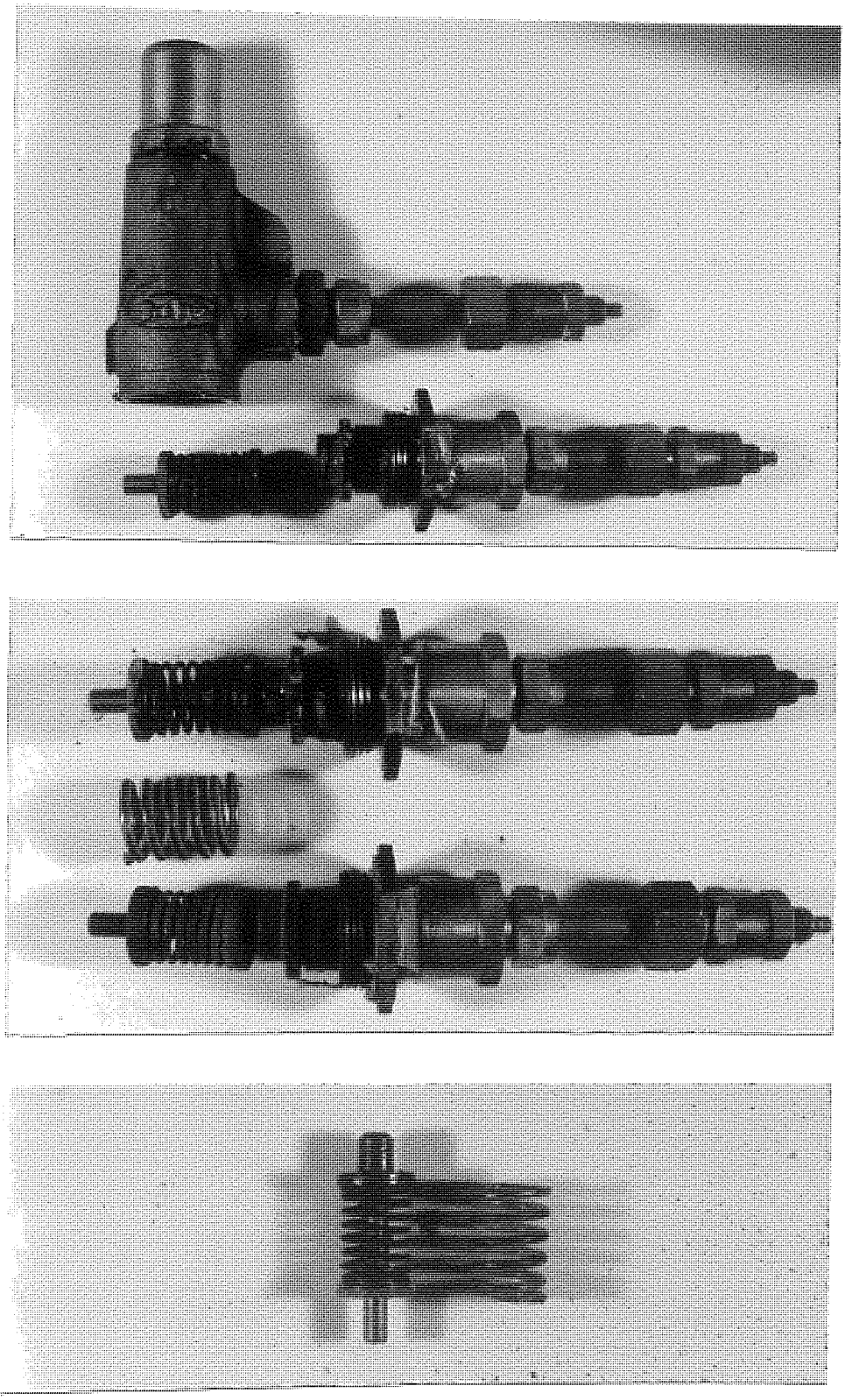
Flowrate - 0-6000 $cm^3.min^{-1}$

Maximum Operating Pressure - 14000 $KN.m^{-2}$

Loading valves were also fitted to the system after the pumps to avoid flowrate fluctuations due to pressure differences in the columns. These were made of brass with steel springs which had to be replaced regularly due to corrosion. Figures 5.9(a),(b) and (c) show pictures of the loading valves with and without corrosion.

All the liquid supply lines were made of Festo PP4 and PP6 polyamide tubing. The tubing for the feed, eluent and glucose product lines were 0.4mm internal diameter and that for the purge and fructose product lines were 0.6mm internal diameter to carry the extra volume. All the lines were colour coded. To minimise the system's dead volume, the columns were mounted so that for two adjacent columns the inlet of one was at the top and for the other at the bottom. Because of this arrangement, it was necessary to have an upper and lower pipe ring for each one of the inlet

Figure 5.9: Pictures of the loading valves also showing corrosion of the springs



and outlet streams. Although the column interconnecting tubes were kept to a minimum, the total dead volume of the system was estimated at 5%. Collection of both products was into plastic containers.

5.2.6 Pressure Control

Three Beta pressure sensors (Loba Ltd, Reigate, Surrey) were fitted - two on the eluent line inlet and one on the purge inlet, to switch the system off in case of a pressure increase in the system. The pressure sensor on the purge line and one on the eluent line were high pressure cut-off switches set to cut power to the SCCR6 if pressure in either line exceeded 550 $KN.m^{-2}$ and the other on the eluent line was the low-pressure cut-off set to cut power at pressure below $10KN.m^{-2}$. In case of an emergency such as a blocked column or stuck valve, the system pressure would increase to above the preset value. This would be detected by the pressure sensor and the electrical supply to the equipment would be broken. Similarly if the pressure dropped to below $10KN.m^{-2}$ due to a leak in the system the power would be switched off by the low pressure sensor.

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

5.2.7 Heating Controls

The deionised water used for the eluent and the purge was heated in a stainless steel heating unit constructed by England (59). The heating unit was connected in series to the SCCR6 equipment and this supplied the hot deionised water to all the experimental units in the lab. The feed was heated on-line before it entered the SCCR6. A 5KW immersion heater was screwed into a 5.0cm.id x 45cm. long stainless steel pipe. Feed entered through one end of the pipe where it was heated. A thermocouple was placed at the exit of the pipe connected to a proportional band controller supplied by Diamond Controls Ltd., Norwich. The hot feed then entered the SCCR6.

The enclosure round the columns and valves was constructed from galvanised steel which was lagged with 50mm. thick glass fibre pads and covered with aluminium foil. The enclosure was heated by a 5KW finned air heater supplied by Eltron Ltd., London, and was controlled by a proportional band controller supplied by Diamond Controls Ltd., Norwich. The input to the controller was via a NiCr/NiAl thermocouple placed inside the enclosure. A safety device was incorporated with the thermocouple which would cut off the electrical power to the heater if the temperature exceeded 70°C. The air was circulated using an airotor.

5.2.8 Product Splitting and Collection

As an attempt was made to regenerate the calcium resin as well as to increase the product concentrations, it was decided to separate the dilute product fractions by fitting two product splitting devices. A 10% calcium nitrate solution was introduced to the purge column when the dilute product fraction was being removed. This is described in detail in chapter 11. The splitting devices were fitted on each of the glucose rich and fructose rich product lines and were operated independently. Each device consisted of a RS346-463 timer (RS Components Ltd, Corby, Northants) and one or two 3-way solenoid valves supplied by A J Foster Ltd, Manchester. The timers were connected directly to corresponding valves. After a preselected time the solenoid valve was switched from one position to the other allowing the splitting of the corresponding product line into two separate lines.

CHAPTER SIX

COLUMN CHARACTERISATION AND THE DETERMINATION OF DISTRIBUTION COEFFICIENTS, Kds

6.1 Introduction

The methods used for the characterisation of the columns and for column packing were similar to those used by previous research students in the department. This enabled direct comparison with previous work. The column characterisation was carried out at very low background sugar concentrations ie. at infinite dilution conditions. The effect of high background sugar concentration was also investigated as this would be the case in practice.

6.2 Ion-Exchange Resins

6.2.2 Properties of ion-exchange resins

Ion-exchange resins are particles consisting of porous matrices having electrically charged functional groups which are covalently bonded. There are four main types of ion-exchangers:

i) synthetic resins

ii) cellulose ion-exchangers

iii) ion-exchange polydextran (Sphadex)

iv) inorganic exchangers - based on aluminium silicates

There are five principle functional group classes found on ion-exchangers:

i) cation exchangers

ii) anion exchangers

iii) chelating ion-exchangers

iv) amphoteric and dipolar ion-exchangers

v) selective ion-exchangers

In chromatography, synthetic ion-exchangers are the type most widely used. This research work was carried out using synthetic cation exchangers which operate by the mechanism as shown:

$$R\text{-}X^+ + Y^+ \rightleftharpoons R\text{-}Y^+ + X^+$$

The forward reaction is known as adsorption and the reverse as desorption, the equilibrium depending on the concentration of solute ions and the relative affinities of the ions for the exchanger.

Chromatographic resins are solid insoluble high molecular weight polyelectrolytes consisting of a three dimensional matrix with a large number of attached ionizable groups. These are produced by the polymerisation of styrene cross-linked with divinylbenzene (DVB) (60).

The degree of cross-linkage is an important factor in chromatography and it determines the mechanical strength of a resin. The cross-linkage can be controlled and varies between 1% and 16%, with the low cross-linkage resins

tending to be more porous and they exchange ions more rapidly, but have the disadvantage of swelling and shrinking which can disturb chromatographic operations. Low cross-linkage resins are also less selective and cannot be subjected to high pressures thereby limiting liquid flowrates.

The choice of an ion-exchange resin for a particular application is influenced by a number of properties. These are:

(i) Capacity - The capacity of an ion exchanger is a measure of the total amount of ions the resin is able to bond to and is usually expressed in terms of milliequivalents per gram of dry resin (in the H^+ or Cl^- form) or in milliequivalents per gram of fully swollen wet resin. The available capacity of an ion-exchange resin is effected by the concentration and ionic strength of the eluent, the pH, the temperature, the accessibility of functional groups and the nature of the counter-ions.

(ii) Affinity - The 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. Equilibrium depends not only on the relative affinities of the 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 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 charge density on a particular ion, ie. small highly charged ions have the greatest exchange potential. The affinity sequence for some of the common cations is:

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

and

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

and the affinity of the divalent ions for ion exchangers is greater than the monovalent ions.

A measure of the affinity is given by the affinity coefficient, β where:

$$\beta = \frac{X_A/Y_A}{X_B/Y_B}$$

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

6.2.3 Particle size of the resin

The resin used for this work was the same as that used by Gould (8) and Thawait (9) and was the ZEROLIT 225 SRC 14 resin supplied by Duolite International, Hounslow,

Middlesex. This resin is now called PUROLITE PCR 833 and is still supplied by the same company.

This resin is a sulphonated polystyrene with 8% cross-linkage with divinylbenzene, giving the resin mechanical strength to withstand pressures greater than 2000 KN.m^{-2}. It can also be used over a temperature range of -10°C to 140°C and is unaffected by pH variations in the range 1-14.

The resin particle size range was quoted by the manufacturer to be 52-100 mesh (150-300μm). A particle size analysis was carried out using BS410 meshes and the results are shown in Table 6.1.

6.2.4 Column packing technique

Three columns were repacked before this research work began. This was because these columns were leaking and some resin was required to fill these columns.

The old packing was removed from the columns and new neoprene gaskets were fitted. Each column was filled with deionised water and its outlet connected to the vacuum line on a cold water tap.

A 50% slurry of the resin and deionised water was added to the column at the same rate that the water was removed by the vacuum suction. This method was used to minimise the segregation of the particles apparent in gravitational settling. The column was tapped at random during the packing. When the packing reached the maximum

height, the inlet plunger was fitted and the column reassembled. Deionised water was passed through the column for two hours to remove any fine particles.

Table 6.1: Sieve analysis for the ZEROLIT 225 resin

BS Sieves (meshes per inch)	Size range (μm)	Mass fraction
+52 mesh	≥ 295	0.03
-52 + 60	251-295	0.48
-60 + 72	210-251	0.21
-70 + 85	178-210	0.14
-85 + 100	152-178	0.11
-100 mesh	≤ 152	0.03

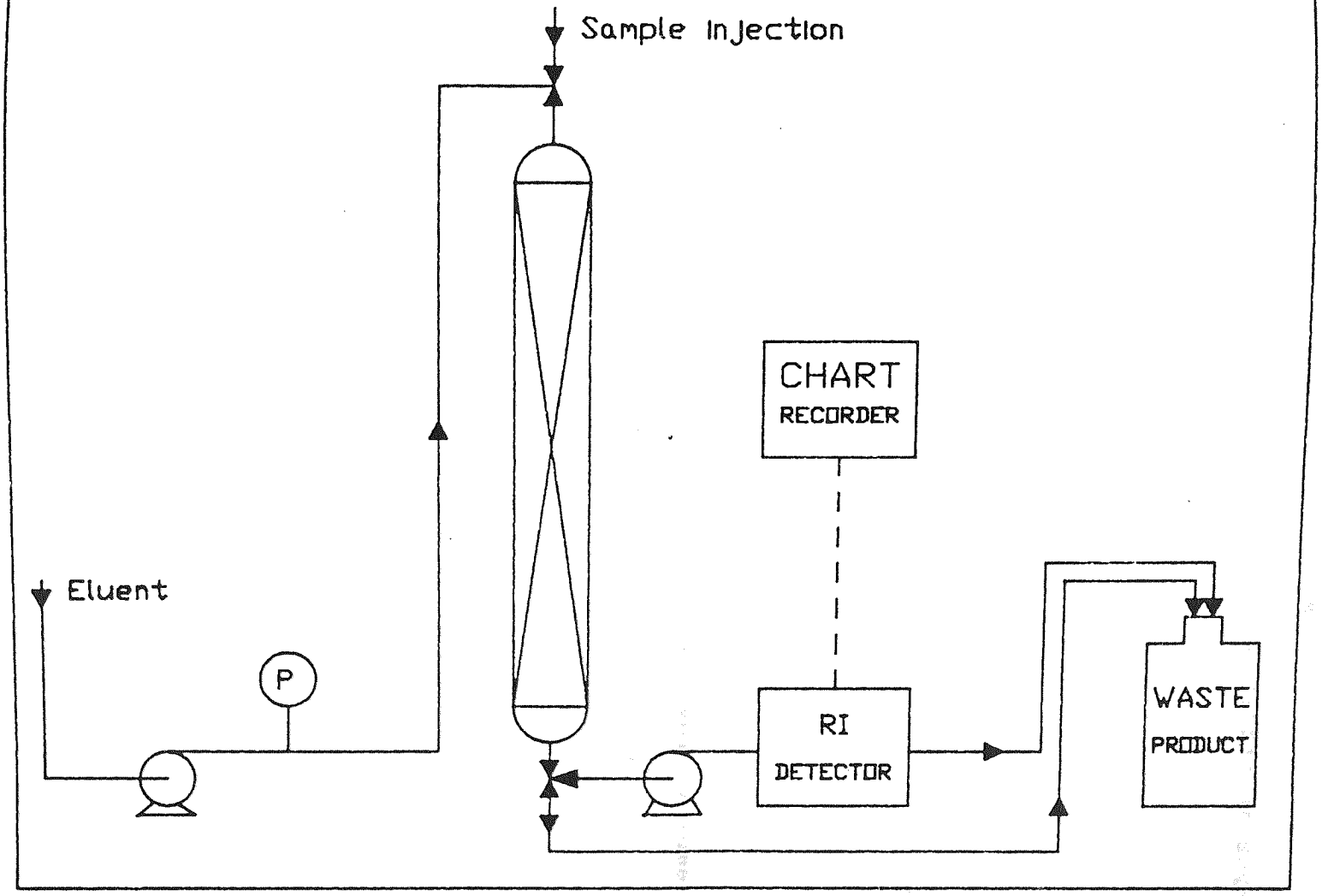
6.3 Experimental Technique for the Characterisation of the Columns

The packed column was linked to the eluent delivery pumphead and a sample detection system as shown in Figure 6.1. A T-piece was connected as close as possible to the column inlet and a silicon rubber septum was fitted to one of the T-piece inlets. The sample was loaded onto the column by inserting a hypodermic needle through the septum.

A similar T-piece was connected to the column outlet. A sampling needle inserted into the rubber septum at the outlet T-piece allowed a small stream of the outlet stream to bypass through the sample detection system which consisted of a peristaltic pump and a Waters refractive index monitor linked to a RICADENKI RO2 chart recorder (BDH Ltd, Atherstone, Warwickshire). The refractometer's outlet stream was connected to a product collection reservoir together with the mainstream outlet from the column.

The eluent pump was set to flow at 105$cm^3.min^{-1}$ as this was the operating flowrate for the SCCR6 system, and the peristaltic pump drew a steady continuous stream of 1.0$cm^3.min^{-1}$ from the outlet stream. Separate 10%w/v solutions of dextran T70, glucose and fructose were made and a 10cm^3 slug of each of these solutions was injected through the inlet rubber septum. A peak was produced on the chart recorder as the sugar was eluted. The chromatograms produced were analysed and the method of determining the

Figure 6.1: Equipment for Column Characterisation



column parameters is described in the next section.

6.4 Column Parameter Calculations

The chromatograms produced for the different sugars are similar to those shown in Figure 2.2. The elution volumes, V_i, of the individual components were calculated by multiplying the respective retention times, t_{Ri}, with the eluent flowrate.

The dextran T70 molecules were too large to enter the inter-particle volume and thus travel only through the interstitial or void volume. Thus, the dextran elution volume was used as the column's void volume, V_o. The individual column volumes were expressed as voidage, ϵ, or void fraction, ie. the ratio of the column void volume to the total empty column volume. A measure of the column's separation potential is the separation factor, α, and for a glucose/fructose mixture is:

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

where:

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

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

The distribution coefficients were determined using the retention equation (61):

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

where:

V_i = retention volume of component i

V_o = column void volume

$V_s = V_T - V_o$

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 in terms of the distribution coefficient gives:

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

By re-expressing Equation 6.3 for glucose and fructose, their infinite dilution coefficients are given by:

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

and

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

The criterion used for comparing the performance of a chromatographic column is generally the height equivalent to a theoretical plate, HETP. This is calculated by

dividing the bed height L by the number of theoretical plates in a column, N. The number of plates is calculated for individual components from the chromatograms and using the equation:

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

where:

N_i = number of theoretical plates based on component i

t_{Ri} = retention time for component i

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

e = logarithmic base

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

6.4.1 Discussion of Results

The theoretical value for the voidage for a bed of spherical particles is about 0.4 (62). The average column voidage for the system was 0.331. This lower value is due to the different packing geometry and the non-uniformity of the packing material. The smaller particles were dispersed and filled the empty space between the larger ones. This was also enhanced by the compression exerted by the piston plunger. The packing of the columns for the SCCR6 system was reasonably consistent with a voidage variation of ±15% about the mean value.

The dextran molecules are totally excluded and are not delayed. The glucose molecules diffuse into and out of the intra-particle space and hence are delayed with respect to the dextran molecules. This results in the larger elution volume for glucose. In addition to that, the fructose molecules are retained further due to the chemical complex formation with the calcium ions thus giving even higher elution volumes. The average elution volumes for glucose and fructose were 2604cm^3 and 3584cm^3 compared to the average column void volume of 1944cm^3.

The average separation factor for a glucose and fructose mixture was found to be 2.48, and the average number of theoretical plates per column was 25 for fructose and 40 for glucose. This gave an average HETP value of 2.58cm for fructose and 1.617cm for glucose. The variations in voidage, HETP's and the distribution coefficients values from column to column are due to the packing non-uniformity. Ideally all these parameters should be constant to ensure similar migration rates for the individual components through successive columns. However, these variations can be tolerated in the SCCR6 system because nine columns are serially linked at any one time and the continuous cyclic operation ensures comparatively constant component migration rates through the system's separation section.

TABLE 6.2 INDIVIDUAL COLUMN PROPERTIES

Column No	Bed Volume (cm3)	Bed Height (cm3)	Void Volume (cm3)	Voidage	Elution Volume (cm3)		HETP's (cm)		Distribution coefficient	
					Fructose	Glucose	Fructose	Glucose	Fructose	Glucose
1	5872	64.1	2258	.38	4077	2838	2.56	2.07	.5	.16
2	5964	65.1	1882	.32	3300	2411	2.41	1.14	.35	.13
3	5936	64.8	1886	.31	3424	2438	3.6	2.59	.38	.14
4	5845	63.8	1950	.33	3509	2671	2.09	0.97	.40	.19
5	5945	64.9	2171	.37	3791	2846	2.60	1.22	.43	.18
6	5781	63.1	1823	.32	3218	2370	2.87	1.11	.35	.14
7	5955	65.0	1659	.28	3622	2520	3.60	2.50	.46	.2
8	5872	64.1	2088	.36	3816	2809	1.64	1.03	.46	.19
9	5936	64.8	1830	.31	3595	2654	2.31	2.09	.41	.15
10	5781	63.1	1912	.33	3488	2495	2.10	1.40	.43	.2
Average	5889	64.28	1944	.331	3584	2604	2.58	1.617	.417	.168

6.5 Determination of the Distribution Coefficients

The distribution coefficient has been described in chapter 2. Previous researchers in the Department of Chemical Engineering and Applied Chemistry at Aston University have identified factors which affected the separating potential of the SCCR system.

These factors are:

- 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.

If these factors are taken into account, the basic design equation (Equation 5.9) becomes:

$$(K_{dg}+\delta_{1g}+\delta_{2g}) < \frac{L_e}{P} < \frac{L_e+L_f}{P} < (K_{df}-\delta_{1f}-\delta_{2f}) \quad .. 6.7$$

or

$$(K_{dg}+\delta_{1g}+\delta_{2g}) < \frac{L_m}{P} < (K_{df}-\delta_{1f}-\delta_{2f}) \quad ..... 6.8$$

where

$$\frac{L_m}{P} = \frac{\left(\frac{L_e}{P} + \frac{L_e+L_f}{P}\right)}{2} \qquad \ldots\ldots 6.9$$

and

δ_{1g} and δ_{1f} - fractional changes due to the effect of concentration on the distribution coefficients.

δ_{2g} and δ_{2f} - fractional changes due to the effects of system characteristics on the distribution coefficients.

The rest of the chapter describes the individual effect of the above mentioned factors on the system and hence assist in the selection of the optimum operating conditions. For the runs using inverted beet molasses, it was assumed that the molasses was a four 'component' mixture made up of fructose, glucose, betaine and ionic non-sugars. The effect of the factors on the distribution coefficients for all these components was investigated. Potassium chloride was found to behave closest to the ionic non-sugar 'component' and was therefore used as the ionic component.

As the effect of the factors mentioned earlier was to be valid for any chromatographic system having different dimensions and characteristics, the distribution coefficient variations were expressed in percentage terms;

the eluent flowrate was expressed as a linear velocity (flowrate divided by the column cross-sectional area) and it was assumed that any system would exhibit analogous K_d changes at similar eluent flowrate variations.

6.5.1 Effect of Eluent Flowrate

One of the columns on the SCCR6 system (Column 5) was isolated and arranged as described in section 6.3 and as shown in Figure 6.1.

The eluent leaving the column was collected over a fixed period, the flowrate determined by the volume collected and then disposed of.

10%w/v individual solutions of dextran T70, glucose, fructose, betaine and potassium chloride were prepared and 5cm^3. slugs were injected into the column using a hypodermic syringe.

Flowrates of 88, 103, 105, 118, 129 and 147 $cm^3.min^{-1}$ were used to observe the effect of flowrates on the K_d's. All these tests were carried out at infinite dilution, ie, water eluent. The derived equations relate the K_d variations to a reference value K_d^* which is calculated for the particular chromatographic system at infinite dilution conditions and at a predetermined linear velocity. This value is normally that derived during the commissioning of the system and thus represents the system's characteristics and configuration. These values for K_d^* are also used in

the equations that relate the effect of temperature and concentration as will be shown later, and were calculated for the SCCR6 system at infinite dilution, at 25°C and at an eluent flowrate of 105cm^3min^{-1}.

The flowrate of 105$cm^3.min^{-1}$ was used as it corresponds to the operating flowrate of the system. These values were used as a basis (100%) during the arrangement of the results as shown in Table 6.3.

Table 6.3: Effect of Eluent Flowrate on the Distribution Coefficients

Flowrate cm^3min^{-1}	Linear velocity (LV) $cm.min^{-1}$	K_d Glucose	% Change in K_{dg}	K_d Fructose	% change in K_{df}
88	0.96	0.273	110	0.521	103.2
103	1.13	0.209	104	0.521	101
105	1.15	0.201	100	0.505	100
118	1.29	-	-	0.498	98.6
129	1.41	0.193	96	0.490	97
147	1.61	0.186	93	0.469	92.9

Flowrate cm^3min^{-1}	Linear velocity (LV) $cm.min^{-1}$	K_d Betaine	% Change in K_{db}	K_d Potassium chloride	% change in K_{dp}
88	0.96	0.713	109.5	0.307	118.5
103	1.13	0.721	111.7	0.276	106.5
105	1.15	0.651	100	0.259	100
118	1.29	0.639	98.15	-	-
129	1.41	0.578	88.7	0.208	80.3
147	1.61	0.547	84	0.193	74.5

By assuming a linear change in the distribution coefficient and fitting regression lines to the experimental data, the following equations were derived relating to the effect of flowrates on the distribution coefficients:

For glucose:

$$K_{dg} = (131.7 - 24.8 \times LV) \times \frac{K^{\infty}_{dg}}{100} \quad \ldots\ldots 6.10$$

For fructose:

$$K_{df} = (118.2 - 15.4 \times LV) \times \frac{K^{\infty}_{df}}{100} \quad \ldots\ldots 6.11$$

For betaine:

$$K_{db} = (153.0 - 43.4 \times LV) \times \frac{K^{\infty}_{db}}{100} \quad \ldots\ldots 6.12$$

For potassium chloride:

$$K_{dp} = (183.1 - 69.6 \times LV) \times \frac{K^{\infty}_{dp}}{100} \quad \ldots\ldots 6.13$$

Therefore, the distribution coefficients for the 'four' components can be modified using the Equations 6.10 - 6.13 to give an estimate of the actual distribution coefficients for the particular eluent flowrate.

It has also been found that the separating performance is also affected by the changes in the eluent to feed ratios. A decrease in the 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 (6).

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.

6.5.2 Effect of Operating Temperature

It has been shown by Ching (6) and Chuah (7) that the increase in operating temperature from ambient to 60°C altered the on-column concentration profiles and the 'cross-over' point moved progressively towards the glucose rich product resulting in reduced GRP purities.

Thawait (9) investigated the individual profiles and revealed that the glucose profile remained unaffected while the fructose profile shifted towards the GRP. This can be explained since 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. It is also known that β-D-fructopyranose is the sweeter fructose tautomer and is the only known form of fructose that forms a complex with the calcium ions. The proportion of this tautomer at equilibrium to other forms is a function of temperature and it decreases with increasing temperature as shown in Table 6.4 (63). This reduces the fraction of fructose available for complexing and increases the amount of fructose moving with the mobile phase.

To establish the relationship between the distribution coefficients and temperature, a series of experiments were carried out on the same system as that described in the

previous section. The column and the eluent were heated and maintained at five different temperatures. A flowrate of 105$cm^3.min^{-1}$ was maintained for the deionised water eluent.

As expected, the effect of temperature on the glucose elution profile showed no significant difference at higher temperatures. The distribution coefficients for potassium chloride and betaine were not affected by the temperature either.

Table 6.5 shows the effect of temperature on the fructose distribution coefficient. Again, 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 points provided the equation relating the temperature to the fructose distribution coefficient:

$$K_{df} = (114.5 - 0.563 \times TEMP) \times \frac{^{25}K_{df}}{100} \quad ..... \quad 6.14$$

Therefore, the fructose distribution coefficient derived at 25°C and at infinite dilution can be modified to give a better estimate of the actual K_{df} for the particular operating temperature. For this research work, the operating temperature was kept at 60°C with the exception of some initial runs which were carried out at ambient temperature (25°C).

Table 6.4: Equilibrium Concentrations of 20% Fructose at Different Temperatures

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

The amounts of Keto-fructose and α-D-Fructopyranose present were very low to be included in the table.

Table 6.5: Effect of Temperature on the Fructose Distribution Coefficient

Temperature °C	Fructose Distribution Coefficient K_{df}	% Variation in K_{df}
25 (Ambient)	0.875	100
33	0.827	94.5
40	0.825	94.3
50	0.762	87.1
65	0.674	77.0

6.5.3 Effect of Concentration

Previous research on the SCCR systems has shown that as the feed concentration to the system was increased, the on-column concentrations also increased, the profiles became broader, the 'cross-over' point 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 (9) quantified the effect and deduced that:

- all the distribution coefficients increased most rapidly in dextran background rather than glucose or fructose background concentrations
- the distribution coefficients of glucose and fructose increase more rapidly in glucose background concentration than in fructose

and the following conclusions were drawn:

- viscosity effect :- increasing the concentration of background sugar increases the viscosity resulting in larger elution volumes. This causes the 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.
- chemical structure effect :- the fraction of β-D-Fructopyranose form of fructose decreases with increased background concentration. Therefore, less fructose is likely to be chemisorbed at high concentration by the calcium ions on the resin causing a decrease in the fructose distribution coefficient.

- concentration gradient effect :- a concentration gradient between the stationary and the mobile phase is created, forcing the glucose to stay with the stationary phase either by diffusion or by osmosis. For fructose, this effect is reduced since it is already chemisorbed by the resin to its limiting capacity.

A series of experiments using different concentration solutions of glucose, fructose and betaine as the eluent were carried out and the effect of these background sugar concentrations on the distribution coefficients are shown in Tables 6.6, 6.7 and 6.8. The background sugar concentrations of up to 0.5g.cm^{-3} were used for glucose and fructose and up to 0.3g.cm^{-3} were used for betaine. The effect of potassium chloride in a background could not be evaluated as the potassium ions in the eluent displaced the calcium from the resin and the elution profiles were not defined and were irregular. The problem of the calcium ions being displaced from the resin by the sodium and potassium ions present in the molasses was solved by regenerating the resin and this is described in detail in Chapter 11.

Table 6.6: Effect of Glucose Background Concentration

Glucose conc^{n} in g.cm^{-3}	K^{G}_{dg}	% change in K^{G}_{dg}	K^{G}_{df}	% change 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

Glucose conc^{n} in g.cm^{-3}	K^{G}_{db}	% change in K^{G}_{db}	K^{G}_{dp}	% change in K^{G}_{dp}
0.0	0.491	100	0.109	100
0.1	0.537	109	0.234	215
0.2	0.598	122	0.378	347
0.3	0.672	137	0.416	382
0.4	0.742	151	0.482	442
0.5	0.813	166	0.533	489

Table 6.7: Effect of Fructose Background Concentration

Fructose concn in g.cm^{-3}	K^F_{dg}	% change in K^F_{dg}	K^F_{df}	% change in K^F_{df}
0.0	0.120	100	0.380	100
0.1	0.160	133	0.400	105
0.2	0.180	150	0.420	111
0.3	0.200	167	0.450	118
0.4	0.230	192	0.430	113
0.5	0.240	200	0.460	121

Fructose concn in g.cm^{-3}	K^F_{db}	% change in K^F_{db}	K^F_{dp}	% change in K^F_{dp}
0.0	0.423	100	0.111	100
0.1	0.469	111	0.181	163
0.2	0.498	118	0.234	211
0.3	0.521	123	0.291	262
0.4	0.547	129	0.316	285
0.5	0.575	136	0.371	334

Table 6.8: Effect of Betaine Background Concentration

Betaine concn in g.cm^{-3}	K^B_{dg}	% change in K^B_{dg}	K^B_{df}	% change in K^B_{df}
0.0	0.113	100	0.367	100
0.1	0.130	115	0.391	107
0.2	0.175	155	0.435	119
0.3	0.237	210	0.463	126

Betaine concn in g.cm^{-3}	K^B_{db}	% change in K^B_{db}	K^B_{dp}	% change in K^B_{dp}
0.0	0.412	100	0.103	100
0.1	0.474	115	0.229	222
0.2	0.558	135	0.308	299
0.3	0.607	147	0.412	400

All the values of K_d's were expressed as a percentage of the corresponding distribution coefficient calculated at infinite dilution conditions, ie. deionised water mobile phase, and to keep the required experimental data to a minimum, the same reference infinite dilution coefficients as those used in the K_d vs flowrate and K_d vs temperature relationships were used. The K_d vs concentration relationships derived from these tables are as shown on the next page. The term C_i is the concentration of component i in the mobile phase.

- For glucose background concentration:

$$K^{G}_{dg} = (102.91 + 320.9 \times C_g) \times \frac{K^{\infty}_{dg}}{100} \quad \ldots\ldots 6.15$$

$$K^{G}_{df} = (95.71 + 173.1 \times C_g) \times \frac{K^{\infty}_{dg}}{100} \quad \ldots\ldots 6.16$$

$$K^{G}_{db} = (96.62 + 139.7 \times C_g) \times \frac{K^{\infty}_{dg}}{100} \quad \ldots\ldots 6.17$$

$$K^{G}_{dp} = (138.9 + 759.9 \times C_g) \times \frac{K^{\infty}_{dg}}{100} \quad \ldots\ldots 6.18$$

- For fructose background concentration:

$$K^{F}_{dg} = (107.43 + 198.3 \times C_f) \times \frac{K^{\infty}_{df}}{100} \quad \ldots\ldots 6.19$$

$$K^{F}_{df} = (101.62 + 194.5 \times C_f) \times \frac{K^{\infty}_{df}}{100} \quad \ldots\ldots 6.20$$

$$K^{F}_{db} = (102.43 + 68.3 \times C^{f}) \times \frac{K^{\infty}_{df}}{100} \quad \ldots\ldots 6.21$$

$$K^{F}_{dp} = (112.47 + 453.4 \times C_f) \times \frac{K^{\infty}_{df}}{100} \quad \ldots\ldots 6.22$$

- For betaine background concentration:

$$K^{B}_{dg} = (89.71 + 366.9 \times C_b) \times \frac{K^{\infty}_{db}}{100} \quad \ldots\ldots 6.23$$

$$K^{B}_{df} = (99.54 + 89.99 \times C_b) \times \frac{K^{\infty}_{db}}{100} \quad \ldots\ldots 6.24$$

$$K^{B}_{db} = (100.1 + 159.9 \times C_b) \times \frac{K^{\infty}_{db}}{100} \quad \ldots\ldots 6.25$$

$$K^{B}_{dp} = (108.7 + 976.9 \times C_b) \times \frac{K^{\infty}_{db}}{100} \quad \ldots\ldots 6.26$$

All these relationships are included in the computer modelling of the system to enable accurate simulation of the system behaviour.

6.6 Summary of the Best Operating Conditions

The reference distribution coefficients for glucose, fructose, betaine and potassium chloride (ionic part of the molasses) K^{∞}_{dg}, K^{∞}_{df}, K^{∞}_{db}, K^{∞}_{dp} and $^{25}K_{df}$ are all evaluated at infinite dilution, ambient temperature (25°C) and at the eluent flowrate of 105 $cm^3.min^{-1}$.

The effect of the potassium chloride in the background cannot be evaluated using the apparatus described earlier and another method has to be used to complete this experiment. An alternative salt to potassium chloride in the calcium form has to be found which would have similar properties to the ionic part of the molasses.

All these relationships have been included in the mathematical model of the SCCR system as described in

Chapter 13 of this thesis. Previous research workers (6, 7, 8) assumed that the K_d's did not change throughout the system and the models they used assumed constant values of K_d's. Thawait (9) set up a model including basic K_d relationships with background concentration variation, but Ganetsos (10) modified the model further to include relationships for glucose and fructose K_d's for variation of flowrates, temperatures and background concentrations.

This work further modified the mathematic model to also include the effect of betaine on the system.

CHAPTER SEVEN

THE ANALYTICAL SYSTEMS

7.1 Introduction

This chapter describes the analytical systems used for the sugar analysis for all the samples generated during the experimental work. The sugar composition and concentration in a sample were determined using high performance liquid chromatography (HPLC) systems. Other analysis carried out on molasses samples included refractive dry substance (RDS) determination, cation and anionic determination, colour determination, conductivity measurements and specific gravities of the samples. Some of this analysis required special equipment and was carried out at the British Sugar Research Labs at Norwich. All these techniques are outlined in this chapter.

7.2 HPLC Systems

The performance and suitability of the three main analytical carbohydrate columns used was as follows.

7.2.1 System Descriptions

Resin based HPLC columns use the mechanisms of ion-exchange, liquid exchange, size exclusion, reverse and normal phase partitioning (64). The applicability of these mechanisms depends on the compounds being analysed as well

as the degree of selectivity required. Reversed phase and ion-pairing HPLC techniques require complex eluent conditions for good separations because they work on the principle of modifying the test sample until it is compatible with the column being used. In the resin based HPLC columns, the column packing material is modified instead to be compatible with the chemical structure of the compound. Therefore resin based columns usually allow the use of an isocratic HPLC system, and thus simplify and speed up sample preparation and analysis.

Two isocratic HPLC systems were used for the analysis of the samples generated during the experiments. Most of the initial analysis was carried out on the first and rather simpler HPLC system consisting of:

- an MPL Series II eluent delivery pump having an operating range of 0-2 $cm^3.min^{-1}$, (MPL Pumps Ltd, Feltham, Middlesex);
- a Waters Associates differential refractometer model type R401, (Millipore UK Ltd, Harrow, Middlesex);
- a Hewlett-Packard 3390A integrator, (Hewlett-Packard Ltd, Altrincham, Cheshire);
- a Specac 6-port loading valve, (Specac Ltd, Orpington, Kent);
- the analytical column was placed inside a glass jacket and was kept at 85°C by recirculating

water using a TECAM C400 circulator, (Techne Ltd, Cambridge);

- the eluent was degassed by keeping the eluent containing glass aspirator in a water bath maintained at 85°C. The eluent contained 0.02gl^{-1} calcium acetate;
- a 30cm length by 0.65cm diameter SUGAR PAK1 (Waters Associates) analytical column. The eluent flowrate was 0.5cm.min^{-1} and the pressure drop was 6900 KN.m^{-2};
- a pressure gauge was fitted before the HPLC column to act as a pressure indicator and a pulsation dampener.

A second analytical system was set up later and this was used for all the runs using molasses feedstocks. This system consisted of:

- a BIO-RAD 1330 Pump (Bio-rad UK Ltd, Watford, Herts). This pump employs a two piston arrangement which operate out of phase by 180° thus minimising pulsations;
- a BIO-RAD column heater;
- a BIO-RAD differential refractometer type 1750;
- a TALBOT ASI-3 Autosampler (Talbot instruments Ltd, Alderley Edge, Cheshire);
- a SPECTRA-PHYSICS integrator type SP4270

(Spectra-physics UK Ltd, St.Albans, Herts);

- an isomantle and a Baird and Tatlock (BDH Chemicals Ltd, Atherstone, Warwickshire) temperature controller was used to degas the eluent;
- an Anachem (Luton, Beds) debubbler was used to trap any air bubbles in the eluent delivery tubing;
- in addition to the SUGAR PAK1 column, a 30cm length by 0.78cm diameter AMINEX HPX-87C (Biorad UK Ltd) and a 30cm length by 0.65cm diameter ALLTECH 700CH (Alltech UK Ltd, Carnforth, Lancashire) columns were used. The pressure drops were 6900 $KN.m^{-2}$ and 8300 $KN.m^{-2}$ for the AMINEX and ALLTECH columns respectively.

7.2.2 Experimental Procedure

The analysis sample was filtered using a 0.45µm GELMAN filter (Gelman Ltd, Brackmills, Northampton) and introduced either manually or via the autosampler into a 20µl injection loop in the injection valve. This sample was then injected and the integrator activated simultaneously. The results were interpreted as areas under plotted peaks on the integrator and these were compared to areas obtained for standard solutions to obtain exact sugar concentrations.

7.2.3 Column Maintenance

The HPLC columns used are microparticulate gel ion exchange beds designed for the chromatographic analysis of sugars. The resins act as a filter and although they are suitable for separating certain solutes, they are also capable of retaining other impurities - thus decreasing column efficiency.

The following procedures were carried out to increase column life:

- distilled deionised water, filtered using a 10μm slip-on filter, was used as the eluent;
- the eluent was heated to 80°C to prevent bacterial growth;
- 0.02g.l^{-1} calcium acetate was added to the eluent to replace any calcium ions lost from the resin due to the presence of other ions and to prevent any on-column sucrose inversion;
- the columns were flushed with 0.02% sodium azide solution when not used for long periods;
- the columns were reversed after every 500-1000 cm^3 of eluent had passed through the bed;
- when a reduction in performance was noted, the columns were cleaned with a 30% acetonitrile solution and then regenerated using 0.1M calcium acetate. The use of calcium chloride, calcium nitrate or any other calcium salt formed from a

strong acid was avoided as this lead to column corrosion and irreversible bed poisoning (65).

- a guard column was used as a pre-column to filter any insoluble matter present in the system. Hibar-lichocart 4-4 cartridges (BDH Chemicals Ltd, Atherstone, Warwickshire) were used and were replaced daily as they clogged easily.

7.2.4 Column Life Expectancy

The SUGAR-PAK1 column was used for over 2000 samples and then a large pressure increase was detected probably due to the pulsations from the single head MPL pump causing the collapse of the packed bed.

The AMINEX and ALLTECH columns were used on the second HPLC system only and over 1800 samples were analysed on the ALLTECH column which is still operating efficiently. The low pH (4.8-5.8) of the eluent caused the packing in the AMINEX column to swell and the problem was solved by adding extra calcium acetate to the eluent to increase the pH to 6.5. Approximately 2200 samples have been analysed on this column and is still being used regularly.

7.2.5 HPLC Column Comparisons

The three HPLC columns are similarly priced and have similar characteristics, i.e 30cm long and have about 10000 theoretical plates each. The experience gained from the

long term usage and the results obtained from the separation of a test solution were used to evaluate the columns.

The test solution used contained:

- a total solids concentration of 4% w/w made up of:

  1% w/w Sucrose

  1% w/w Glucose

  1% w/w Fructose

  1% w/w Betaine

The tests were carried out on the second HPLC system under identical conditions of:

Eluent flowrate - $0.5cm^3.min^{-1}$

Column temperature - 85°C

Sample injection volume - 20µl

Refractive Index monitor range - 16

Integrator attenuation - 128

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

The result of the analysis is shown in Table 7.1 and the chromatograms are shown in Figures 7.1 to 7.3. The retention times were similar for the SUGAR PAK1 and ALLTECH columns but were 30% longer with the AMINEX column. The AMINEX column gave the best glucose/fructose resolution and the peaks were flatter and broader. The base line stability was not obtained for the ALLTECH column and was drifting

significantly towards the end of the analysis. Overall the AMINEX gave a better resolution for the monosaccharide separations and is therefore recommended.

Table 7.1: Analysis of Test Solution

The table shows the Retention Times (in minutes) for each component in the three columns.

COMPONENT	AMINEX	SUGAR PAK1	ALLTECH
Sucrose	10.41	8.15	7.42
Glucose	12.63	9.90	9.27
Fructose	15.73	12.21	11.46
Betaine	36.92	26.99	25.32

Figure 7.1: HPLC profile for test sample using the AMINEX column

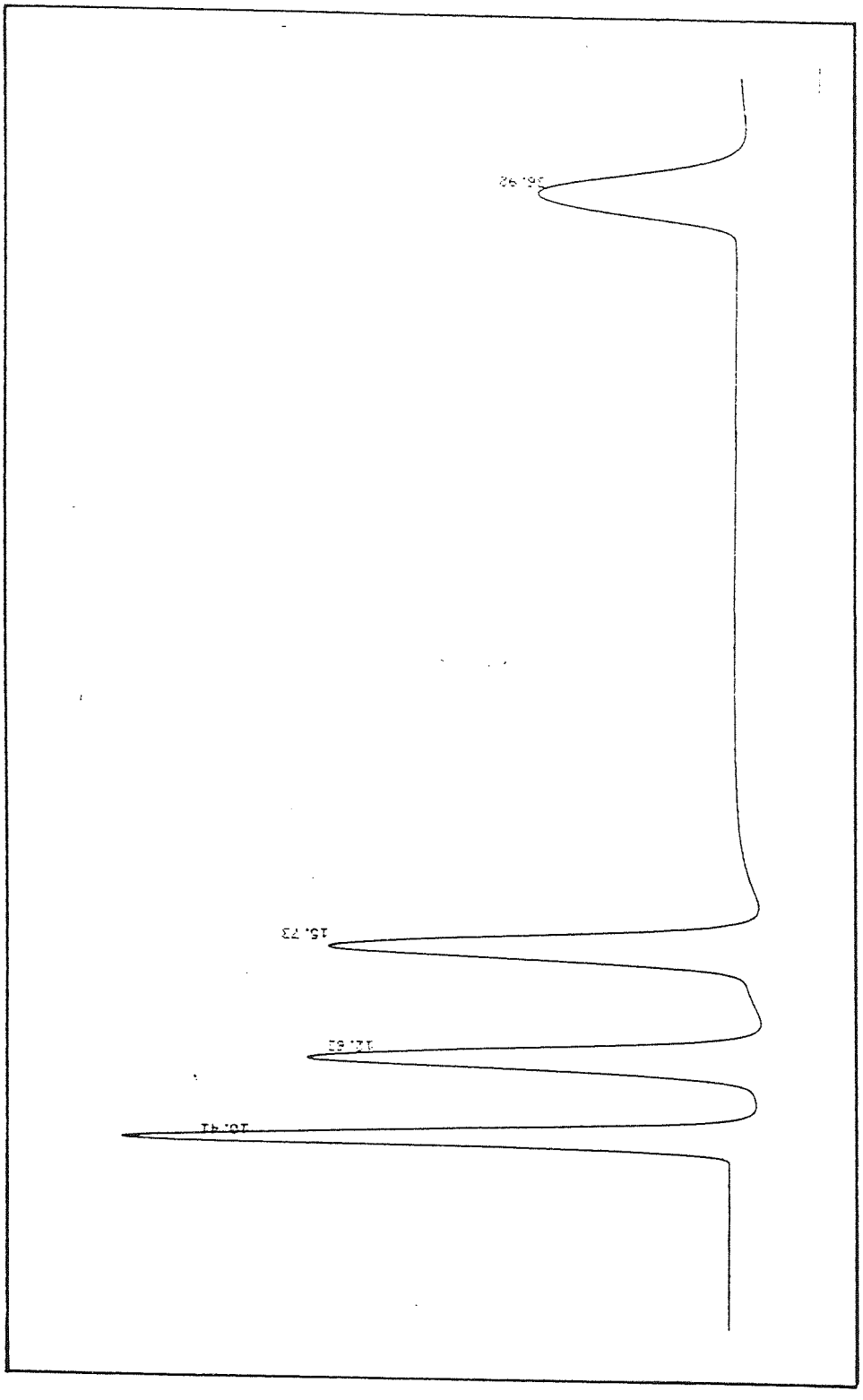


Figure 7.2: HPLC profile of the Test Sample using the SUGAR PAK1 column

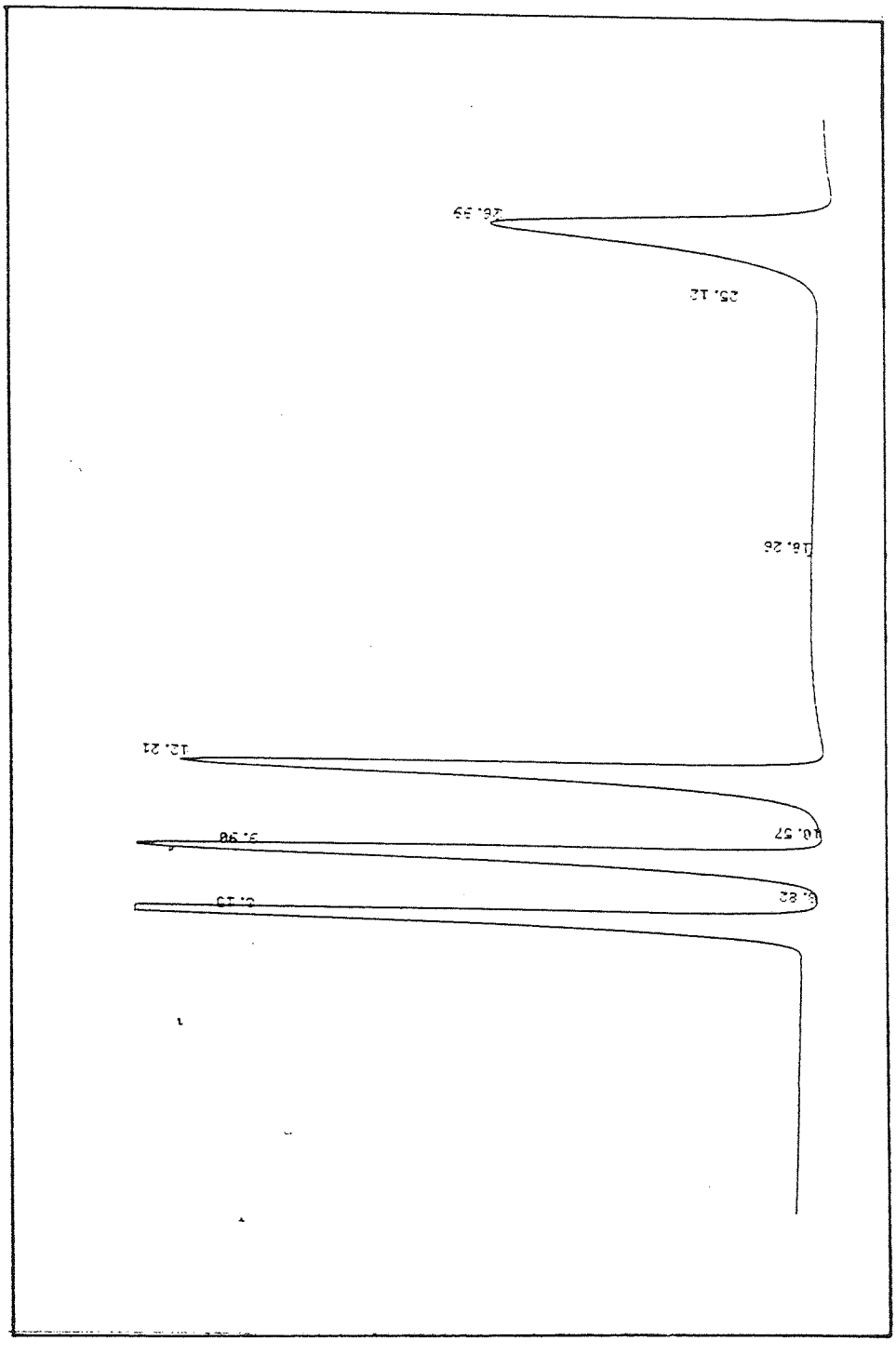
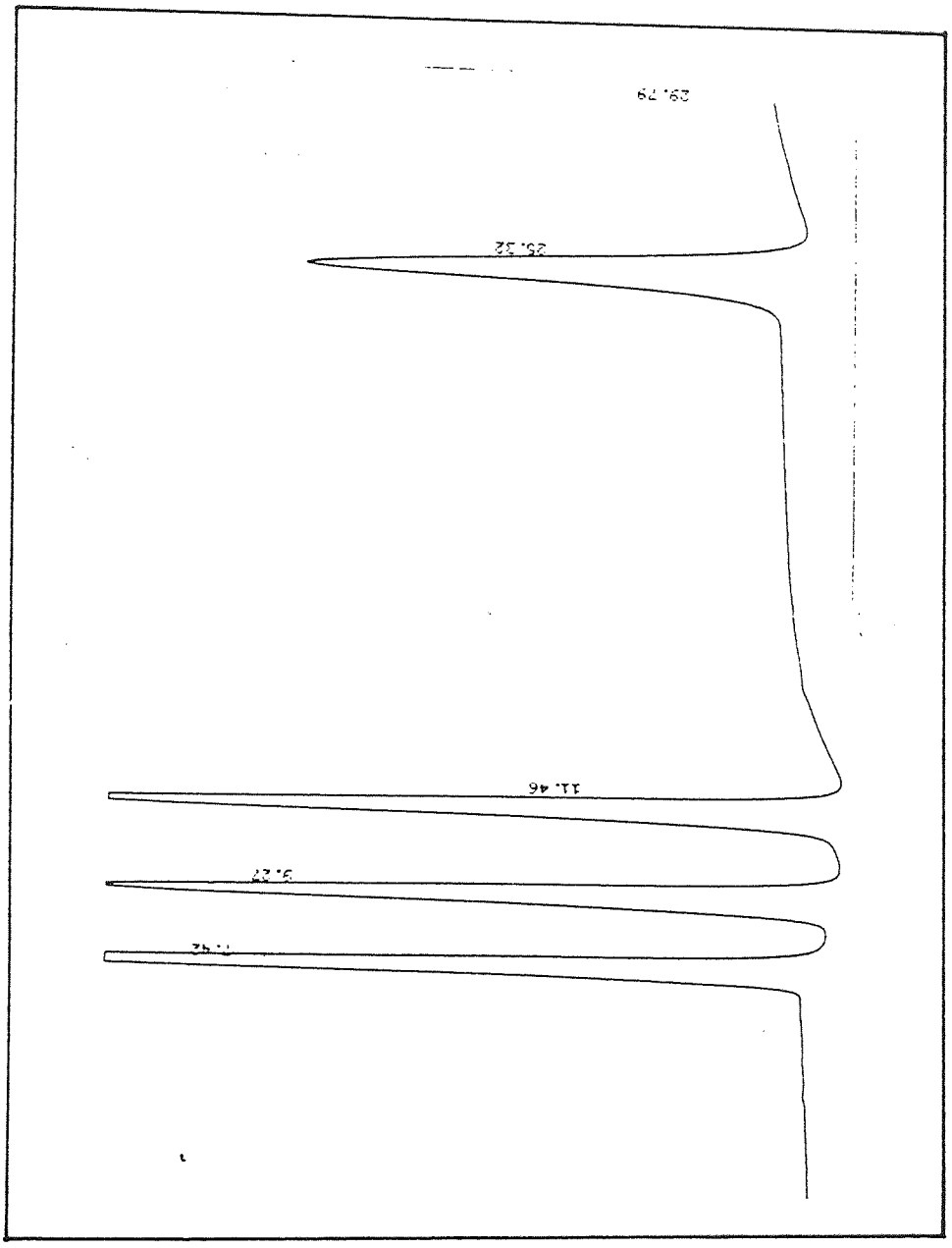


Figure 7.3: HPLC profile of the Test Sample using the ALLTECH column



7.3 Analysis Of Sugar Beet Molasses

Sugar beet molasses is a very complex mixture of inorganic and organic components. The composition of beet molasses has been described earlier in Chapter 4. This section describes the various techniques used to analyse the molasses products during the experimental program.

7.3.1 HPLC Analysis Of Beet Molasses

The HPLC analysis of beet molasses has indicated in the past (66), that other components besides the sugars can be detected as peaks on the chromatogram. The analysis of beet molasses was carried out on the second HPLC system. No pretreatment of the molasses was required. The molasses was diluted to a solid concentration of less than 5% w/w and filtered before injection.

Experiments carried out by British Sugar at Norwich suggests that almost all molasses components can contribute to the HPLC chromatogram. Table 7.2 shows the relative retention times of some of the important molasses components taking sucrose as having a relative retention time of one. Figures 7.4 and 7.5 show typical HPLC chromatograms for diluted beet molasses and INVERTASE enzyme inverted beet molasses. From the table, it is seen that substances with shorter retention times than sucrose include inorganic compounds (eg. potassium chloride and sodium sulphate), trisaccharides (mainly raffinose in beet

molasses) and organic acids. Substances with longer retention times than sucrose include monosaccharides (eg. glucose and fructose) and non-ionic low molecular weight compounds (eg. betaine).

The results were obtained using a SUGAR PAK1 column but should be similar for all calcium charged carbohydrate columns.

Table 7.2: HPLC times of molasses components

	Relative Retention time (sucrose = 1)	Concentration (typical) %w/w
Potassium chloride	0.65	0.7
Pyrollidone carboxylic acid	0.70	3.5
Disodium citrate	0.71	0.3
Citric acid	0.72	0.3
Sodium sulphate	0.74	1.0
Raffinose	0.80	1.0
Aspartic acid	0.82	<0.5
Glutamic acid	0.83	0.5
Lactic acid	0.97	1.1
Sucrose	1.00	62.0
Glucose	1.22	0.5
Fructose	1.52	0.5
Betaine	3.19	6.0
Glutamine	3.26	<0.5
Alanine	3.57	<0.5
Glycine	3.64	<0.5
Leucine	5.15	<0.5
Adenosine	6-8	<0.5

Figure 7.4:HPLC profile of beet molasses on the SUGAR PAK1 column

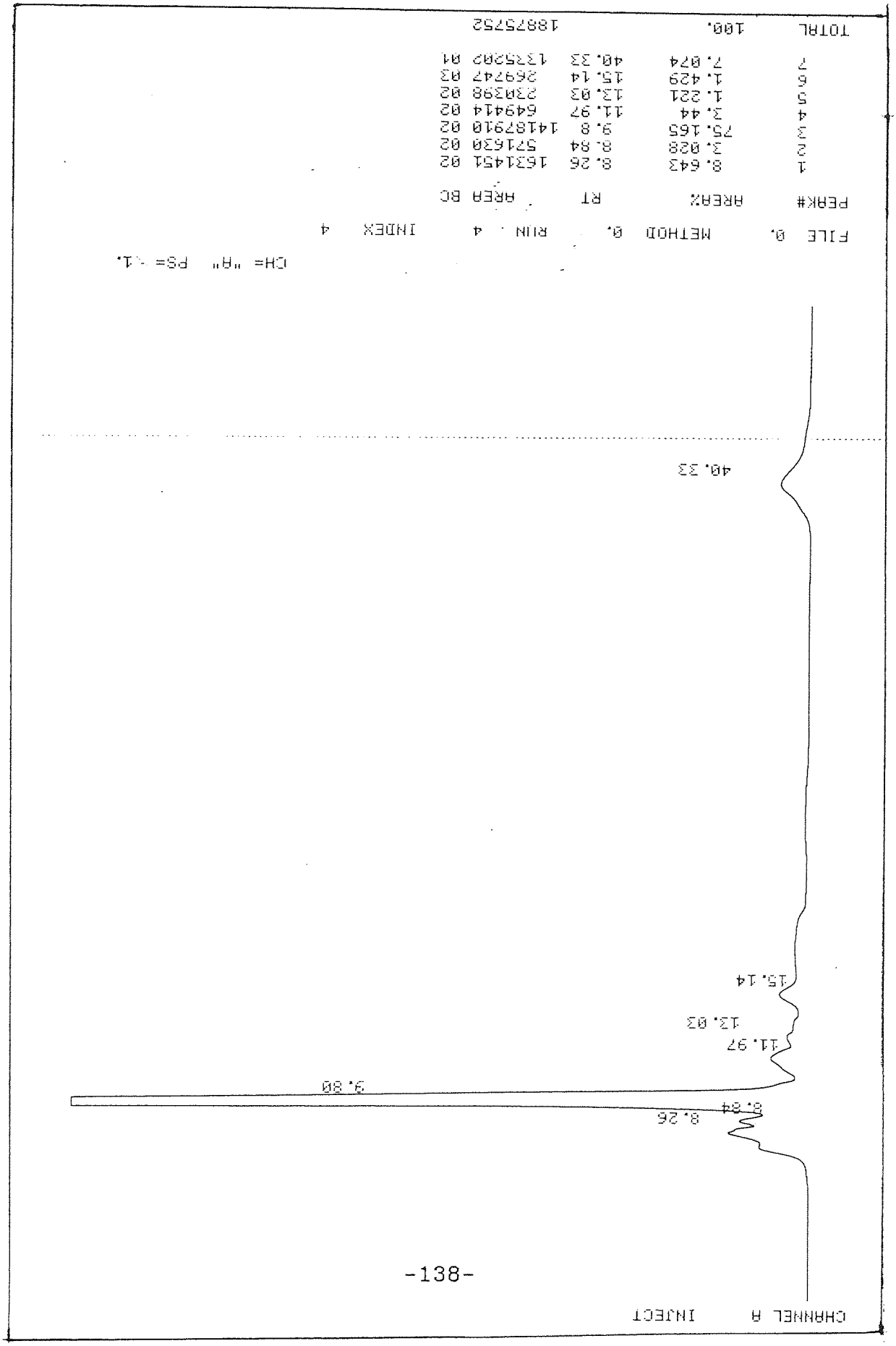
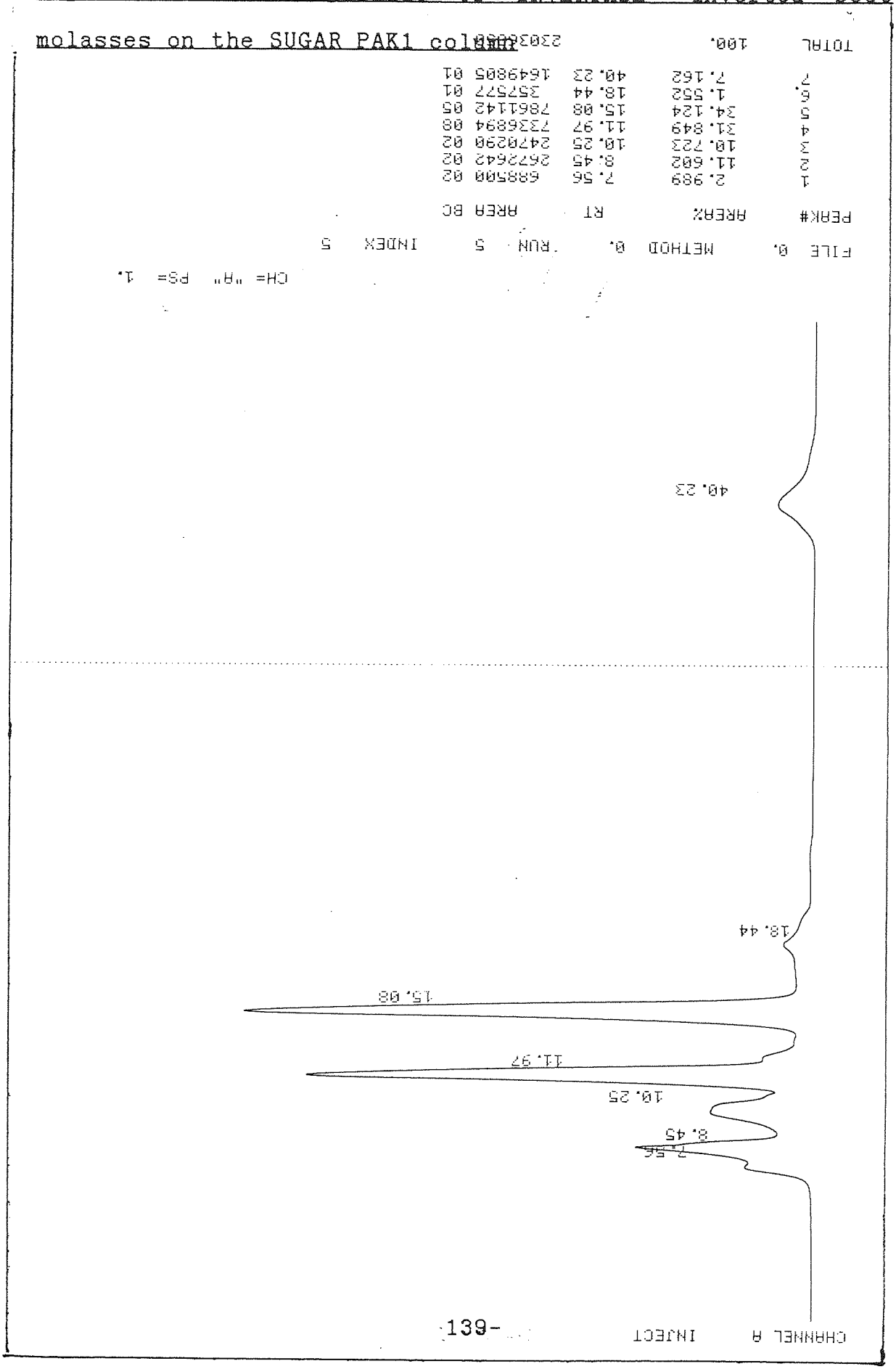


Figure 7.5: HPLC profile of INVERTASE inverted beet molasses on the SUGAR PAK1 column



7.3.2 Refractive Dry Substance (RDS)

The refractive dry substance (RDS) of a sample is the percentage total solids in the sample on a dry basis and the determination was carried out at Aston University using a refractometer (Bellingham and Stanley Ltd, London).

A shaded sodium lamp was used to eliminate any extraneous light. Water at 20°C was circulated through the prism jackets. Drops of the sample were placed on the lower face of the prism and the two prisms brought together slowly and clamped. The prism was then rotated until the edge of the shadow passed exactly through the cross-wires when viewed through the eyepiece. This gave a reading in degrees on the vernier scale. Tables were then used to obtain the refractive index value of the sample. Further tables gave a relationship between the refractive index and the RDS values. The prisms were then cleaned and dried before reuse.

7.3.3 Other analytical procedures used

Detailed analysis was carried out on only two runs as some analysis required use of expensive and delicate equipment at British Sugar at Norwich. The analysis included:

- Colour absorbance of the sample using a UV spectrophotometer set at 420nm in a 1cm cell.
- potassium and sodium ion determination using

flame photometry.

- Calcium ion determination using atomic absorption spectrometry.
- Anion determination using Dionex ion chromatography.

CHAPTER EIGHT

PREPARATION OF FEEDSTOCKS

8.1 Introduction

The experimental programme for this research as far as the type of feedstock used for the experiments using the SCCR6 unit was divided into three main sections:

(i) synthetic glucose/fructose mixtures

(ii) sucrose inverted to glucose and fructose using three different methods of inversion

(iii) sucrose in the beet molasses inverted to glucose and fructose using the optimum inversion technique derived from (ii) above.

These different types of feeds required different methods of preparation for the experimental work on the SCCR6 and are described in detail in this chapter.

The average volume of feed necessary for each run varied between 100 and 150 litres.

8.2 Synthetic Feedstock Preparation

The fructose was purchased from Roche Products Ltd (Dunstable, Beds) in 25Kg bags marketed under the trade name Fructofin C. This was pure food grade crystalline fructose (67). The glucose was purchased in 10Kg bags from Sigma Chemical Co Ltd (Poole, Dorset). This was the anhydrous crystalline food grade product.

The feed solution of the required concentrations was prepared by dissolving the calculated weight of the sugars in deionised water. For sugar concentrations greater than 30%w/v the water was heated to increase the solubility of the sugars in the water. The glucose had to be dissolved with continual stirring of the water to prevent lumps being formed. The fructose dissolved easily. After both the sugars were dissolved, a sample of the mixture was analysed using HPLC to determine the exact concentration of the sugars.

For synthetic feedstocks, the quantity required for the complete run was prepared in one batch. To prevent any microbial growth in the feed storage tank, tubings or in the columns, a solution of 0.02%w/v sodium azide, NaN_3, was used. This was particularly necessary for ambient temperature and low concentration runs.

The prepared feedstock was analysed daily to ensure that the feed composition and concentration did not change.

8.3 Inverted Sucrose Feedstock Preparation

Sucrose inversion is defined as the hydrolysis of sucrose, a disaccharide, to form invert sugar an equi-molar mixture of fructose and glucose, two monosaccharides as shown:

$$\underset{\text{(sucrose)}}{C_{12}H_{22}O_{11}} + H_2O \text{ ------> } \underset{\text{(fructose)}}{C_6H_{12}O_6} + \underset{\text{(glucose)}}{C_6H_{12}O_6}$$

The terms invert or inversion are derived from the changes that occur in the optical rotation of sucrose solutions (68),

$$\underset{(\alpha)^{20}_D=66.5^\circ}{\text{sucrose}} \longrightarrow \underset{(\alpha)^{20}_D=+52.2^\circ}{\text{glucose}} + \underset{(\alpha)^{20}_D=-93^\circ}{\text{fructose}}$$

$$\text{INVERT } (\alpha)^{20}_D = -20.4^\circ$$

ie. invert sugar rotates polarized light in a direction that is opposite to that obtained with sucrose.

From a thermodynamic point of view, sucrose is not stable in water or aqueous solution; the thermodynamic stable phase is really invert sugar. The fact that one is able to dissolve sucrose in water and recover it as sucrose crystals is due to the slow rate in which sucrose normally inverts in water. The inversion reaction, however, may be catalyzed by hydrogen ion addition or enzymatically by invertase (sucrase).

Both these types of catalytic reactions are classic examples of reactions that follow the monomolecular rate law:

$$k = \frac{2.303}{t} \log \frac{c}{c-x}$$

where: k = rate constant

t = time in minutes

c = initial mole % sucrose

x = mole % sucrose remaining at time, t

The inversion of sucrose for this research was carried out using three different methods of inversion:

- enzyme inversion using INVERTASE enzyme (supplied by Biocon UK Ltd, Eardiston, Worcestershire);
- acid inversion using HYDROCHLORIC ACID and then neutralising the invert solution with CALCIUM HYDROXIDE;
- resin inversion using AMBERLITE IR118 which is a polystyrene ion-exchange resin charged in the hydrogen form (supplied by Rohm and Haas Ltd, Harrow, Middlesex).

The method of preparation of the feedstock using these three methods is outlined below.

8.3.1 Preparation of enzyme inverted feedstock

The enzyme used for the inversion of sucrose to fructose and glucose was INVERTASE, which is a commercially available clear concentrated solution produced by the extraction and purification of the enzyme from a selected strain of yeast under carefully controlled conditions. The enzyme breaks the C-O-C (glycosidic) bond in the sucrose molecule with the C-O- group remaining with the glucose half of the molecule and the C- group with the fructose half of the molecule. The H-O- part of the water then links with the fructose part to give a fructose molecule and the -H part with the glucose part to give a glucose molecule as

shown in Figure 8.1.

Figures 8.2 and 8.3 show the temperature and pH curves for the enzyme and from these it can be seen that the optimum temperature for the inversion is 55-60°C and the optimum pH is between 4 and 5, although substantial activity is exhibited between 3.5 and 5.5. The incubation temperature affects the rate of hydrolysis of sucrose considerably and an increase of 6% per degree is normal between 25 and 35°C. The enzyme is destroyed when heated to above 65°C. The activity of invertase falls rapidly in alkaline media.

The manufacturers of the enzyme suggested that the optimum conditions for the enzyme to operate were to prepare a 70% sucrose solution at pH 4.5, boil to sterilize, cool to 50°C and add invertase with stirring, holding at 50°C until the desired degree of inversion has occurred and heat to 80°C to stop the reaction (69). As an indication of the rate of activity, the manufacturers suggested that the addition of 0.16% invertase would, over 12 hours, produce at a temperature of 50°C and pH 4.5, an 80% invert sugar solution. The enzyme was supplied at an activity of 11.6 x 10^3 units per cm^3.

Figure 8.1: The Inversion of Sucrose Using Invertase Enzyme

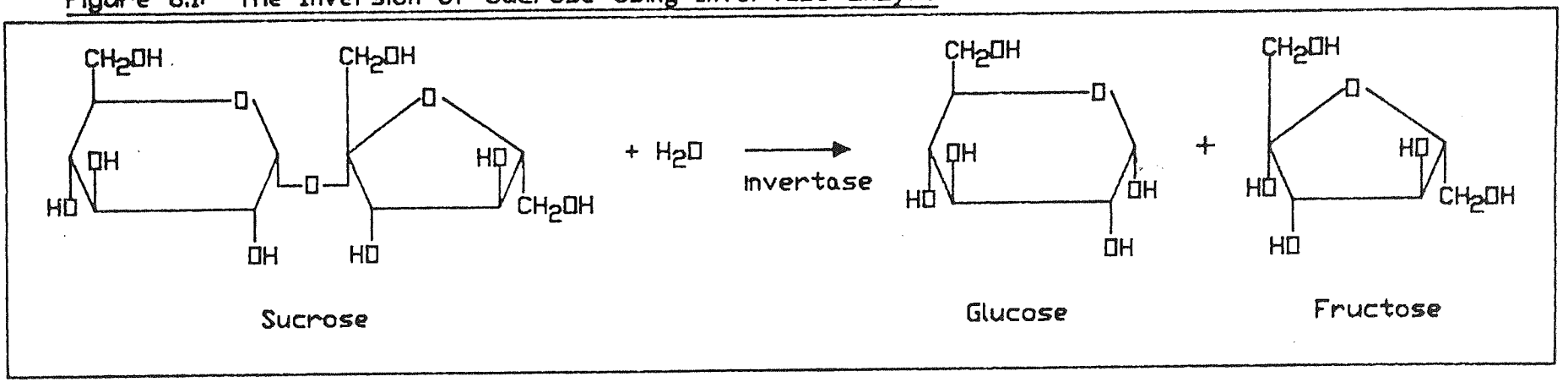
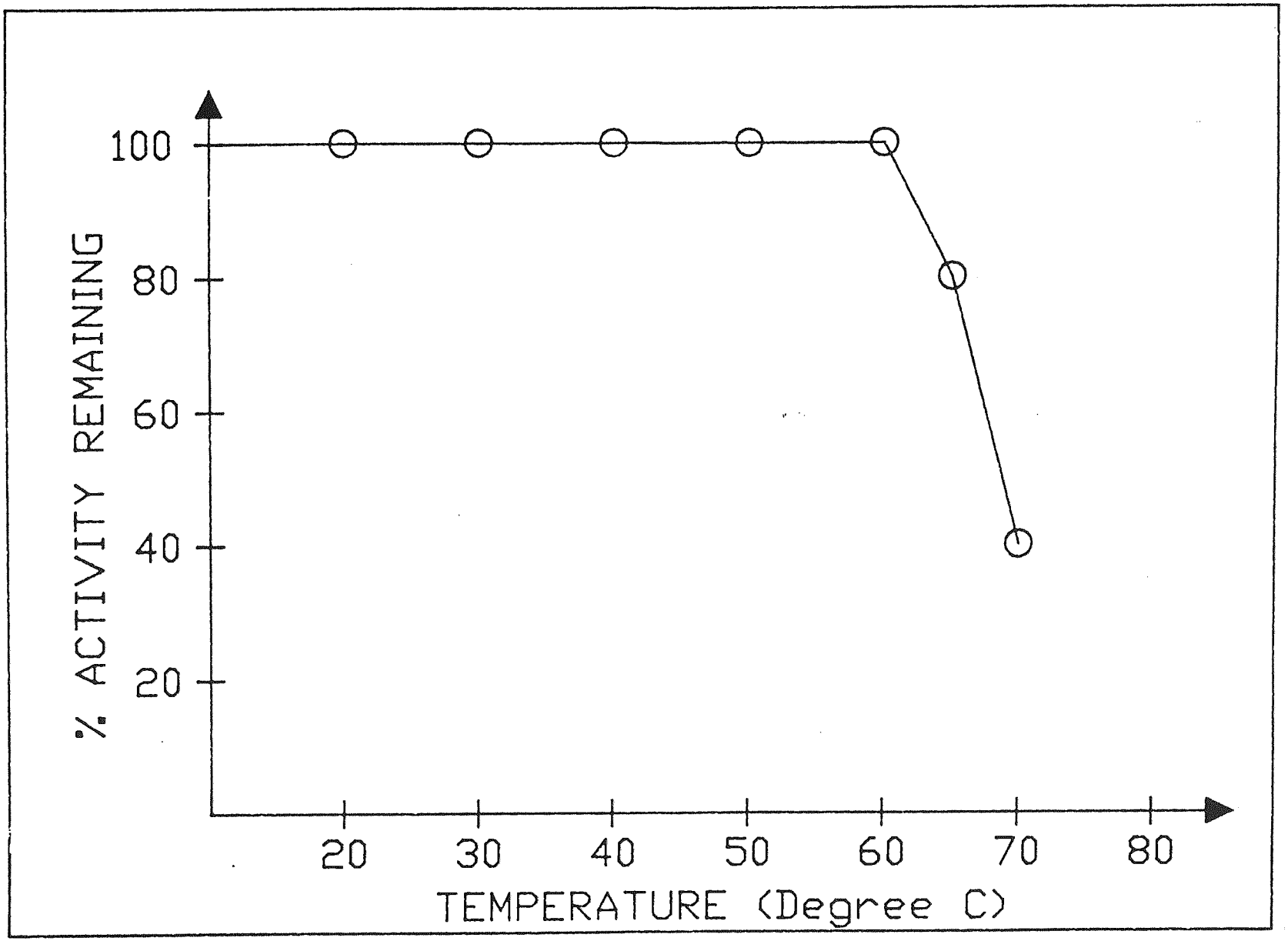


Figure 8.2: Effect of Temperature on Enzyme Activity



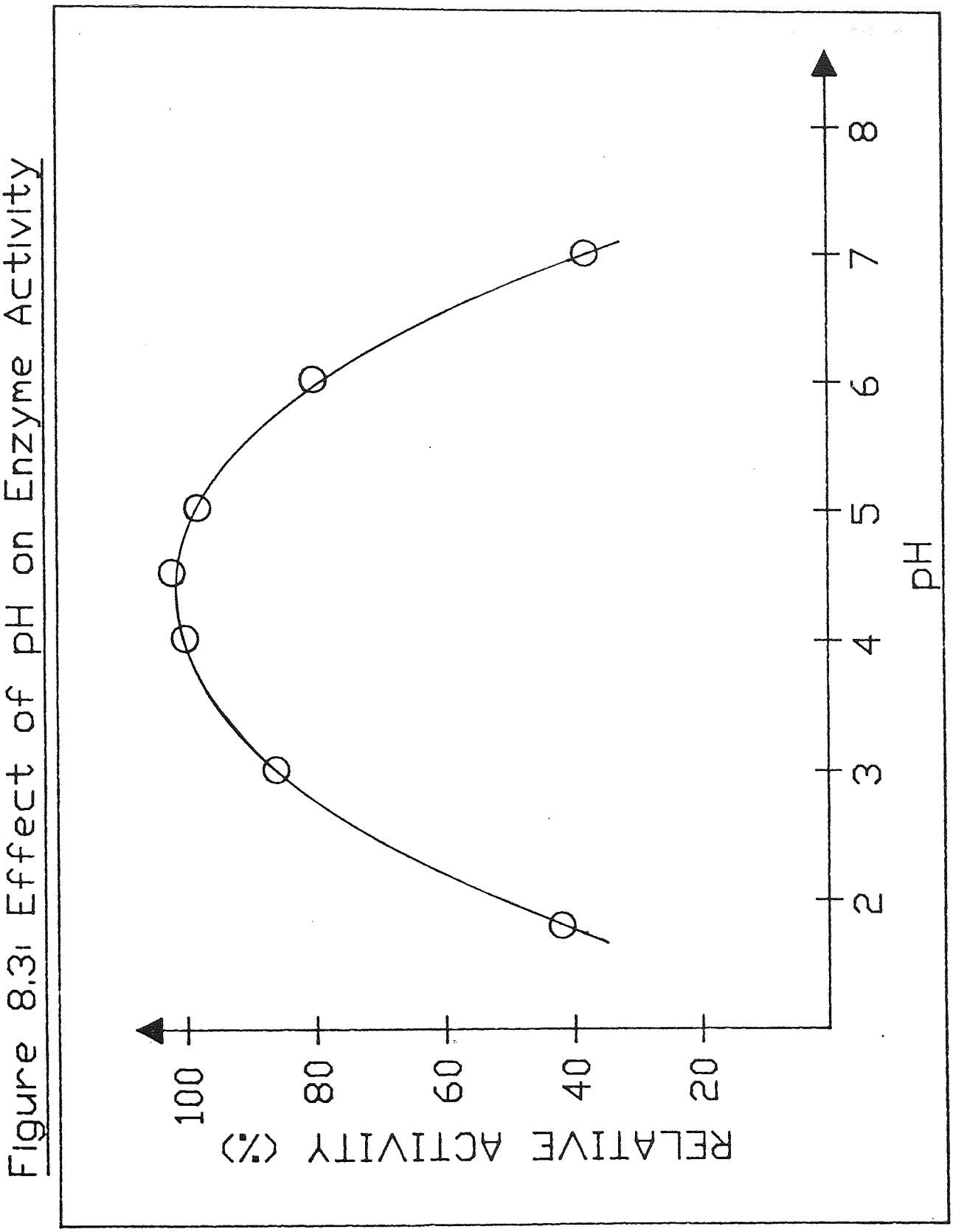


Figure 8.3: Effect of pH on Enzyme Activity

The enzyme is extremely stable and there is no loss of activity when stored in a sealed container at room temperature for 12 months. The specific gravity of the enzyme is 1.16.

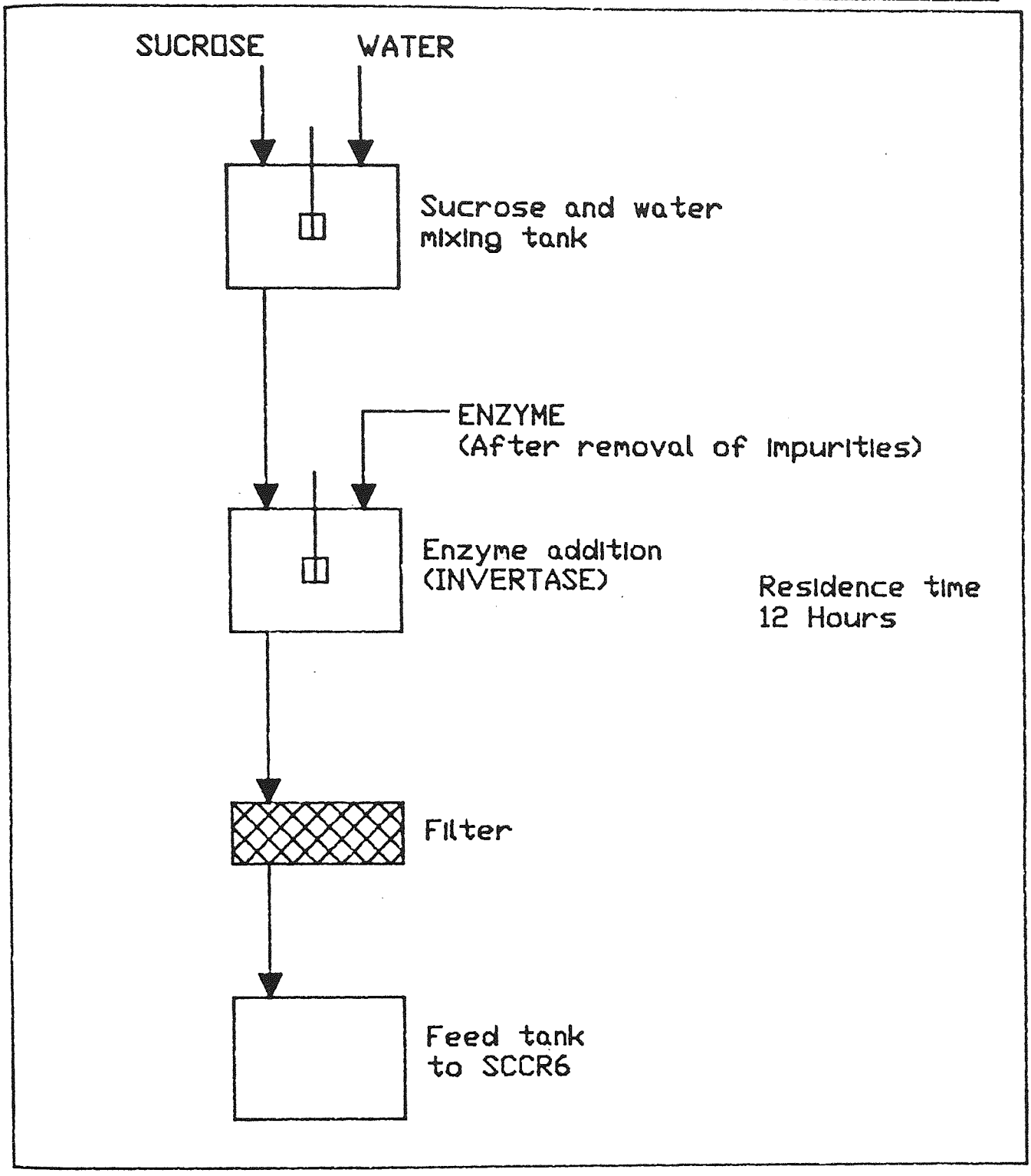
The inverted sucrose feedstock was prepared using the following method:

- The sucrose was dissolved in 120 litres of deionised water in a large polypropylene vessel to make up a 20%w/v sucrose solution.
- The solution was heated to 50°C using a hot water jacket to sterilise the solution.
- 0.2%w/v invertase was added to the heated solution and the mixture stirred continuously. The extra 20% was added to ensure complete inversion as the temperature could not be maintained constant at 50°C. The temperature varied between 20°C and 30°C.
- The mixture was left overnight for complete inversion.
- The pH of the mixture was 6.
- The analysis of the mixture after approximately 18 hours showed complete inversion with 49.43%w/v glucose and 50.50%w/v fructose.

The flow diagram for the process route for the inversion of sucrose using invertase enzyme is shown in Figure 8.4.

This feedstock was now ready for use in the SCCR6 equipment and the run carried out using this feedstock is described in chapter 10.

Figure 8.4: Inversion of Sucrose Using Enzyme



8.3.2 Preparation of Acid Inverted Feedstock

Sucrose is easily hydrolysed by acids to form glucose and fructose and the rate of this reaction depends on the concentration of acid used and the temperature.

Typical inversions of sucrose are carried out at a constant temperature of 35°C and at an acidity of pH 1.0. The reaction is complete in about 16 hours.

The inversions can also be carried out at higher temperatures and thus in a shorter time, but there is a second reaction which occurs at these conditions which results in the production of coloured compounds from the invert sugars. This reaction is also dependent on the temperature and whilst it is feasible to carry out rapid inversions at higher temperatures, the problems involved in cooling and neutralising the reactants in a relative short time so that no undue colour is formed lead to the recommendations to carry out inversion at not more than 35°C (70).

Both sulphuric acid and hydrochloric acid could be used for the inversion. Sulphuric acid has the advantage that it can be almost completely removed from the final product by precipitation as calcium sulphate by either calcium hydroxide or calcium carbonate. The major disadvantage of this technique is that calcium sulphate is partially soluble (approximately $2gm.l^{-1}$) and tends to be retained in solution, above its saturation concentration,

and precipitates slowly over a long period. Because of this it would be possible for the resin columns to become fouled by precipitated calcium sulphate during subsequent processing of the invert.

The use of hydrochloric acid avoids this problem as the neutralisation by means of either sodium hydroxide or calcium hydroxide results in the production of very soluble sodium or calcium chloride salts.

Hydrochloric acid was used for the inversion of sucrose for this research and the invert solution was neutralised using calcium hydroxide. The calcium cations present in the invert solution did not affect the ion exchange system of the SCCR6 as the resin was in the calcium form anyway.

The inverted sucrose feedstock was prepared using the following method:

- A 67%w/v sucrose solution was made and heated to 35°C while stirring.
- Concentrated hydrochloric acid (36%w/w) was added in the proportion of 1 part by weight of acid per 100 parts of sucrose solution ie. 1.5%w/w acid on sucrose dry solids.
- The mixture was left for 115 hours and frequently analysed. (A small sample of the mixture was neutralised before analysis). The inversion seemed to have peaked at approximately 96%;

leaving the inversion mixture longer did not seem to complete the inversion of all the sucrose.

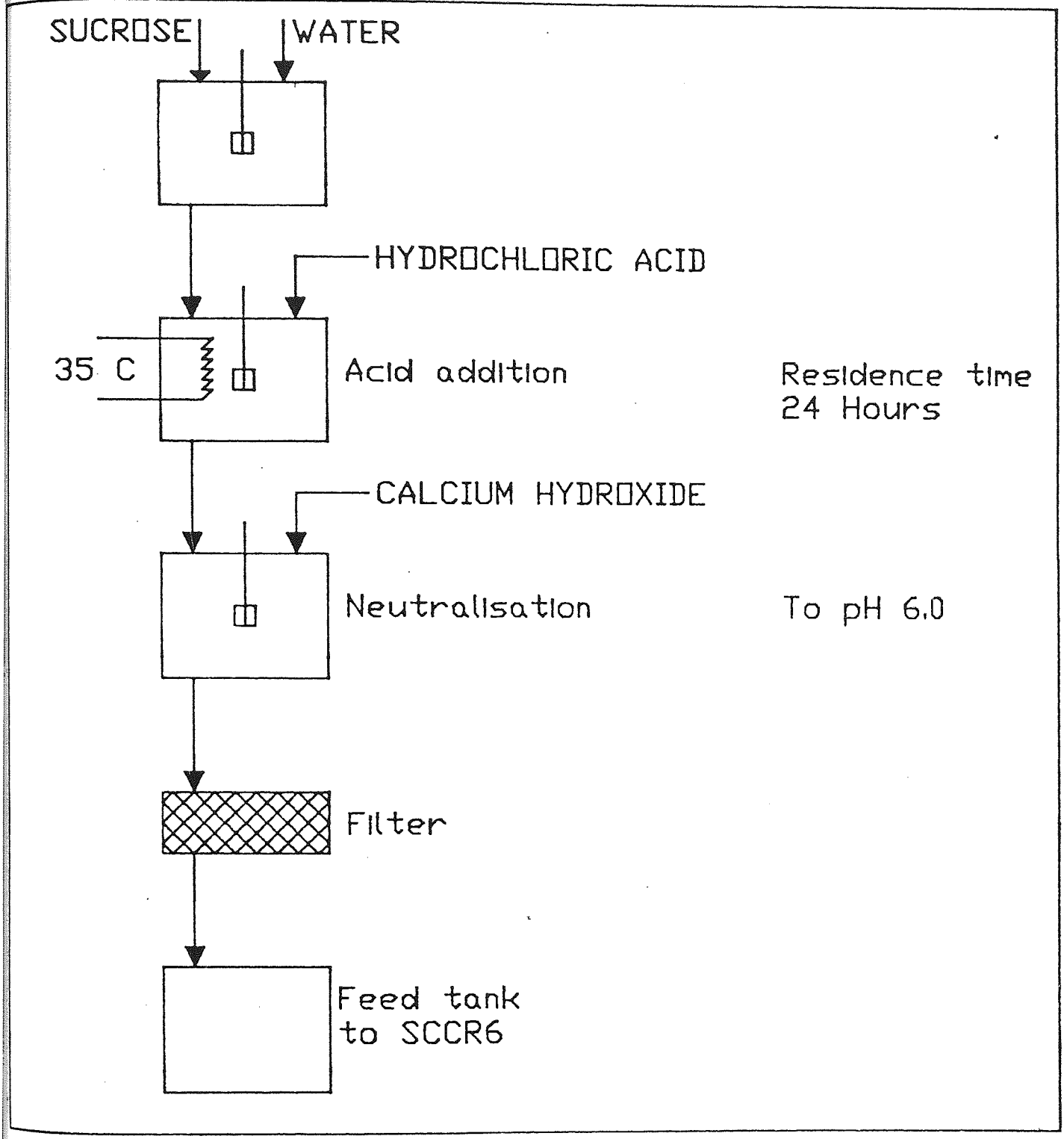
- Calcium hydroxide was added to the mixture until the pH was 5.8 and diluted to the total solids concentration of 20%w/v. The analysis of the feed showed the following composition:

- an ionic mixture peak	3 - 5.5%
- sucrose	< 1.2%
- glucose	approx 49%
- fructose	approx 47%

The flow diagram for the process route for the inversion of sucrose using hydrochloric acid is shown in Figure 8.5.

This feedstock was now ready for use in the SCCR6 equipment and the runs carried out using this feedstock are described in chapter 10.

Figure 8.5: Inversion of Sucrose Using Acid



8.3.3 Preparation of H^+ Resin Inverted Feedstock

The use of ion exchange for the inversion of sucrose is a classical example of ion exchange catalysis since it clearly illustrates the basic advantage of the technique which is the ability to catalyse a reaction without introducing impurities into the products of the reaction and thereby minimise undesirable side reactions.

For this research, the resin used was AMBERLITE IR118 which is a crosslinked polystyrene resin in the hydrogen form and was supplied by Rohm and Haas, Harrow, Middlesex. The inverted sucrose feedstock was prepared by passing the sugar syrup through a column packed with the resin without adding any impurities to the syrup. The degree of inversion can be varied under controlled conditions by means of flowrate and temperature. As the ion exchange resin becomes exhausted during the exchange of cations for hydrogen ions, the degree of inversion decreases in proportion to the degree of resin exhaustion (68).

The inverted sucrose feedstock was prepared using the following method:

- A 50%w/v sucrose solution was prepared and pumped through a QVF column packed with the resin. The column was 5.6cm id and 60cm long, and the resin voidage was 0.3. The sugar solution was pumped from the bottom of the column at a flowrate of $167cm^3.min^{-1}$ and the system temperature was

maintained at 60°C.

- After 8 days of continuous recycling of the mixture, the sucrose had been reduced to approximately 5% concentration.
- A similar column was added in series with the first column in an attempt to obtain complete inversion. The volume of resin used was 3.5 litres. The pH of the final solution was 5.9.

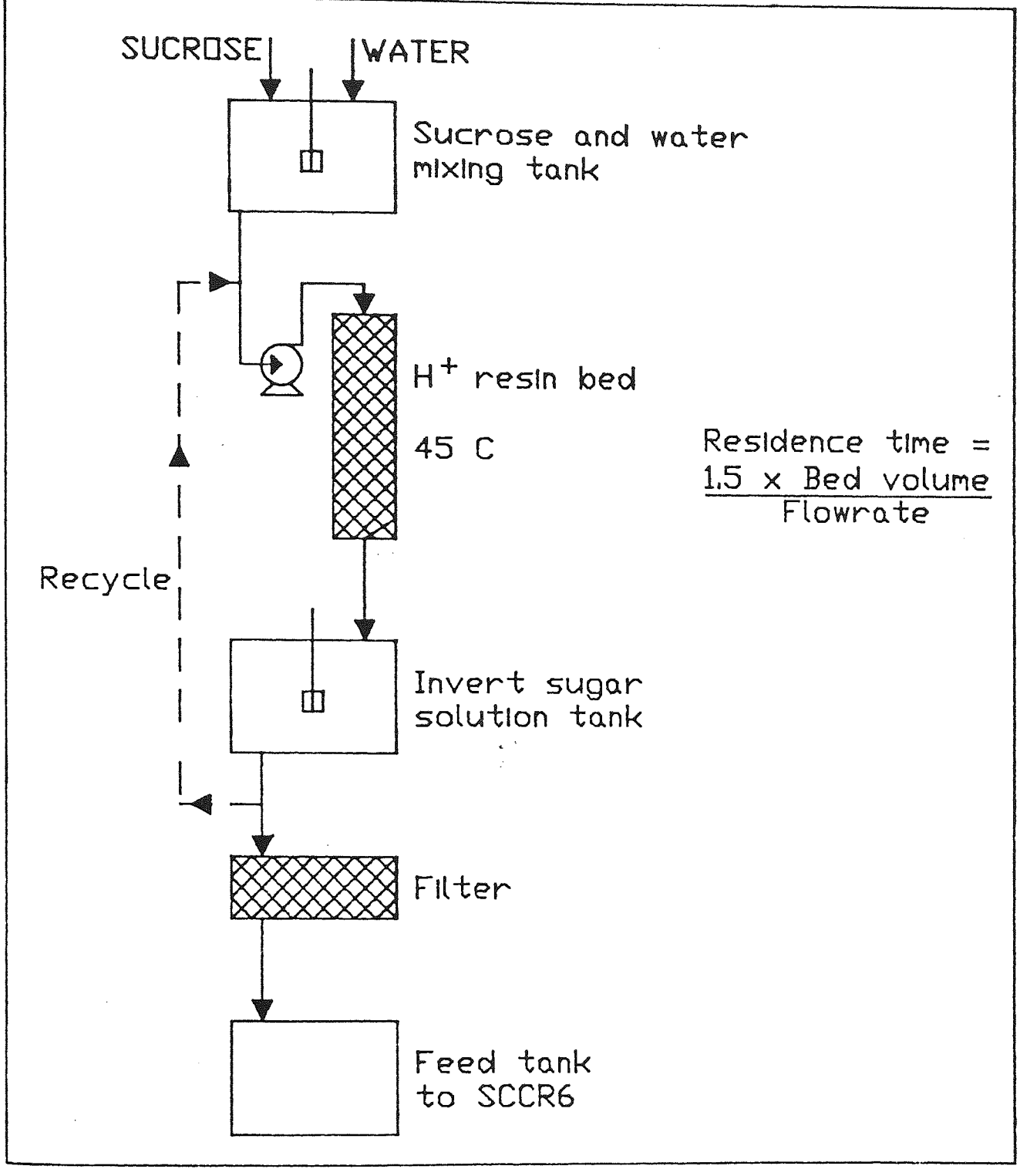
The residence time recommended for the inversion by the resin manufacturer's was given by:

$$\text{Residence time} = \frac{1.5 \times \text{Bed volume}}{\text{Flowrate}}$$

- After further 48 hours of pumping the mixture through both columns, there was no decrease in the sucrose concentration and it was decided to carry out a run on the SCCR6 using this feedstock.

The run carried out using this feedstock is described in chapter 10. The flow diagram for the process route for the inversion of sucrose using the H^+ ion resin is shown in Figure 8.6.

Figure 8.6: Inversion of Sucrose Using H^+ Resin



8.4 Selection of the Best Inversion Technique

Figures 8.4, 8.5 and 8.6 show the flow diagrams for the three possible inversion routes being considered. The inversion of sucrose using the enzyme requires the least amount of ancillary equipment and therefore the capital cost of a plant using enzyme for the inversion would be less than that using either acid or resin for the inversion.

From the manufacturer's literature for the resin, it can be seen that the resin is very stable as long as there are no other cations in the sugar solutions being passed through the resin. The inversion route chosen was eventually to be used for the inversion of sucrose in beet molasses and therefore the resin could not be used successfully without periodic regeneration due to the presence of various cations and anions in the molasses. Also, the inversion carried out did not reach completion and 5.5% of the sucrose was uninverted. Thus complete inversion of the sucrose in beet molasses was not suitable using resin in the H^+ form.

The inversion of sucrose using hydrochloric acid required more ancillary equipment than if the enzyme was used. The inversion required careful temperature control and an additional step of neutralising the acid was involved using calcium hydroxide. The material costs for the acid and the calcium hydroxide was greater than that

for any other route and thus it was decided that this method of inversion was not economically favoured.

The inversion of the sucrose using enzyme required the least amount of ancillary equipment and the inversion was easier to carry out. The cost of the enzyme used was less than the cost of the acid or the resin regenerant as the quantity of enzyme used was less than 2% of the mixture compared to 5% of the mixture for the acid and the calcium hydroxide and over 10% calcium salt for the resin regenerant.

Thus, based on the ease of the inversion, the completion of the inversion and the limited ancillary equipment required, it was decided to follow the enzymatic inversion of sucrose in molasses route in this research programme.

8.5 Inversion of Sucrose in Sugar Beet Molasses

The inversion of sucrose in beet molasses was carried out at British Sugar research laboratories at Norwich as they had the equipment to invert the molasses in large batches.

The concentration of raw molasses is approximately 78%w/w total solids (or 78 Brix (Bx) or 78 RDS). The inversion of the sucrose in this molasses could not be carried out because of the high concentration of the total solids. Thus the molasses was diluted using deionised water

to approximately 51 brix before the inversion. Due to the nature of molasses, 1% by volume of enzyme was added per unit weight of molasses ie. 10cm^3 of enzyme per kilo of molasses.

The inverting molasses was left stirring for 17 hours and analysed to ensure complete inversion.

As the molasses was not used straight away but split into smaller containers for shipment to Aston University for use later, it was necessary to concentrate the molasses up to 65 brix to prevent any microbial growth in the molasses. This was carried out using low temperature vacuum distillation.

The molasses feedstock was diluted to the required concentration as required for every cycle to prevent microbial growth in the feed and the equipment.

CHAPTER NINE

EXPERIMENTAL OPERATION OF THE SCCR6 SYSTEM

9.1 Introduction

In order to ensure that the system operated safely, some operating procedures had to be carried out before, during and after a run. This chapter outlines these procedures necessary for the safe operation of the SCCR6 equipment.

9.2 Preliminary Checks and Start-up Procedures

For safe operation of the system, the following procedures were carried out before a run commenced.

The feed had to be prepared as described in chapter 8 and placed in the feed tank connected to the equipment. This tank held enough feed for one cycle and had to be refilled at the end of every cycle.

The desired temperature settings were selected and the column oven heater, feed heater and the deionised water heaters were switched on. The air circulating fan in the oven was also switched on to distribute the heat evenly in the oven.

The digital timer controlling the switch time was set to the required value for the run. This timer triggered the cam-shaft motor to activate/deactivate the 10 pneumatic valve controllers.

All the inlet and outlet valves were opened and the product collecting containers were positioned. For the latter part of the research when the automatic product splitting system was used, the appropriate timers were set to the proper values.

The eluent, feed and purge pumps were set to the required flowrates and were switched on. Again, for the latter part of the research when the calcium nitrate regenerant was used, this pump was switched on at the same time.

When the correct operating pressure was reached the automatic shut-off mechanism was activated and all other controllers were checked.

Finally, the experimental run was started by resetting all the timers simultaneously.

9.3 Procedures During an Experimental Run

During the run, the eluent, feed and purge flowrates were checked frequently to ensure constant operating conditions. The feed and eluent flowrates were measured at the inlet using the calibrated measuring device and the purge flowrate was measured by weighing the outlet stream over a switch period. The pressure drop in the three streams and the temperature inside the enclosure were recorded during each cycle.

Two minutes before the end 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 ten switches of a cycle, each column served every function ie. as a feed, eluent entry, purge column or any other column of the separating length.

At the end of each cycle the product collecting vessels were weighed and analysed and a mass balance carried out. When a pseudo-equilibrium was reached, usually after 5 or 6 cycles, the results of the last two cycles were used to indicate the systems performance. The run was usually continued to cycle 9 to monitor the operation of the system under pseudo-equilibrium conditions.

At the end of the last cycle, all the pumps were switched off together with the feed heater. The control system was switched to manual and a new purging period was set on the digital timer. This was usually 15 minutes. The flowrate of the purge pump was increased to 400 $cm^3.min^{-1}$. The purge pump was switched on and the product collected in a separate container, thoroughly mixed and analysed. The procedure was repeated until all columns were purged out. The plotting of the analysed data gave the purging concentration profiles for glucose and fructose and represent the average carbohydrate concentrations in each column for the particular operating conditions. It should be noted that the on-column concentration profiles are

point values whereas the purge concentration profiles are based on average column concentrations. The operating conditions and results of each experimental run were defined by a set of five figures. For example, the run 20-35-105-30-60 corresponds to:

- 20 = feed concentration (%w/v solids)
- 35 = feed flowrate ($cm^3.min^{-1}$)
- 105 = eluent flowrate ($cm^3.min^{-1}$)
- 30 = switch time (min)
- 60 = temperature (°C)

As the SCCR6 operates in a semi-continuous mode, only a 'pseudo-equilibrium' state can be achieved and not a 'true' equilibrium state like in any other continuous counter-current mass transfer processes like distillation. When the on-column concentration profiles of two consecutive cycles were almost identical and within experimental accuracy, the 'pseudo-equilibrium' state was said to have been reached. The reference points chosen to determine if a pseudo-equilibrium state had been reached were the relative concentrations in the same column one cycle apart and the position of the 'cross-over' point, ie. the point of intersection of the glucose and fructose profiles. Figures 10.2 and 10.3 in Chapter 10 show two consecutive on-column profiles for the same run and these show the 'pseudo-equilibrium' state having been reached. Figure 9.1 shows a typical on-column concentration profile

for glucose and fructose in the SCCR6 unit.

9.4 Shut-down Procedure

The pumps were switched off and the outlet lines blocked to prevent any liquid leaving the system and the packing drying out.

The heaters for the deionised water, feed and the enclosure were switched off. The rotary fan was also turned off.

The digital timers and the valves on the water inlets to the system were switched off.

The compressor air supply was closed and the electrical power to the system was turned off.

After every run it was necessary to pump 0.02%w/v sodium azide through the system to prevent any microbial growth.

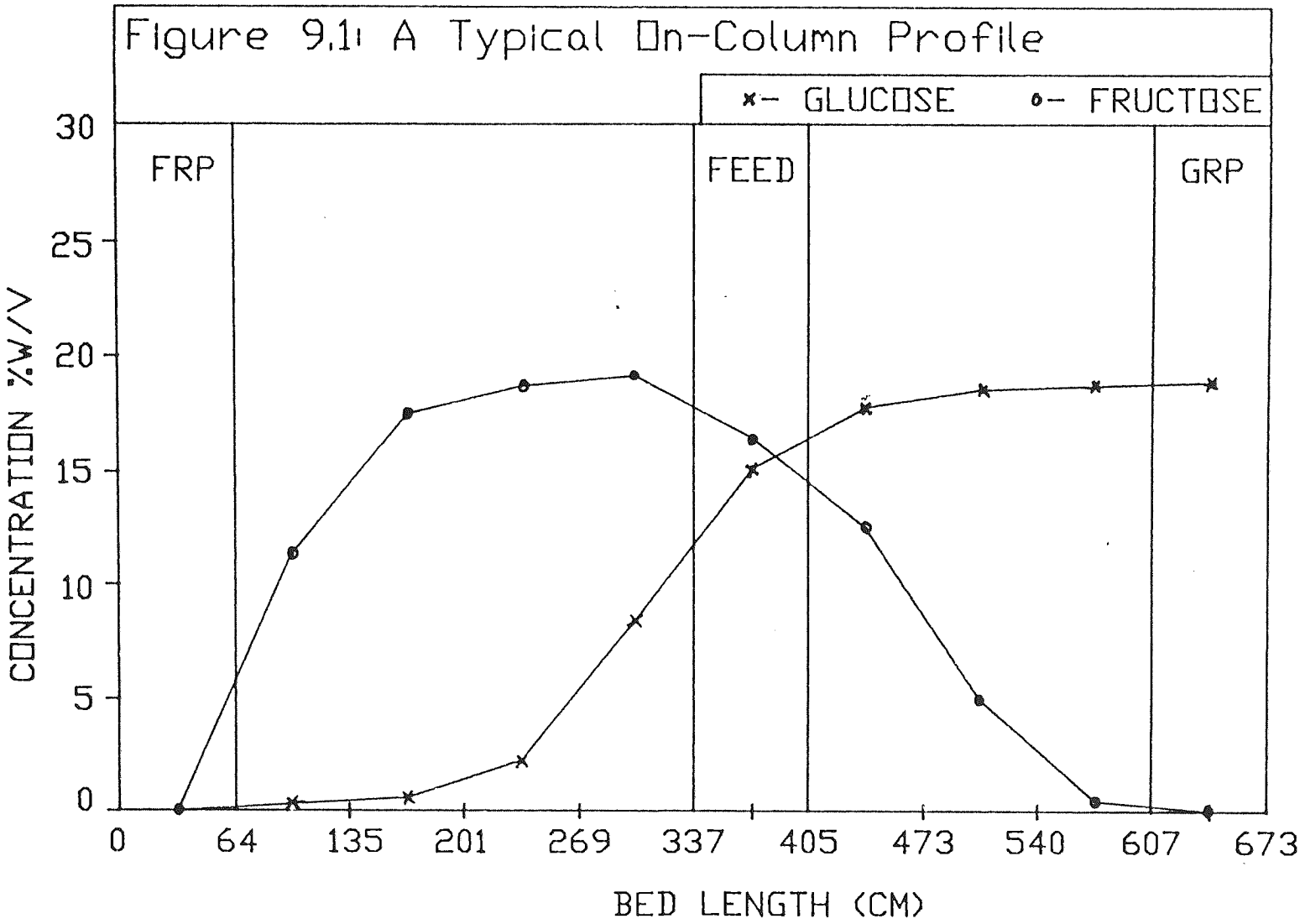


Figure 9.1: A Typical On-Column Profile
x- GLUCOSE
o- FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
30
25
20
15
10
5
0
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

CHAPTER TEN

THE CONTINUOUS SEPARATION OF INVERTED SUCROSE USING THE SCCR6 SYSTEM

10.1 Introduction

The majority of the experimental runs carried out by previous research students on the SCCR6 systems were based on synthetic feedstocks having a 50:50 ratio of glucose and fructose. In this experimental program, only the base runs were carried out using synthetic glucose:fructose mixtures to ensure that the equipment functioned well and that the runs were reproducible with those carried out by previous workers.

As explained in chapter 8, the initial part of the programme was to carry out runs on the SCCR6 unit using inverted sucrose feedstocks prepared as shown in that chapter. This chapter outlines the initial runs carried out on the SCCR6 system using synthetic feedstocks as well as those carried out using inverted sucrose feedstocks prepared as described earlier. A comparison of all these runs is carried out in section 10.7.

The numbering of the runs as defined in chapter 9 has been slightly modified to take account of the different feedstocks being used. The synthetic runs were labelled SYN** where the ** represented the run number using synthetic feedstock. The enzyme inverted runs were labelled

ENZ**; the resin inverted runs were labelled RESIN** and the acid inverted runs were labelled ACID**. Within a set of runs using the same feedstock, any variations in the operating parameters were indicated using the numbering system described in chapter 9.

10.2 Initial Commissioning Synthetic Feedstock Runs on the SCCR6

The initial runs on the SCCR6 were carried out under the following conditions to compare the results obtained with those obtained by Thawait (9):

- Feed concentration 40%w/v total sugar solids consisting of a 50:50 ratio of glucose and fructose.
- Feed flowrate 35 $cm^3.min^{-1}$
- Eluent flowrate 105 $cm^3.min^{-1}$
- Purge flowrate 400 $cm^3.min^{-1}$
- Run temperature 60°C

The first run on the SCCR6 was carried out over 9 cycles with a switch time of 30 minutes. The SCCR6 equipment had been standing for over 7 months without being used and it was found that both the product purities for the first run (Run SYN1) were only 60% compared to 99.9% obtained by Thawait (9). The purge pump was leaking and not operating satisfactorily. It was assumed at this stage that the resin was contaminated or the resin required

regeneration and therefore the sugars were not separating on the resin. The system was cleaned with a 0.02%w/v sodium azide solution to eliminate any microbial growth in the columns and the resin regenerated with a 2.0%w/v calcium acetate solution. The system was checked for leaks and the purge pump repaired before the next run.

Run SYN2 was carried out under identical conditions to run SYN1. The run was abandoned after 5 cycles as the temperature controller for the heater supplying the water to the eluent and purge lines failed causing the water temperature to be raised to 80°C. Some of the tubing in the SCCR6 melted causing the run to be abandoned. A new temperature controller was fitted and temperature sensors were added to the system to switch the heaters off if the temperature exceeded 70°C.

Another problem observed during the run was that, as the deionised water storage tanks were approximately 3 metres above the SCCR6 unit, there was a flow due to gravity through the unit and therefore there were inaccuracies in the eluent and the purge streams flowrates. To overcome this, loading valves were fitted in the three inlet lines just after the pumps to stop water flow into the system due to gravity.

Run SYN3 was carried out under identical conditions to the previous two runs except for the feed concentration which was reduced to 20%w/v sugar solids. This was a

continuous run over 9 cycles and the analysis of the final products showed that very little separation had occurred since purities of only 58% fructose rich product and 53% glucose rich product were obtained. Figure 10.1 shows the purge concentration profile for this run. This shows that there was no separation in the post-feed section of the system and very little separation in the pre-feed section.

After thorough checks to the system, it was found that some of the pneumatic valves were not operating satisfactorily and some mixing was occurring between the columns and product lines. It was found that the locking nuts securing the poppet valves to the diaphragm in 25 of the valves were loose causing the valves to remain open. Therefore all the valves were removed one by one and a locking washer was fitted to the poppet stem to secure the nut. New neoprene diaphragms were also fitted and all the valves were tested using a timer connected to a pneumatic line for rapid opening and shutting and the valves that failed the test were replaced with new ones. Figure 5.8 shows the picture of the pneumatic valve with all the components exposed.

As it had been impossible to check for leaking valves, it was necessary to replace all the tubing in the SCCR6 with clear polypropylene tubing so that visible tracers could be added to the system and any faulty valves identified and rectified before a run commenced.

Having carried out these modifications, the equipment was tested with a blue dextran solution as feed to check visibly for any mixing in the system. These tests showed that the system was operational again and it was possible to carry out another run. This completed the commissioning runs on the SCCR6 system and the system was ready for operation again.

Run SYN4 was carried out under the following conditions:

- Feed concentration 20%w/v total sugar solids consisting of a 50:50 ratio of glucose and fructose.
- Feed flowrate 35 $cm^3.min^{-1}$
- Eluent flowrate 105 $cm^3.min^{-1}$
- Purge flowrate 400 $cm^3.min^{-1}$
- Run temperature 20°C
- Switch time 30 minutes

The run was over 8 cycles and the analysis of the last three cycles showed that the purity of the glucose rich product (GRP) was 99.9% whereas that of the fructose rich product (FRP) was 93%. To improve the FRP purity, another run (SYN5) was carried out under the same conditions for 8 cycles except that the switch time which was increased to 34 minutes. For this run, it was expected that the FRP purity would increase and the GRP purity decrease slightly. This was because by increasing the switch time, extra time

is given for all the glucose to be carried with the mobile phase leaving a purer FRP. This principle is better explained by Equation 5.9 which indicates that by increasing the switch time, the value P in the ratio L_s/P increases, and the separation potential is reduced due to the ratio being very close to the operating limits as defined by the distribution coefficients. The FRP purity for this run was 99.9% whereas the GRP purity was decreased to 98%. The on-column concentration profiles for the last two cycles and the purge concentration profile for the run are as shown in Figures 10.2, 10.3 and 10.4 respectively.

This run was similar to that carried out by Thawait and the results obtained were within experimental and analytical inaccuracies. Thawait had obtained 99.9% purities for both the products. Having obtained a base run which was similar to the one carried out by Thawait, it was established that the system now functioned satisfactorily and the behaviour of the system when using inverted sucrose feedstocks could now be investigated.

10.3 Enzyme Inverted Sucrose Feedstock Runs

The feedstock was prepared for these runs using the method as described in Chapter 8. The 20%w/v feed solution was totally inverted giving a mixture of 49.43%w/v glucose and 50.50%w/v fructose. The first run (run ENZ1) was carried out over 8 cycles and the analysis of the final

products showed that the purity of the FRP was 96.4% and that for the GRP was 92.9%. The reason for the decrease in the product purities is explained later. The on-column concentration profiles for the last two cycles and the purge concentration profile for this run are shown in Figures 10.5, 10.6 and 10.7 respectively. It should be noted that the cross-over point had moved to the right compared to the base run. Section 10.7 shows the comparison of this run with the base run and the other runs in the series and the results of the comparison are explained. A similar run (ENZ2) was carried out to confirm the results obtained and this gave identical profiles to run ENZ1.

10.4 Acid Inverted Sucrose Feedstock Runs

An 8 cycle run (run ACID1) using sucrose inverted by acid as described in Chapter 8 was carried out under identical conditions to those in the base run. The feed contained 20%w/v solids which included approximately 49% glucose, 47% fructose and 6% impurities including uninverted sucrose. The pH of the feed was 5.8.

The on-column concentration profiles for the last two cycles and the purge concentration profile for this run are shown in Figures 10.8, 10.9 and 10.10 respectively. For this run the cross-over point was in the GRP column giving a purity of the FRP of 90.6% and that of the GRP of 80.9%. This run is also discussed further in Section 10.7.

As there was some feed left over from the previous run, it was decided to carry out another run under identical conditions and the profiles obtained from this run were similar to those from run ACID1.

10.5 Resin Inverted Sucrose Feedstock Runs

An 8 cycle run (run RESIN1) using sucrose inverted by passing a 50%w/v sucrose solution through a column packed with an ion-exchange resin in the H^+ form as described in Chapter 8 was carried out under identical conditions to those in the base run. The feed contained 20%w/v solids which included approximately 5% uninverted sucrose. The pH of the feed was 5.9.

The on-column concentration profiles for the last two cycles and the purge concentration profile for the run RESIN1 are shown in Figures 10.11, 10.12 and 10.13 respectively. For this run the cross-over point was in the GRP column giving a FRP purity of 95.8% and a GRP purity of 68.4%. This run is also discussed further in Section 10.7.

The cross-over point had moved even further to the right compared to the profiles for the previous runs. From the profiles, it can be seen that the shift in the cross-over point is due to the changes in the fructose concentration in the columns and not the glucose concentration. This result is similar to that found by Abusabah (71), who worked on a similar system to the SCCR6,

containing the same form of resin. This run is discussed later in Section 10.7. As the purity of the final products was diminishing from run to run, it was decided to carry out another run using a synthetic feed of glucose and fructose similar to the one used in run SYN5 so as to compare the purities obtained with those in run SYN5.

10.6 Synthetic Feedstock Runs on the SCCR6

Run SYN6 was carried out using a 20%w/v synthetic feed similar to that used for run SYN5 and was carried out under identical conditions to the previous run. This was an 8 cycle continuous run and the purities obtained were 98.5% for the FRP and 87.9% for the GRP. The run did not produce identical results to those obtained in run SYN5 and therefore the pneumatic valves were checked again to ensure that none were sticking open or shut causing mixing between the two products, though more serious effects would be expected if the valves were faulty. The valves were found to be functioning well and therefore it was decided to regenerate the resin in the columns. It was suspected that the ion-exchange capacity of the resin was reduced ie. the calcium ions had been displaced from the resin and therefore the resin needed regeneration. It appeared that the resin was not retarding the fructose due to lack of calcium ions and therefore some fructose moved with the mobile phase giving a less pure GRP product.

A 0.2%w/v sodium azide solution was passed through the columns to sterilise the equipment and the resin was then regenerated with a 10%w/v solution of calcium acetate. Figures 10.14, 10.15 and 10.16 show the on-column concentration profiles for the last two cycles of run SYN6 and the purge concentration profile for the run respectively.

Two more runs were carried out on the equipment using the same feedstock and under identical conditions as those used for run SYN6. Runs SYN6 and SYN7 gave almost identical product purities but the GRP purity in these runs (approximately 87%) was lower than the purity of 98.45% found for run MOL5.

It can be deduced that one or all the runs using the inverted sucrose feedstocks affected the column packing by calcium ion replacement and the resin was not retarding the fructose in preference to the glucose.

From Section 6.2.2, it can be noted that any free cations in the mobile phase would displace the calcium from the resin provided that their concentration is higher in the mobile phase than in the stationary phase. As the pH of the feedstocks prepared by acid hydrolysis and by resin hydrolysis was less than 6, the hydrogen ions in the feed prepared by these routes could have replaced the calcium ions on the SCCR6 resin.

It was decided to regenerate the resin again but to

use a more fully dissociated calcium salt rather than calcium acetate. A 10%w/v solution of calcium chloride was pumped through the columns at a flowrate of approximately 3-4 bed volumes per hour as recommended by the resin manufacturers. This regeneration was carried out and the system subsequently flushed with deionised water.

Run SYN8 was then carried out over 8 cycles continuously and the end products analysed. The FRP purity was now 99.9% and the GRP purity increased to 95.5%. At this stage of the programme, it was decided to leave the synthetic feedstock runs and move on to runs on the SCCR6 using inverted molasses as feedstocks. The on-column and purge concentration profiles for runs SYN7 and SYN8 are shown in Figures 10.17 to 10.22 respectively.

10.7 Discussion and Comparison of the Runs

The three inverted sucrose feedstock runs and four synthetic feedstock runs are tabulated in Table 10.1. From the table, it can be seen that the results for the acid inverted feed and the resin inverted feed were the least effective with the GRP purity decreasing to 67.45% and 68.4% respectively. Yet, for the run ACID1, the product concentration for the GRP is the highest compared to it being the lowest for the FRP. This shows that the carbohydrates have moved with the mobile phase and the fructose has not been retarded by the resin. This indicates

that the feedstock was not neutralised adequately and there were free hydrogen ions in the mobile phase which displaced some of the calcium ions from the resin resulting in the fructose not being retarded. The loss of some calcium ions from the resin can account for the low GRP purity for the run. As the run using H^+ resin inverted sucrose was carried out after the acid inverted sucrose run, this would also account for the low GRP purity for run RESIN1. But as the FRP purity was still high, the displacement of calcium from the resin was not extreme and there was sufficient calcium on the resin to retard some fructose.

The product purities and concentrations for the enzyme inverted feed runs are comparable with the synthetic feed runs giving results which are closest to those for synthetic feeds.

Abusabah (71) carried out a series of experiments on a SCCR system similar to the one being used. He used the SCCR4 unit and found that the system behaved differently when separating H^+ resin inverted sucrose syrups than when separating synthetic feed mixtures of glucose and fructose. The difference was apparent in the shift to the left by three columns of the cross-over point of the concentration profiles and the changes in the glucose product purity. The work carried out for this research gave a similar shift in the cross-over point of the concentration profiles but in this case the shift was to the right ie. in the opposite

direction to that obtained by Abusabah.

The shift in the cross-over point and the loss of purity of the GRP in the runs ACID1 and RESIN1 can also be explained from the tautomeric equilibria of the two sugars. The β-D-fructopyranose is the only tautomer which forms the complex with the calcium. If more of this tautomer is available, more fructose would be retained on the resin. During the inversion of sucrose, the fructose formed consists of four tautomers. If less of the β-D-fructopyranose is formed a substantial amount of the fructose will travel with the mobile phase resulting in a less pure GRP. It is possible that the different inversion techniques may produce less of the β-D-fructopyranose.

A simpler explanation could be that the cross-over point shift could be due to the reduced fructose retention due to the displacement of the calcium ions by the hydrogen ions from the acid inverted feed which results in poor separation.

The effect of the various feedstocks on the SCCR6 unit should be investigated further to clarify some of the questions still not answered. This would involve regenerating the resin after each run to ensure that the system was operating satisfactorily before the change in feed could be investigated. The desired product purities can be obtained by changing the switch time. A reduction in switch time would move the cross-over point back towards

the feed column . The effect of varying switch time on synthetic feedstocks has been investigated by Ganetsos (10) and the effect of switch time on inverted molasses feedstock has been investigated and reported in Chapter 11.

Table 10.1: Comparison of the Runs

Run Number	Average Flow Rates ($Cm^3.min^{-1}$) Eluent Flow	Feed Flow	Purge Flow	Feed Composition G	F	Switch Period (Min.)	Temp (°C)
SYN5	105	35	400	51.1	48.9	34	20
ENZ1	105	35	400	49.43	50.56	34	20
ACID1	105	35	400	51.75	48.24	34	20
RESIN1	105	35	400	42.32	52.15	34	20
SYN6	105	35	400	47.33	52.96	34	20
SYN7	105	35	400	46.9	53.5	34	20
SYN8	105	35	400	47.57	52.42	34	20

Run Number	FRUCTOSE RICH PRODUCT Purity %	Product Concn (%w/v)	GLUCOSE RICH PRODUCT Purity %	Product Concn (%w/v)
SYN5	99.1	0.76	98.45	1.89
ENZ1	96.4	0.72	92.9	2.12
ACID1	96.6	0.41	67.45	2.53
RESIN1	95.79	0.49	68.4	2.08
SYN6	98.5	0.52	87.99	2.11
SYN7	99.9	0.48	87.2	2.10
SYN8	99.9	0.58	95.5	2.11

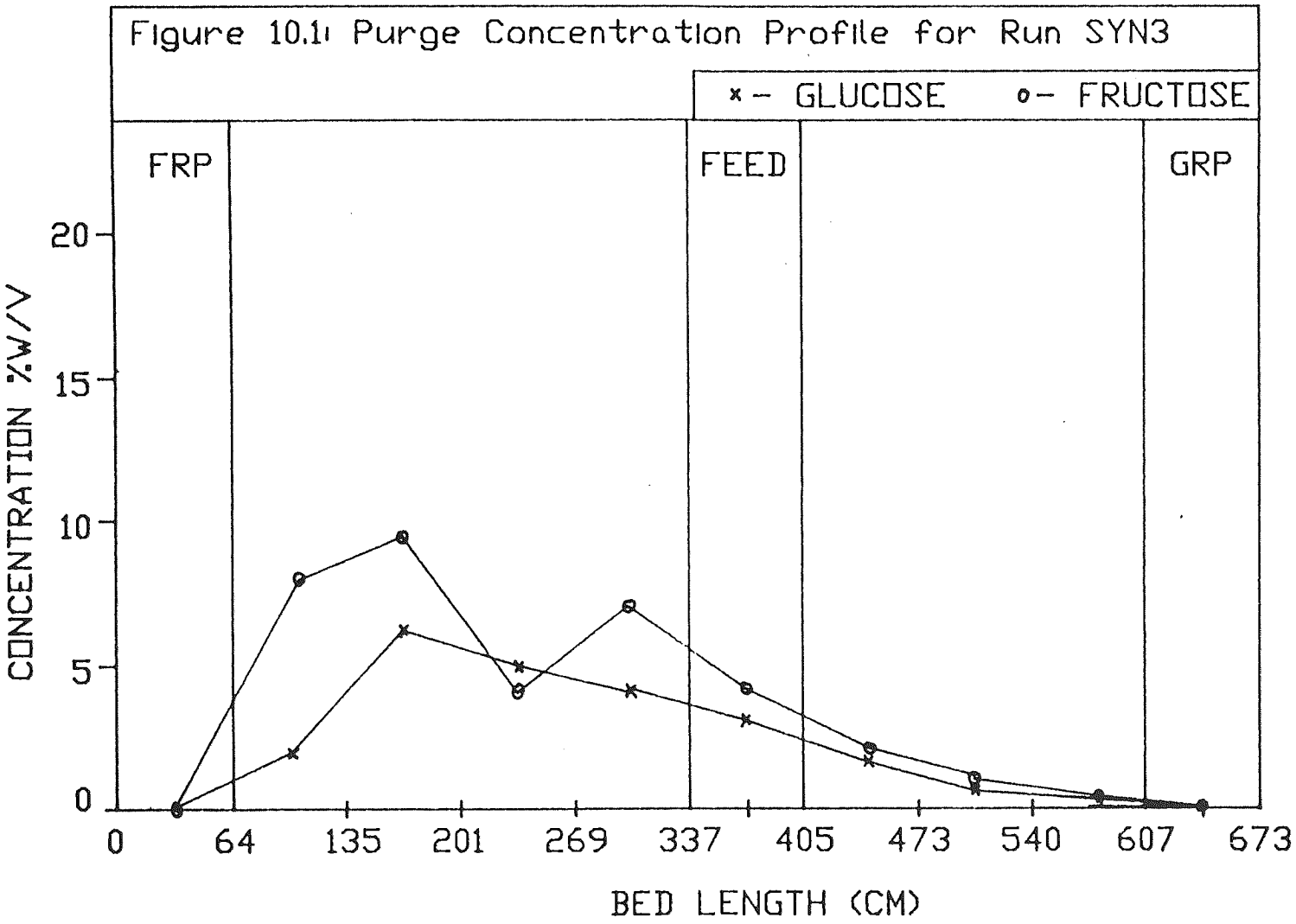


Figure 10.1: Purge Concentration Profile for Run SYN3
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

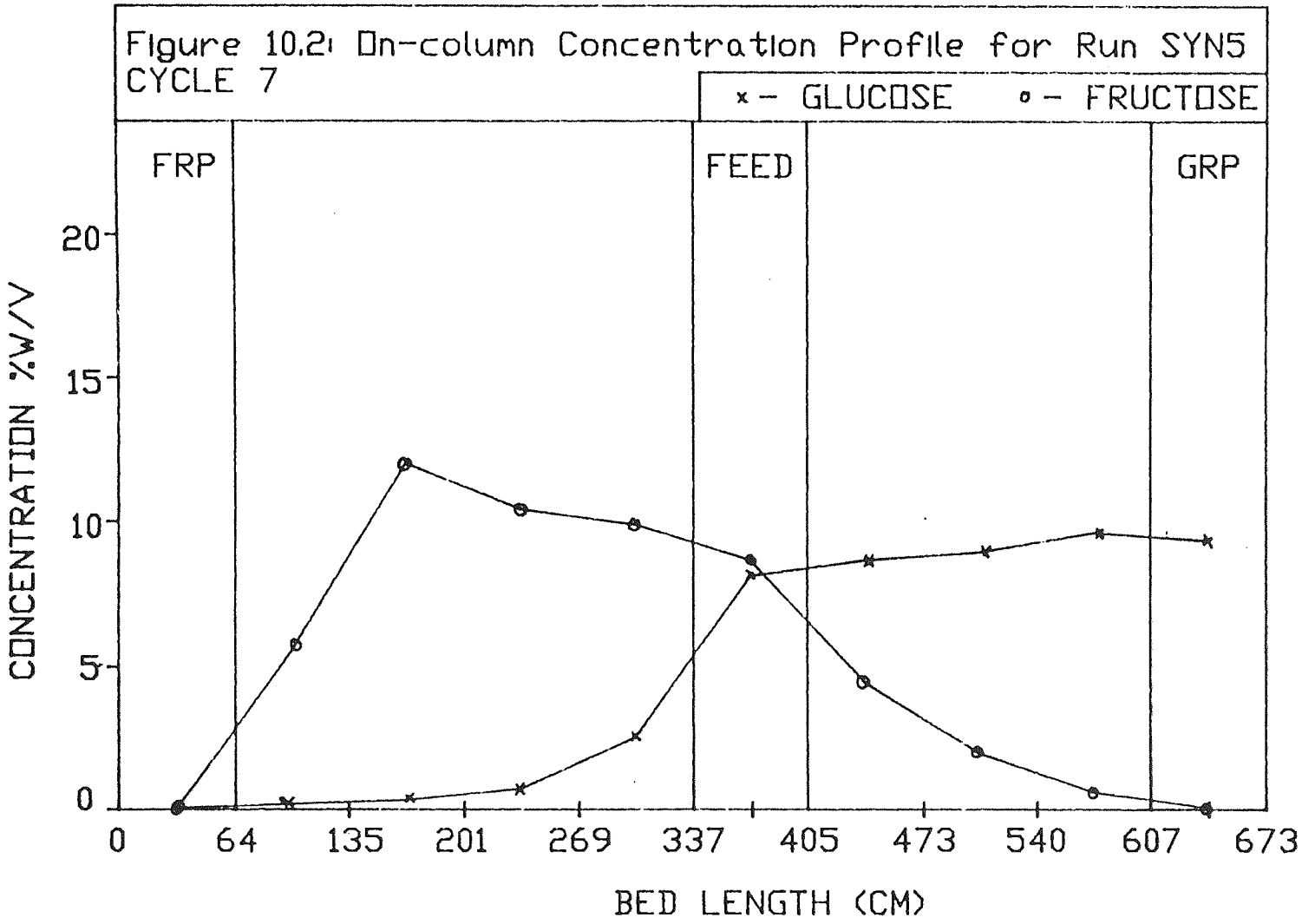


Figure 10.2: On-column Concentration Profile for Run SYN5
CYCLE 7
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

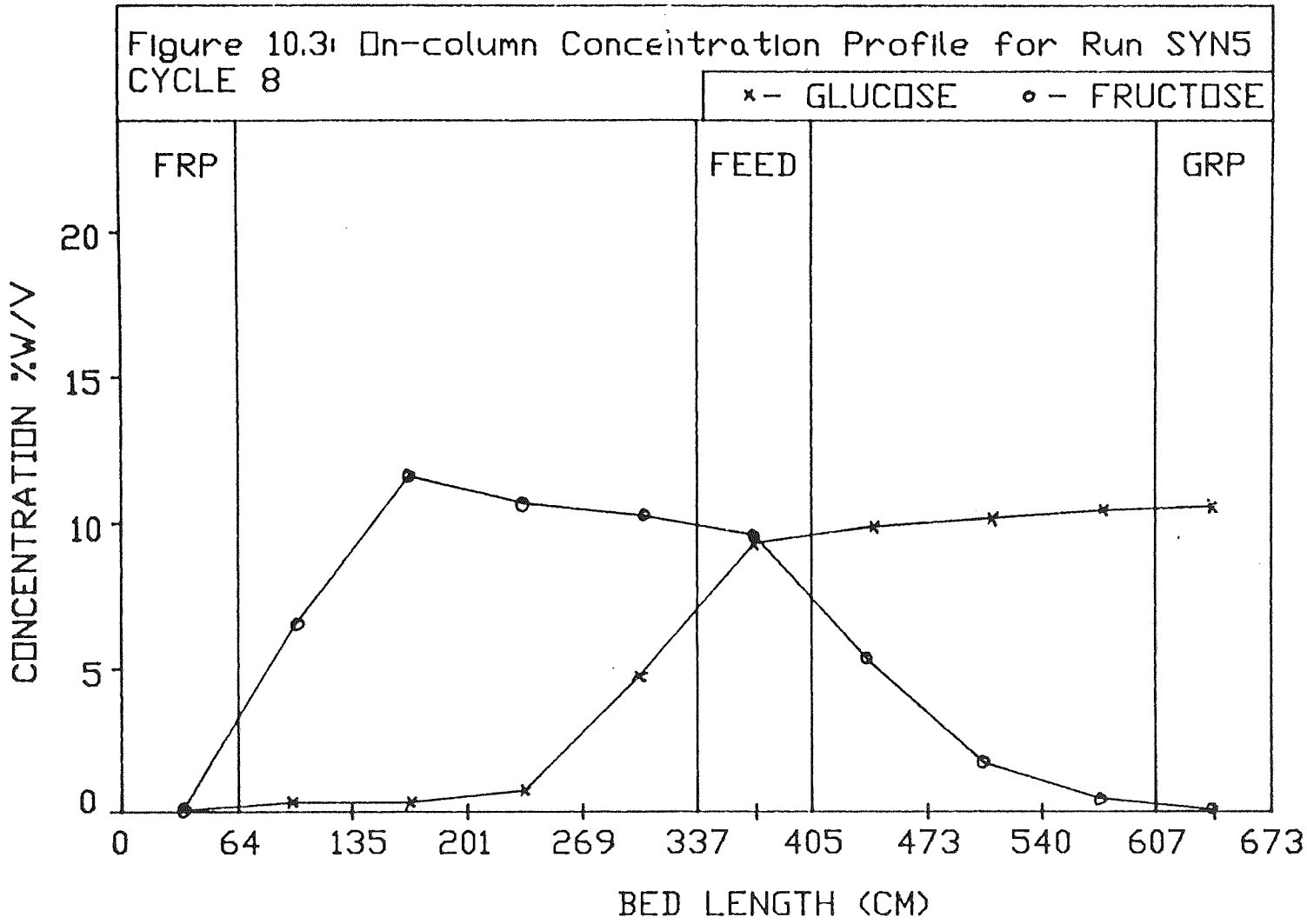


Figure 10.3: On-column Concentration Profile for Run SYN5
CYCLE 8
x- GLUCOSE
o- FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

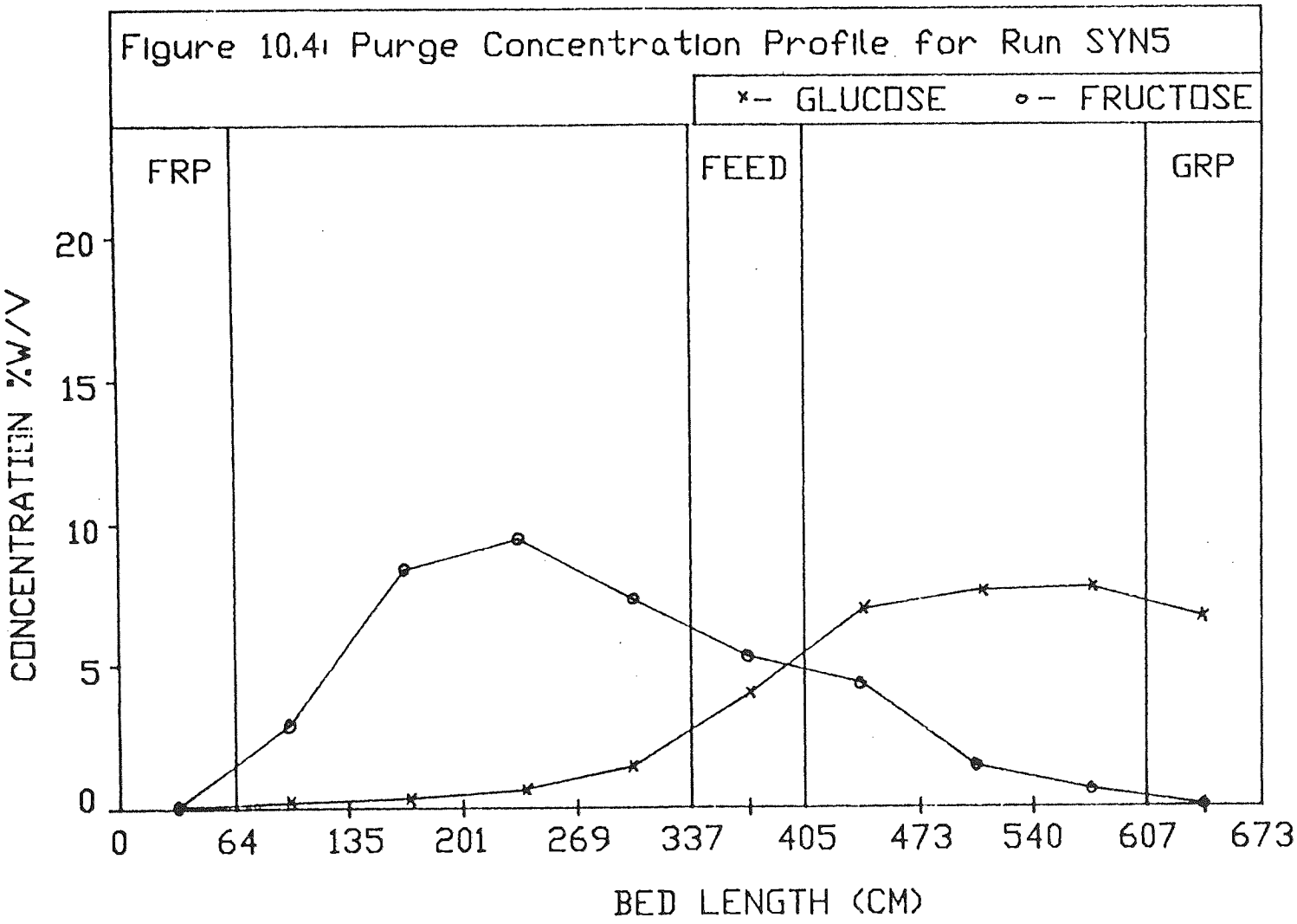


Figure 10.4: Purge Concentration Profile for Run SYN5
x- GLUCOSE
o- FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

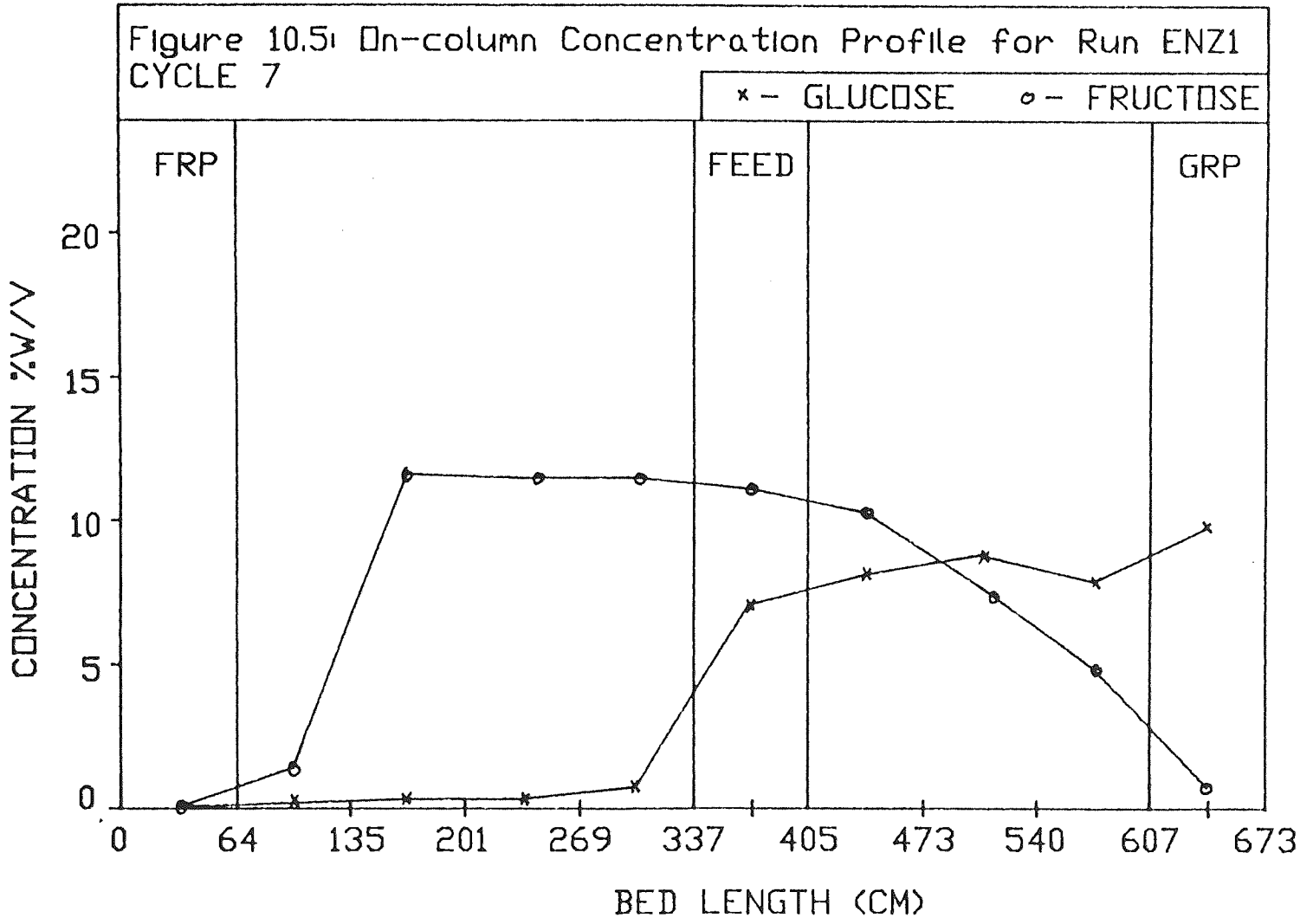


Figure 10.5: On-column Concentration Profile for Run ENZ1
CYCLE 7
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

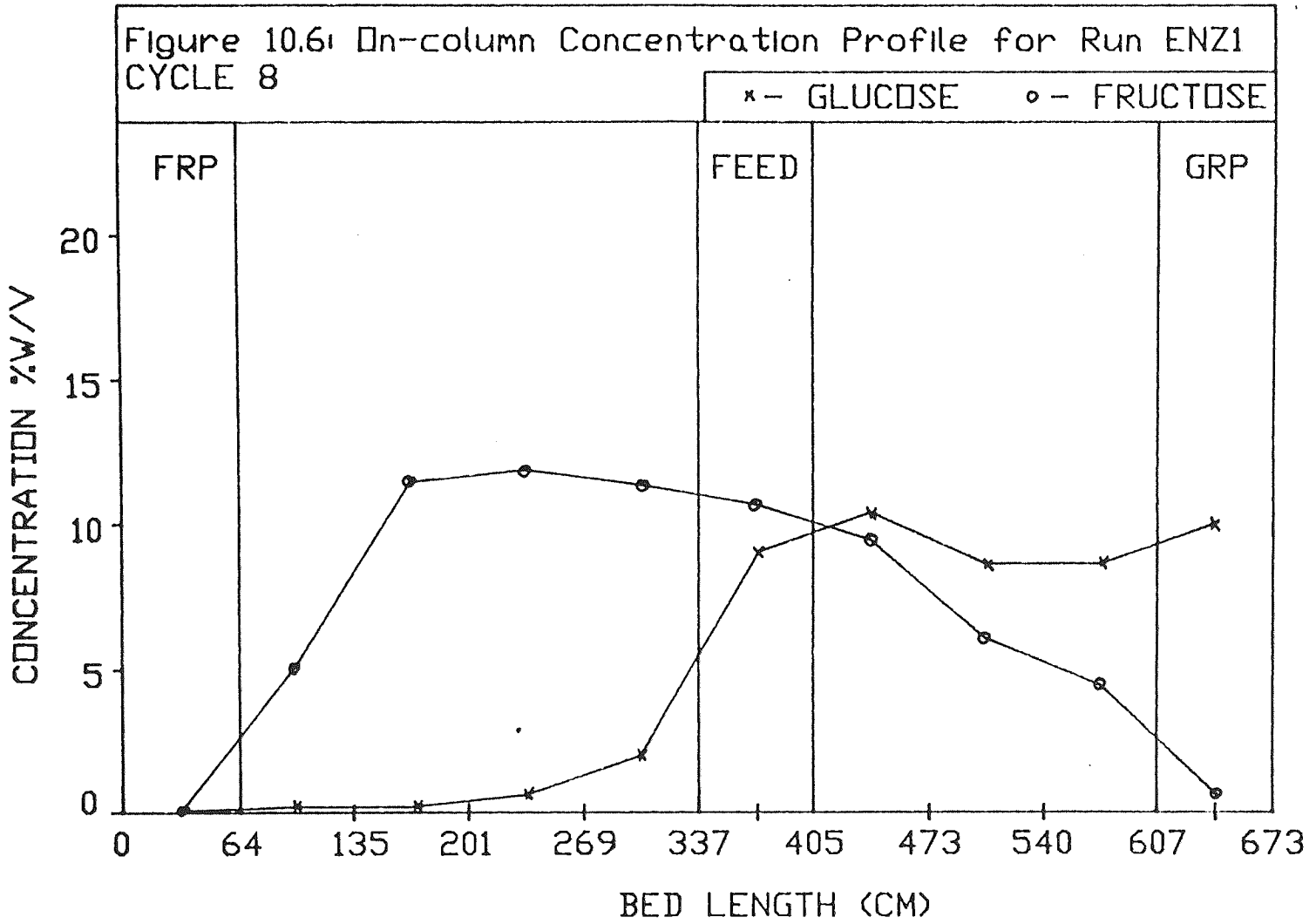


Figure 10.6: On-column Concentration Profile for Run ENZ1
CYCLE 8
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

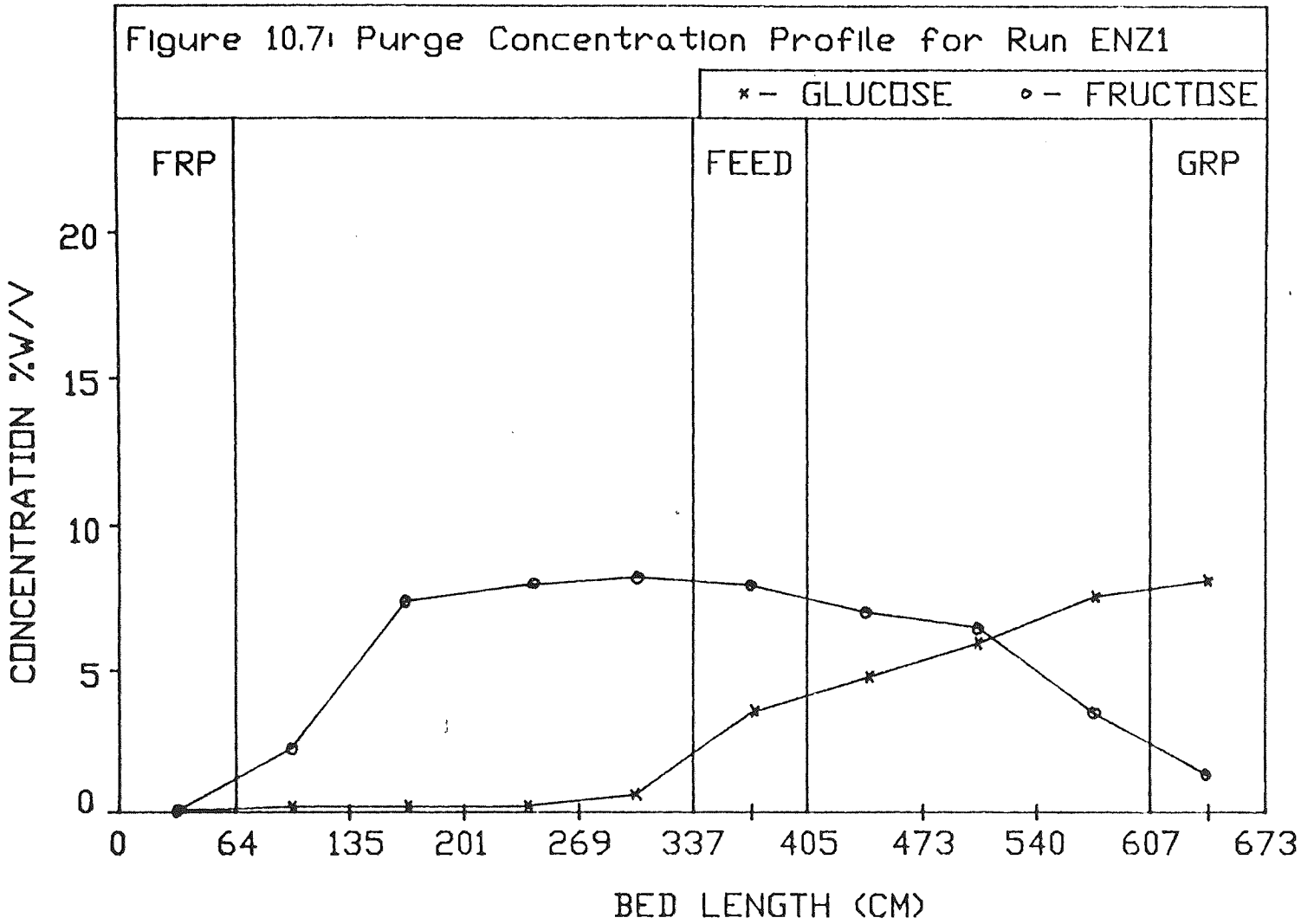


Figure 10.7: Purge Concentration Profile for Run ENZ1
* - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

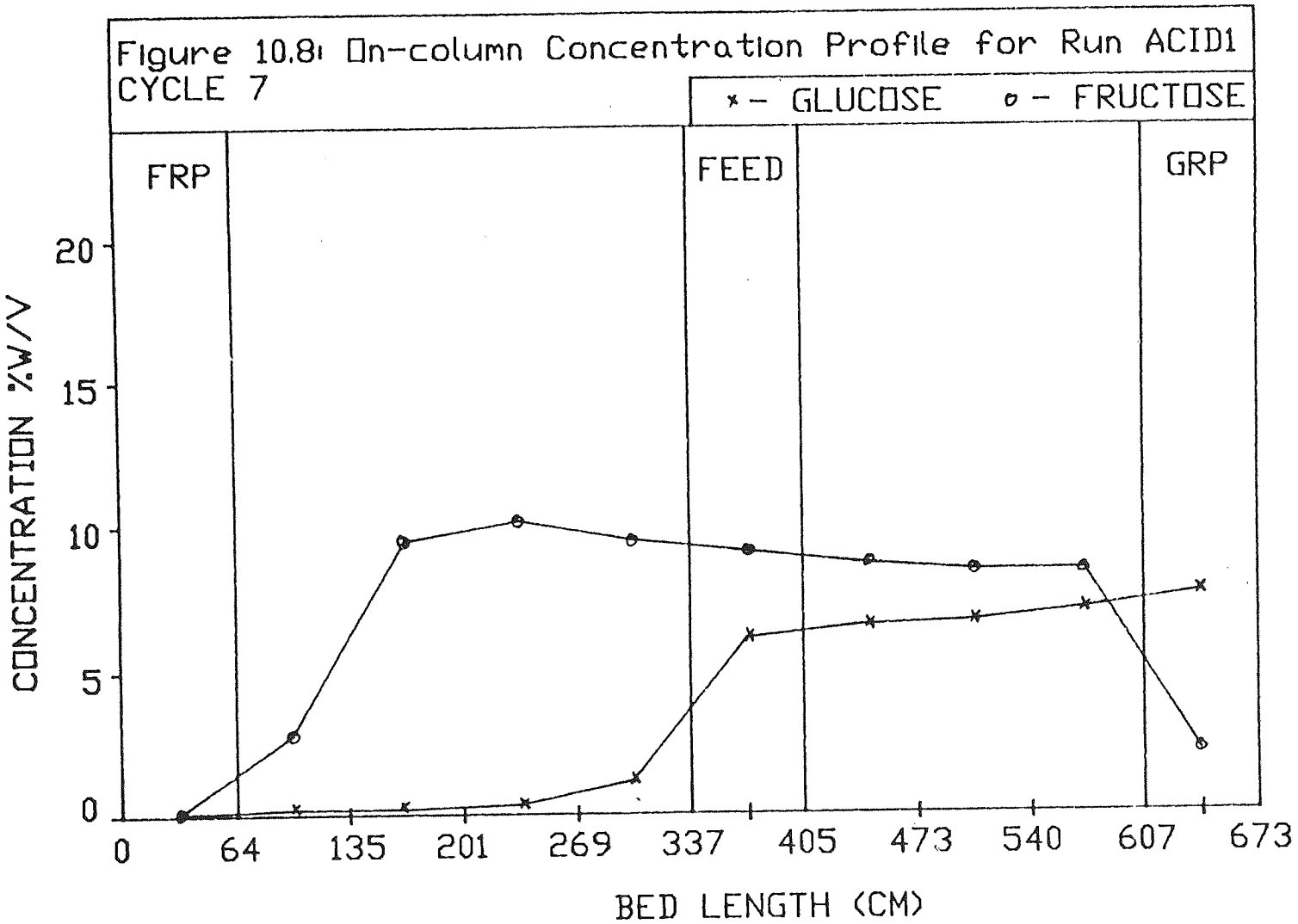


Figure 10.8: On-column Concentration Profile for Run ACID1
CYCLE 7
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

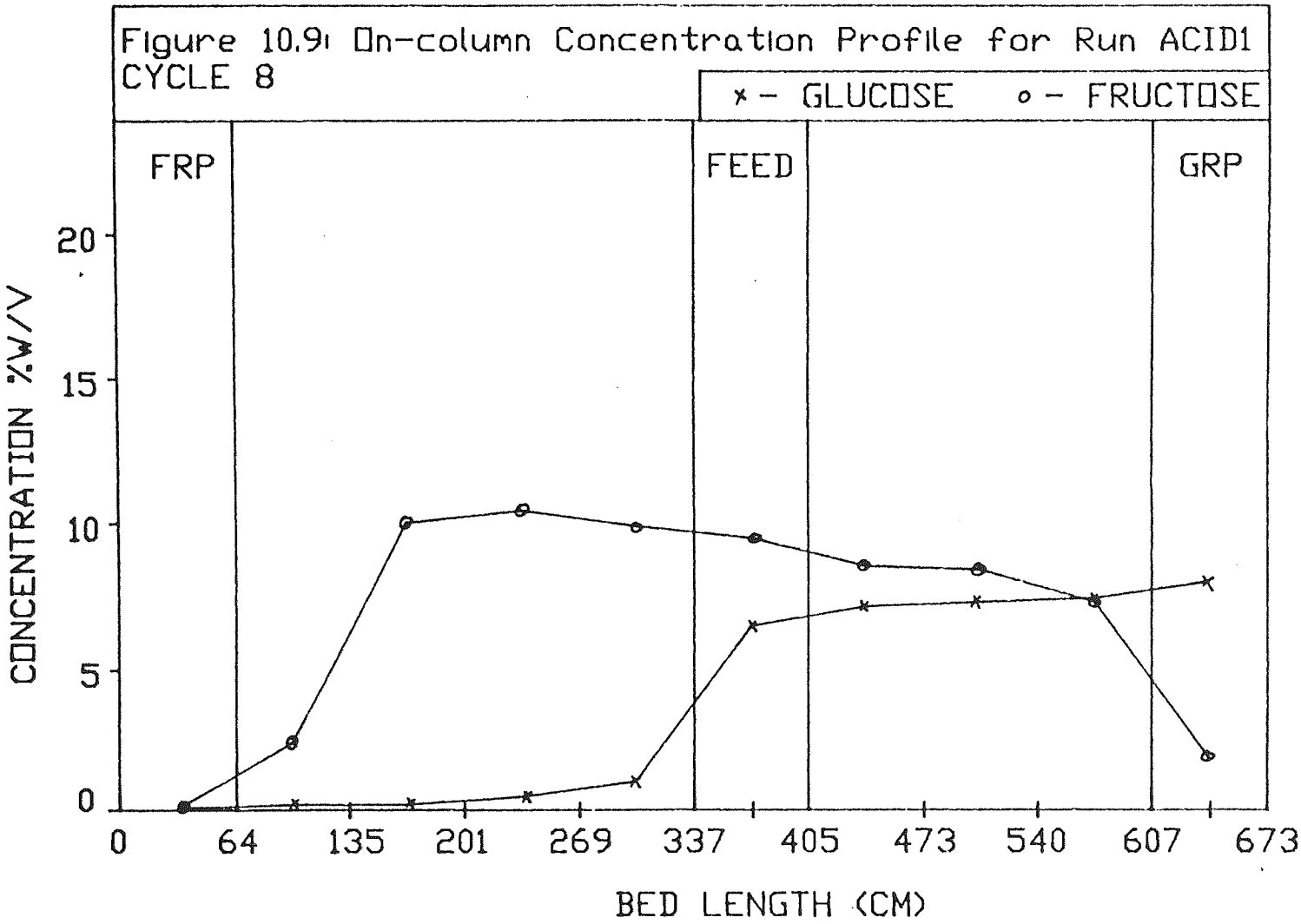


Figure 10.9: On-column Concentration Profile for Run ACID1
CYCLE 8
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

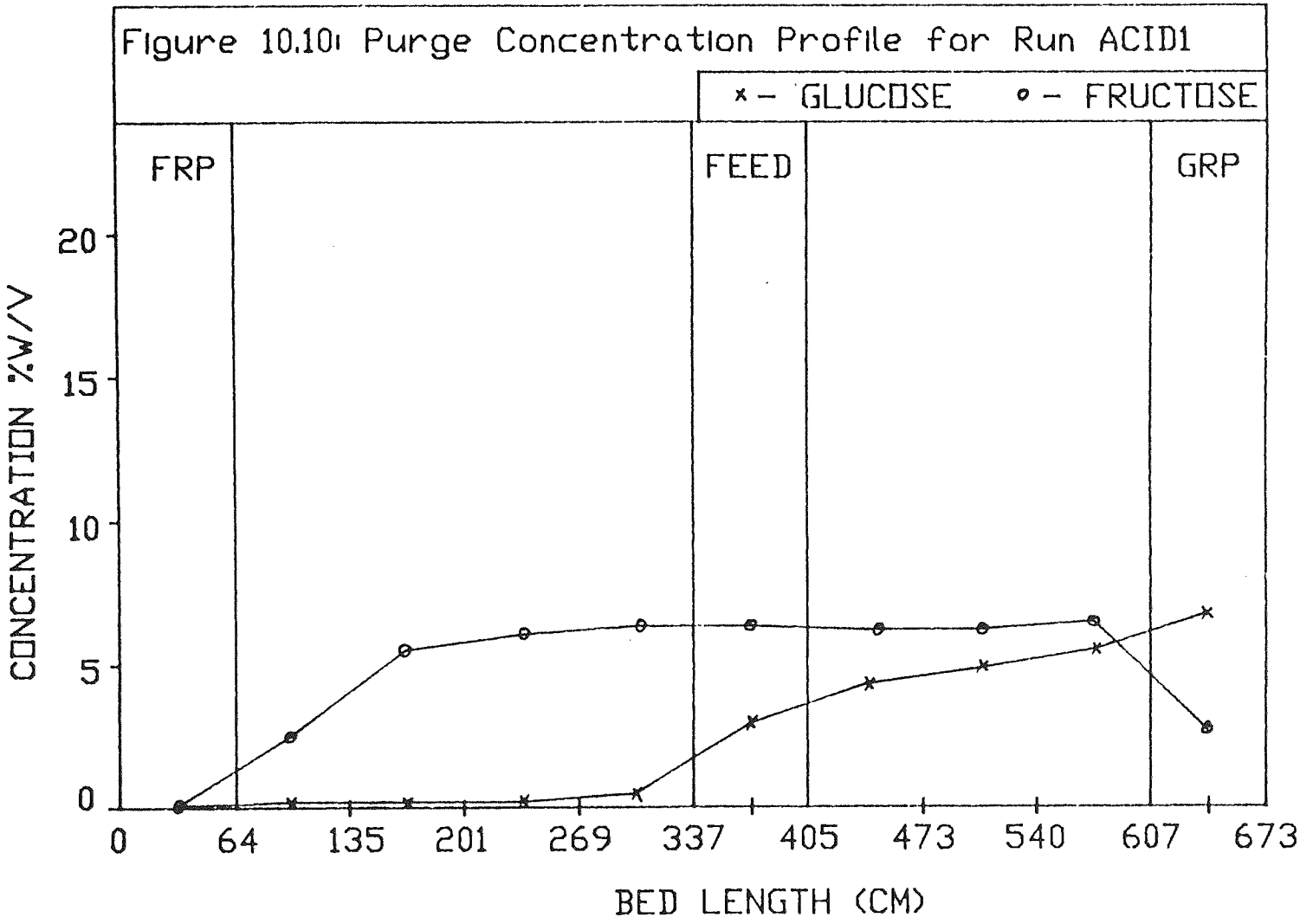


Figure 10.10: Purge Concentration Profile for Run ACID1
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

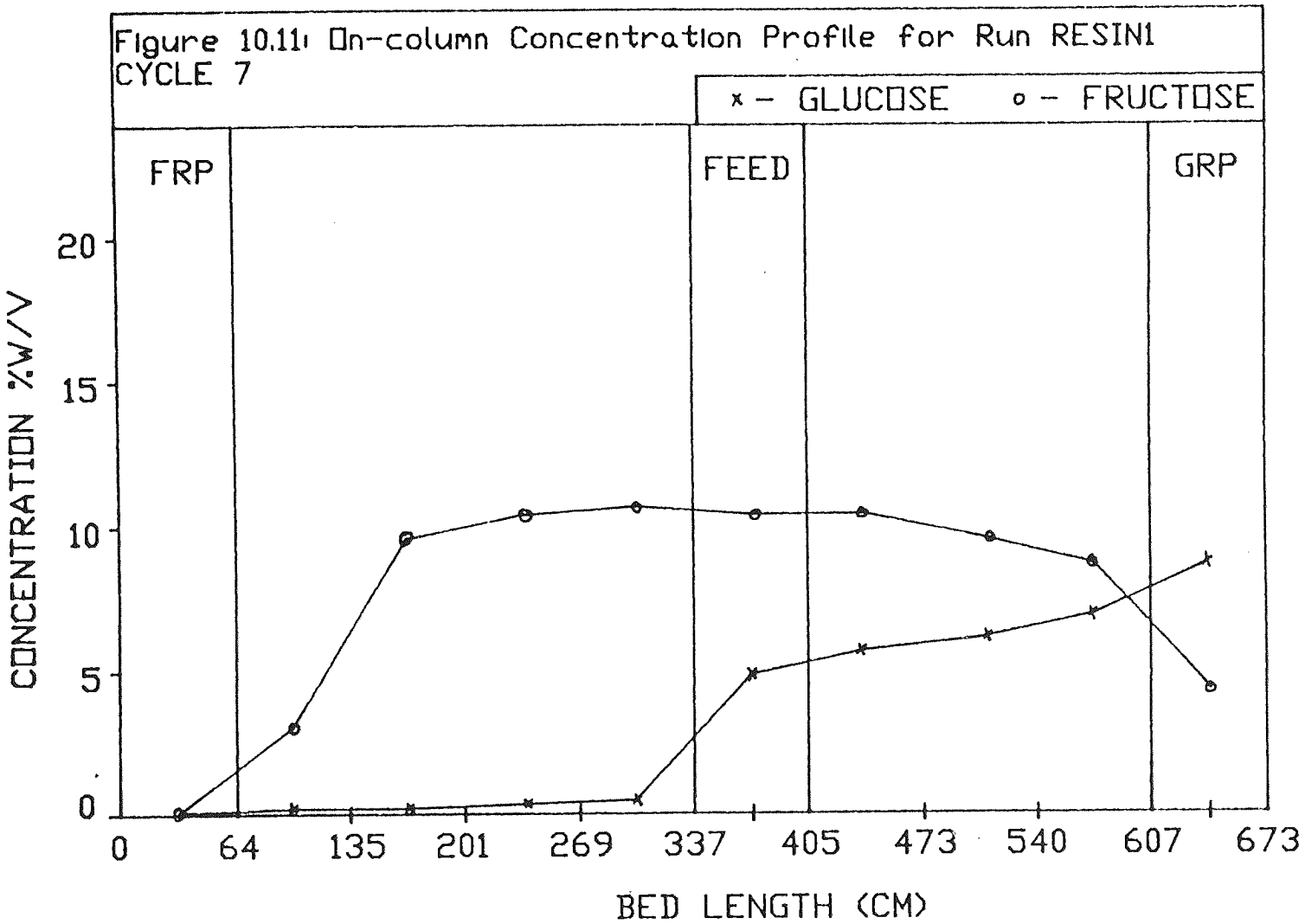


Figure 10.11: On-column Concentration Profile for Run RESIN1
CYCLE 7
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

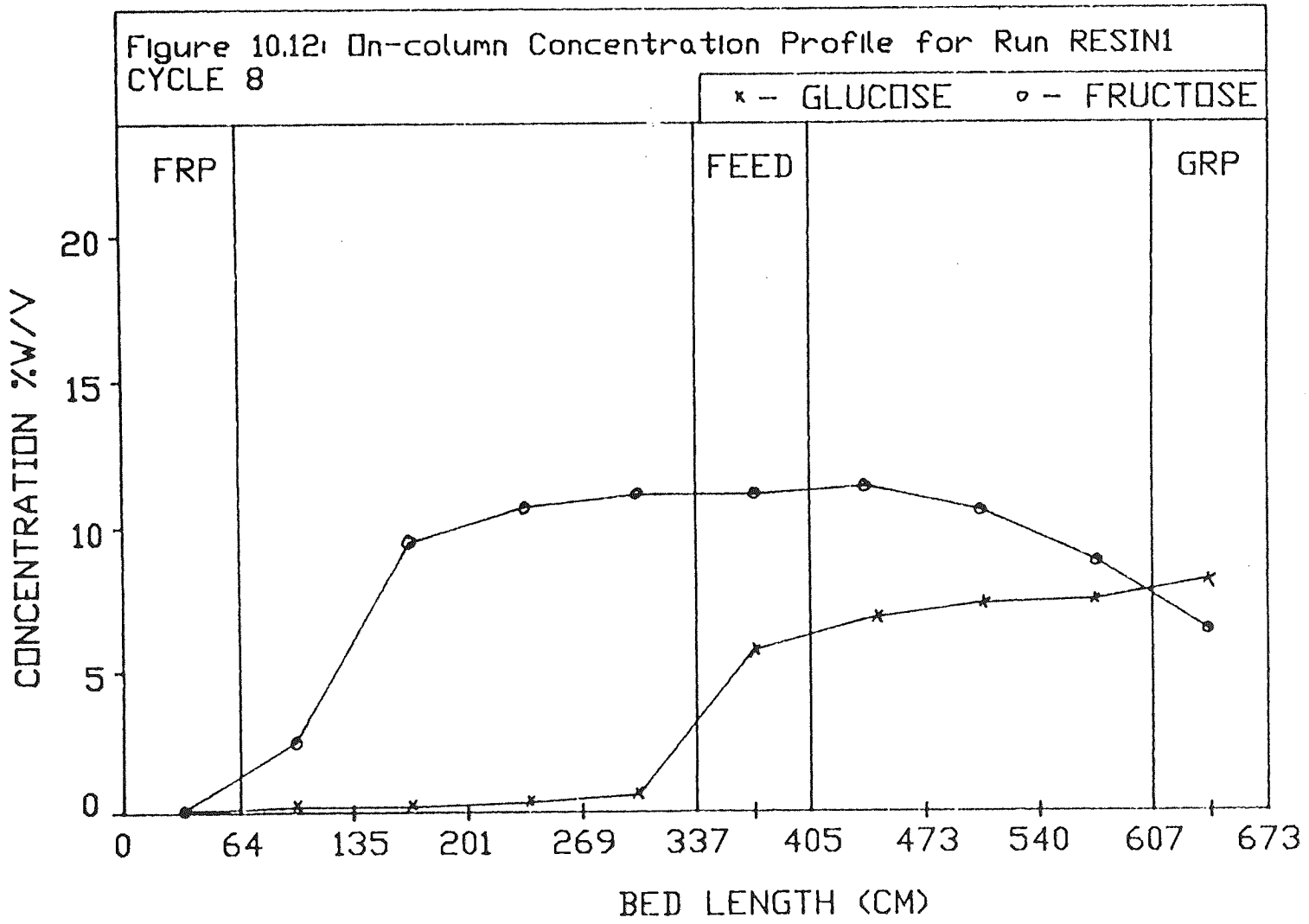


Figure 10.12: On-column Concentration Profile for Run RESIN1
CYCLE 8
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

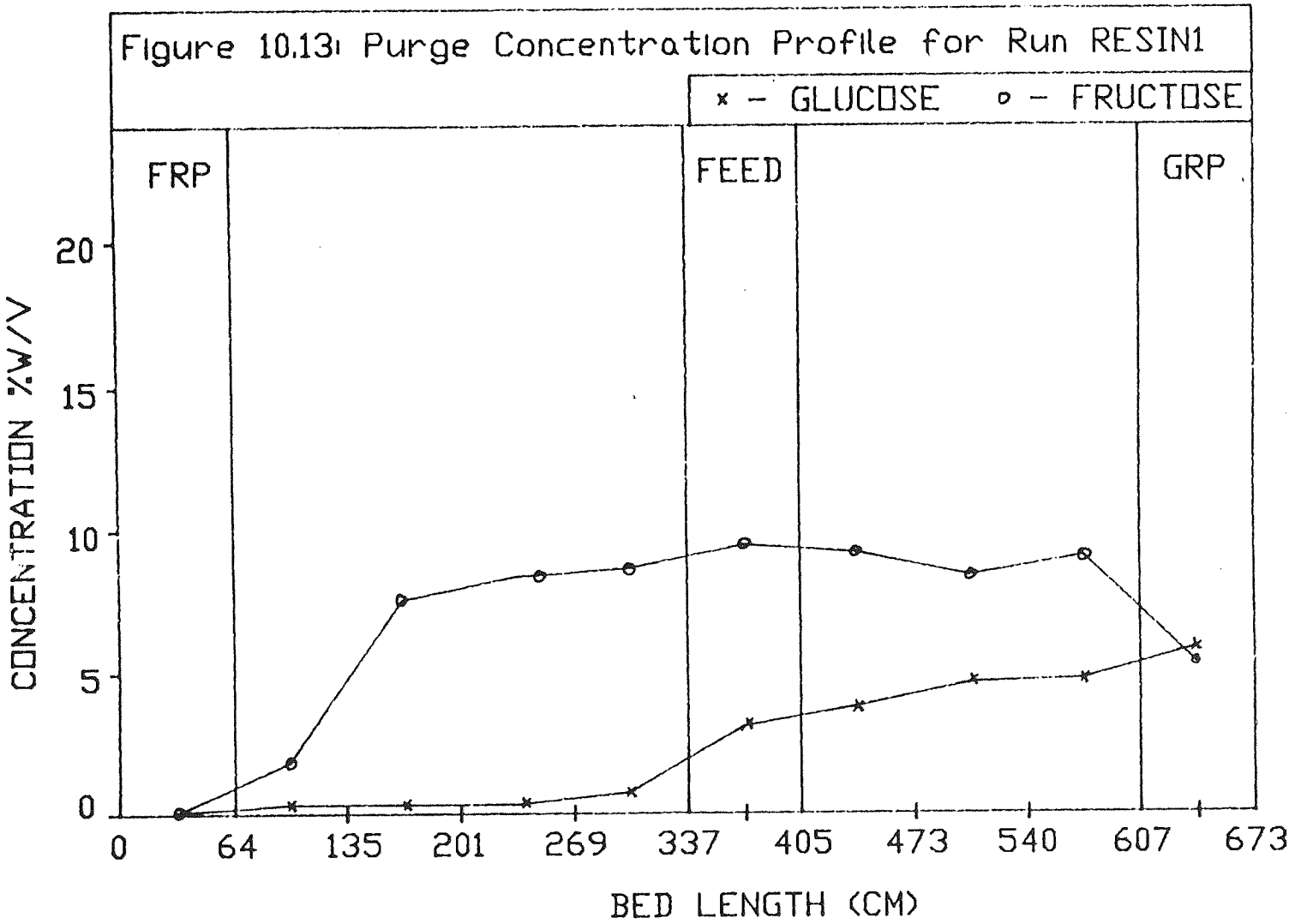


Figure 10.13: Purge Concentration Profile for Run RESIN1
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

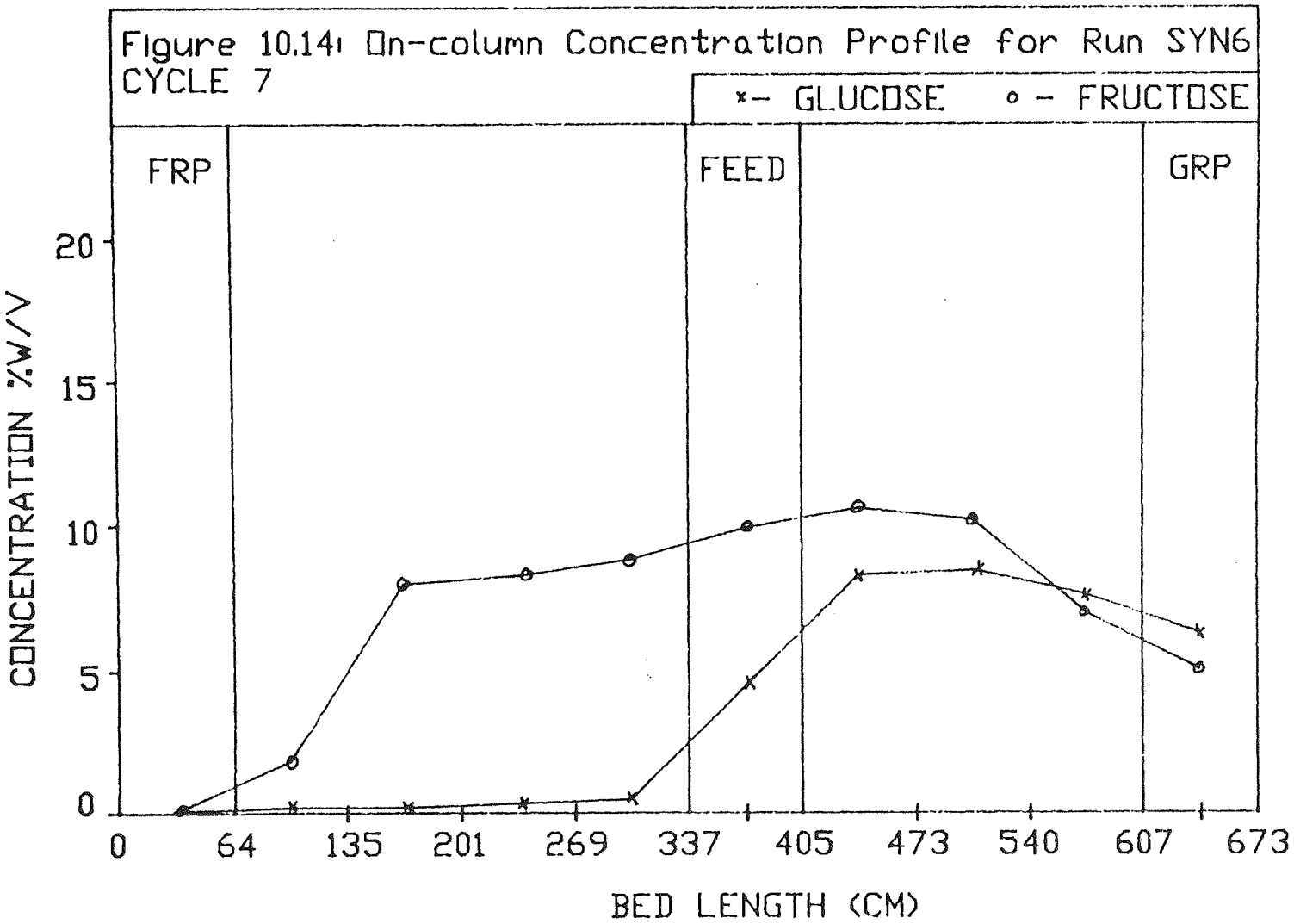


Figure 10.14: On-column Concentration Profile for Run SYN6
CYCLE 7
x- GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

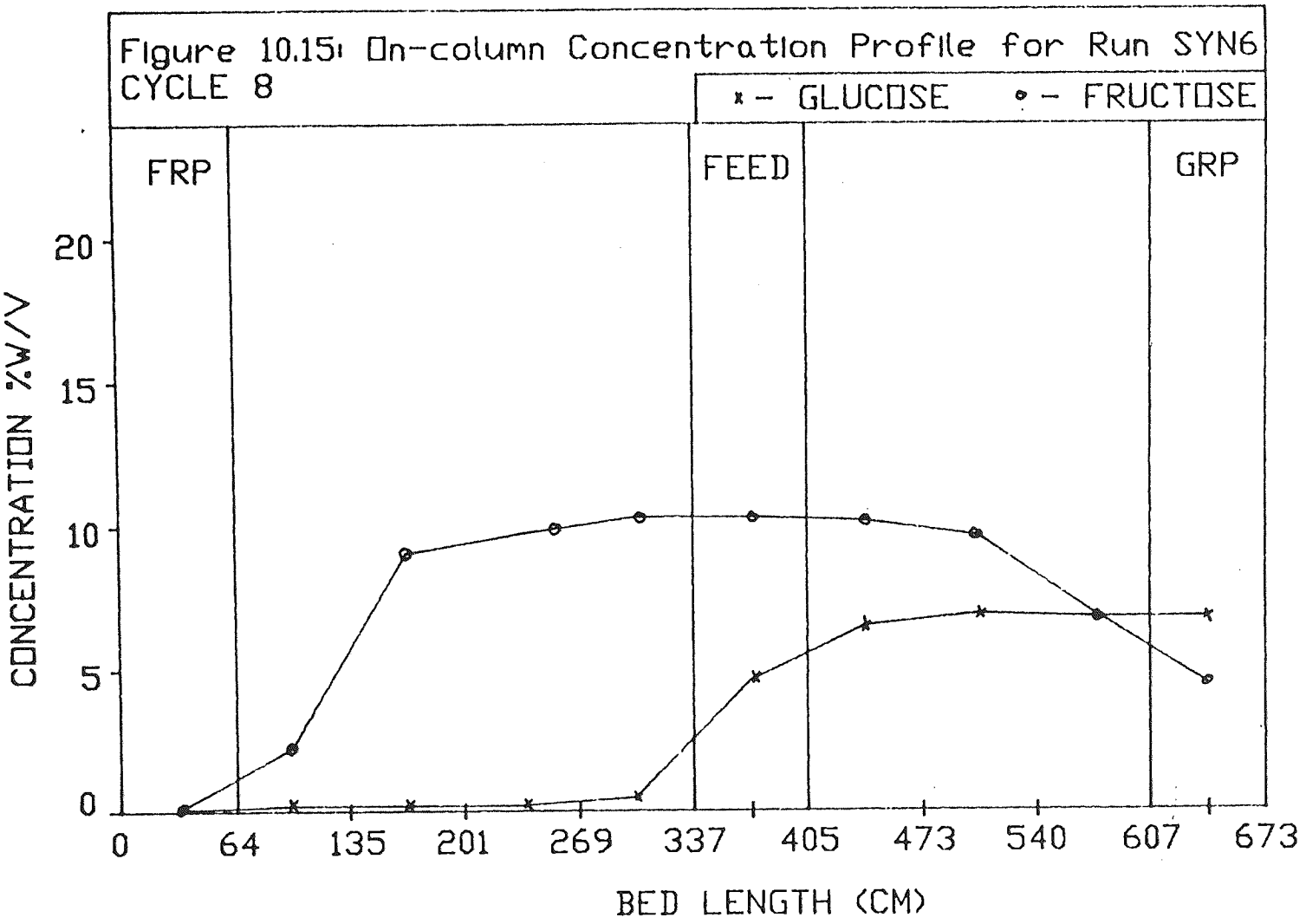


Figure 10.15: On-column Concentration Profile for Run SYN6
CYCLE 8
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

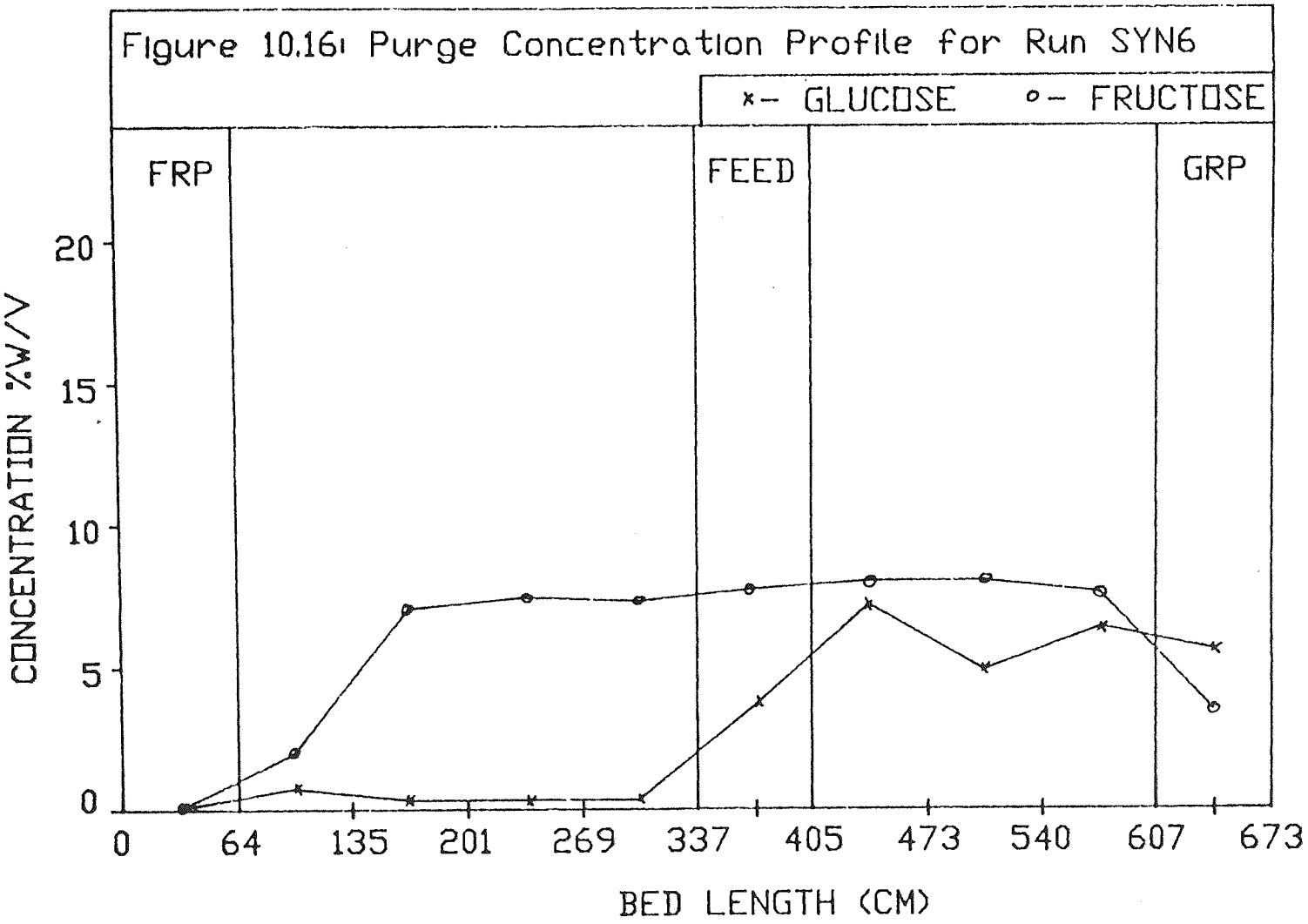


Figure 10.16: Purge Concentration Profile for Run SYN6
x- GLUCOSE
o- FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

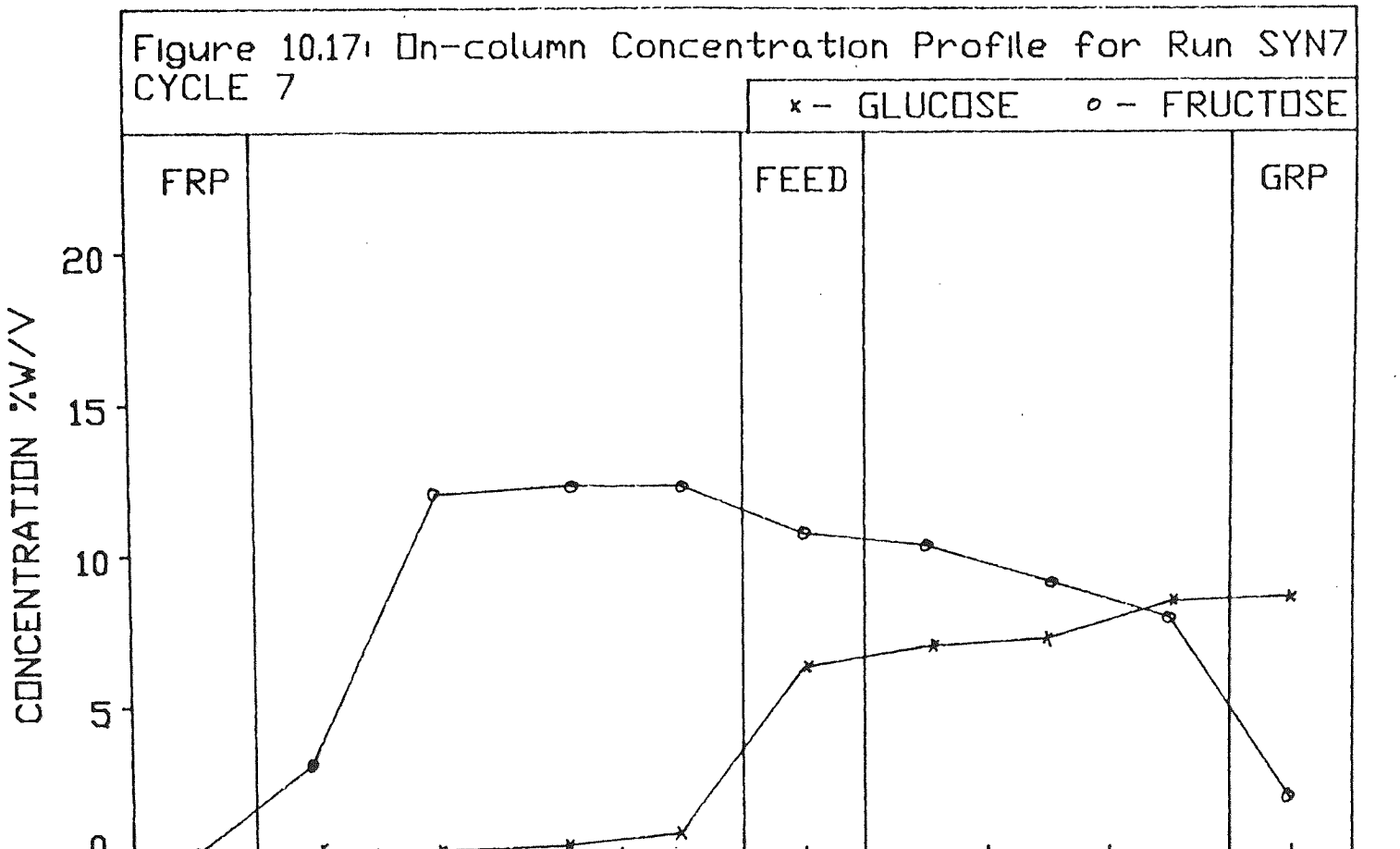


Figure 10.17: On-column Concentration Profile for Run SYN7
CYCLE 7
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

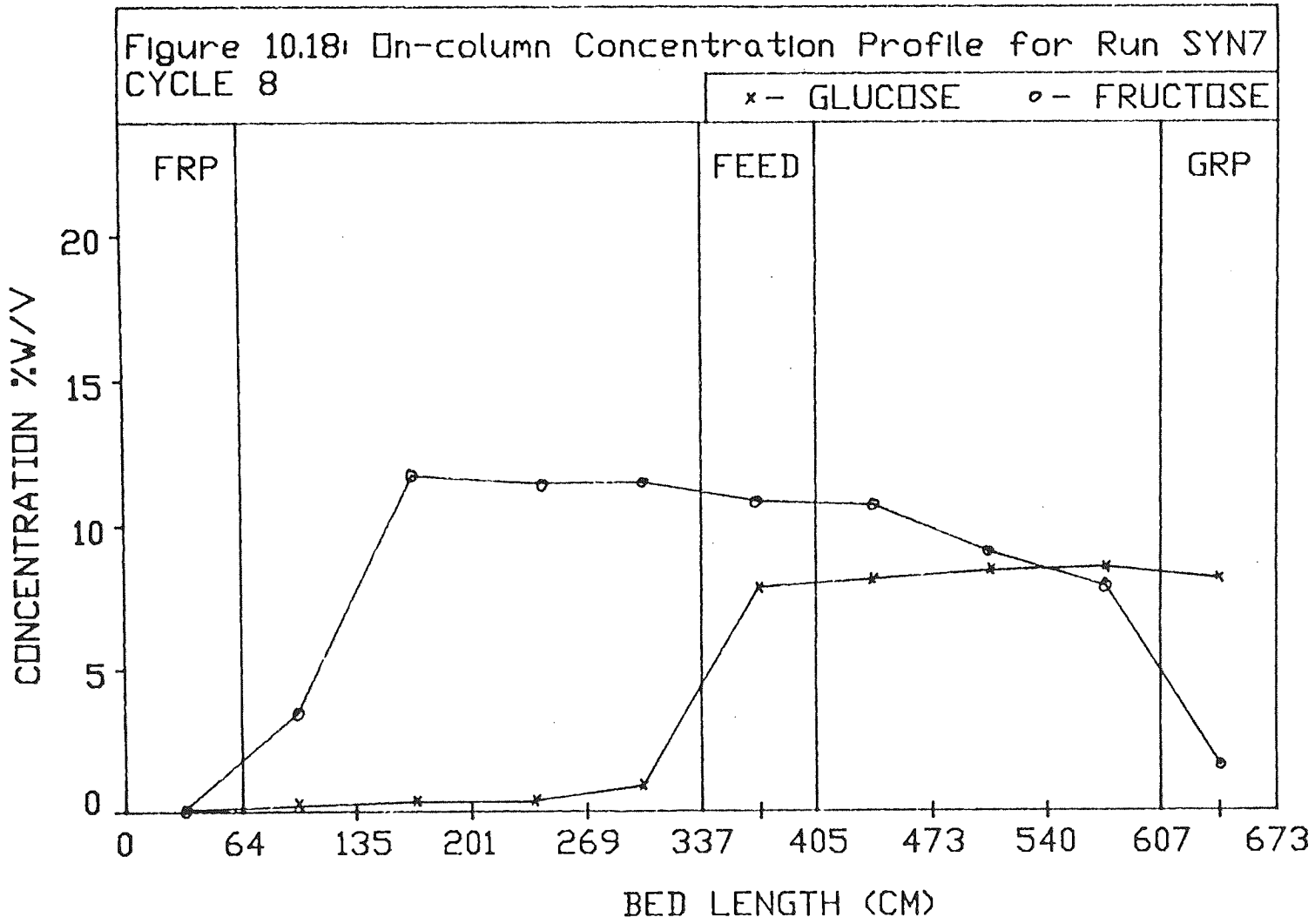


Figure 10.18: On-column Concentration Profile for Run SYN7
CYCLE 8
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

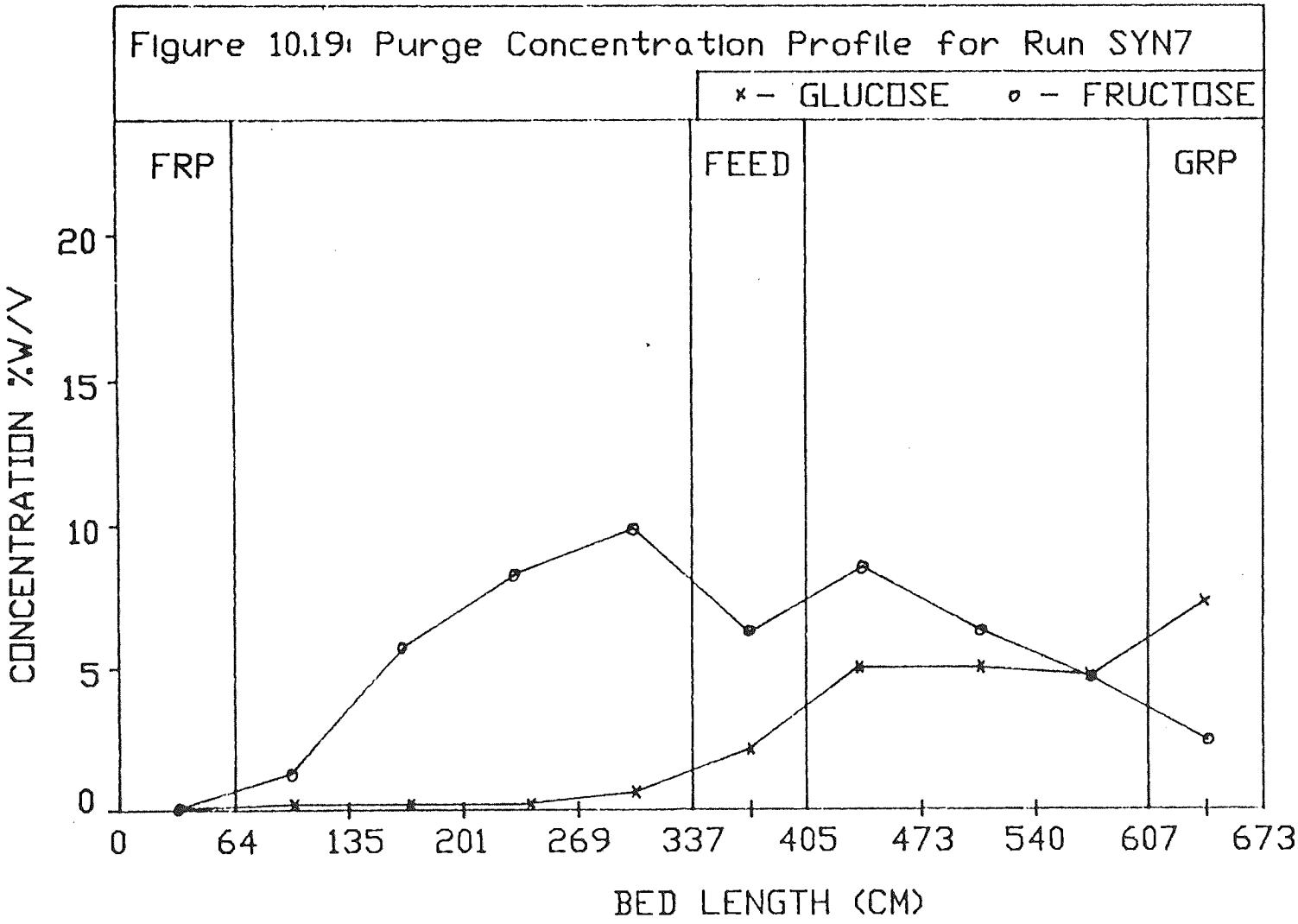


Figure 10.19: Purge Concentration Profile for Run SYN7
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

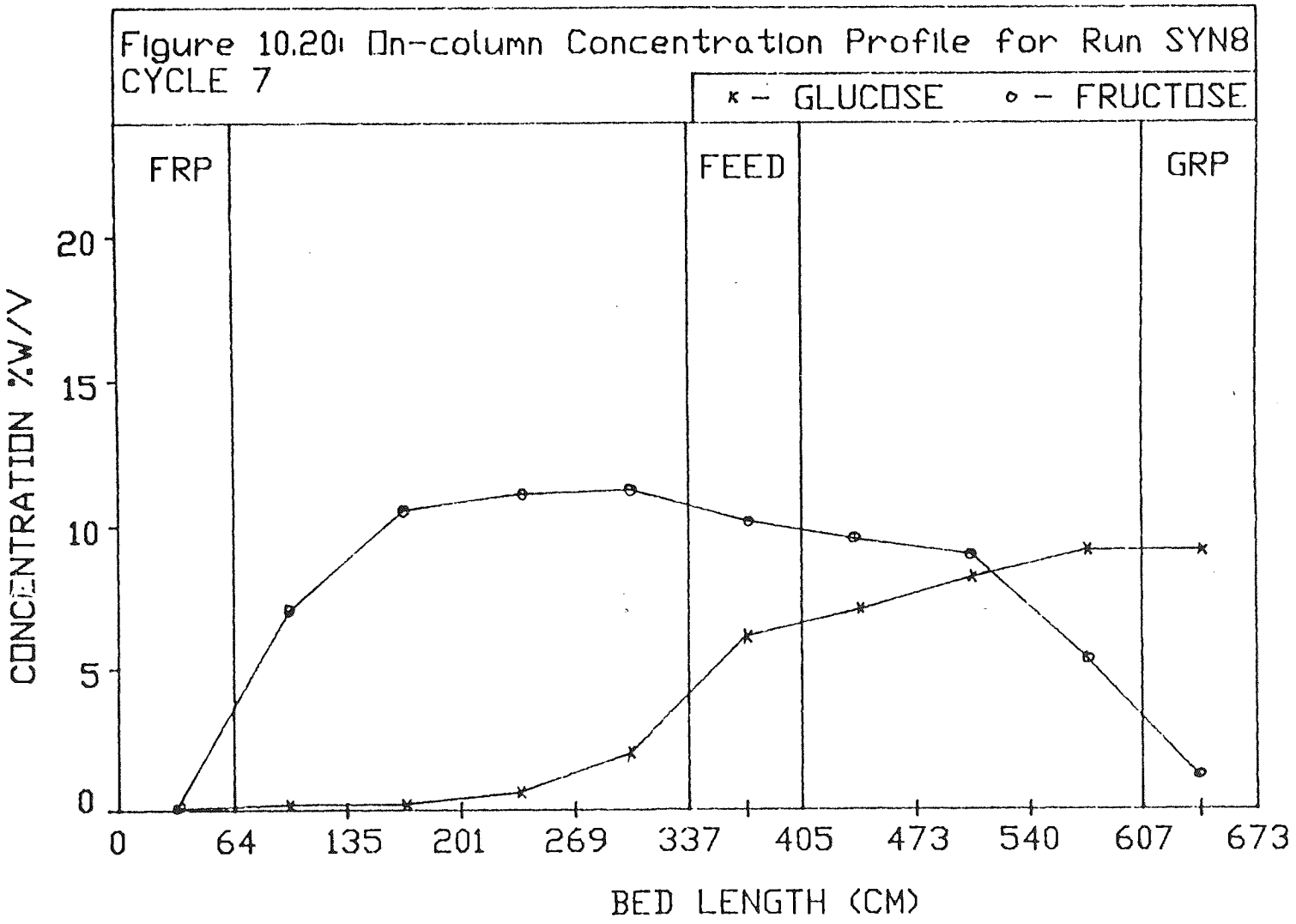


Figure 10.20: On-column Concentration Profile for Run SYN8
CYCLE 7
* - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

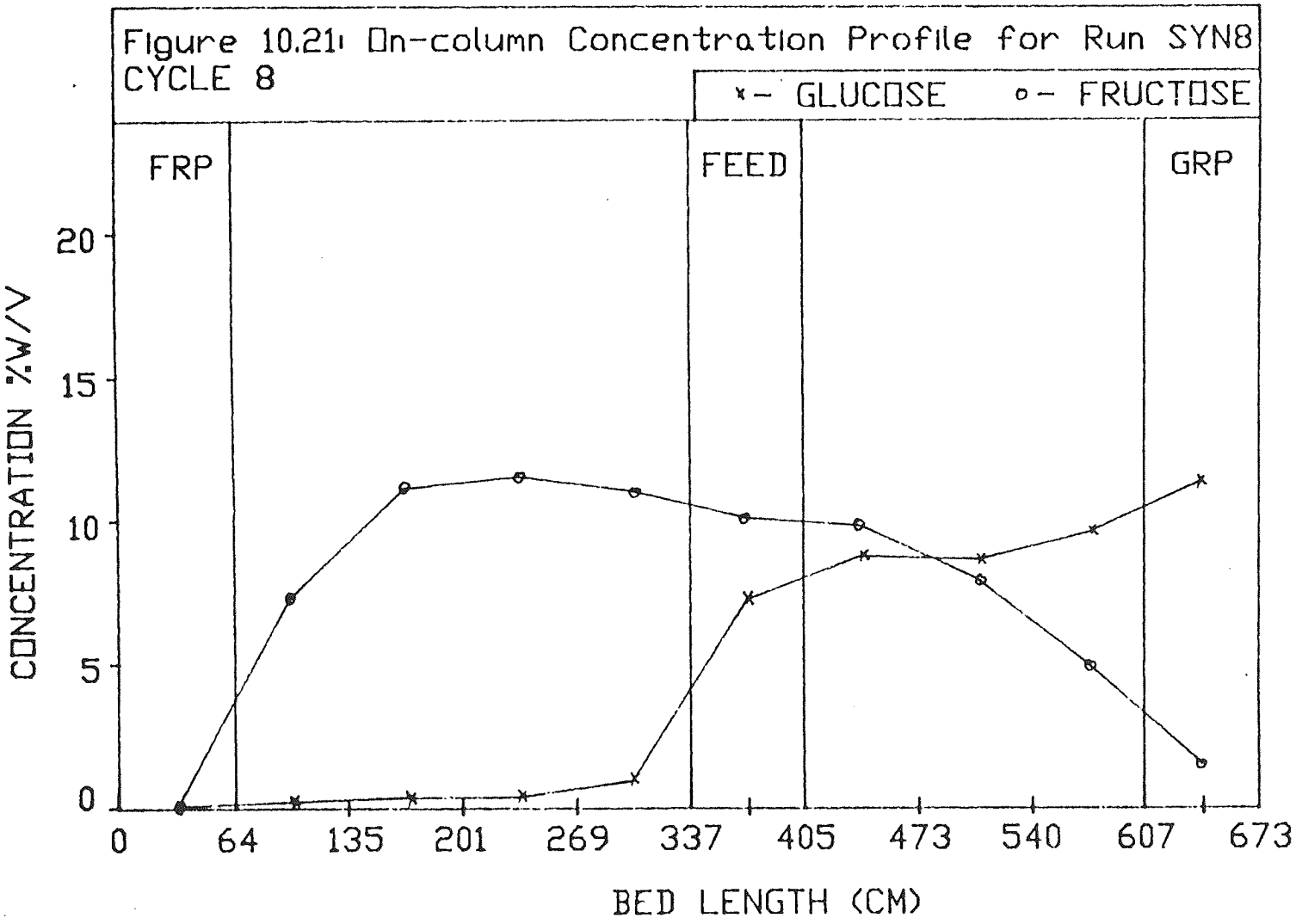


Figure 10.21: On-column Concentration Profile for Run SYN8
CYCLE 8
x- GLUCOSE o- FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

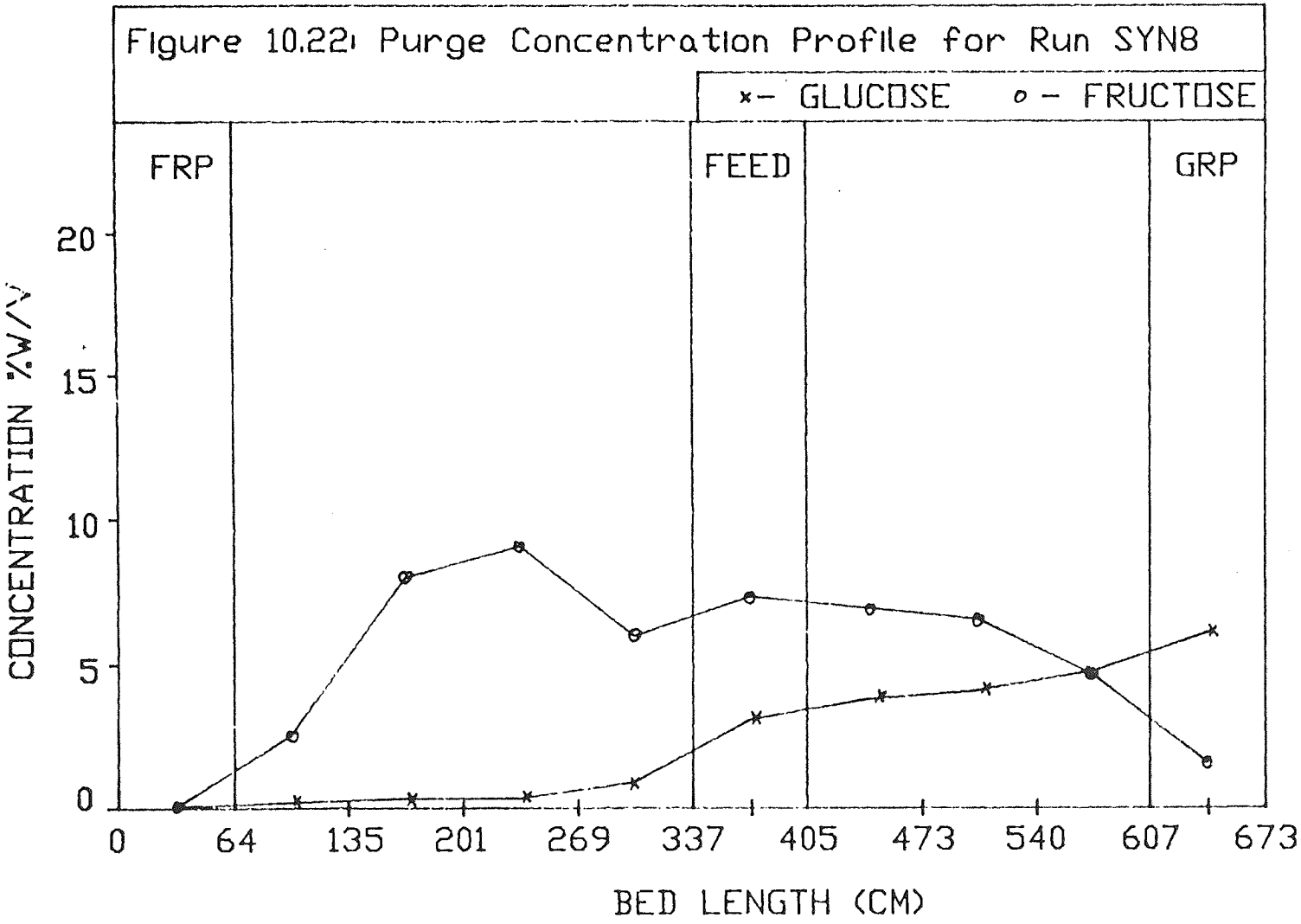


Figure 10.22: Purge Concentration Profile for Run SYN8
x- GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
20
15
10
5
0
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

CHAPTER ELEVEN

THE CONTINUOUS SEPARATION OF INVERTED SUCROSE IN BEET MOLASSES USING THE SCCR6 SYSTEM

11.1 Introduction

This chapter records the runs carried out on the SCCR6 system using the inverted molasses as feedstock. The effect of variation of switch time, calcium regeneration, feed concentration, temperature and product concentration on the system have been investigated to obtain optimum operating conditions for the recovery of fructose from inverted beet molasses using the SCCR6 system.

The experimental method to isomerise the glucose recovered to fructose using immobilised GLUCOSE ISOMERASE enzyme is also outlined in this chapter.

11.2 Commissioning Run Using Inverted Molasses as Feed

As explained in the last chapter, the sucrose in the molasses was inverted by British Sugar plc staff at the Norwich Research Laboratories using the enzyme invertase. The molasses supplied, when diluted as suggested, gave an approximately 20%w/v sugar solids concentration. Fresh feed was prepared for each cycle to reduce the opportunity for microbial growth in the feed and the equipment.

The first run, Run MOL1 was an 8 cycle run using approximately 20%w/v sugar solids in molasses as feedstock

and was carried out under the following conditions:

Feed flowrate	$35cm^3.min^{-1}$
Eluent flowrate	$105cm^3.min^{-1}$
Purge flowrate	$400cm^3.min^{-1}$
Operating temperature	60°C
Switch time	34 minutes

As this was the commissioning run using inverted molasses on the SCCR6 equipment it was necessary to monitor the bulk products from the beginning of the run. The purities of the bulk products were initially monitored on the basis of the separation of glucose and fructose only rather than on the total solids. This provided a basis for comparison with the inverted sucrose feed runs as well as with synthetic feed runs. Later, the purity of a product with respect to the total solids was determined for some runs. The purity of the bulk FRP and GRP products over each cycle for run MOL1 are as shown in Table 11.1.

The on-column concentration profiles for cycles 7 and 8 and the purge profile for this run are shown as Figures 11.1, 11.2 and 11.3 respectively. The profiles shown are for glucose and fructose only as the concentration profiles for the other components could not be evaluated individually as molasses consists of over 30 components with concentrations greater than 1%. From the experience of British Sugar personnel at Norwich it was suggested that the inverted molasses should be treated as a four component

mixture consisting of glucose, fructose, betaine and ionics. This was because the first three components were easily identified using HPLC and all the other smaller components were treated as one component which had similar properties to potassium chloride.

Table 11.1: Product Purities for Run MOL1

Cycle Number	%w/w fructose in FRP stream	%w/w glucose in GRP stream
2	85.96	69.47
3	96.49	81.10
4	94.77	74.51
5	98.91	71.22
6	98.12	67.67
7	97.50	63.19
8	95.95	61.22

Figures 11.4, 11.5 and 11.6 show the HPLC profiles for the feed, FRP and GRP for the final cycle for this run. The HPLC analysis was carried out on the ALLTECH 700CH carbohydrate column which is in the calcium form. As expected, the FRP consisted mainly of fructose together with some components of molasses which have a longer retention time than fructose on a calcium charged resin. The betaine eluted with the FRP. The GRP consisted mainly

of glucose together with some higher molecular weight compounds which were not retained by the resin. Most of the coloured components eluted with the GRP. Table 11.2 outlines the feed and products compositions and concentrations for this run.

The system was regenerated using a 10%w/v solution of calcium chloride. A sample of the spent regenerant was sent to the British Sugar Laboratories at Norwich for analysis and it was found to contain 3.1 grams of potassium ions and 1.3 grams of sodium ions per litre of the spent regenerant. As these values were higher than expected, it was decided that the resin be regenerated after every run which used molasses as feedstock. The potassium and sodium ions present in the molasses were displacing the calcium from the resin during a run, as like the hydrogen ion, they were in a higher concentration in the mobile phase and for equilibrium had to replace some calcium on the resin.

Because of the corrosion damage to the stainless steel parts of the system by the chloride ions, it was decided to replace the regenerant with calcium nitrate. From the resin manufacturers recommendations, it was observed that the regeneration has to be carried out until there are two equivalents of calcium ions per litre of resin ie. two moles of calcium ions per litre of resin. From the amount of calcium displaced, it was noted that a flowrate of $300cm^3.min^{-1}$ through each column for 30 minutes was

sufficient for the regeneration to be carried out. This was based on the regenerant being a 10%w/v solution of calcium nitrate. The calcium nitrate used was a general purpose reagent which was 99.8% pure. The calcium nitrate used was in the tetrahydrate form.

Table 11.2 Operating Conditions for Run MOL1

Run Number	Average Flow Rates cm^3/min			Feed Concentration % w/w			G:F Ratio in Feed	Switch Time Minutes	Cycle Number
	Feed	Eluent	Purge	G	F	Others			
MOL1	35	105	400	9.74	10.52		1 : 1.06	34	8

Experimental Results from Run MOL1

Run Number	Feed Throughput (sugar solids) $Kg\ h^{-1}$	Glucose Rich Product					Fructose Rich Product				
		Glucose Purity %	% of Glucose in Feed Recovered	Total Product conc^n % w/v	Impurities % F	Others	Fructose Purity %	% of Fructose in Feed Recovered	Total Product conc^n % w/v	Impurities % G	Others
MOL1	0.399	61.22	89.2	3.67	3026	8·5	95.95	101.93	0.51	-	4·05

11.3 Effect of Changing the Switch Time

11.3.1 Experimental Results and Discussion

The criterion for separation on an SCCR system indicates that the switch time is a critical operating parameter. The effect of switch time on the equipment's performance was studied by using molasses feedstocks containing approximately 20%w/v sugar solids and the experimental conditions and results are shown in Table 11.3. The three runs were carried out under identical conditions of operation except for the switch time which was different for each run. Switch times of 34, 30 and 31 minutes were used for the three runs respectively.

It was observed that a high switch time resulted in a loss of purity in the GRP stream and a purer FRP product. This was because the cross-over point had moved to column 10 which is the GRP column. This indicates that the stationary phase effective movement was too slow, causing some of the fructose to be carried forward with the mobile phase and contaminating the GRP. As a result, all the columns had more fructose than glucose in them. This can be seen from the on-column concentration profiles for the last two cycles and purge concentration profile for run MOL1 in Figures 11.1, 11.2 and 11.3.

Table 11.3 Comparison of runs MOL1, MOL2, MOL3,

Run Number	Average Flow Rates cm^3/min			Feed Concentration % w/w			G:F Ratio in Feed	Switch Time Minutes	Cycle Number
	Feed	Eluent	Purge	G	F	Others			
MOL1	35	105	400	9.74	10.52		1 : 1.08	34	8
MOL2	35	105	400	9.51	10.78		1 : 1.13	30	8
MOL3	35	105	400	9.63	10.58		1 : 1.09	31	8

Experimental Results of the Three Runs

Run Number	Feed Throughput (sugar solids) $Kg\ h^{-1}$	Glucose Rich Product					Fructose Rich Product				
		Glucose Purity %	% of Glucose in Feed Recovered	Total Product conc^n % w/v	Impurities % F	Others	Fructose Purity %	% of Fructose in Feed Recovered	Total Product conc^n % w/v	Impurities % G	Others
MOL1	0.399	61.22	89.2	3.67	30.26	8.5	95.95	101.93	0.51	–	4.05
MOL2	0.426	94.92	120.17	1.99	2.20	2.87	63.25	89.99	2.05	27.27	9.48
MOL3	0.616	87.53	97.16	2.55	9.52	2.96	87.10	79.93	1.24	16.6	-3.76

As the switch time was reduced to 30 minutes from 34 minutes, this resulted in the FRP being less pure than the GRP. The purity of the GRP increased from 61.22% to 84.92% whereas the FRP purity decreased from 95.95% to 63.25%. 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 product. Therefore only 7 columns were used effectively in the separation and the cross-over point was in the third column. Figures 11.7, 11.8 and 11.9 show the on-column concentration profiles for the last two cycles and the purge concentration profile for this run (Run MOL2) respectively.

Thus, for optimum separation of the fructose and glucose in the molasses feedstock, the switch time was between 30 and 34 minutes. As the cross-over point was nearer the feed column for the run with switch time of 30 minutes, the optimum switch time would be nearer 30 minutes rather than 34 minutes.

Run MOL3 was carried out with a switch time of 31 minutes. After reaching pseudo-equilibrium state, it was observed that the cross-over point was in the feed column with all the columns being used for the separation. The purities for the products were 87.53% for the GRP and 87.10% for the FRP. The on-column and purge concentration profiles for this run are as shown in Figures 11.10, 11.11

and 11.12 respectively.

Thus for the run conditions described earlier, the optimum switch time for the separation of glucose and fructose in beet molasses was 31 minutes. The results of these three runs using equimolar feed indicate the significance of the switch time. A comparison of the three purge concentration profiles, Figures 11.3, 11.9 and 11.12, 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 of the fructose profile is a direct result of the change in the switch time. As the switch time increases, the stationary phase effective flowrate, P, decreases resulting in increasing L/P ratios. These L/P ratios are too high, especially for the post-feed L/P in comparison to the actual K_{dF}, resulting in greater amounts of fructose moving with the mobile phase. A switch time of 31 minutes was found to be the right one giving a 87.10% pure FRP and a GRP purity of 87.53%. For this run, all the systems length was used effectively for the separation and the cross-over point was in the feed column.

11.3.2 Conclusion

The product purities and the concentration profiles clearly show the effect of switch time. To adjust the composition of the products, this operating technique

requires no location changes of the relative inlet feed or eluent positions and no equipment modifications. This technique can also be applied at any stage of the operation to improve the systems performance according to the operators requirements. The approximate value for the switch time can be obtained from the separation criterion relationship (Equation 5.9) using the distribution coefficients and the feed and mobile phase flowrates.

11.4 Modifications to the System

The purities of the three runs described above were lower than those achieved with synthetic and inverted sucrose feedstocks. This was because it was observed that as the runs progressed, the purities of the two products reached a maximum and then decreased rapidly. This was because molasses contains 5.8% potassium and 1.7% sodium expressed as a %w/w on dry substance. Therefore a feed solution of approximately 20% sugar solids contains 0.7 equivalents of potassium and sodium per kilogram of feed solution. These ions displaced the calcium from the resin reducing the amount of calcium ions on the resin with which the fructose could form a complex. Therefore the fructose would be carried with the glucose in the mobile phase.

The purities of the bulk FRP and GRP products over each cycle for the runs MOL2 and MOL3 are shown in Tables 11.4 and 11.5 respectively. The purities for the two

products for each cycle for Run MOL1 were shown in Table 11.1 earlier. From these three tables, it can be seen that the purities reached a maximum at cycle 4 or 5 and then decreased towards the end of the run. From the analysis of the feed and products for all the cycles and a sample of resin after each run for the presence of sodium and potassium ions, it was noted that the calcium ion concentration on the resin was reduced to less than 2 equivalents/litre of resin by cycle 3. This loss of calcium ions on the resin was sufficient to prevent some of the fructose from forming a complex and therefore it was carried with the mobile phase. This resulted in lower purities for the FRP and the GRP as the runs proceeded. Figures 11.13, 11.14 and 11.15 show the % purity of the bulk products against the cycle number for the runs MOL1, MOL2 and MOL3 respectively and all these show a drop in purity as the run proceeds.

Table 11.4: Product Purities for Run MOL2

Cycle Number	%w/w fructose in FRP stream	%w/w glucose in GRP stream
2	95.84	95.87
3	94.94	97.58
4	90.75	94.35
5	80.92	88.62
6	76.73	87.79
7	73.28	86.05
8	63.25	84.92

Table 11.5: Product Purities for Run MOL3

Cycle Number	%w/w fructose in FRP stream	%w/w glucose in GRP stream
2	66.54	88.56
3	97.54	88.24
4	93.37	93.03
5	95.00	91.60
6	92.77	89.18
7	97.07	85.00
8	87.10	87.53
9	86.85	86.98
10	72.58	78.75

As the purities of the bulk products decreased from around cycle 4 or 5 in each run, the system could not be operated continuously without modifications to the operating procedures to prevent the loss of calcium ions from the resin. Two techniques of addition of the calcium to the resin were developed. The first involved the addition of calcium nitrate to the eluent and feed to maintain a balance of calcium ions on the resin. The second involved regenerating the resin in the purge column after the fructose had been purged off the resin. This process required modifications to the system in terms of additional vessels, pumps, timers and valves.

These procedures are described in detail in the next section.

It was observed that if the system was stopped overnight and then restarted in the morning, polysaccharide gums formed in the columns overnight resulting in a large pressure gradient across the columns. This caused the lines to burst resulting in abandoned runs. From the experience of the research personnel at the British Sugar Laboratories at Norwich in the problem of gum formations in equipments, it was recommended that the runs were carried out continuously without any stoppages at night to reduce the chance of these gums being formed in the equipment.

11.5 Effect of Addition of Calcium Nitrate

As mentioned earlier, the purities of the two bulk products was decreasing as the run progressed due to the loss of calcium ions on the resin. Two techniques of calcium addition to the resin were developed and tested over the period of this research.

11.5.1 Addition of Calcium to the Eluent and Feed

The calcium ions were added to the eluent and the feed to prevent significant loss of calcium ions from the resin. The salt used was calcium nitrate as it was very soluble in water and the nitrate salts generated when in contact with other cations in the molasses were soluble as well. This prevented the system from being blocked by any solid precipitates formed.

A solution of calcium nitrate was made up which when mixed with the molasses during its dilution, gave a 0.5%w/v solution of calcium nitrate in the feed.

A 20%w/v solution of calcium nitrate was made up and pumped at a flowrate of 5.25$cm^3.min^{-1}$ into the eluent stream using a metering pump. The eluent pump was pumping deionised water at 99.75$cm^3.min^{-1}$ and the combined stream gave a 1%w/v solution of calcium nitrate in the eluent stream pumping at 105$cm^3.min^{-1}$ into the system.

Thus enough calcium ions were being added to the system to maintain a balance of calcium ions on the resin.

This series of runs were carried out at 60°C.

Run MOL4 was carried out continuously over 8 cycles and the purity of the bulk FRP and GRP products over each cycle are as shown in Table 11.6 below. The on-column concentration profiles for cycles 7 and 8 and the purge concentration profile for this run are shown as Figures 11.16, 11.17 and 11.18 respectively. Table 11.7 shows the operating details for this run. The system developed faulty valves during the purging process resulting in an unrepresentative purge concentration profile. The purities and profiles shown are based on glucose and fructose only.

Table 11.6: Product Purities for Run MOL4

Cycle Number	%w/w fructose in FRP stream	%w/w glucose in GRP stream
2	97.01	98.73
3	74.64	96.20
4	85.96	98.52
5	81.09	98.04
6	78.75	98.37
7	78.12	97.86
8	74.99	98.37

The addition of the calcium nitrate to the eluent and feed streams seems to have stabilised the decrease in

Table 11.7 Operating Conditions for Run MOL4

Run Number	Average Flow Rates cm^3/min			Feed Concentration % w/w			G:F Ratio in Feed	Switch Time Minutes	Cycle Number
	Feed	Eluent	Purge	G	F	Others			
MOL4	35	105	400	16.88	16.88		1 : 1	31	10

Experimental Results from Run MOL4

Run Number	Feed Throughput (sugar solids) $Kg\ h^{-1}$	Glucose Rich Product					Fructose Rich Product				
		Glucose Purity %	% of Glucose in Feed Recovered	Total Product conc^n % w/v	Impurities % F	Others	Fructose Purity %	% of Fructose in Feed Recovered	Total Product conc^n % w/v	Impurities % G	Others
MOL4	0.709	57.05	64.82	2.20	41.31	1.64	71.47	39.57	1.00	21.75	6.78

purity of the products. The switch time had to be adjusted again to give a higher purity FRP and a slightly less pure GRP. The on-column profile for cycle 8 shows the cross-over point to be at column 3. Thus to get a purer FRP, it was necessary to move the cross-over point nearer to the feed column. Therefore the switch time had to be increased to 32 minutes for the next run.

The faulty valves mentioned earlier were opened and the brass fittings that were in contact with the molasses inside the valve had a copper film deposited on them. This was assumed to have been caused by a reaction with the acidic molasses (pH 5.6). This was tested and later confirmed to be the case. Thus from this run onwards, the inverted molasses was neutralised to pH 7.0 before using it on the SCCR6.

The addition of calcium nitrate to the system in the eluent and feed streams moved the cross-over point to the pre-feed section of the system resulting in a less pure FRP. This was probably due to some of the fructose complexing with the calcium ions in the mobile phase and being carried forward with the mobile phase resulting in less fructose being left on the resin. This would result in the overall FRP product having less fructose in it. Thus the purity of the FRP would be low as far as the amount of fructose present in relation to the other components is concerned.

It seemed that the fructose in the mobile phase was not being carried through the entire length of the system but was being left on the resin in the middle columns. This occurs because as the calcium ions are being displaced from the resin by the sodium and potassium ions, these two ions are again displaced by the calcium in the mobile phase. Some of this calcium is complexed to the fructose in a high concentration zone of fructose in the middle section of the separating unit. This has to be the case because the GRP is pure with less than 2% fructose present.

Run MOL5 was carried out under similar conditions to run MOL4 except for the switch time which was increased to 32 minutes and the feed molasses neutralised to pH 7.0. This run was carried out continuously for 10 cycles. The calcium nitrate was added to the system in similar proportions as those used for run MOL4.

The bulk products were analysed for each cycle and the purities of the products are shown in Table 11.8. From these purities, it can be seen that the addition of calcium nitrate to the system seems to have reduced the decrease in purity as the run proceeds and could have stabilised the product purities.

The on-column concentration profiles for cycles 7, 8, 9 and 10 and the purge concentration profile for the run MOL5 are shown in Figures 11.19 to 11.23 respectively. Table 11.9 shows the operating details for the run.

Table 11.8: Product Purities for Run MOL5

Cycle Number	%w/w fructose in FRP stream	%w/w glucose in GRP stream
2	100.00	98.86
3	100.00	98.18
4	88.96	98.23
5	83.25	96.46
6	84.32	94.86
7	82.95	93.13
8	86.04	90.87
9	87.62	90.82
10	86.84	88.39

The increase in switch time to 32 minutes has increased the FRP purity slightly and brought the cross-over point to column 5.

To confirm that the addition of calcium nitrate to the eluent and feed streams in the concentrations stated stabilises the decrease in purity of the products, it was necessary to analyse the purge samples obtained from each column at the end of the run for the presence of calcium, potassium and sodium ions. The concentration of each of these ions in each column helps to determine the columns most susceptible to ion displacement.

Table 11.9 Operating Conditions for Run MOL5

Run Number	Average Flow Rates cm^3/min			Feed Concentration % w/w			G:F Ratio in Feed	Switch Time Minutes	Cycle Number
	Feed	Eluent	Purge	G	F	Others			
MOL5	35	105	380	13.18	12.94		1 : 0.98	32	10

Experimental Results from Run MOL5

Run Number	Feed Throughput (sugar solids) $Kg\ h^{-1}$	Glucose Rich Product					Fructose Rich Product				
		Glucose Purity %	% of Glucose in Feed Recovered	Total Product conc^n % w/v	Impurities % F	Others	Fructose Purity %	% of Fructose in Feed Recovered	Total Product conc^n % w/v	Impurities % G	Others
MOL5	0.549	88.39	98.89	2.56	2.32	9.29	86.84	82.01	1.02	2.84	10.32

Table 11.10 shows the distribution of these ions in the system at the end of the run. From the table, it can be seen that there was a high concentration of calcium ions in columns 4 and 6. The potassium ion concentration was high in the last four columns. Therefore in these columns the calcium on the resin and in solution was keeping the potassium in solution rather than being displaced on the resin. There was a significant rise in sodium ion concentration in column 4. This column also has the highest calcium concentration in solution for the system.

To prove that the system had stabilised, it was necessary to carry out a further run of at least 12 cycles continuously to observe any loss of purity in the two products after cycle 8. For this run the switch time had to be further increased to bring the cross-over point to the feed column and thereby increasing the FRP purity further.

Run MOL6 was carried out under similar conditions as for the previous two runs except for the switch time which was increased to 33 minutes. The products were analysed after every cycle and the purities obtained are as shown in Table 11.11. From these purities, it can be seen that the addition of calcium nitrate has stabilised the decrease in product purity as the run proceeds. The amount of calcium nitrate consumed for this and the previous two runs was $80g.h^{-1}$.

Table 11.10: Distribution of cations for Run MOL5

Column Number	CALCIUM		SODIUM		POTASSIUM	
	mg/l	% on DS	mg/l	% on DS	mg/l	% on DS
1 FRP	170	5.7	44	1.5	50	1.7
2	895	2.4	456	1.2	1800	4.8
3	3635	2.3	800	0.5	2275	1.5
4	6848	2.7	1300	0.5	2625	1.0
5	3312	2.3	775	0.5	1813	1.3
6	5780	2.9	788	0.4	2338	1.2
7	4540	2.6	725	0.4	3063	1.7
8	4700	2.7	756	0.4	2875	1.1
9	4040	2.7	738	0.5	3188	2.1
10 GRP	2560	2.2	913	0.8	3313	2.9

DS = Dry solids basis

Table 11.11: Product Purities for the Run MOL6

Cycle Number	%w/w fructose in FRP stream	%w/w glucose in GRP stream
2	59.53	100.00
3	88.47	95.64
4	90.56	93.13
5	94.05	86.96
6	92.12	77.14
7	94.31	75.85
8	93.75	73.73
9	96.84	80.77
10	92.54	82.81
11	92.05	89.75
12	94.05	87.17

The on-column concentration profiles for cycles 7, 8, 9, 10, 11 and 12 and the purge concentration profile for this run MOL6 are shown as Figures 11.24 to 11.30 respectively. The profiles for cycles 7 and 8 are included as a comparison with the previous runs which were carried out up to cycle 8 only and the other profiles show the system stability from cycle 9 up to cycle 12.

From these profiles it can be seen that by increasing the switch time to 33 minutes from the previous runs, which were carried out at 31 and 32 minutes, the cross-over point

was now in the feed column and purities of about 90% were obtained for the two products.

The operating details for this run MOL6 are shown in Table 11.12. The mass balances for the run are shown in Table 11.13. From the product concentrations, compositions and densities, the refractive dry substance (RDS) values for the two products were evaluated and are shown in Table 11.14. These RDS values indicated the actual solids concentration in the products thereby giving the actual fructose and glucose purities with respect to the total solids.

It can be seen that the optimum conditions for the operation of the SCCR6 system to obtain around 90% purities (based on glucose and fructose only) for both products from inverted molasses are as those used for run MOL6.

As the concentration of the bulk products was low, it was possible to collect the products over a part of a switch only. The theory and necessary modifications required for the system to carry this out are described in Section 11.5.3.

Table 11.12 Operating Conditions for Run MOL6

Run Number	Average Flow Rates cm^3/min			Feed Concentration % w/w			G:F Ratio in Feed	Switch Time Minutes	Cycle Number
	Feed	Eluent	Purge	G	F	Others			
MOL6	35	105	380	11.70	11.98		1 : 1.02	33	12

Experimental Results from Run MOL6

Run Number	Feed Throughput (sugar solids) $Kg\ h^{-1}$	Glucose Rich Product					Fructose Rich Product				
		Glucose Purity %	% of Glucose in Feed Recovered	Total Product conc^n % w/v	Impurities % F	Others	Fructose Purity %	% of Fructose in Feed Recovered	Total Product conc^n % w/v	Impurities % G	Others
MOL6	0.497	87.17	80.09	3.88	2.30	10.53	94.05	80.74	1.27	2.35	3.60

Table 11.13: Mass balances for Cycles 7-12 for Run MOL6

Cycle No.	Feed inlet %w/v G	Feed inlet %w/v F	Total mass IN (kg) G	Total mass IN (kg) F	Mass of FRP collect Kg	FRP out %w/v G	FRP out %w/v F	Mass OUT in FRP G	Mass OUT in FRP F
7	12.46	12.59	1.264	1.227	108.9	0.08	0.55	0.087	0.599
8	14.12	13.66	1.432	1.385	115.8	0.04	0.67	0.046	0.776
9	14.86	14.21	1.482	1.417	111.2	0.04	1.47	0.044	1.653
10	12.40	12.63	1.257	1.281	113.5	0.13	1.65	0.148	1.873
11	11.36	11.49	1.152	1.165	119.1	0.07	0.86	0.084	1.031
12	11.70	11.98	1.180	1.208	115.8	0.20	1.07	0.232	1.239

Cycle No.	GRP out %w/v G	GRP out %w/v F	Mass OUT in GRP G	Mass OUT in GRP F	Mass of GRP collect Kg	Total mass OUT (kg) G	Total mass OUT (kg) F	% out in G	% out in F
7	1.85	1.07	0.771	0.446	41.7	0.858	1.045	67.9	85.2
8	2.87	1.02	1.225	0.436	42.7	1.271	1.212	88.8	87.5
9	1.78	0.42	0.767	0.181	43.1	0.811	1.816	54.7	128.2
10	1.40	0.29	0.591	0.122	42.2	0.739	1.997	58.8	155.9
11	3.75	0.42	1.601	0.179	42.7	1.685	1.210	146.3	103.9
12	3.39	0.49	1.386	0.200	40.9	1.618	1.439	137.1	119.1

Table 11.14: RDS of Feed and Products for Run MOL6

SAMPLE	RDS OF SAMPLE
Feed	33.23
FRP	0.71
GRP	4.82

11.5.2 Discussion and Conclusion

All these experiments have been repeated and similar results within the accuracy of the experiments were obtained.

The addition of calcium nitrate to the system in the eluent and feed streams affected the separation of the fructose and glucose.

The switch time had to be increased from 31 minutes when no calcium ions were added to the system to 33 minutes when calcium ions were added to achieve similar product purities.

From the purities of the two products obtained in run MOL6, it can be seen that the FRP purity was constant from around cycle 5 at approximately 92%w/w fructose content and the GRP purity dropped around cycle 8 to 73%w/w glucose content but stabilised later around 87%w/w glucose in the product. This showed that there was enough calcium ions in the system to replace those being displaced from the resin

by the potassium and sodium ions in the molasses.

The need to increase the switch time by 2 minutes could be explained by the fact that some of the fructose was forming complexes with the calcium ions in the mobile phase and was being carried forward with the mobile phase rather than being left on the resin. Therefore not enough fructose was being held on the resin indicating that the overall FRP product had less fructose in it resulting in a less pure FRP.

A major problem with this technique of regeneration of the resin was that the two products were contaminated with the added calcium nitrate. Therefore another method to regenerate the resin during the run was required where the calcium nitrate was not mixed with the products.

It was known that the bulk of the solids in the FRP stream were eluted in the first part of a switch and the latter part contained mainly the purge water. Similarly, for the GRP stream, the first part of a switch contained mainly water with the solids eluted in the latter part of the switch. For the SCCR6 system, to evaluate the exact solids elution profiles, it was necessary to collect each of the products over a two minutes interval as they were eluted at the end of the corresponding product lines and analysed. The individual elution profiles were obtained by plotting the total sugar concentrations against time of elution. The total solids concentration would have a

similar profile as all the non-sugar solids are eluted at similar times as the sugars. These profiles provided the time intervals after which the products were being diluted. The elution profiles for the FRP and GRP for the final cycle in run MOL6 are shown in Figures 11.31 and 11.32 respectively. From Figure 11.31, it can be seen that all the sugars are eluted in the first 20 minutes of the switch. The rest of the switch is adding water to the product diluting it further. Similarly, from Figure 11.32, it can be seen that the sugars start eluting after the first 16 minutes of the switch. This means that for the first 16 minutes, the GRP is being diluted with water. Both these profiles are 'delayed' with respect to the 'actual' ones which are obtained from the column exit ports rather than the system exit ports. This was due to the relatively long length of the system's product line network between the column exit ports and the actual product collection port. Thus the concentration values for the first 2 minutes in both profiles are incorrect. 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 dead volumes of the respective networks.

This 'product splitting' technique can be used to increase the concentration of the two products. It can also be used to regenerate the resin without the regenerant mixing with the 'separation part' of the system. This procedure is explained in the next section.

11.5.3 Addition of Calcium to the Purge Column

As most of the sugars eluted in the first 16 minutes of the switch in the FRP stream, it was possible to stop collecting the product and use the remainder of the switch to pump calcium nitrate through the column to regenerate the resin. As the purge column became the GRP column in the next switch, it was possible to flush the calcium nitrate off the column before the product eluted after the first 16 minutes of the switch. To do this the system had to be modified as shown in Figure 11.33. Two 3-way solenoid valves were connected to a timer to control the calcium nitrate intake and the FRP splitting. One 3-way solenoid valve was connected to a timer to control the product splitting on the GRP line. Both these timers were synchronised to switch on or off 16.5 minutes into a switch. Due to limitations on the timers, they could operate on and off for the same period ie. on for 16.5 minutes and off for 16.5 minutes. Thus it was not possible to have independent switch periods for the on and off positions on these timers. As the switch time was 33

minutes the switch period for product splitting had to be 16.5 minutes for the timers to be in synchronisation with the main SCCR6 timer.

The system was started with the valve on the calcium nitrate reservoir in the closed position allowing the deionised water into the purge column. The valve on the FRP outlet was open in the FRP collection period and closed on the calcium nitrate effluent reservoir. After 16.5 minutes, the timer switched both these valves 'off' in that the calcium nitrate was now pumped into the purge column and the effluent reservoir opened. This reset to the initial setting after another 16.5 minutes. Similarly for the first 16.5 minutes of a switch, the valve on the GRP line was positioned in the GRP 'drain' line and switched on to the GRP collection after 16.5 minutes. So, for the first 16.5 minutes of the switch the calcium nitrate in the column was being flushed out. In this way the regeneration could be carried out semicontinuously to one column at a time without any contamination of the products.

It should be noted that the elution profiles for the two products would be similar for different switch times for the system. The elution profiles would change only with a change in the flowrates of the eluent, feed or purge entering the system.

Four runs were carried out using this method of resin regeneration.Run MOL13 was carried out under the following

conditions of operation:

- Feed concentration 50% total solids
- Temperature 60°C
- Feed flowrate $35cm^3.min^{-1}$
- Eluent flowrate $105cm^3.min^{-1}$
- Purge flowrate $300cm^3.min^{-1}$
- Switch time 36 minutes
- FRP switch over time 18 minutes
- GRP switch over time 18 minutes

For this run the feed concentration had been increased from 30% total solids to 50% total solids. The 3 minute increase in switch time was to account for the increase in feed concentration. If this method of regeneration was not significantly different from the first method, then the product purities for this run would be similar to those obtained for run MOL6. Run MOL13 was carried out over 9 cycles continuously and the products analysed after each cycle. Table 11.15 below shows the product purities for this run in terms of glucose and fructose only. From the table, it can be seen that the fructose rich product purity has decreased from 87% in run MOL6 to 52.73% for this run. The GRP purity has increased from 94% in run MOL6 to 96.95% for this run. Thus the addition of calcium nitrate in the purge column has affected the separation of the sugars resulting in a purer GRP. The glucose was not being carried

forward with the mobile phase as fast as for the previous runs, resulting in a less pure FRP.

Table 11.15: Product Purities for the Run MOL13

Cycle Number	%w/w fructose in FRP stream	%w/w glucose in GRP stream
2	61.93	90.75
3	63.67	95.61
4	57.81	99.14
5	56.36	97.77
6	56.92	97.52
7	54.38	98.13
8	54.19	98.01
9	52.73	96.95

The operational conditions and details for the run MOL13 are shown in Table 11.16. The RDS values for the feed and the 'four' products (both parts for each product) for the run MOL13 are shown in Table 11.17. The on-column concentration profiles for cycles 8 and 9 and the purge concentration profile for the run are shown as Figures 11.34, 11.35 and 11.36 respectively. From these figures it can be seen that the cross-over point was well to the left in the FRP section which accounted for the less pure FRP product. Thus an increase in switch time is necessary to

return the cross-over point to the feed column.

Table 11.16 Operating Conditions for Run MOL13

Run Number	Average Flow Rates cm^3/min			Feed Concentration % w/w			G:F Ratio in Feed	Switch Time	Cycle Number
	Feed	Eluent	Purge	G	F	Others			
MOL13	35	105	300	22.33	22.75		1 : 1.02	36	9

Experimental Results from Run MOL13

Run Number	Feed Throughput (sugar solids) $Kg\ h^{-1}$	Glucose Rich Product					Fructose Rich Product				
		Glucose Purity %	% of Glucose in Feed Recovered	Total Product conc^n % w/v	Impurities % F	Others	Fructose Purity %	% of Fructose in Feed Recovered	Total Product conc^n % w/v	Impurities % G	Others
MOL13	0.947	96.95	89.70	4.32	1.59	1.46	52.73	92.98	9.29	22.29	24.98
			"Drained" →	0.24				"Drained" →	0.44		

Table 11.17: RDS of Feed and Products for Run MOL13

SAMPLE	RDS OF SAMPLE
Feed	52.76
FRP 'collect'	10.31
FRP 'drained'	0.64
GRP 'collect'	8.16
GRP 'drained'	0.43

The products are labelled 'collected' and 'drained' depending on whether it was the concentrated part of the product or was the calcium nitrate contaminated part respectively.

The mass balances for the final cycle gave a 100.05% value for total solids mass balance and a total sugar mass balance of 95.05%.

Further runs were carried out on the SCCR6 system using this system of resin regeneration but with increasing switch time to return the cross-over point to the feed column.

Run MOL16 was carried out under identical conditions as for run MOL13 except for the switch time which was increased to 42 minutes. This run was carried out over nine cycles and the product purities for the final cycle were 97% glucose in the GRP and 96% fructose in the FRP. Both the FRP and the GRP had purities above 95% and thus the correct switch time and other operating parameters had been obtained for a 50% total solids molasses feedstock separation.

The product purities for each cycle for the run MOL16 are shown in Table 11.18. From this table, it can be seen that the product purities are stable and there is no significant loss of purities as the run progressed. Figures 11.37, 11.38 and 11.39 show the on-column concentration profiles for cycles 8 and 9 and the purge concentration profile for the run respectively. Table 11.19 shows the operating details for the run MOL16. Table 11.20 shows the product concentrations for the 'four' products and the feed for the run.

Table 11.18: Product Purities for the Run MOL16

Cycle Number	%w/w fructose in FRP stream	%w/w glucose in GRP stream
2	98.10	96.86
3	96.96	94.13
4	97.54	96.40
5	97.72	96.94
6	97.15	98.07
7	96.38	98.22
8	96.23	98.76
9	95.84	97.00

The mass balances for this run were 100% for both the solids and the sugars balance.

Table 11.19 Operating Conditions for Run MOL16

Run Number	Average Flow Rates cm^3/min			Feed Concentration % w/w			G:F Ratio in Feed	Switch Time	Cycle Number
	Feed	Eluent	Purge	G	F	Others			
MOL16	35	105	300	25.31	25.74		1 : 1.02	42	9

Experimental Results from Run MOL16

Run Number	Feed Throughput (sugar solids) $Kg\ h^{-1}$	Glucose Rich Product					Fructose Rich Product				
		Glucose Purity %	% of Glucose in Feed Recovered	Total Product conc^n % w/v	Impurities % F	Others	Fructose Purity %	% of Fructose in Feed Recovered	Total Product conc^n % w/v	Impurities % G	Others
MOL16	1.072	97.00	97.91	4.68	1.26	1.74	95.84	95.10	10.11	0.52	3.64
			"Drained" →	0.47				"Drained" →	0.50		

Table 11.20: RDS of Feed and Products for Run MOL16

SAMPLE	RDS OF SAMPLE
Feed	53.51
FRP 'collect'	10.93
FRP 'drained'	0.48
GRP 'collect'	10.07
GRP 'drained'	0.44

11.5.4 Discussion and Conclusion

Three methods of resin regeneration have been studied:-

- regeneration of the resin after every run ie. after nine cycles of operation.
- regeneration of the resin continuously by the addition of calcium in the eluent and feed inlet streams.
- regeneration of the resin semicontinuously by the addition of calcium to the purge column after the purge products have been removed from the column.

The first method did not help to maintain enough calcium ions on the resin to enable the fructose and glucose separation. The second method worked well and enough calcium ions were on the resin to enable the fructose to form the complex for separation. But the drawback with this method was the resultant contaminated products which would require further treatment in a commercial plant.

The third method resulted in both products having purities of over 95% and there was no contamination due to the addition of calcium nitrate to the system. This was proved by analysing the products for the presence of calcium ions.

The change in the calcium nitrate addition to the system resulted in an increase in switch time from 30

minutes for run MOL3 to 33 minutes for run MOL6 to 42 minutes for run MOL16. Except for the feed concentration increase in run MOL16, the operating parameters were consistent for all these runs. The reason for the increase in switch time between runs MOL3 and MOL6 was discussed earlier in section 11.5.2. The increase switch time was also due to the increased feed concentration. The switch time used for run MOL16 was the ideal switch time for a 50% total solids molasses feedstock separation. For this run the resin was fully charged in the calcium form and the regeneration was not affecting the separation section of the system.

11.6 Effect of Change of Feed Concentration

The throughput increases if the feed concentration increases. This however has a detrimental effect on the separating efficiency of the system as the solids concentration increases and leads eventually to equipment overload. As described in Chapter 6, the drop in separation efficiency is a direct result of the increasing background sugar concentrations on the distribution coefficients K_d's. The separation efficiency is also affected by the increased concentration gradient and the viscosity.

Thawait (9) carried out a series of experiments on the SCCR6 using synthetic feedstocks of increasing concentrations and the results she obtained are shown in Figure 11.40. Table 11.22 shows a summary of the conditions and results of the experiments used for this evaluation. The figure shows the variation in the glucose concentration profiles with increasing concentration using a 50:50 fructose and glucose mixture operating at 20°C and keeping the switch time constant at 30 minutes. As the concentration of the feed increases the cross-over point moves towards the FRP column. The general shape of the glucose and fructose profiles individually remain similar.

The effect of increasing the feed concentration was not studied specifically during this research program as significant work has been carried out in the past using synthetic feeds to study the effect. Molasses feedstocks of

approximately 20% sugar solids and 65% sugar solids in molasses were used during this research and the trend obtained was similar to that obtained for synthetic feedstocks. A comparison of the runs using these different feed concentrations gave similar results to those obtained by Thawait (9).

Table 11.22: Run Conditions for Varying Feed Concentration (9)

Run Number	Average Flowrates $cm^3.min^{-1}$			Feed Concn %w/v		Switch Period min
	Eluent	Feed	Purge	Glucose	Fructose	
1	105	35	550	10.0	10.0	30
2	105	35	550	15.0	15.0	30
3	105	35	550	17.5	17.5	30
4	105	35	550	20.0	20.0	30
5	105	35	550	25.0	25.0	30
6	105	35	550	30.0	30.0	30

Run Number	Feed T'put Kg/hr	Fructose Rich Product Purity %	Fructose Rich Product Product concn	Glucose Rich Product Purity %	Glucose Rich Product Product concn
1	0.42	99.9	0.64	99.9	2.37
2	0.63	99.9	0.94	99.9	3.59
3	0.735	99.9	1.28	99.9	4.2
4	0.84	88.0	1.29	99.9	3.6
5	1.05	82.0	1.74	99.9	4.5
6	1.26	77.0	1.79	99.9	5.0

11.7 Effect of Change of Temperature

From Chapter 6 it was shown that the effect of temperature on the SCCR6 system would affect the fructose concentration on the resin but not the glucose concentration. Thawait (9) carried out a series of experiments on the SCCR6 using synthetic feed mixtures at temperatures of 20°, 30°, 45° and 60°C and the fructose profiles for these runs are shown in Figure 11.41. Table 11.23 summarises the operating conditions and results for the experiments she used to investigate the effect of temperature on the separation capability of the SCCR6.

As the temperature of operation increases, the cross-over point moves to the right towards the GRP column. No significant difference is observed if the glucose profile alone is studied and the glucose concentration in the GRP remains the same. However, when the fructose profile is studied, it can be seen that a significant amount of fructose has travelled with the mobile phase and into the post feed section. As the temperature is increased, the amount of fructose travelling with the mobile phase is enhanced and consequently a contamination in the GRP occurs.

As explained in chapter 4 , the isomeric form of fructose that forms the complex with calcium is β-D-fructopyranose. The concentration of this form of fructose in equilibrium decreases with increasing temperature. Hence

fewer fructose molecules are able to form a complex with the calcium ions which reduces the retention of fructose, hence more fructose travels with the mobile phase.

The effect of temperature on the SCCR6 system using molasses feedstocks is expecter to behave in a similar way. This is because from the K_d determination work reported in Chapter 6, it was seen that none of the components of inverted molasses except fructose was affected by temperature as was the case using synthetic feedstocks.

Table 11.23: Run Conditions for the Effect of Temperature (9)

Run Number	Average Flowrates $cm^3.min^{-1}$			Feed Concn %w/v		Temp. °C
	Eluent	Feed	Purge	Glucose	Fructose	
1	105	35	550	10.1	10.2	20
2	105	35	550	10.1	10.1	30
3	105	35	550	9.8	10.2	45
4	105	35	550	9.9	10.3	60

Switch time for all the runs 30 minutes

Run Number	Feed T'put Kg/hr	Fructose Rich Product Purity %	Fructose Rich Product Product concn	Glucose Rich Product Purity %	Glucose Rich Product Product concn
1	0.42	99.9	0.64	99.9	2.37
2	0.42	99.9	0.65	91.0	2.69
3	0.42	99.9	0.65	91.0	2.69
4	0.42	99.9	0.80	62.0	3.31

11.8 Effect of Increasing Product Concentration

As mentioned earlier, the product concentration could be increased by increasing the feed flowrate or by increasing the feed concentration but this would lead to increased on-column concentrations and eventually to a loss of product purities.

To improve the commercial viability of the system, it was necessary to increase the product concentrations to reduce the operating and capital costs of any additional processes like evaporation and crystallisation.

Thus the product splitting mentioned earlier in section 11.5 could be used to increase the product concentration. For the runs where no product splitting was carried out the concentration of the FRP did not exceed 2 RDS and that for the GRP was around 5 RDS for feeds of about 50 RDS. With the product splitting, these concentrations were increased to 10.93 RDS for the FRP and 10.07 RDS for the GRP for feeds of 53 RDS. Thus a concentration increase of five times was achieved for the FRP and two times for the GRP.

These concentrations could be further increased if the timers used could be switched on and off for different time intervals. The timers used for this research could be switched on and off for the same time interval ie. for a switch time of 42 minutes, the timer could be switched on for 22 minutes and off for 22 minutes. But as the elution

profiles show in Figures 11.31 and 11.32, the bulk of the sugars for the FRP were eluted in the first 16 minutes. This meant that the product was being diluted for a further 6 minutes before the collection was switched off. Similarly, for the GRP, the product emerged after the first 14 minutes but the product could be concentrated further if collected for the last 20 minutes only ie. after 22 minutes into the switch. A further 20% increase in product concentration could be achieved in this way.

The products could also be concentrated slightly by minimising the 'dead volume' in the system. As this was not easily possible, a time lag before the products were collected could be introduced as explained in Section 11.5.

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's and an economic evaluation would determine the exact lengths of the splitting periods.

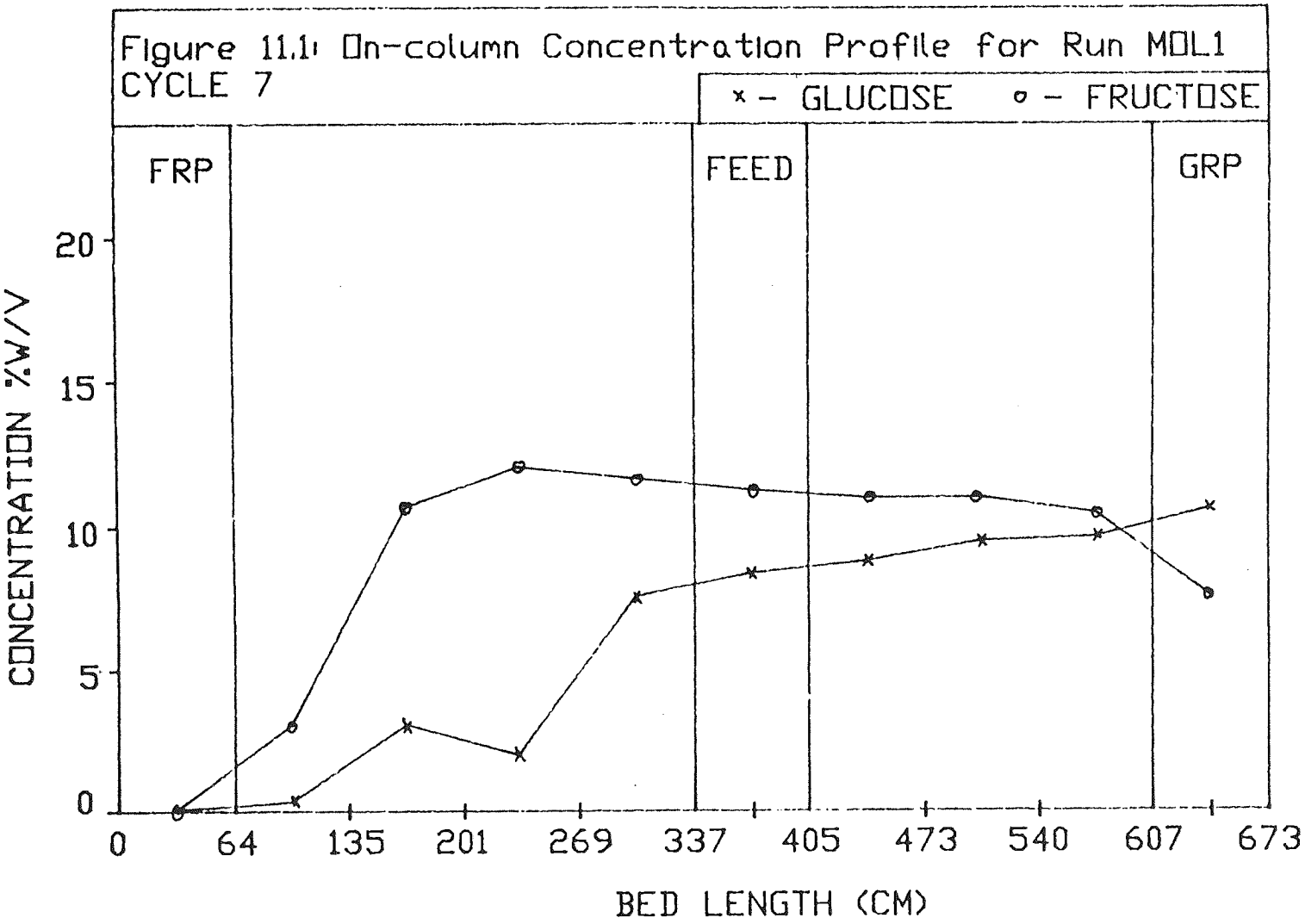


Figure 11.1: On-column Concentration Profile for Run MOL1
CYCLE 7
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

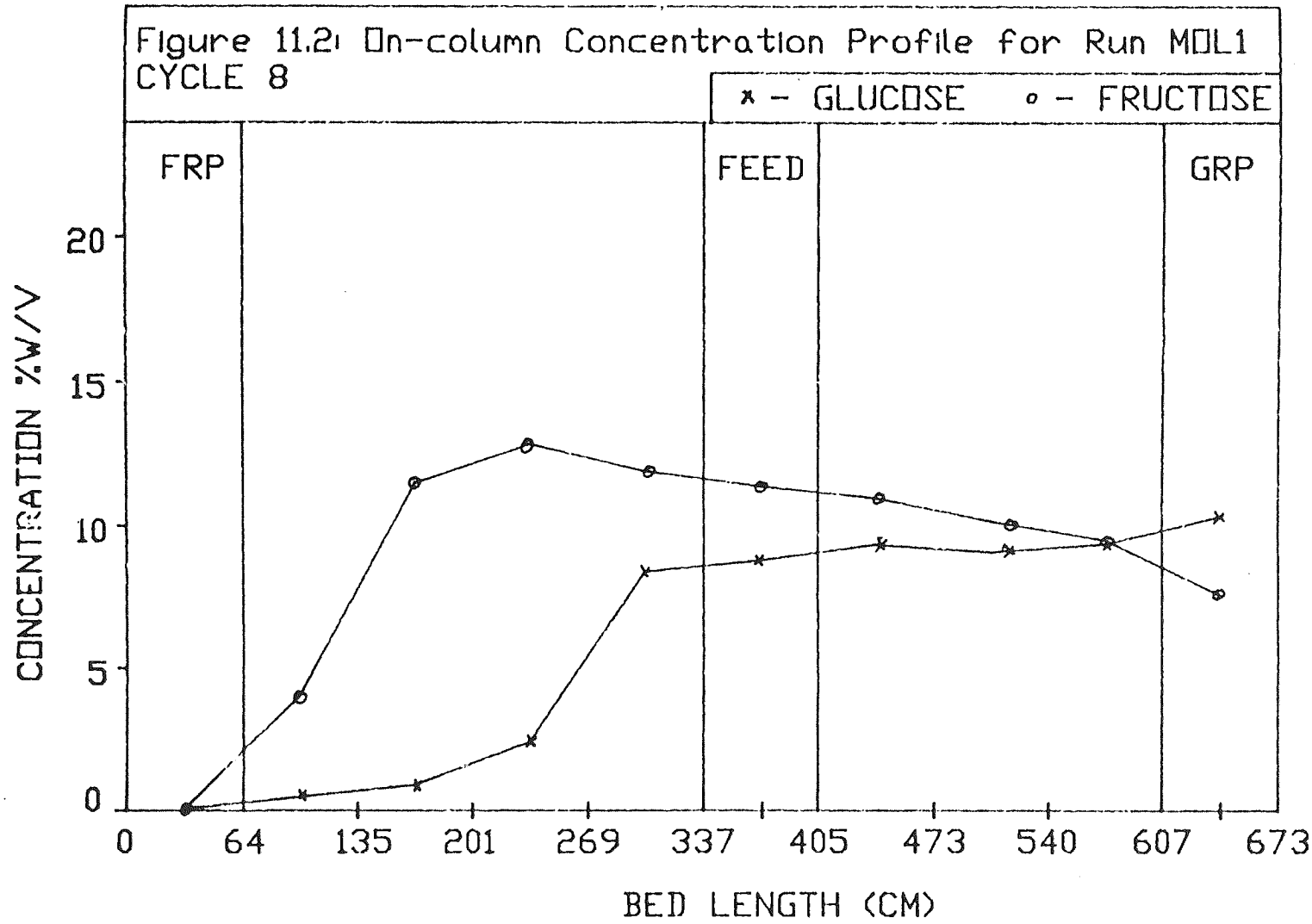


Figure 11.2: On-column Concentration Profile for Run MOL1
CYCLE 8
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

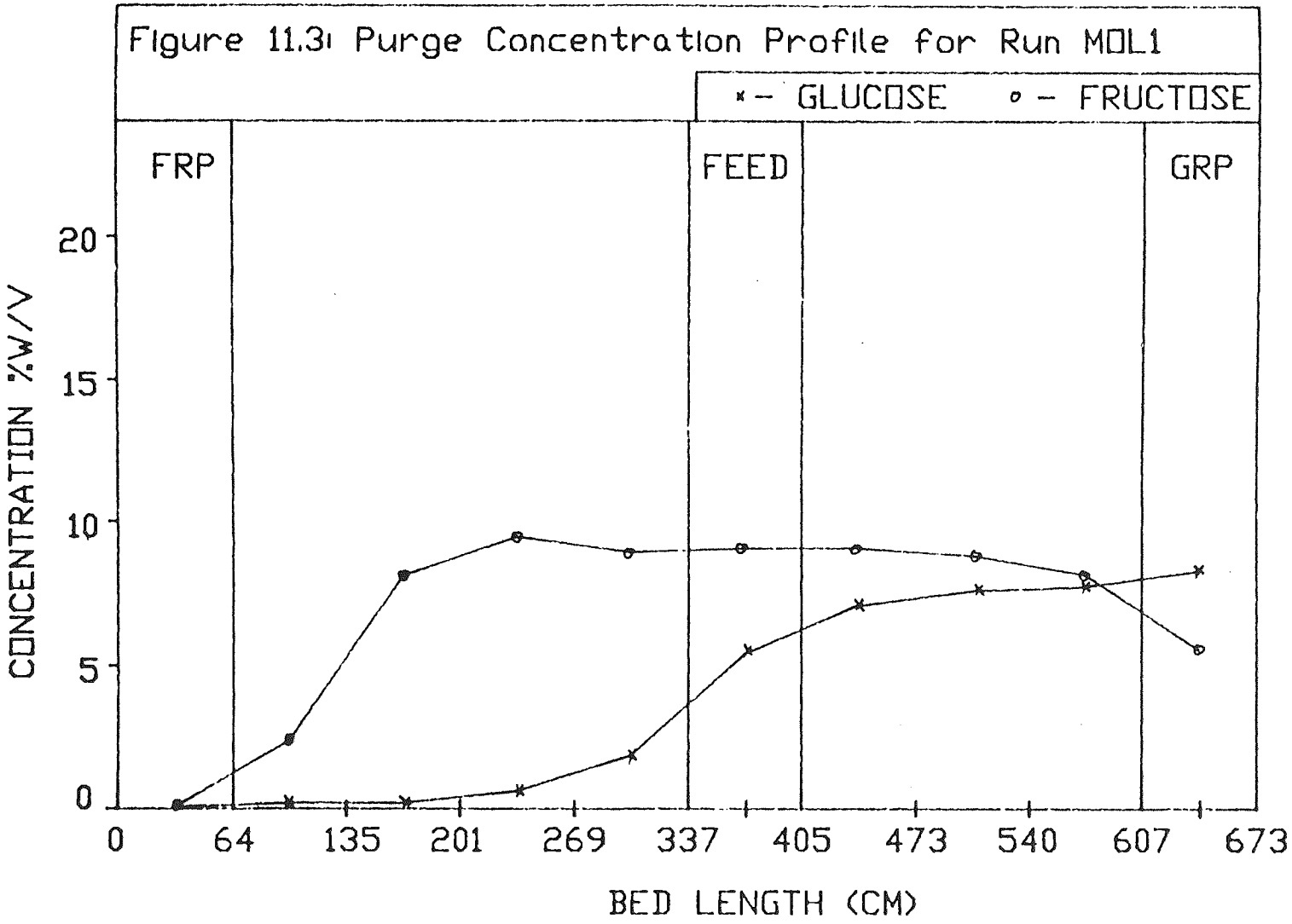


Figure 11.3: Purge Concentration Profile for Run MOL1
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

Figure 11.4: HPLC Profile For the Feed for the Final Cycle for Run MOL1

Figure 11.5: HPLC Profile For the FRP for the Final Cycle for Run MOL1

Figure 11.6: HPLC Profiles For the GRP for the Final Cycle for Run MOL1

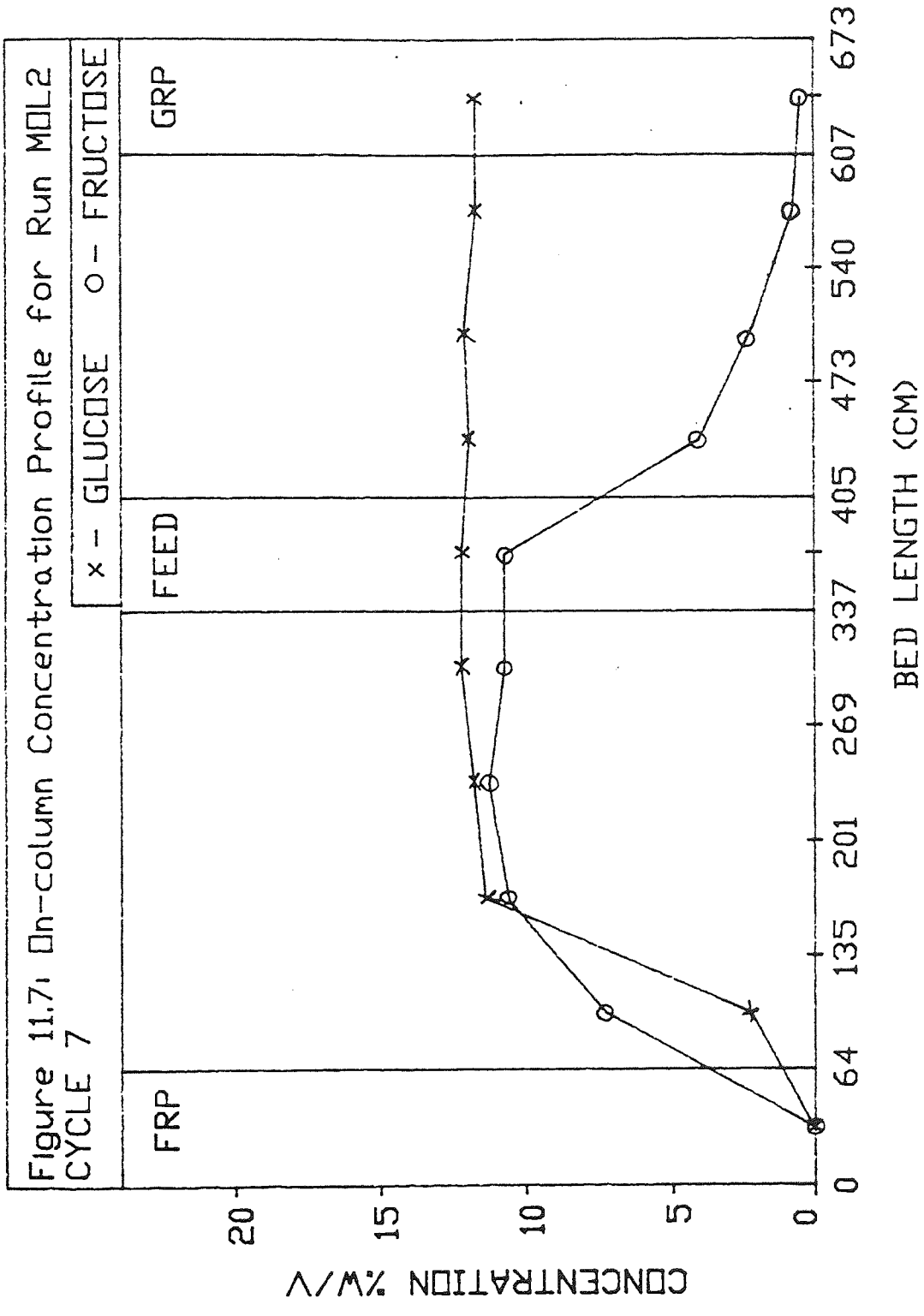


Figure 11.7: On-column Concentration Profile for Run MOL2
CYCLE 7
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

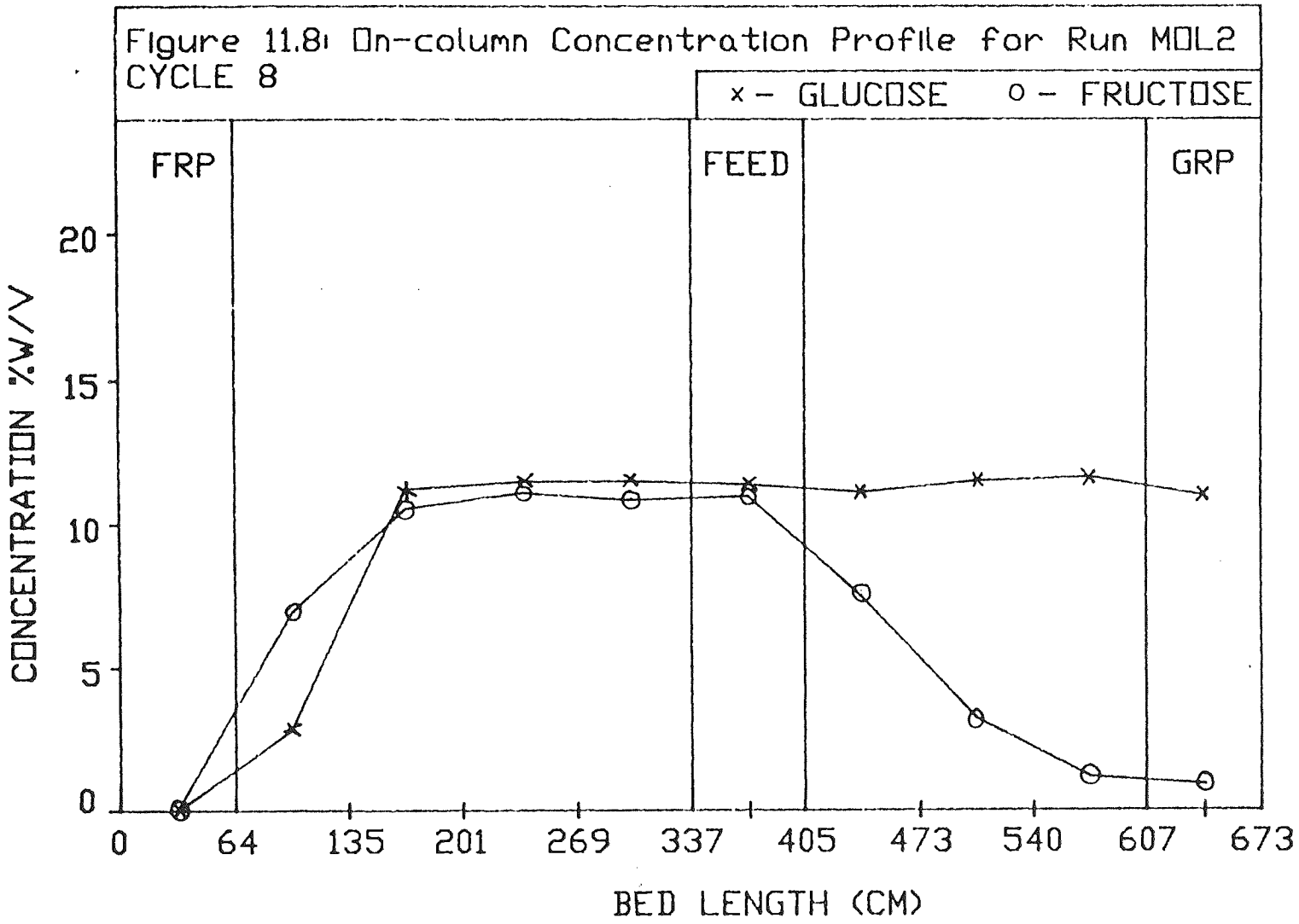


Figure 11.8: On-column Concentration Profile for Run MOL2
CYCLE 8
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

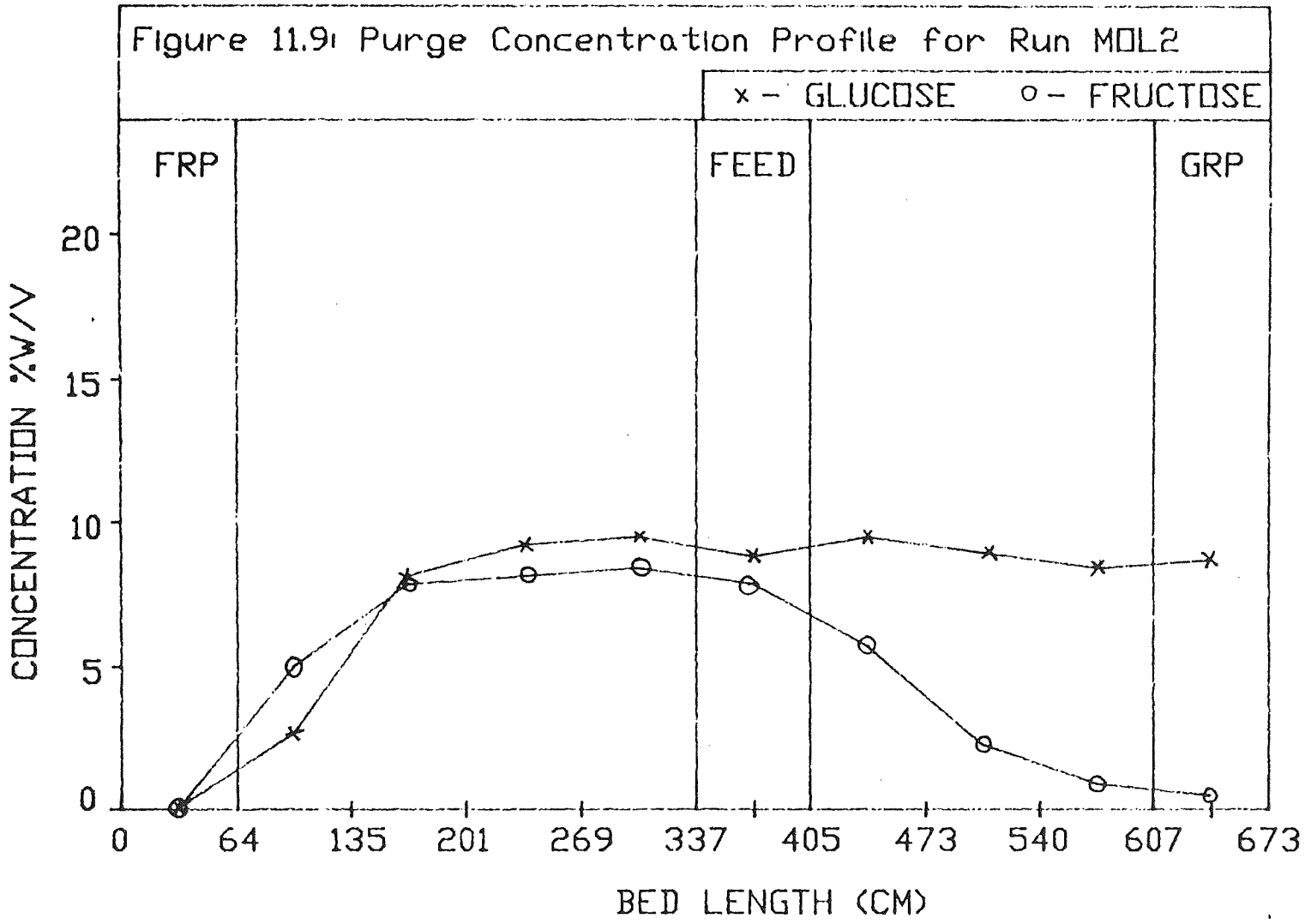


Figure 11.9: Purge Concentration Profile for Run MOL2
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

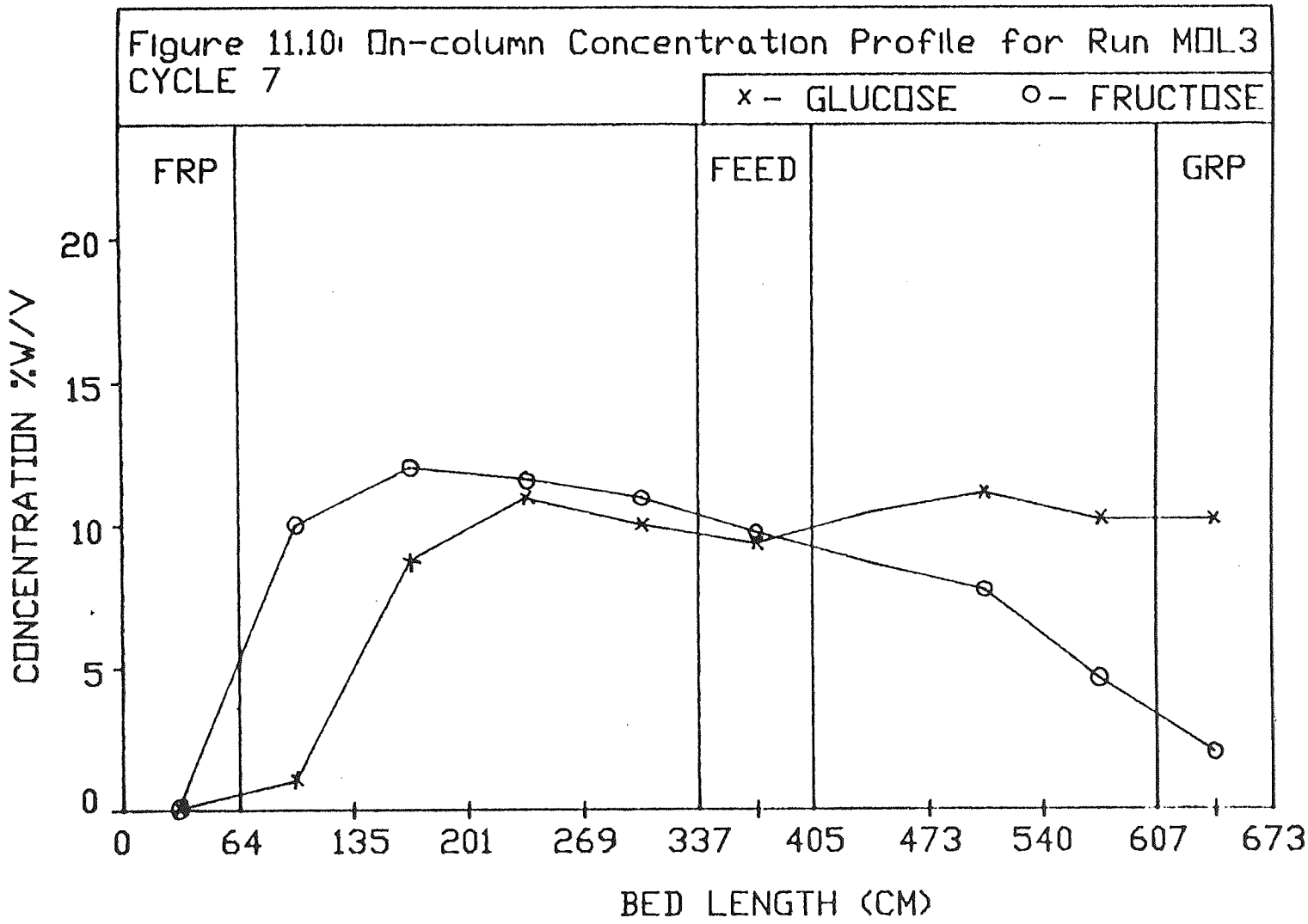


Figure 11.10: On-column Concentration Profile for Run MOL3
CYCLE 7
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

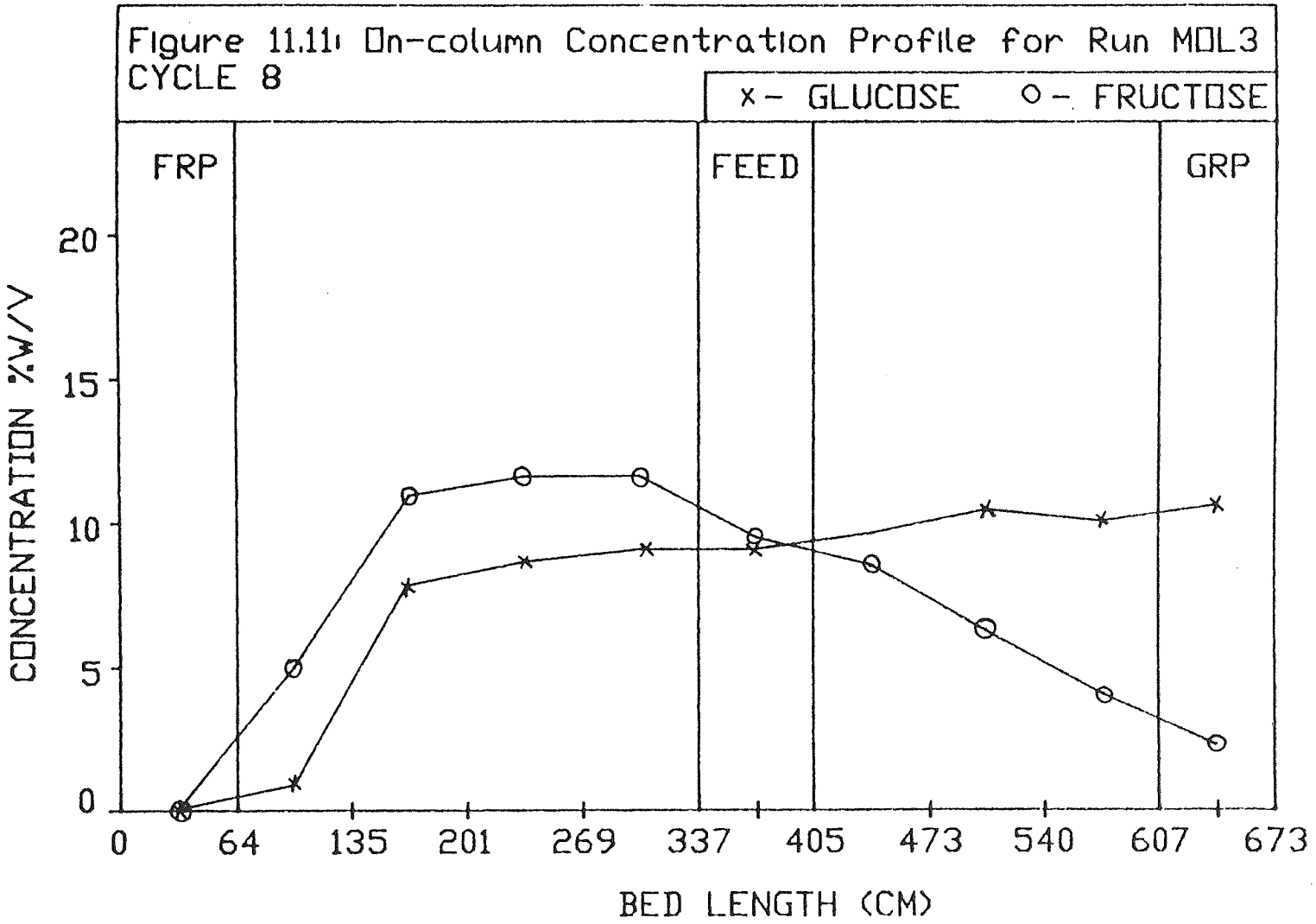


Figure 11.11: On-column Concentration Profile for Run MOL3
CYCLE 8
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

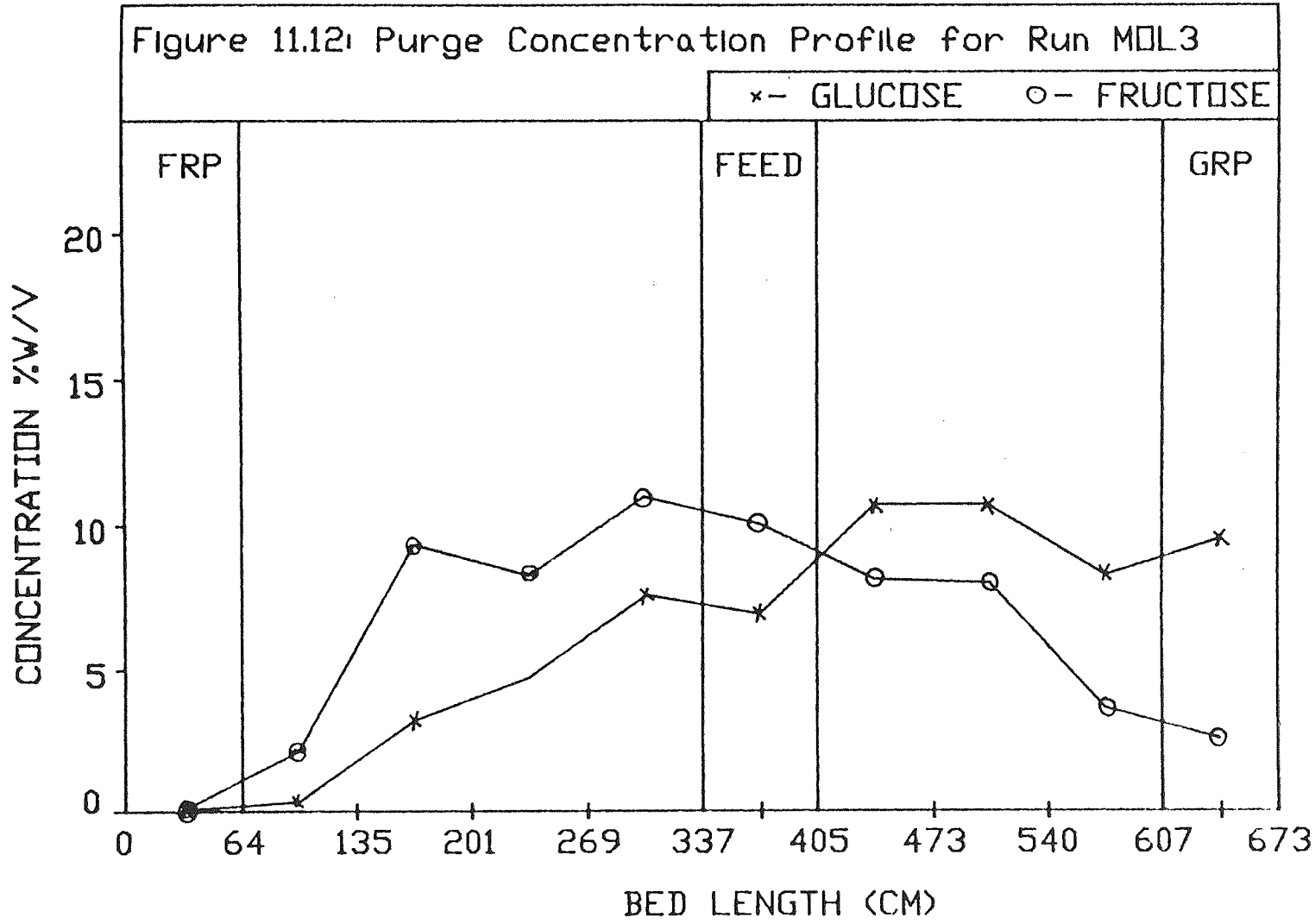


Figure 11.12: Purge Concentration Profile for Run MOL3
×- GLUCOSE
○- FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

Figure 11.13: Product Purity vs Cycle Number for Run MOL1

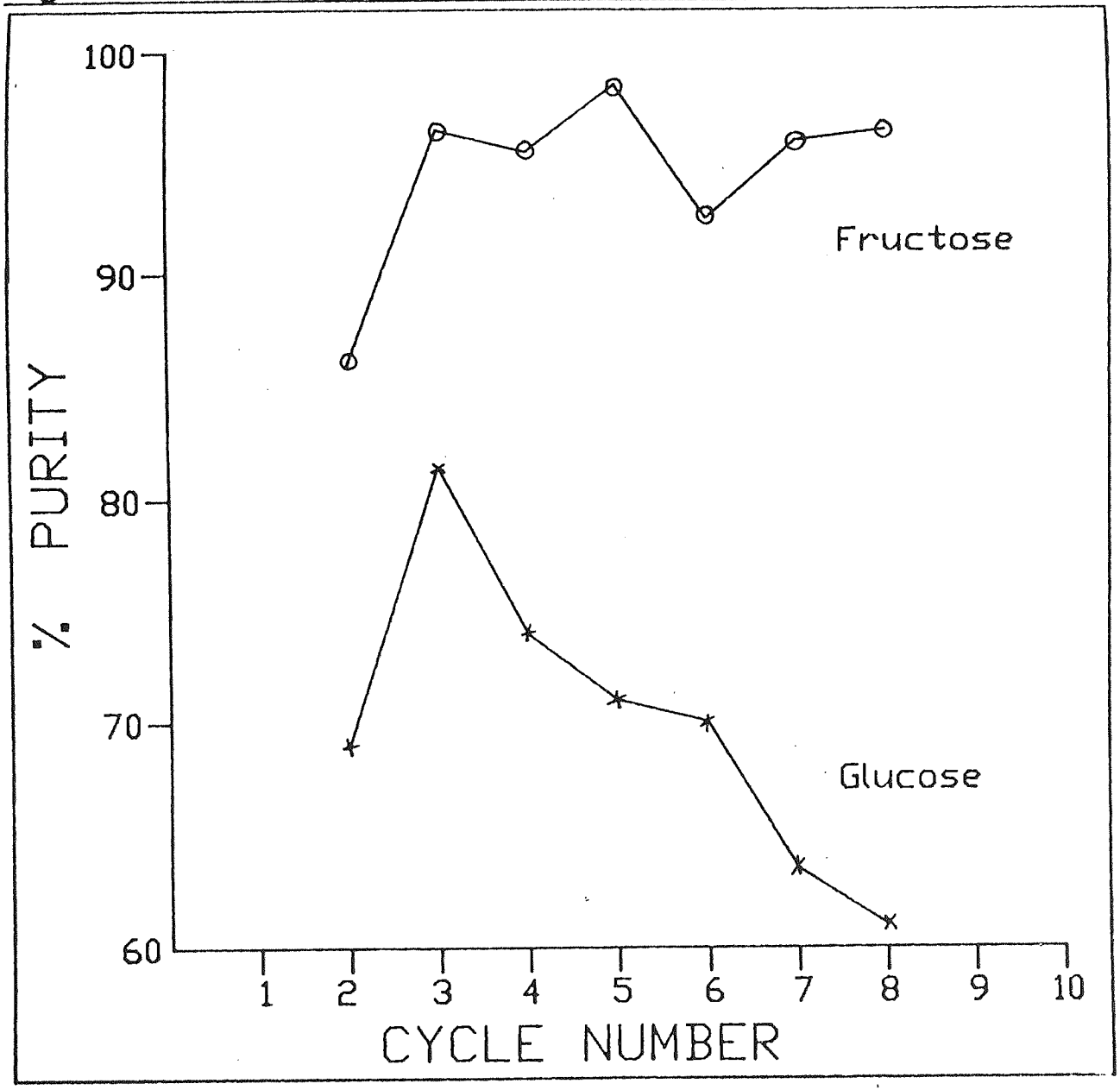


Figure 11.14: Product Purity vs Cycle Number for Run M□L2

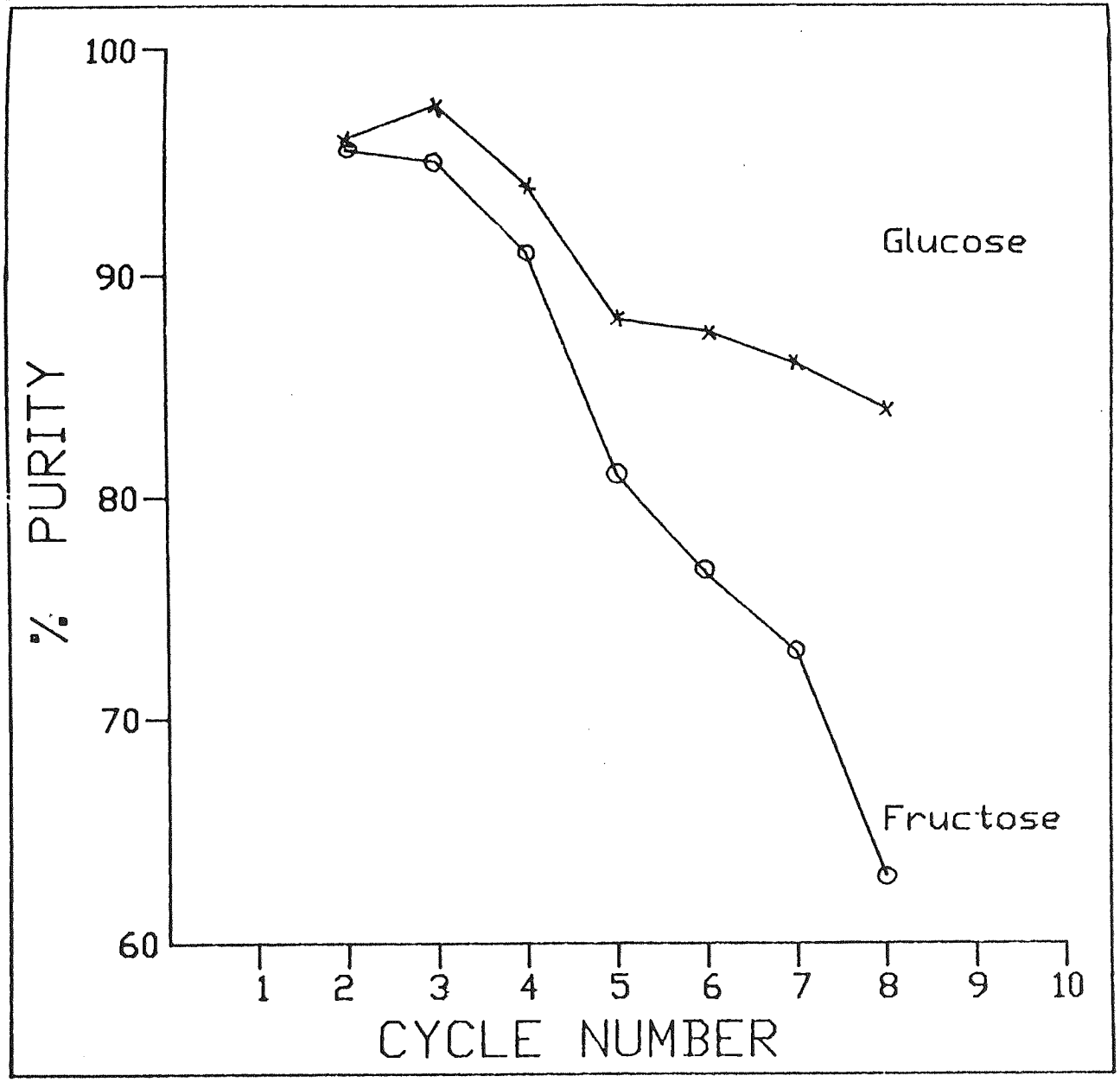
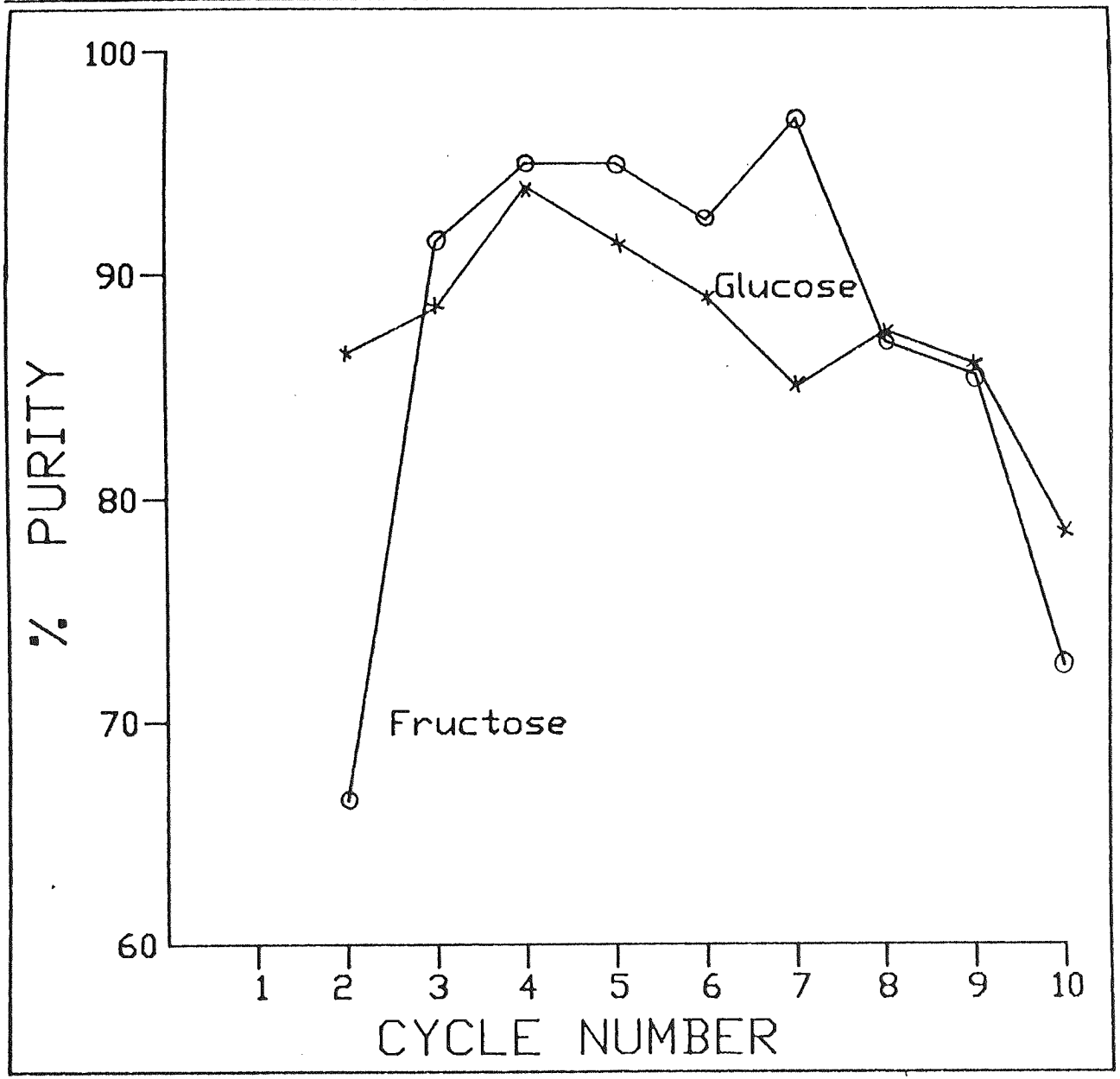


Figure 11.15: Product Purity vs Cycle Number for Run MOL3



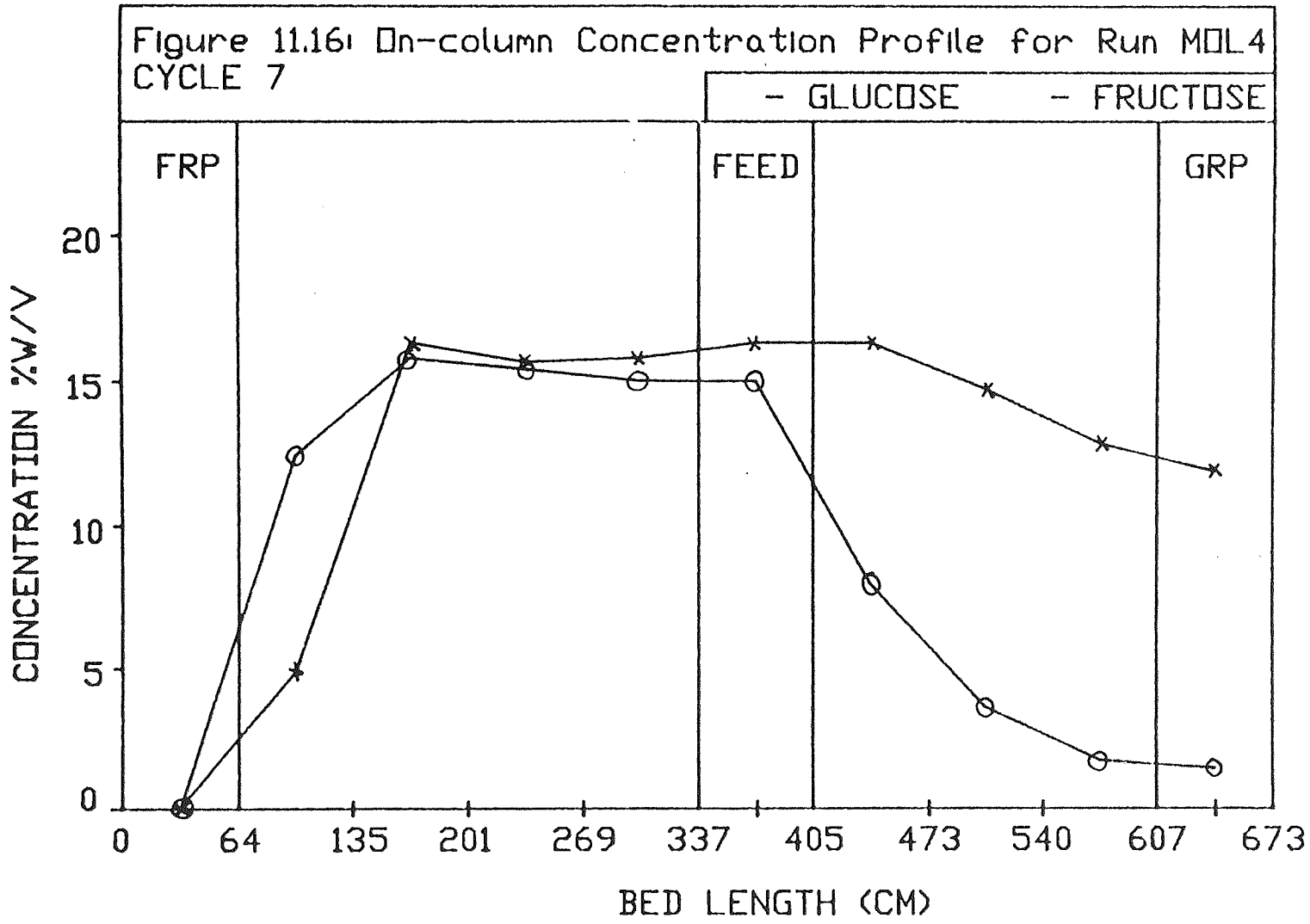


Figure 11.16: On-column Concentration Profile for Run MOL4
CYCLE 7
- GLUCOSE
- FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

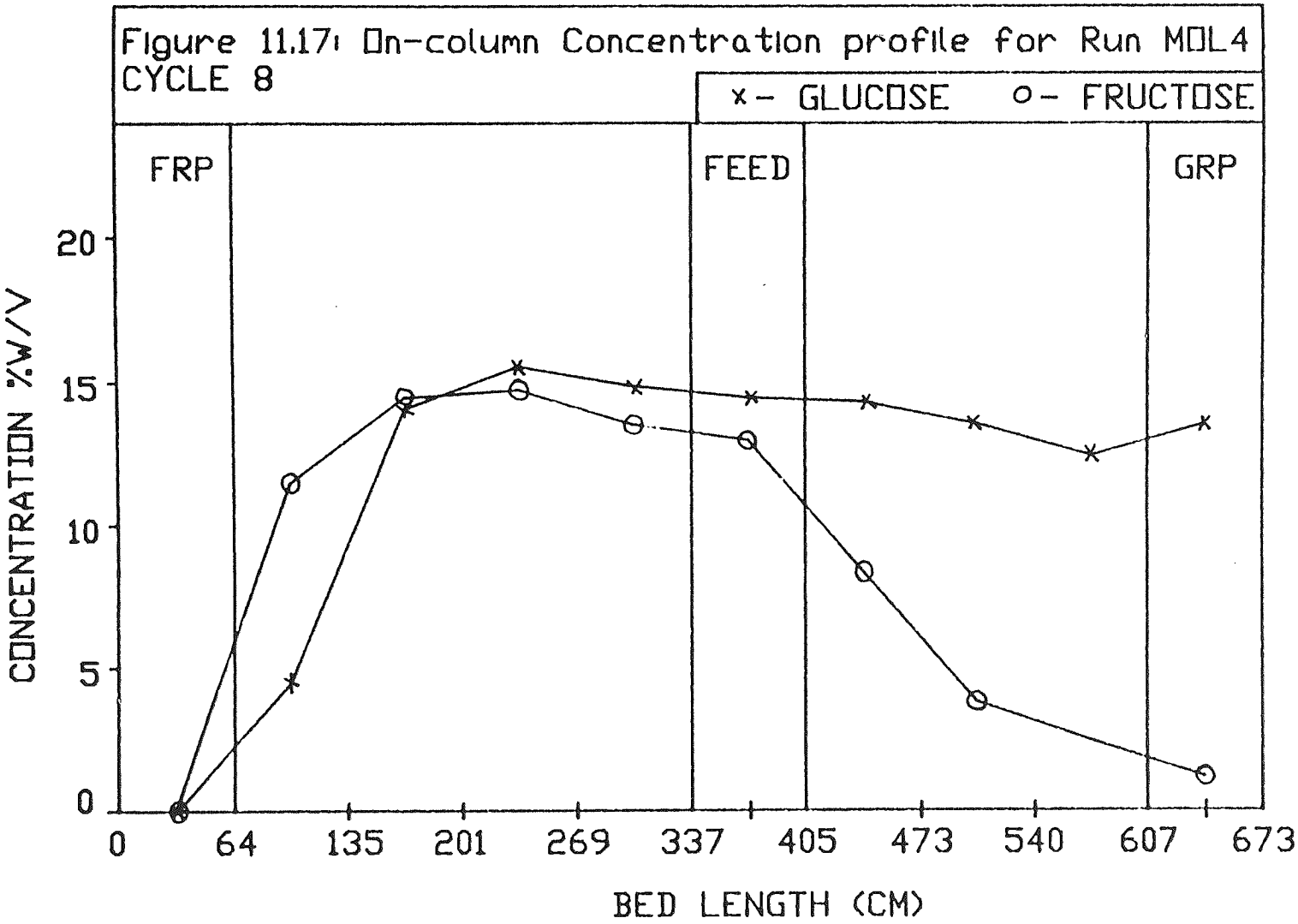


Figure 11.17: On-column Concentration profile for Run MOL4
CYCLE 8
x- GLUCOSE o- FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

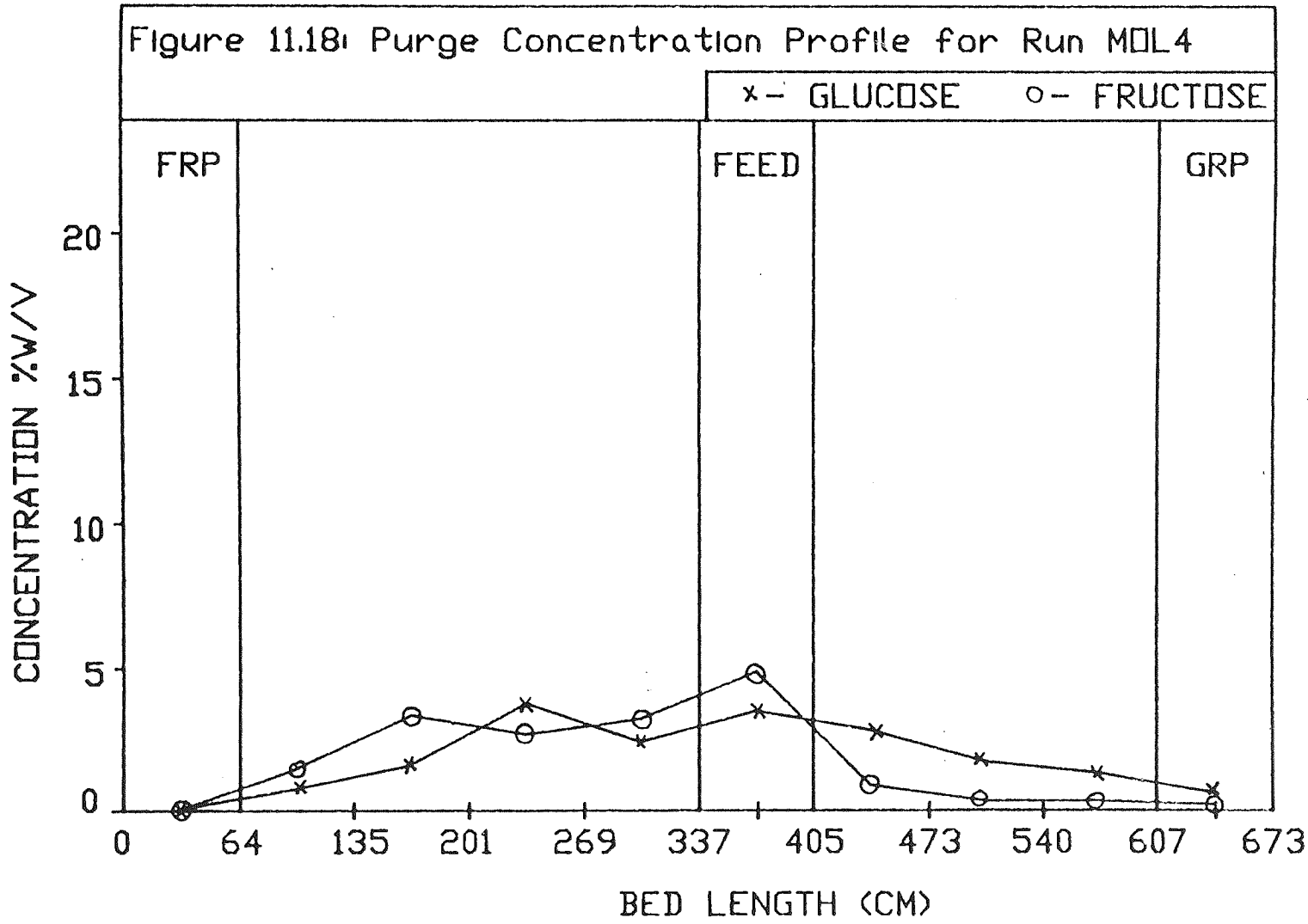


Figure 11.18: Purge Concentration Profile for Run MOL4
x- GLUCOSE
o- FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

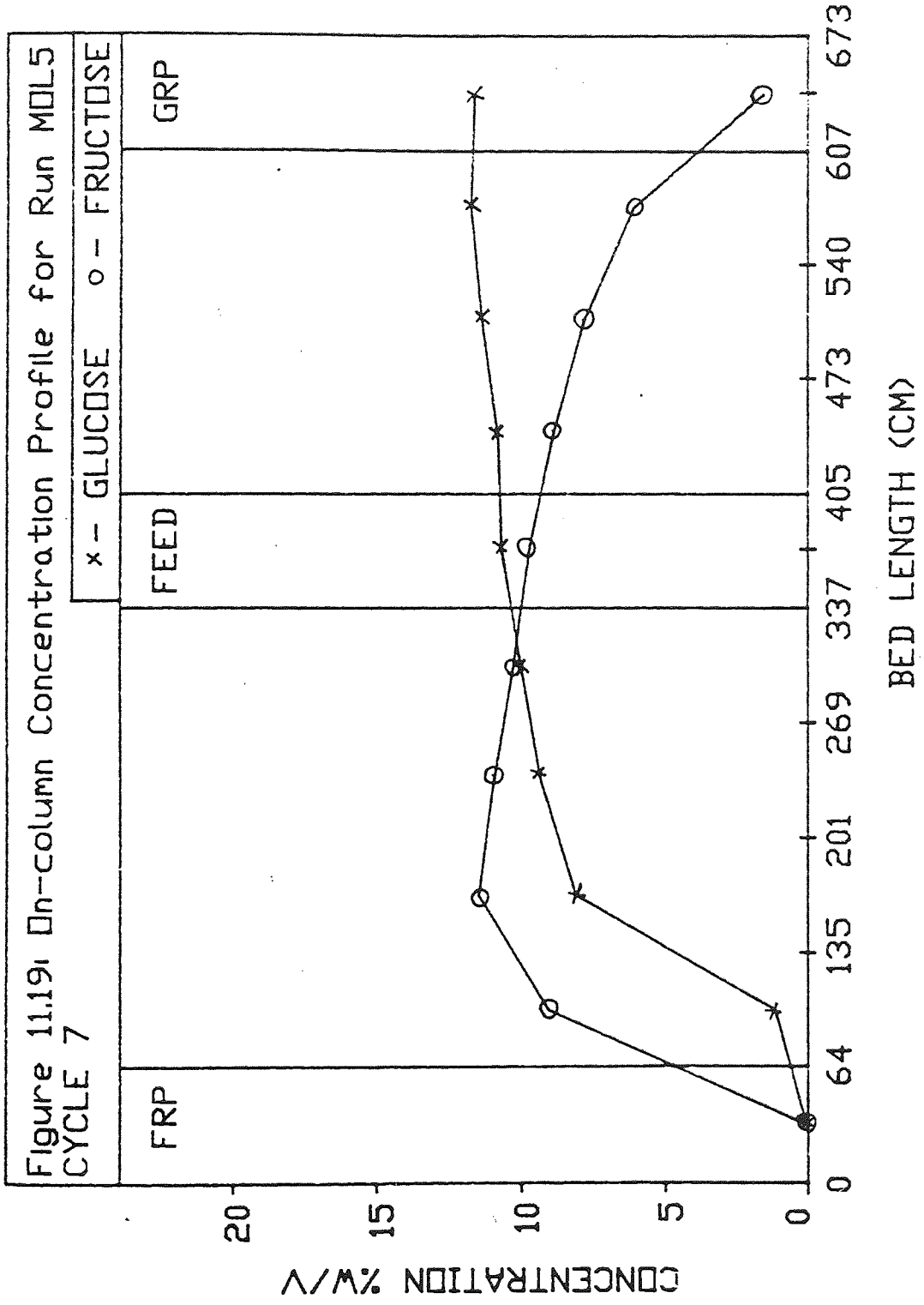


Figure 11.19: On-column Concentration Profile for Run MOL5
CYCLE 7
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

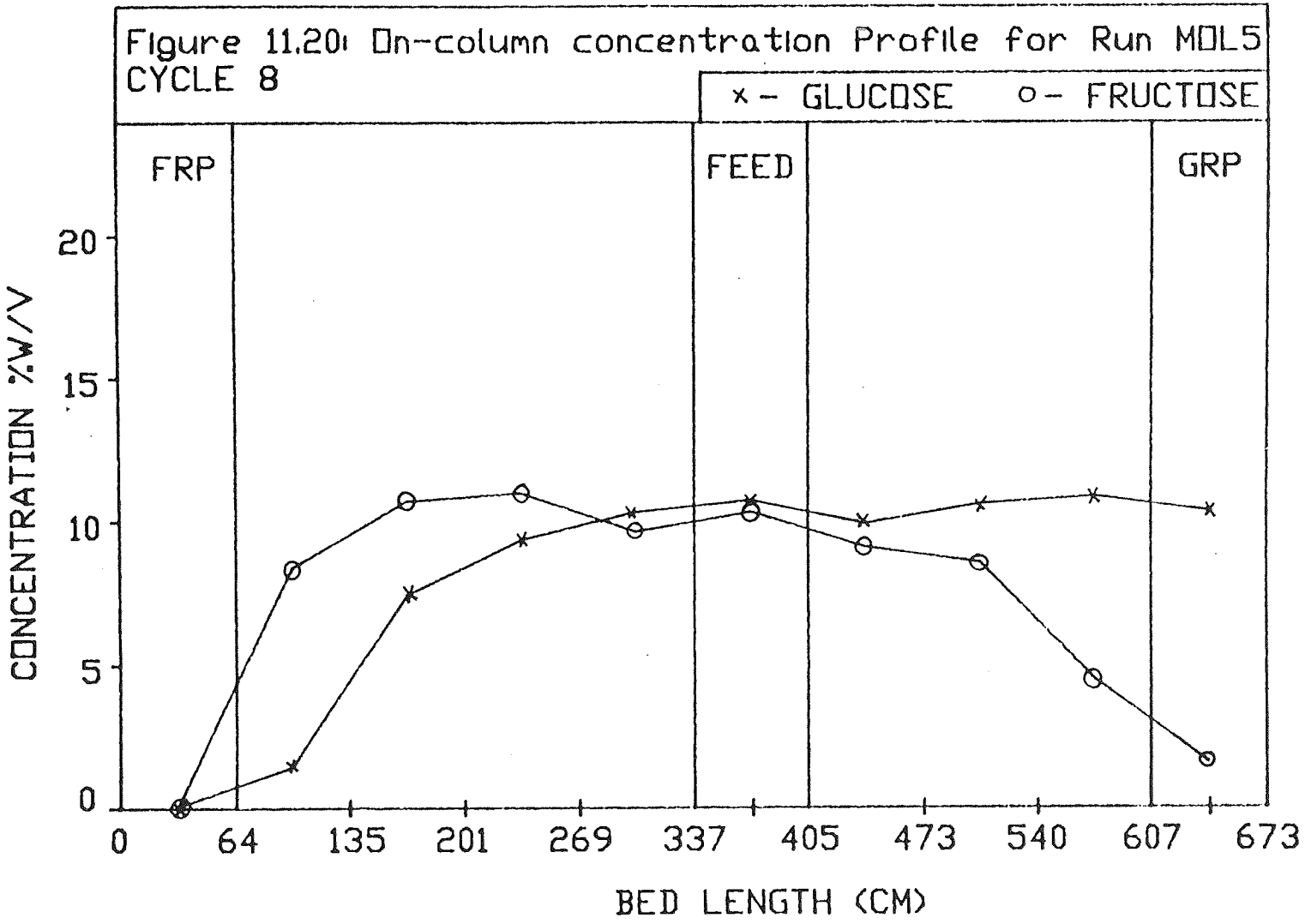


Figure 11.20: On-column concentration Profile for Run MOL5
CYCLE 8
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

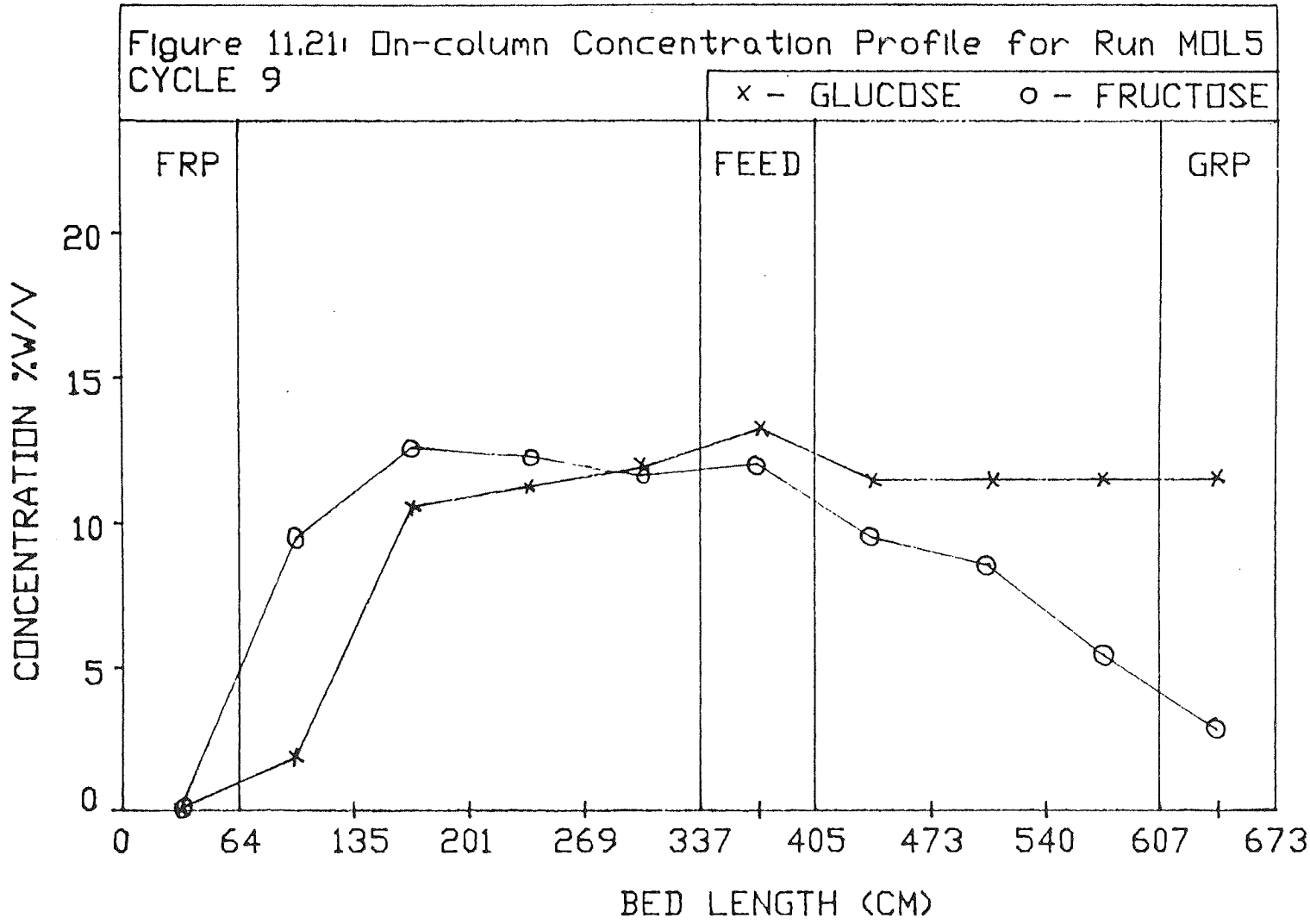


Figure 11.21: On-column Concentration Profile for Run MOL5
CYCLE 9
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

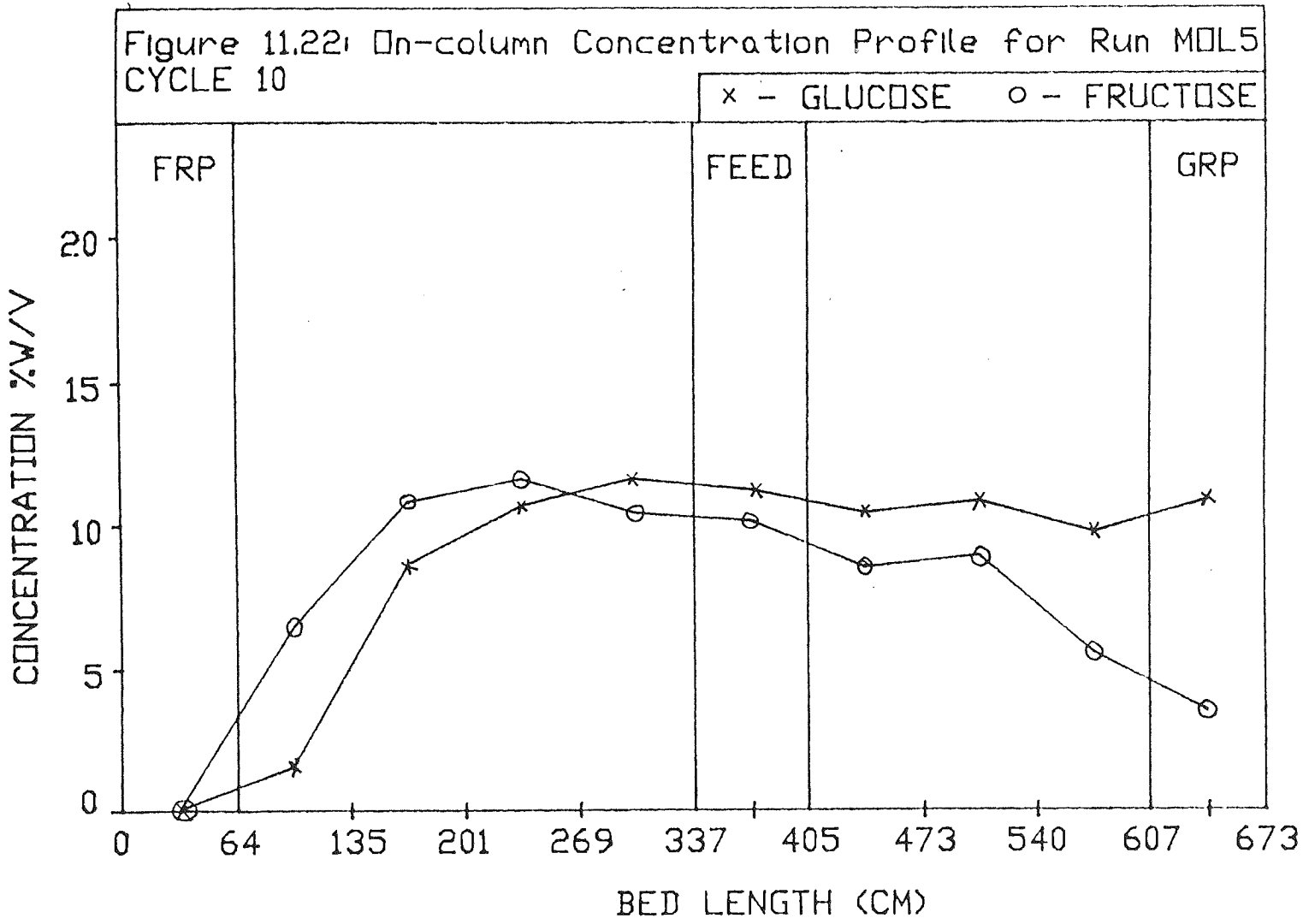


Figure 11.22: On-column Concentration Profile for Run MOL5
CYCLE 10
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

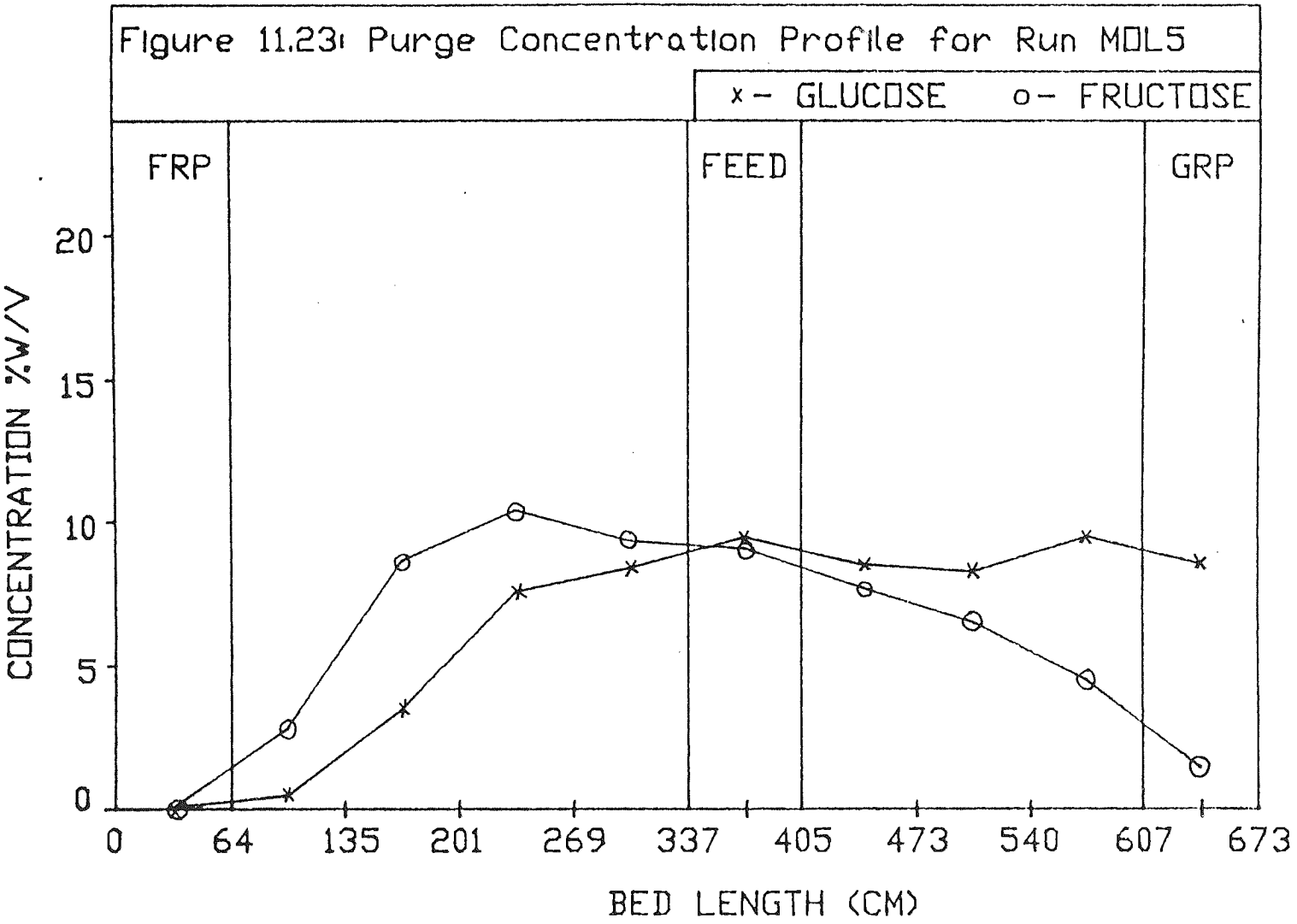


Figure 11.23: Purge Concentration Profile for Run MOL5
x- GLUCOSE o- FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

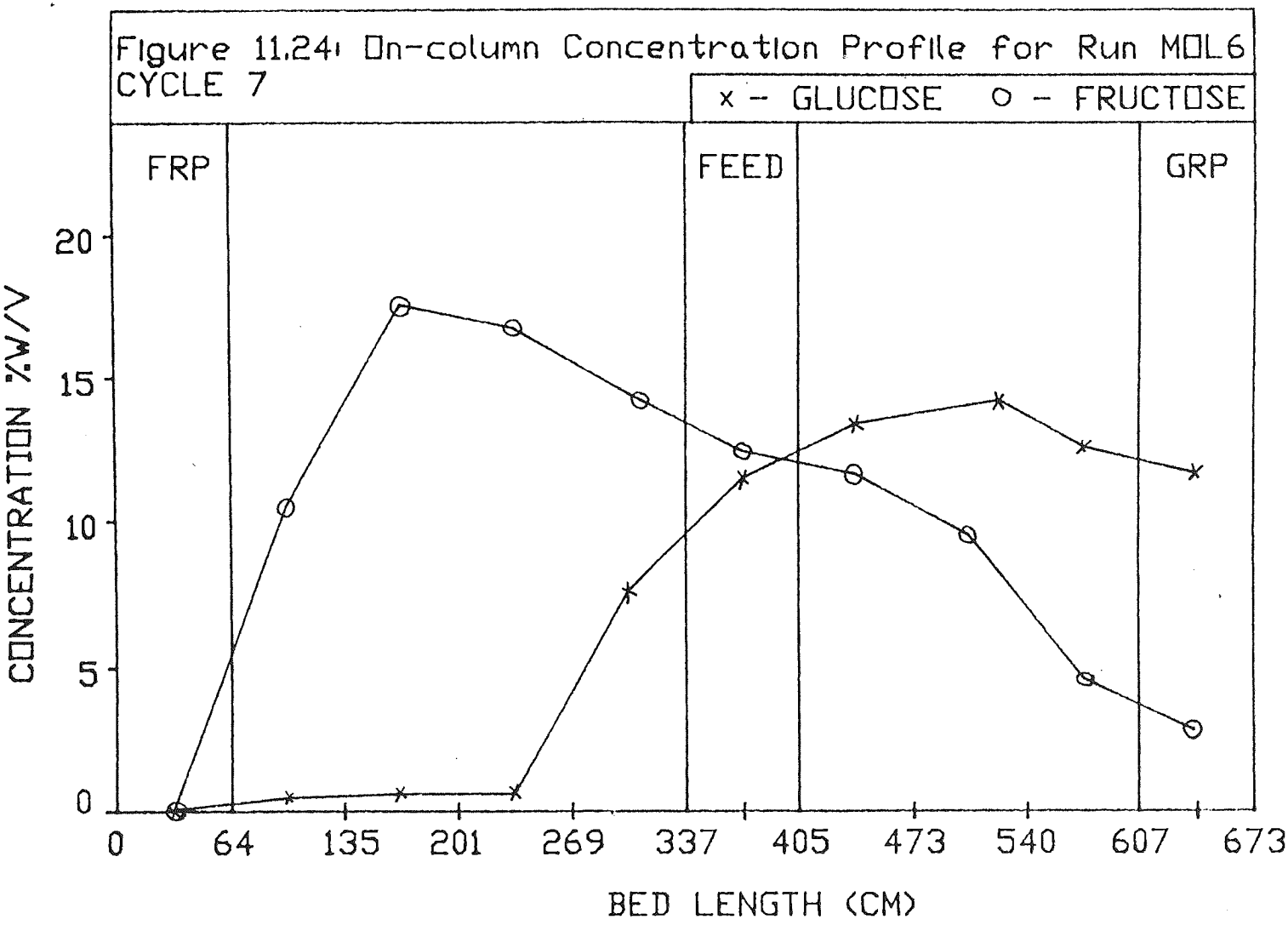


Figure 11.24: On-column Concentration Profile for Run MOL6
CYCLE 7
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

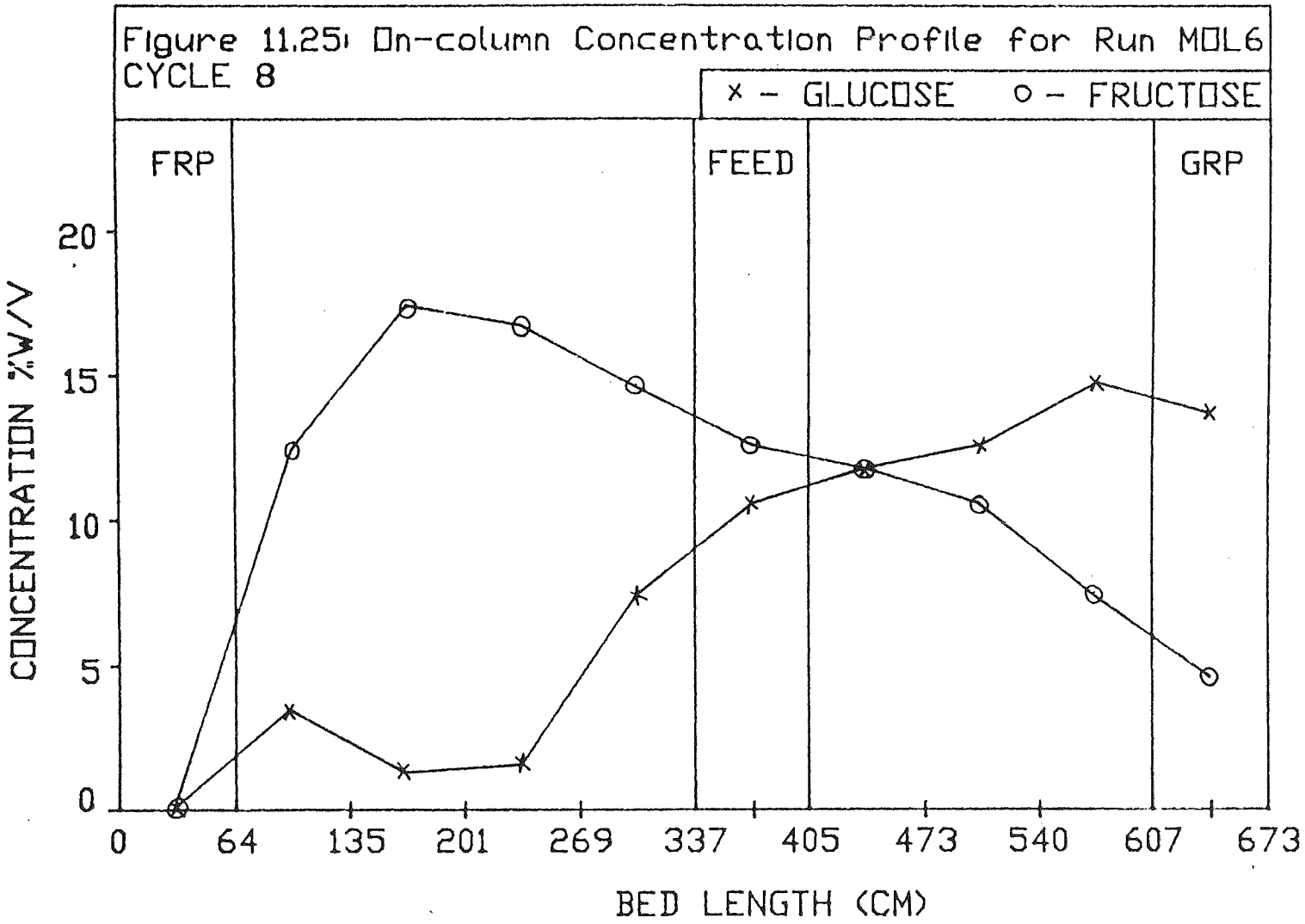


Figure 11.25: On-column Concentration Profile for Run MOL6
CYCLE 8
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

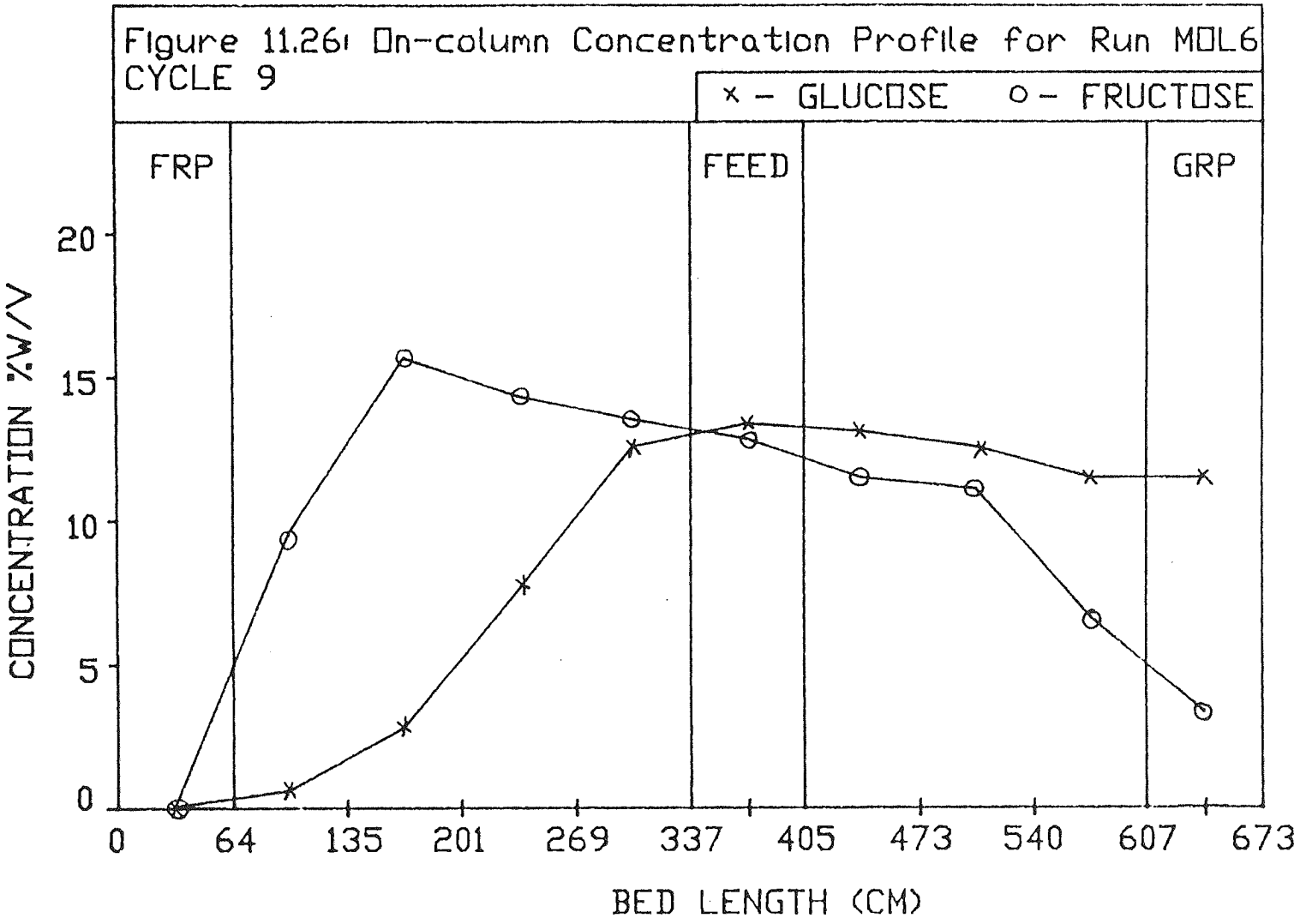


Figure 11.26: On-column Concentration Profile for Run MOL6
CYCLE 9
× - GLUCOSE
○ - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

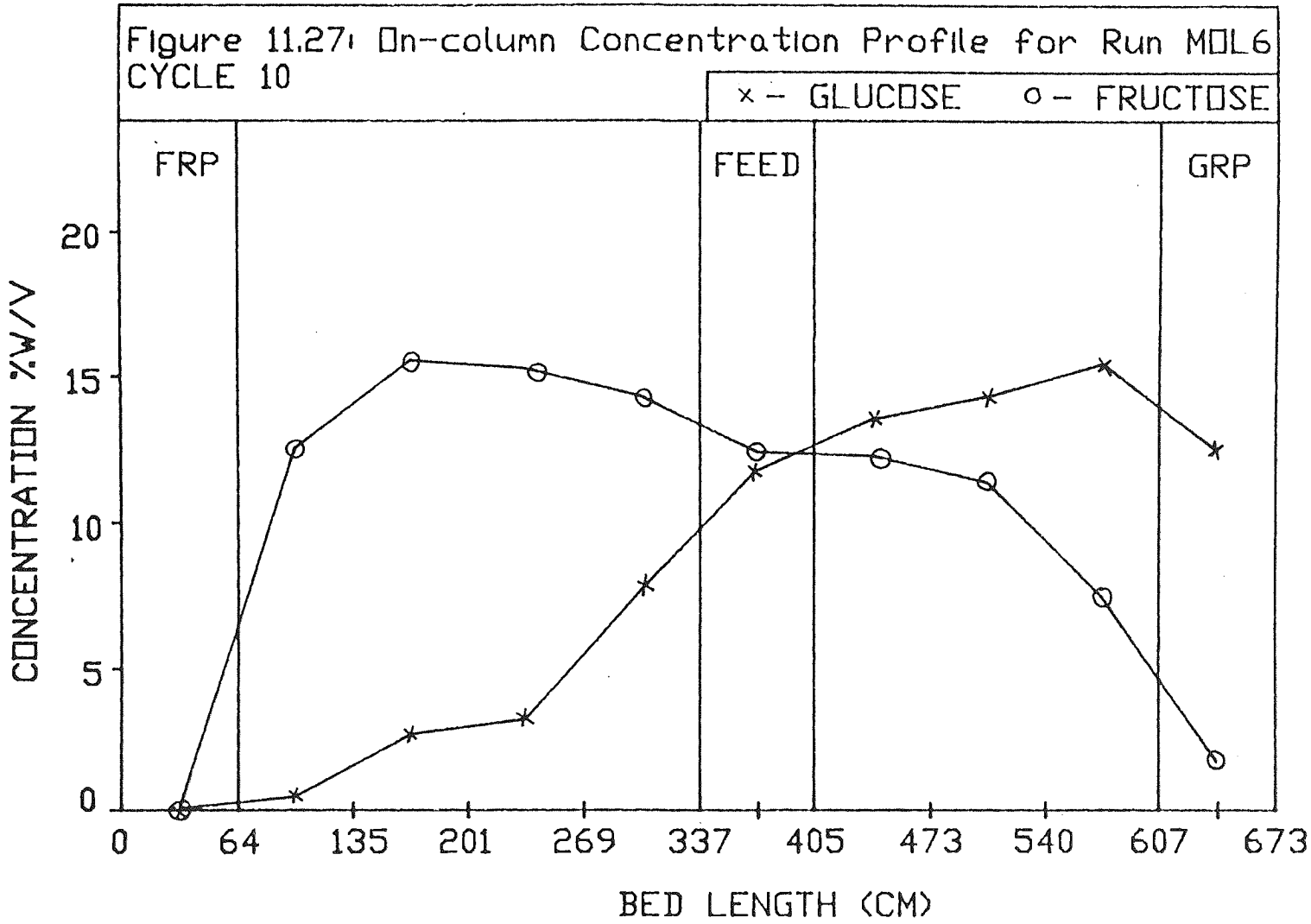


Figure 11.27: On-column Concentration Profile for Run MOL6
CYCLE 10
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

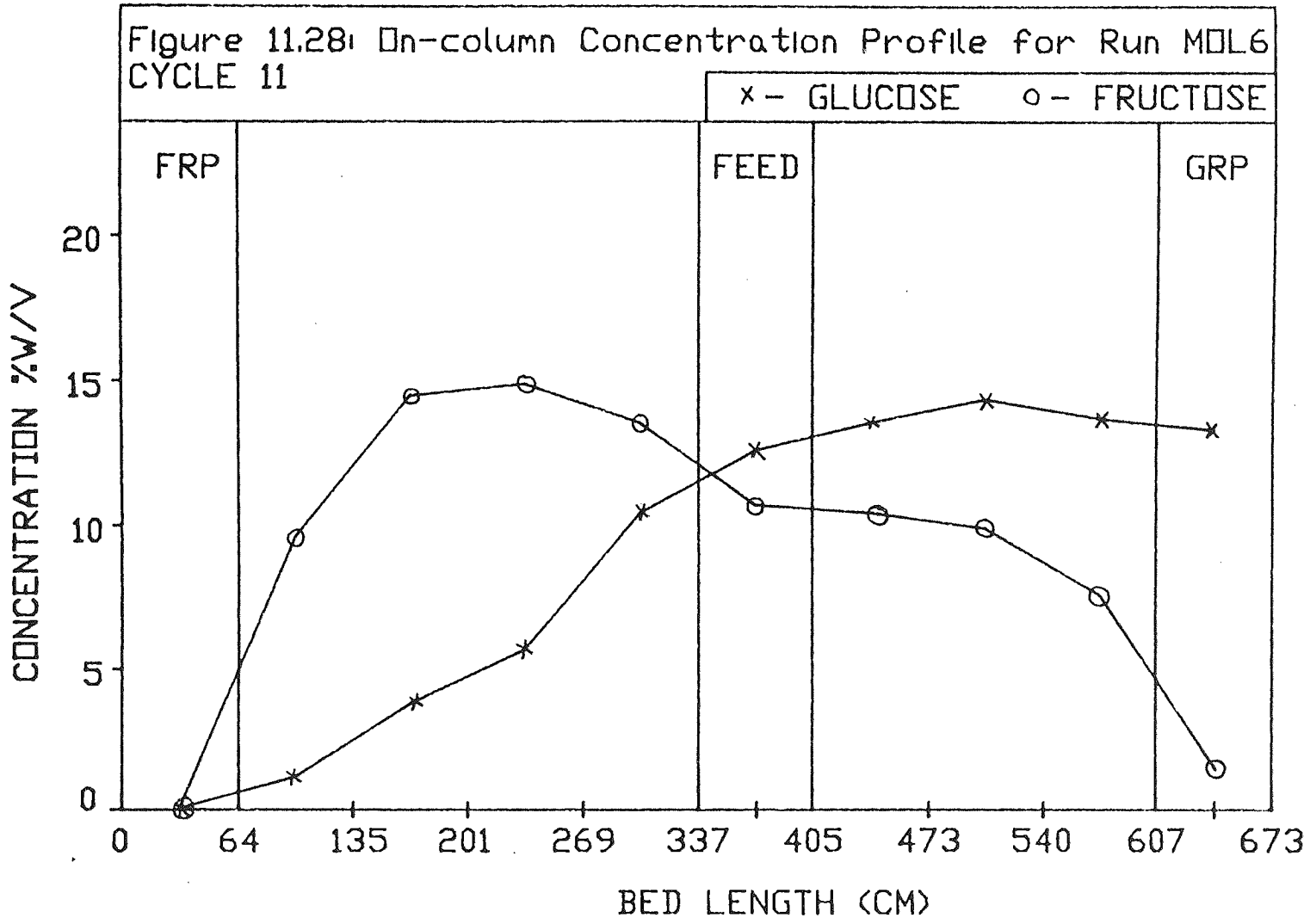


Figure 11.28: On-column Concentration Profile for Run MOL6
CYCLE 11
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

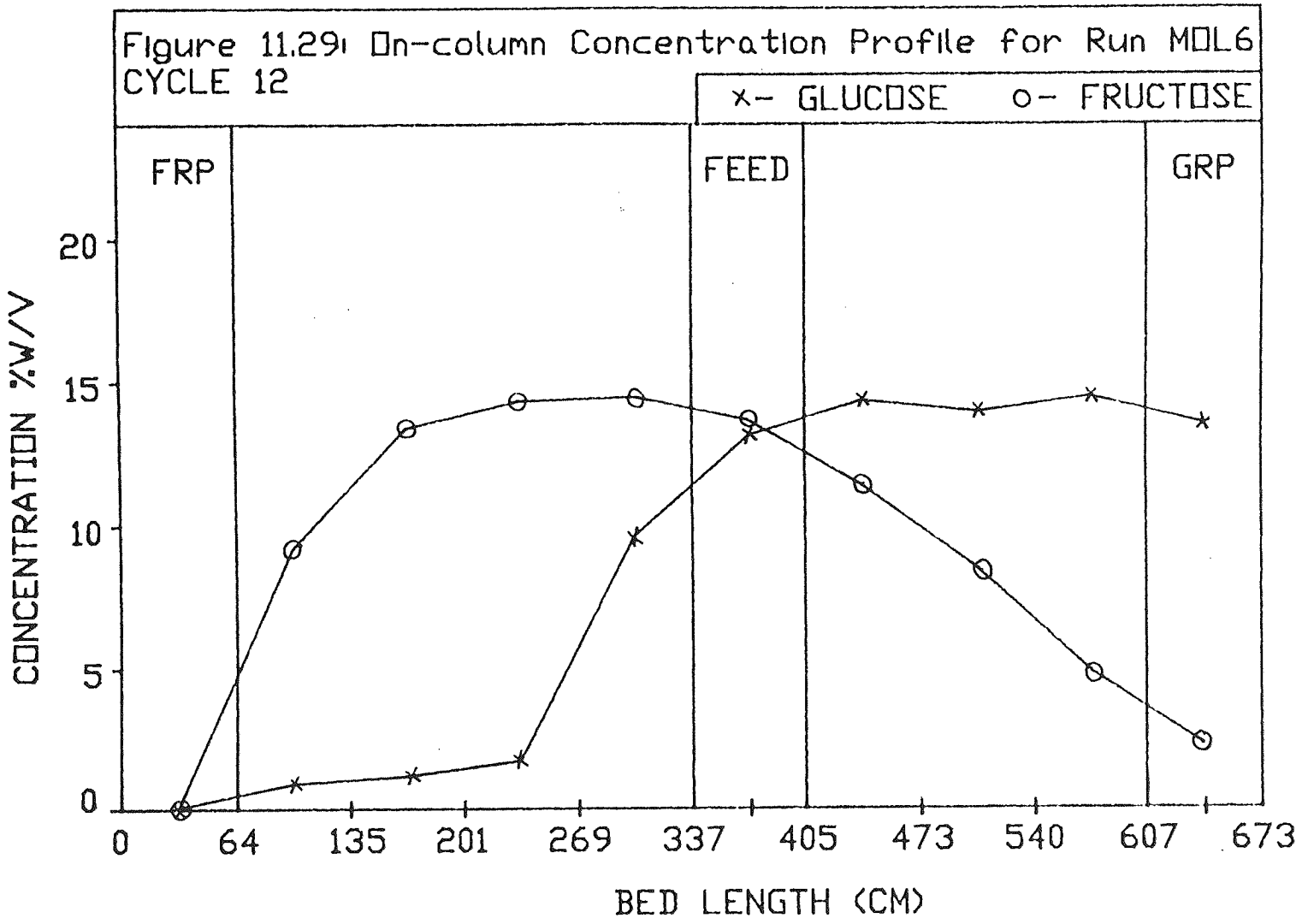


Figure 11.29: On-column Concentration Profile for Run MOL6
CYCLE 12
x- GLUCOSE o- FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

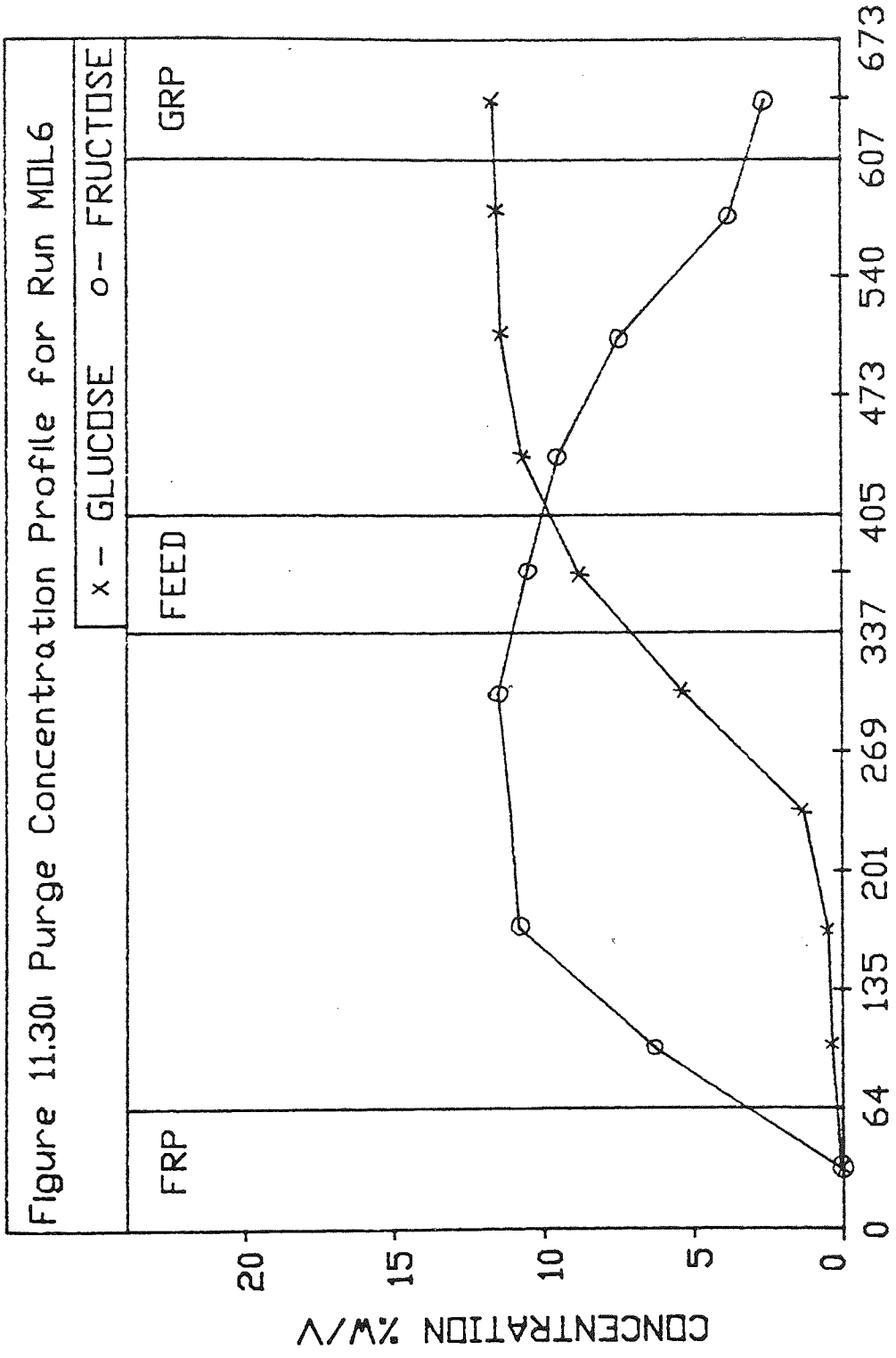


Figure 11.30: Purge Concentration Profile for Run MOL6
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

Figure 11.31: FRP Concentration over a Switch Period Run MOL6

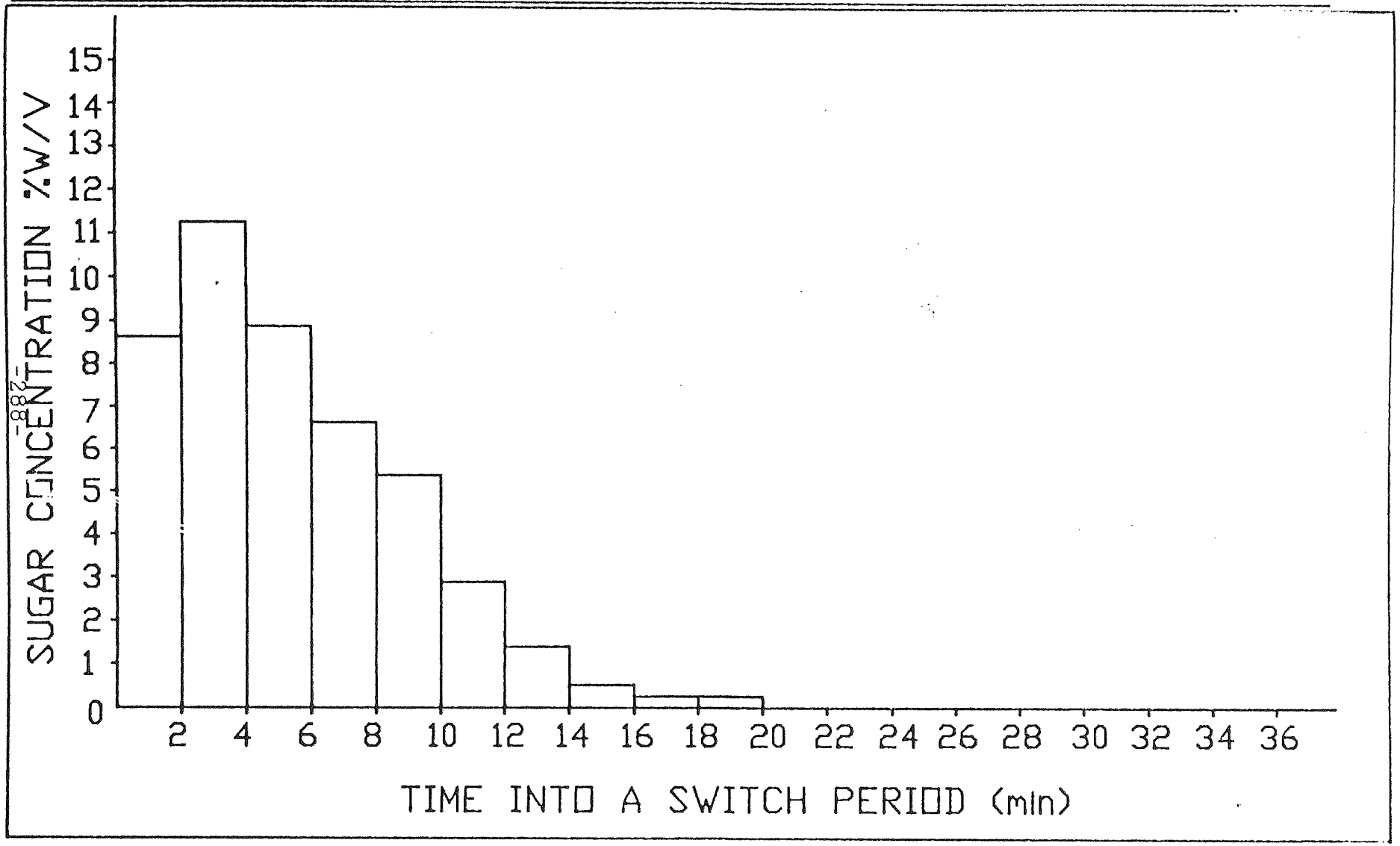


Figure 11.32: GRP Concentration over a Switch Period Run MOL6

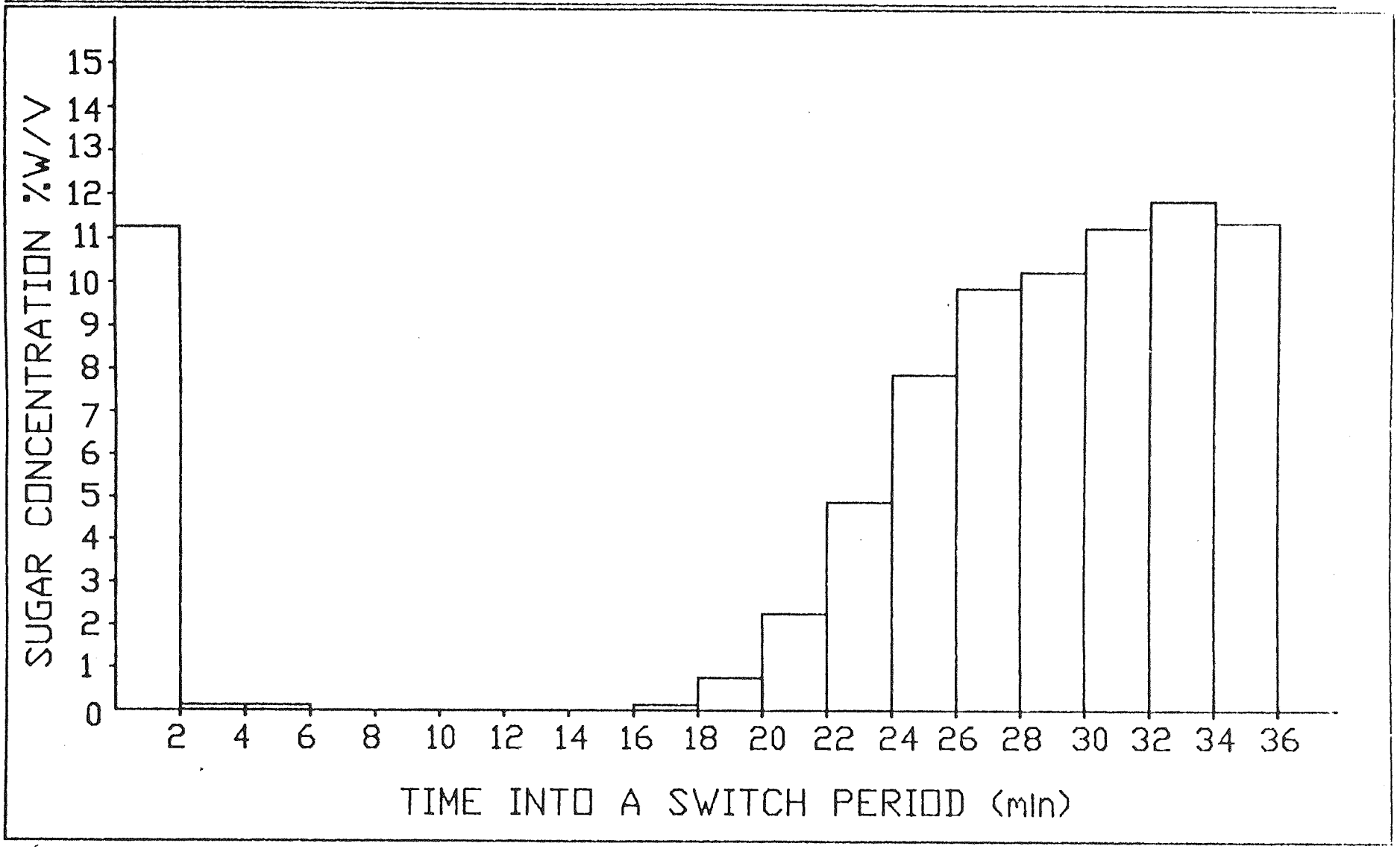
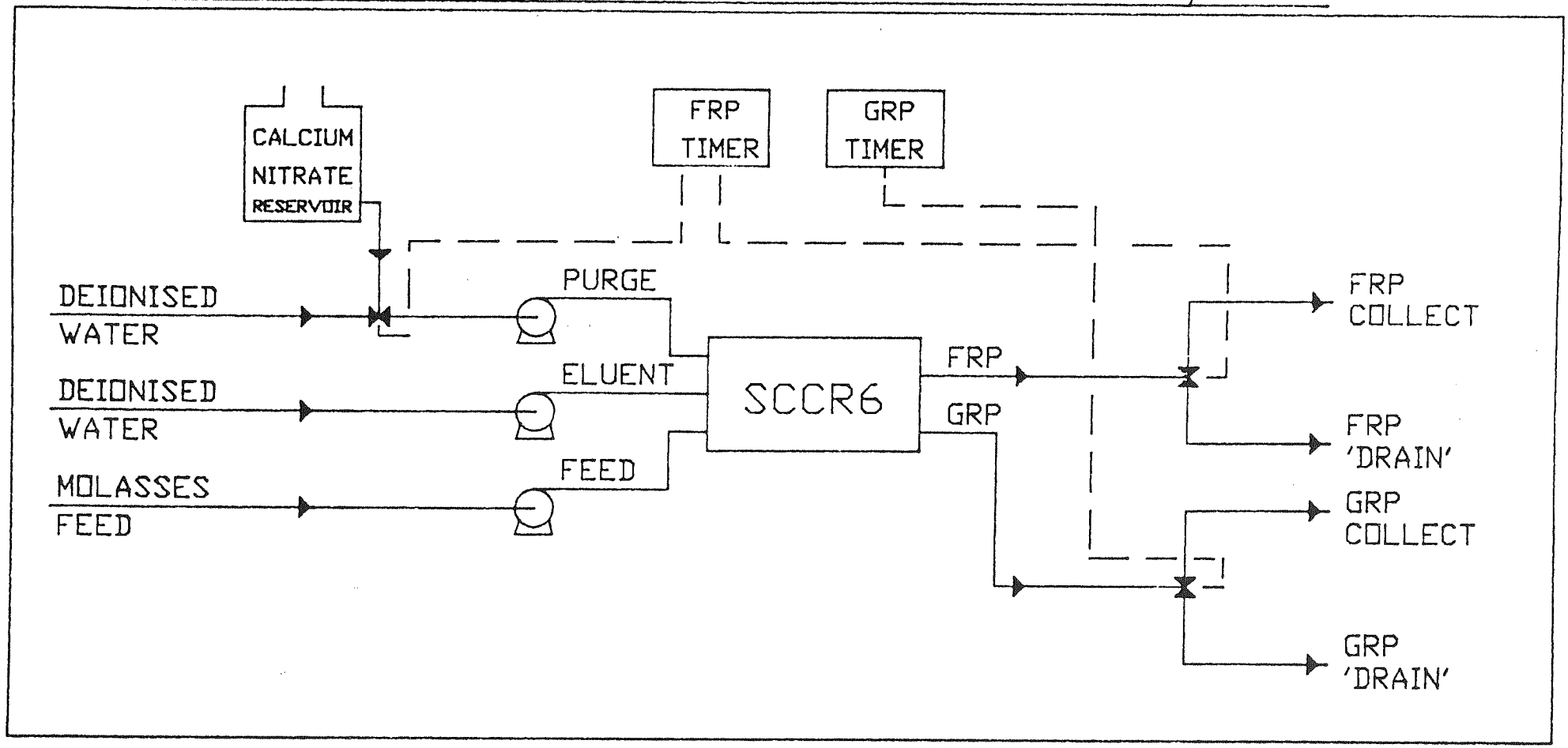


Figure 11.33: Modifications to the SCCR6 System



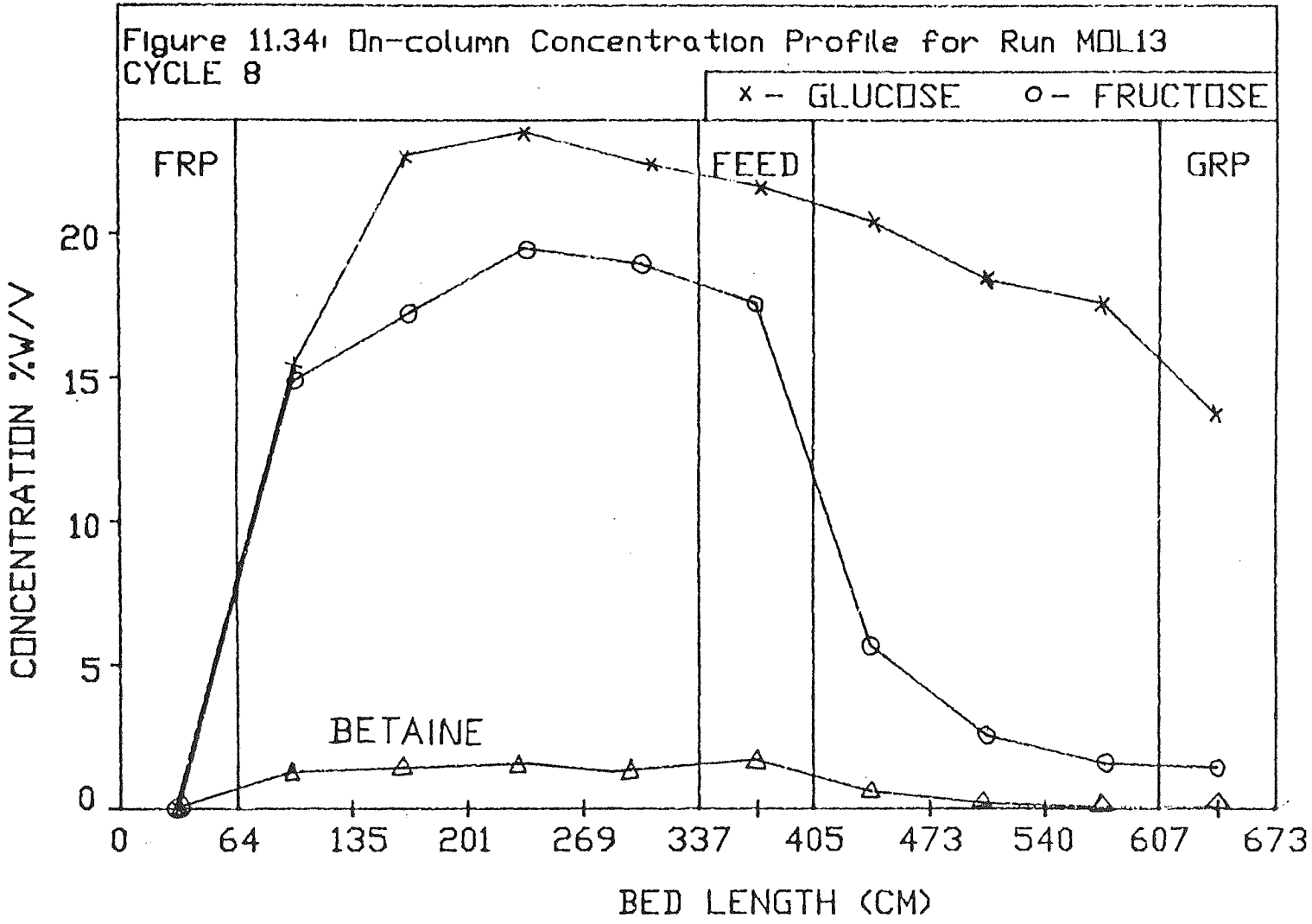


Figure 11.34: On-column Concentration Profile for Run MOL13
CYCLE 8
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
BETAINE
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

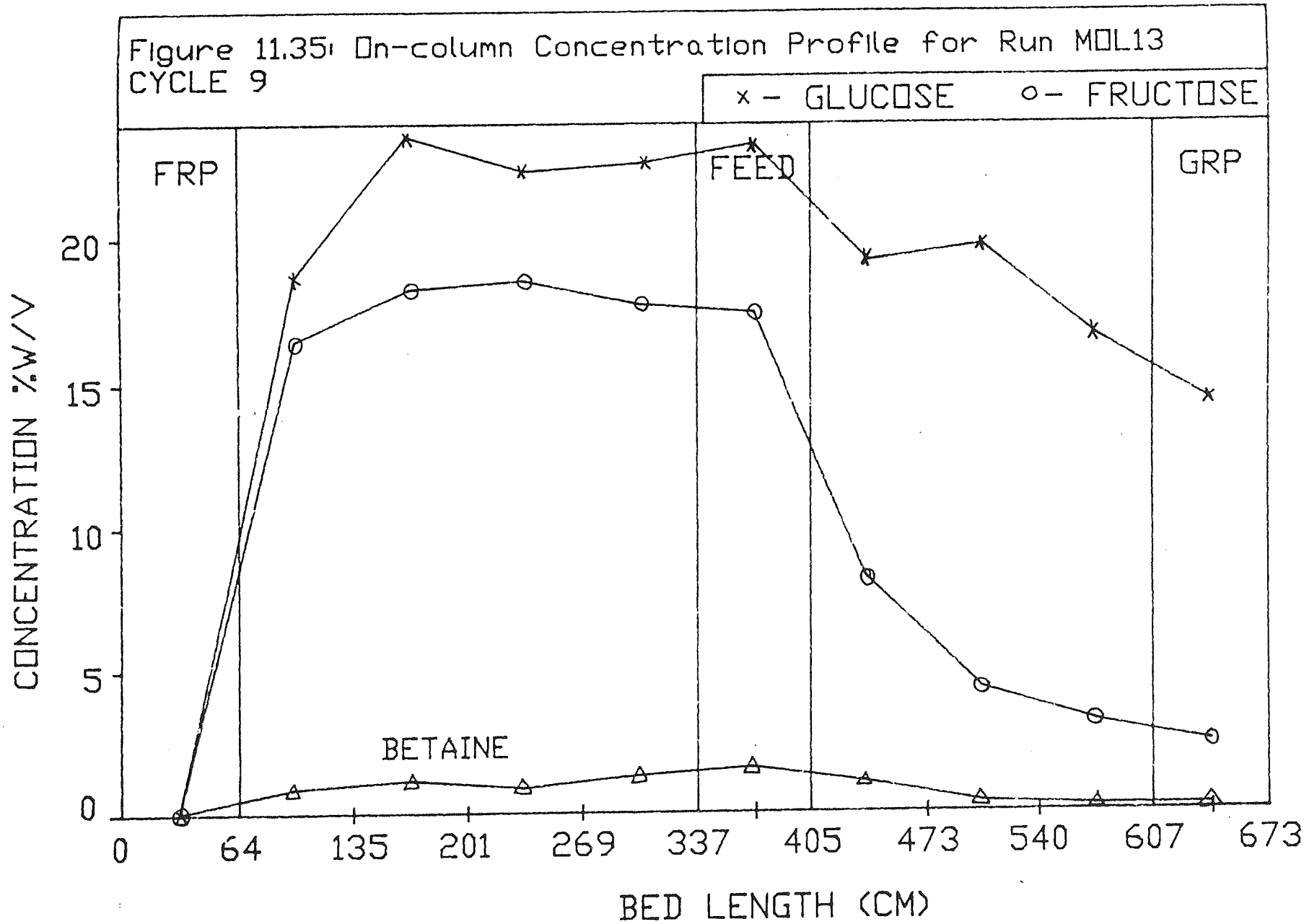


Figure 11.35: On-column Concentration Profile for Run MOL13
CYCLE 9
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
BETAINE
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

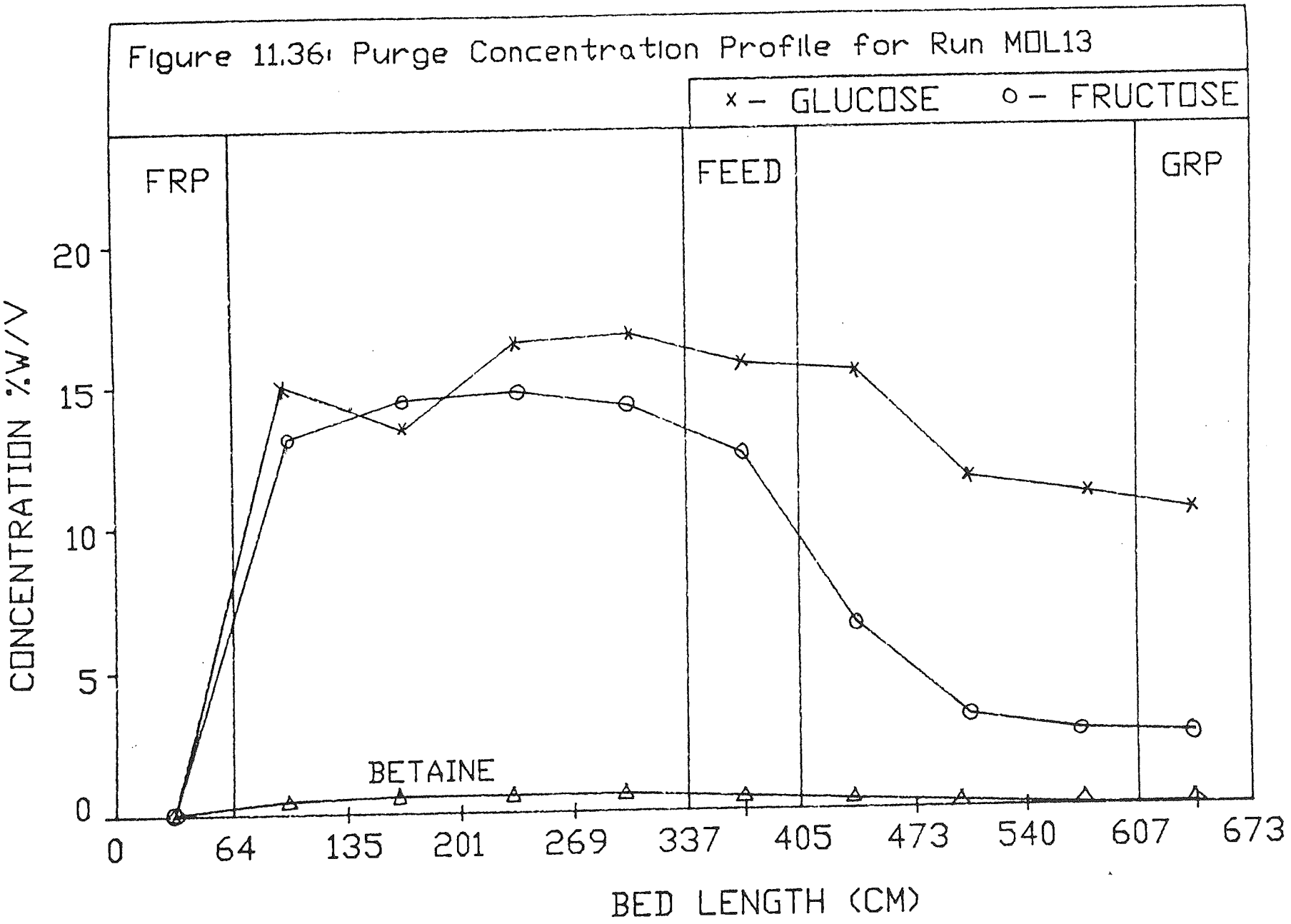


Figure 11.36: Purge Concentration Profile for Run MOL13
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
CONCENTRATION %W/V
20
15
10
5
0
BETAINE
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

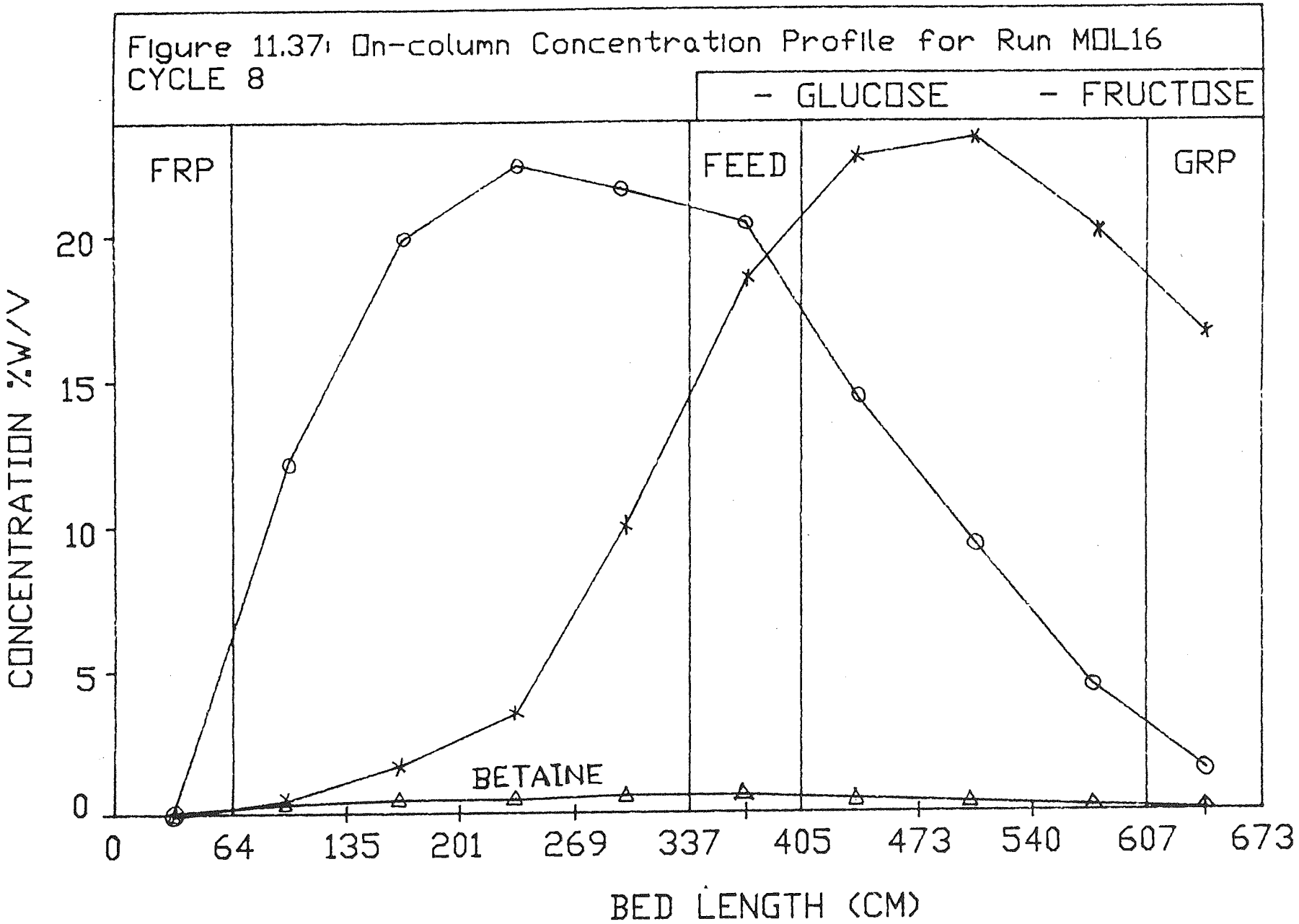


Figure 11.37: On-column Concentration Profile for Run MOL16
CYCLE 8
- GLUCOSE
- FRUCTOSE
FRP
FEED
GRP
BETAINE
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

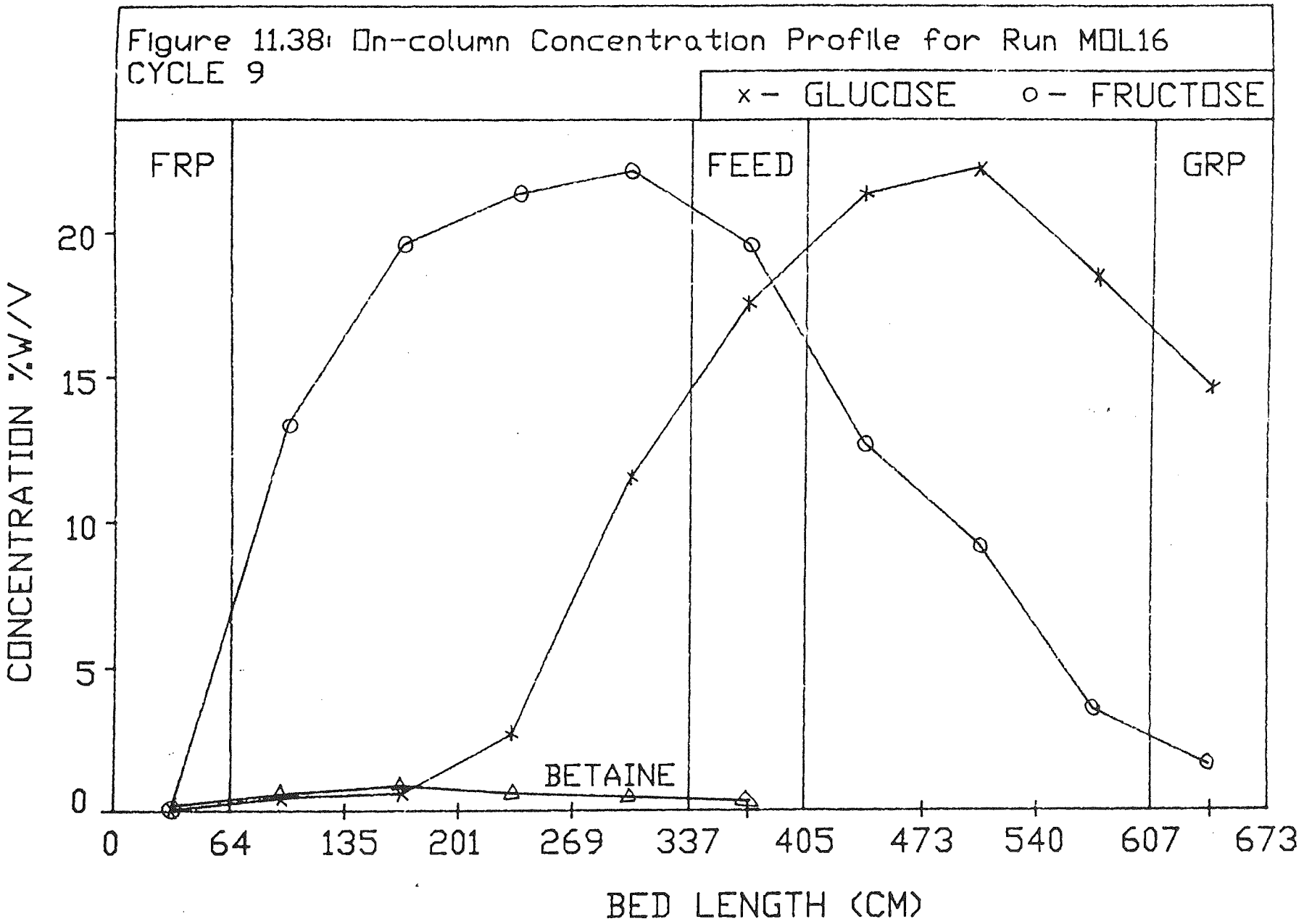


Figure 11.38: On-column Concentration Profile for Run MOL16
CYCLE 9
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
BETAINE
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

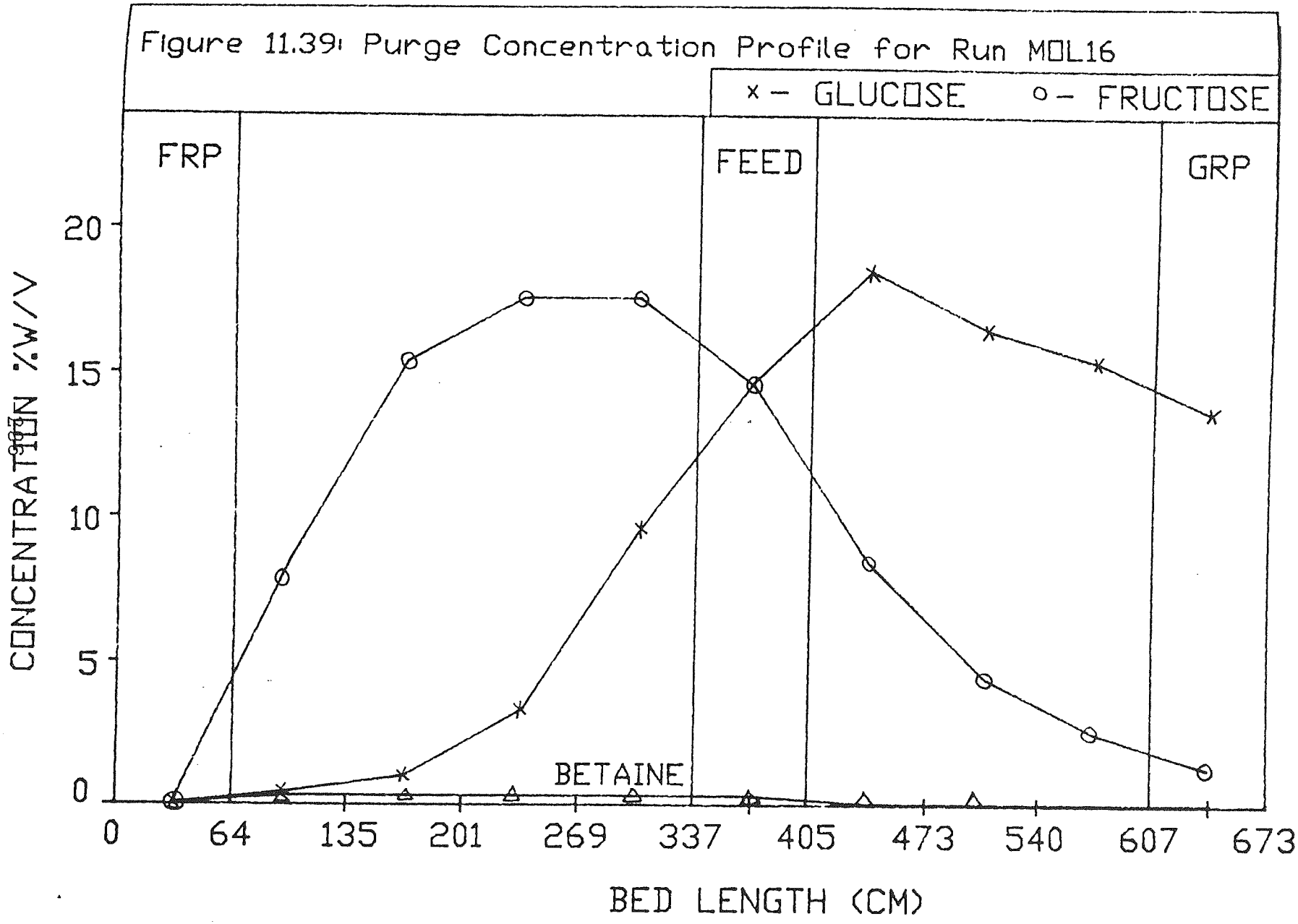


Figure 11.39: Purge Concentration Profile for Run MOL16
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
BETAINE
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
673
BED LENGTH (CM)

Figure 11.40: Individual Glucose Profiles for Varying Feed Concentration experiments

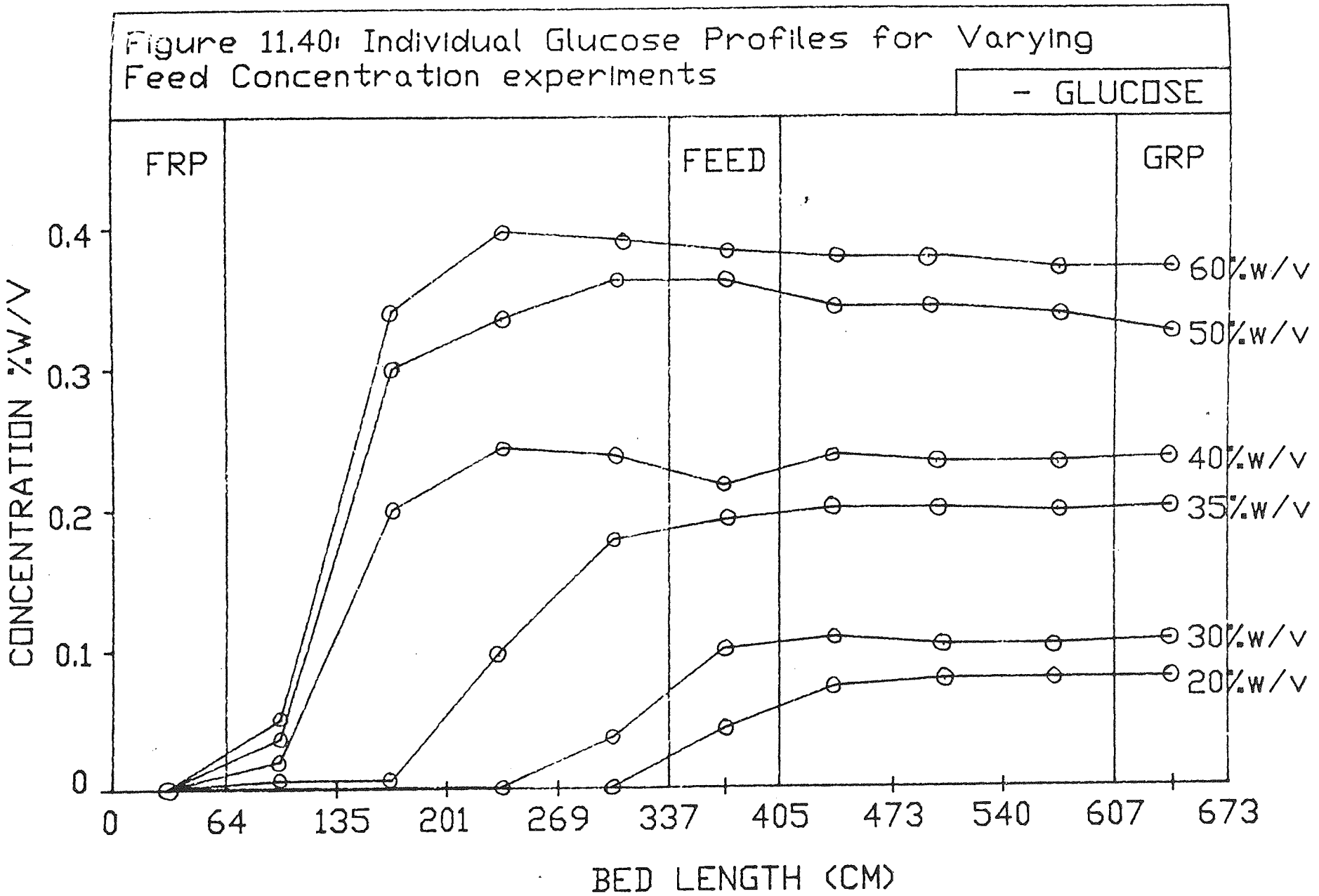
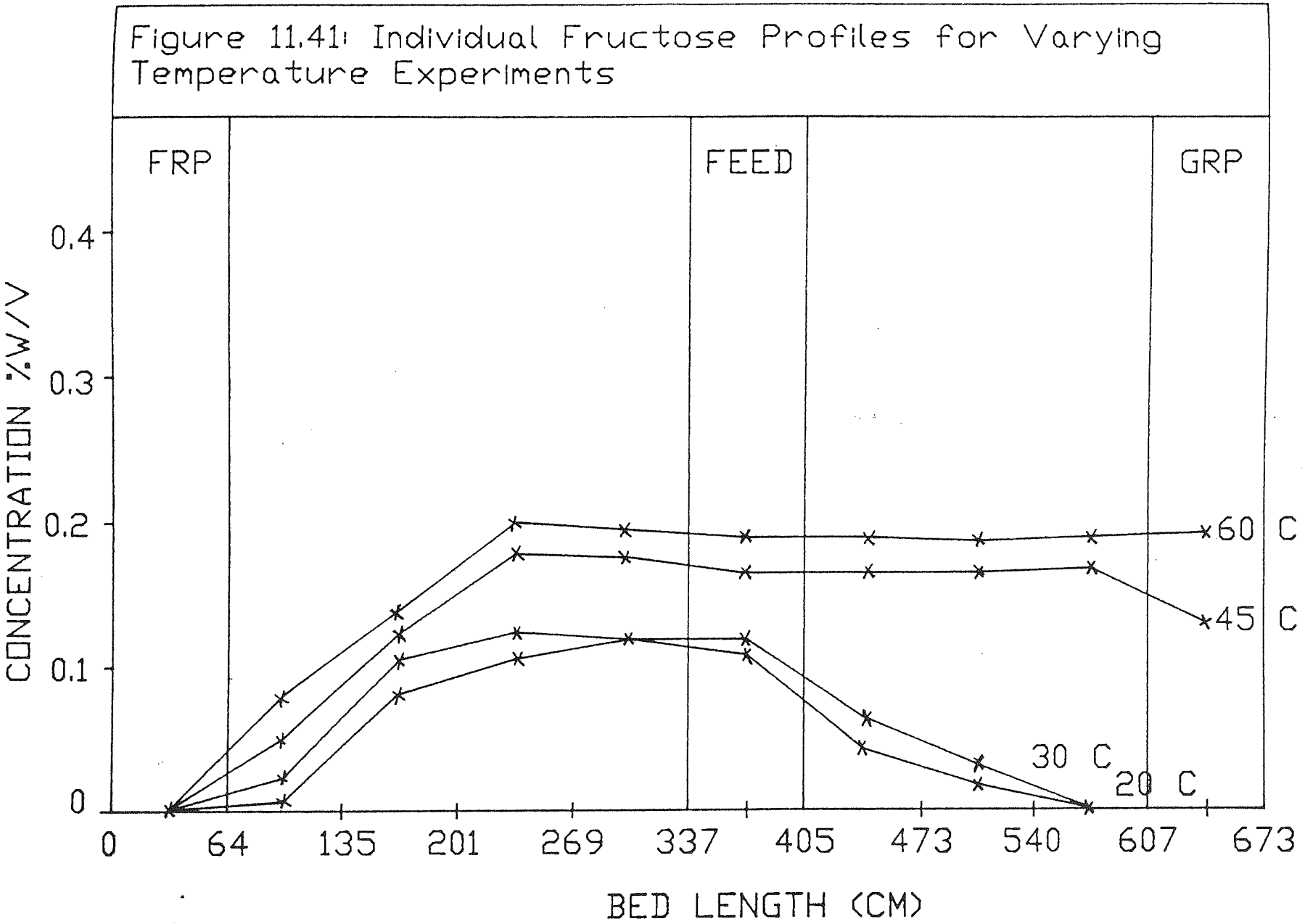


Figure 11.41: Individual Fructose Profiles for Varying Temperature Experiments



CHAPTER TWELVE

ISOMERISATION OF GLUCOSE TO FRUCTOSE USING IMMOBILISED GLUCOSE ISOMERASE

12.1 Introduction

The enzyme used for the isomerisation of glucose to fructose was glucose isomerase which was in the immobilised form. The main difference between a free and an immobilised enzyme is that, once immobilised, the enzyme is no longer completely surrounded by an aqueous environment (72). The immobilised form of an enzyme is the generally preferred type as it is more economical to operate a fixed bed bio-reactor compared to batch operation with stirred tank reactors.

Glucose isomerase is one of the most important enzymes used for commercial processing in the food industry. The enzyme is an intracellular enzyme produced from a selected strain of 'Bacillus coagulans' and is difficult and expensive to produce. The enzyme is available in both the immobilised and free forms (73).

The next section outline two possible ways the SCCR6 could be modified to give higher yields of fructose from inverted beet molasses using beds of immobilised glucose isomerase. The section also records the experiments carried out in a column packed with glucose isomerase for the isomerisation of glucose to fructose with synthetic

mixtures of glucose and fructose and also a GRP from an inverted molasses feedstock experiment.

12.2 Isomerisation and Separation Using the SCCR6

As the SCCR6 system operates in a cyclic mode where all the columns carry out the same operation once in a complete cycle, it is not possible to use some columns as enzyme reactors and some as separators. However there are two methods to carry out the isomerisation and separation of the glucose and fructose on the SCCR6 unit without changing the equipments' operating principle (74).

Figure 12.1 shows the first method which is the simplest of the two requiring no modifications to the SCCR6 separating section. The glucose rich product (GRP) could be passed through a reactor column (R) packed with the immobilised enzyme to isomerise some of the glucose to fructose. The reaction products could then be used to dilute the feed solution and hence be recycled back to the SCCR6 unit for further separation. A purge stream may be necessary to prevent the build up of unwanted components when using some carbohydrate feedstocks.

The advantages for this type of system are that no major modifications are required to the SCCR6 system. Also, by diluting the feed with the partially converted glucose, the fructose/glucose ratio would increase resulting in an easier separation. Thus greater yields of fructose would be

possible due to the isomerisation step.

A disadvantage of this type of system is that a maximum of 55% of the glucose present in the GRP can be isomerised in one pass through the enzyme reactor (75). Further isomerisation requires the removal of fructose from this product before passing the mixture again through the reactor column. Thus additional columns could be added downstream in series alternatively as a reactor - separator - reactor - separator, requiring this downstream section to operate as a repetitive batch process.

Figure 12.2 shows the second method of isomerisation of glucose to fructose using a bio-reactor column linked into the SCCR6 system. This method involves the solution to be repetitively passed from the transfer line on the post-feed section of the SCCR6 unit, through a reactor column and then recycled as a reaction product back into the transfer line. In this way, a fraction of the glucose would be isomerised to fructose and the newly produced fructose would be retained on the next column. From Figure 12.3, it can be seen that two three-way valves are used on the transfer line between columns 7 and 8. Valves A2 and B1 are closed whilst valves A1, A3, B2 and B3 are open. The solution from column 7 passes through the reactor and reactor products re-enter a transfer line back to column 8.

A number of such reactor columns could be used and their dimensions would depend on the expected switch time

and reaction kinetics. The exact positioning of the reactor columns would be determined by the on-column concentration profiles and the number of separating columns required to retain the newly produced fructose. An additional reactor could also be added at the end of the system to allow the GRP to be further isomerised if required. A purge stream may be required to remove any unwanted components from the system. The final product would be rich in fructose and could be used as an eluent for the system.

If three reactor columns are used and on the basis that 55% of the glucose would be isomerised per reactor, then up to 87% of the glucose present could be isomerised to fructose. This value is based on the assumption that all the fructose produced in the reactor columns is retained on the last two separating columns.

The advantages of this system are that the eluent requirement would be minimised and the fructose production rate increased. Also, the operation would still be continuous.

The disadvantages are that as the maximum isomerisation is 55% of the glucose, the use of the reaction products as eluent would result in contamination of the FRP. Also, the system requires significant modifications increasing the complexity of the system. The systems 'dead volume' is increased and it would take longer to reach pseudo-equilibrium. It would require 24 new three-

way valves and two new pipe-lines around the system for every reactor used as shown in Figure 12.4.

12.3 Glucose Isomerase Reactor Test Runs

Runs were carried out on a column packed with immobilised glucose isomerase enzyme to study the isomerisation of glucose to fructose under varying conditions. Figure 12.5 shows the layout of the equipment used for these runs.

A jacketed glass column 1.75m in length and average 1.94cm ID was packed with the immobilised enzyme supplied by Novo Enzymes Ltd under the trade name SWEETZYME Q. The enzyme was immobilised on polystyrene beads. The voidage in the column was 0.55. A heater/cooler unit was used to keep the temperature of the enzyme column at 3°C when not in use and 60°C when isomerisation tests were being carried out.

A peristaltic pump was used to pump the feed through the enzyme. The product was analysed using HPLC to monitor the isomerisation rates.

For the initial runs, a synthetic mixture of glucose/fructose was used. The test feed solution containing 20% sugar solids (90% glucose + 10% fructose in composition) was used for all the runs. This was to match a typical GRP product leaving the SCCR6 in composition and concentration.

The initial experiments were performed to study the

effect of flowrates on the level of isomerisation achieved. Five runs were carried out using the same feed and system at flowrates of 3, 5, 15, 30 and 40$cm^3.min^{-1}$. The feed was pumped through the column and the products analysed to see the amount of glucose isomerised to fructose at these flowrates. Table 12.1 shows the % glucose isomerised at the different flowrates. This value is defined as the ratio of the amount of glucose isomerised to the amount of glucose present in the feed.

Table 12.1: Flowrate vs Rate of Glucose Isomerisation

Flowrate $cm^3.min^{-1}$	INPUT		OUTPUT		% glucose isomerised
	% glucose	% fructose	% glucose	% fructose	
40	85.98	11.16	72.32	26.70	15.88
30	85.95	11.14	65.91	30.98	23.32
15	85.81	11.16	51.79	45.04	39.65
5	89.95	9.75	49.77	50.23	44.67
3	83.75	12.19	38.20	60.86	54.39

From the table, it can be seen that the isomerisation is at a maximum at a flowrate of 3$cm^3.min^{-1}$. This corresponds to a linear velocity through the column of 0.009$cm.min^{-1}$. As the experimental program was limited to the type of equipment available, it was not possible to carry out further experiments using another column with a

larger cross-sectional area thereby enabling larger volumes of solution to pass through the column while maintaining the overall isomerisation rate.

Having obtained a set of results using a synthetic feedstock, further runs were carried out on the same column but using a GRP product from a previous SCCR6 run as feedstock. The GRP was pumped at a flowrate of $10cm^3.min^{-1}$ through the column and after a single pass, the product was collected and analysed. Table 12.2 shows the isomerisation of glucose obtained for a synthetic mixture as well as for the GRP at the same flowrate. It can be seen from the table that the % glucose isomerised by the enzyme has decreased by 50% when using the GRP from an inverted molasses run as feed. After communication with the enzyme manufacturers, it became apparent that the enzyme was sensitive to the presence of calcium ions and the activity was greatly suppressed if the calcium ion concentration present was greater than 1 part per million. The presence of any magnesium ions in the feed syrup also enhances the activity of the enzyme and stabilises the loss of activity. Therefore any isomerisation of the glucose in the GRP (from inverted molasses) to fructose using immobilised glucose isomerase would require a pretreatment with an ion-exchange resin bed to replace the calcium ions in the feed by magnesium ions. Alternately, a weight ratio between magnesium and calcium ions of 12 should be provided for.

Table 12.2: Comparison of the Two Feedstocks

Feed Type	INPUT % glucose	INPUT % fructose	OUTPUT % glucose	OUTPUT % fructose	% glucose isomerised
Synthetic	89.93	9.97	52.08	43.87	42.09
GRP	88.33	11.67	69.74	30.26	21.04

This section of the experimental programme was terminated as the ion-exchange resins to replace the calcium with the magnesium were not available readily in the UK and had to be imported from USA by the suppliers. As this would take over three months it was decided to stop further research in this area and concentrate on the rest of the inverted molasses programme.

Figure 12.1: SCCR6 Modifications for GI Reactor - 1

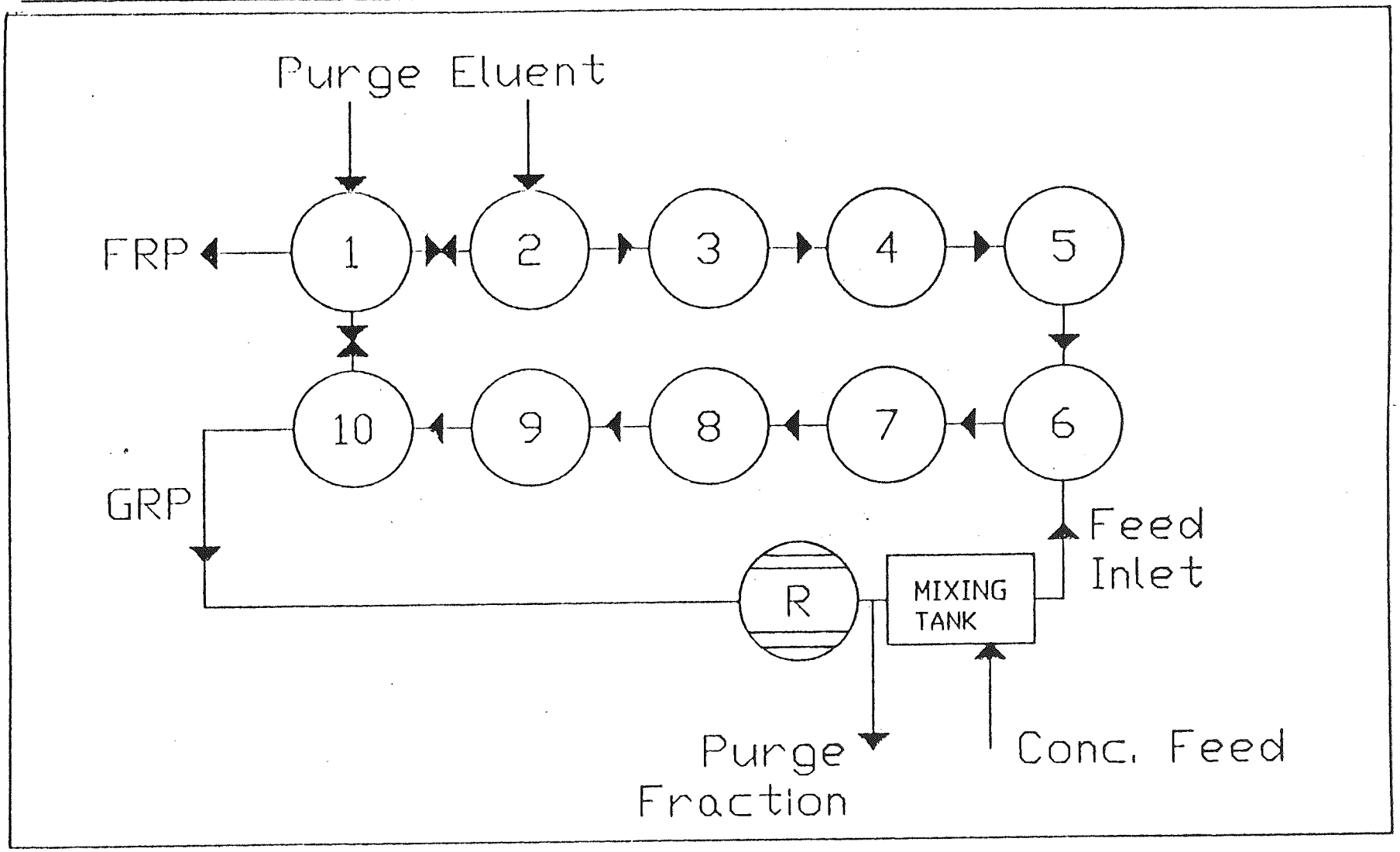


Figure 12.2: SCCR6 Modifications for GI Reactor - 2

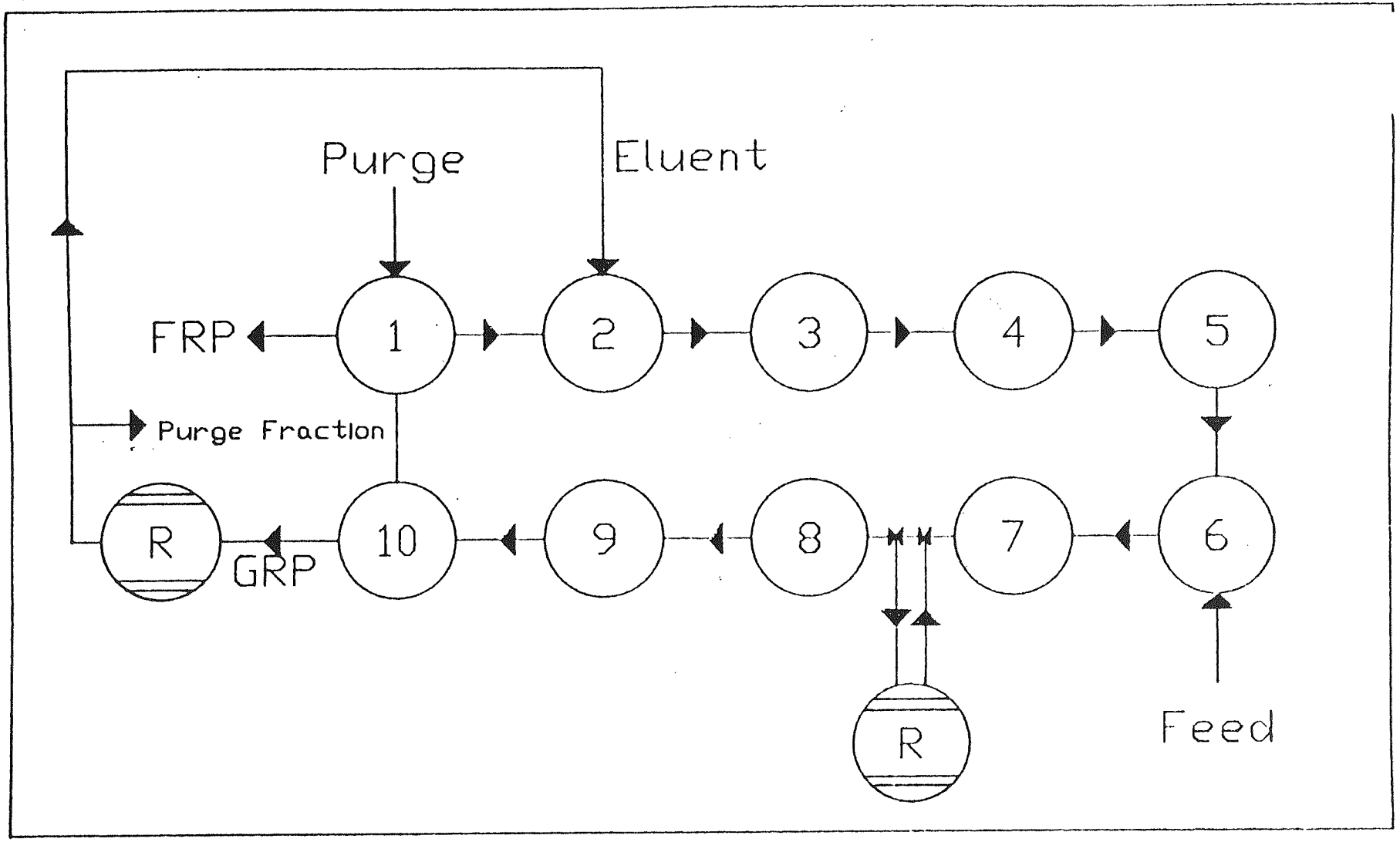
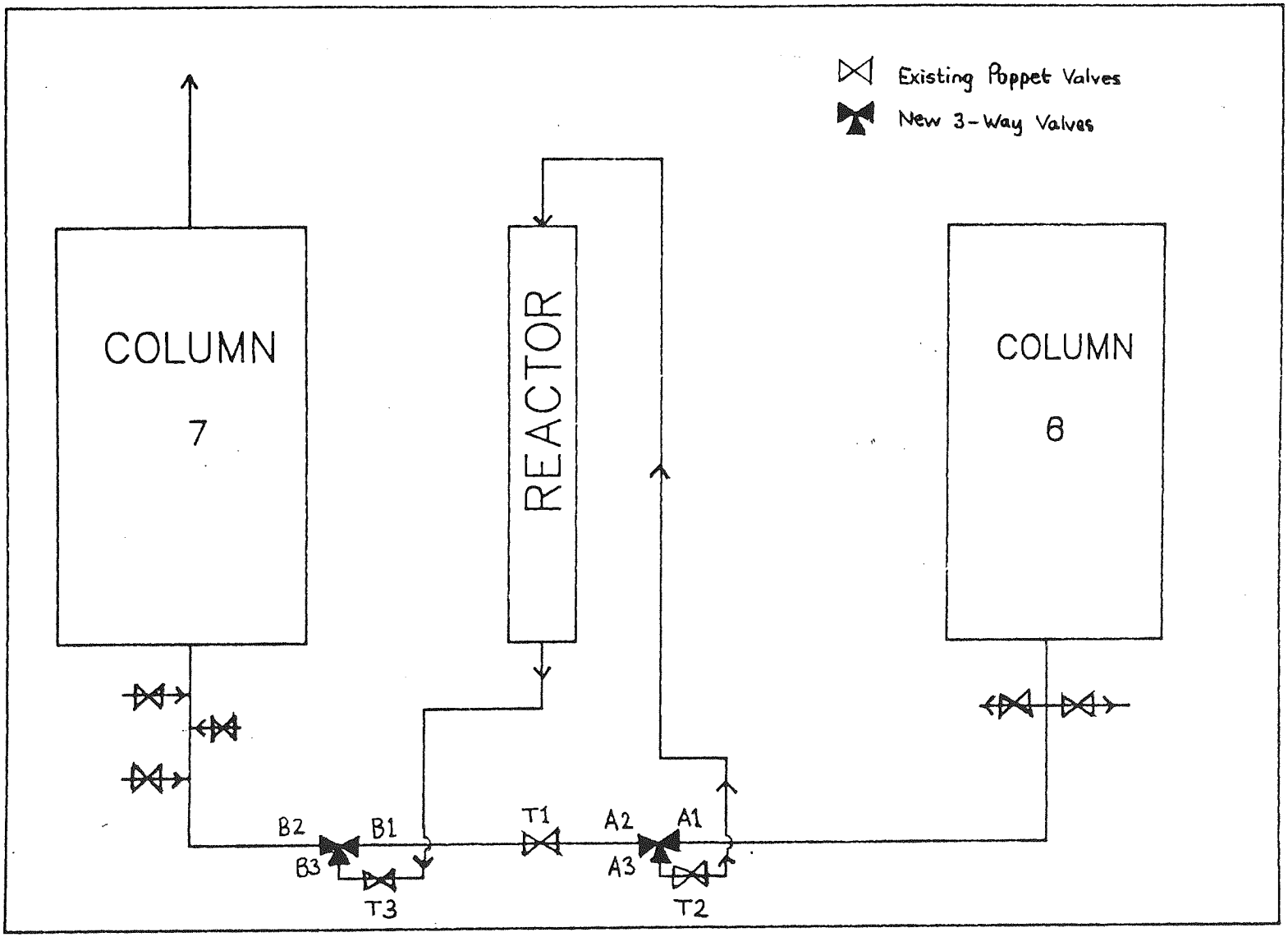


Figure 12.3: Close up of Columns 6 and 7



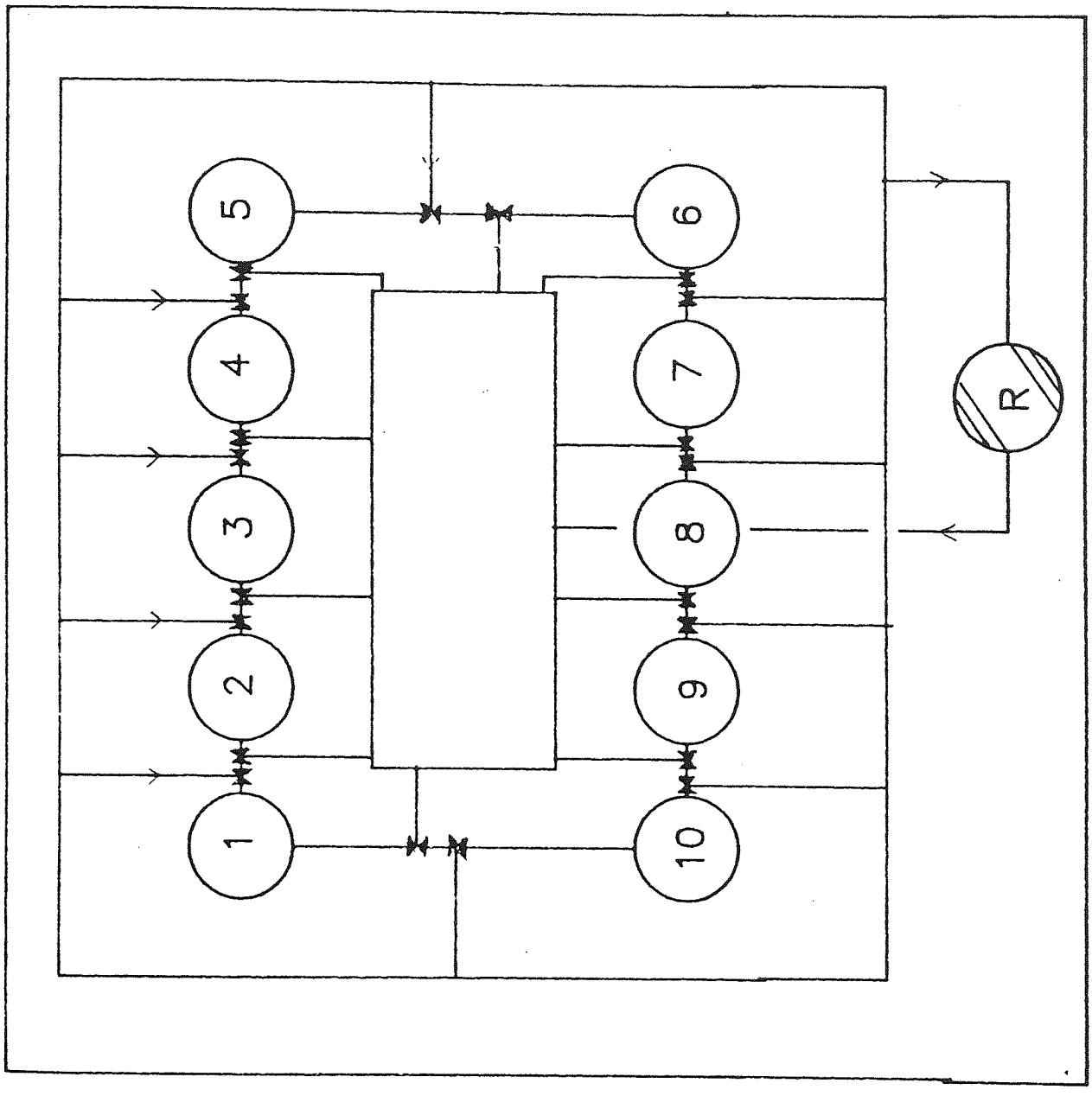


Figure 12.4: Pipe Network per Reactor Column

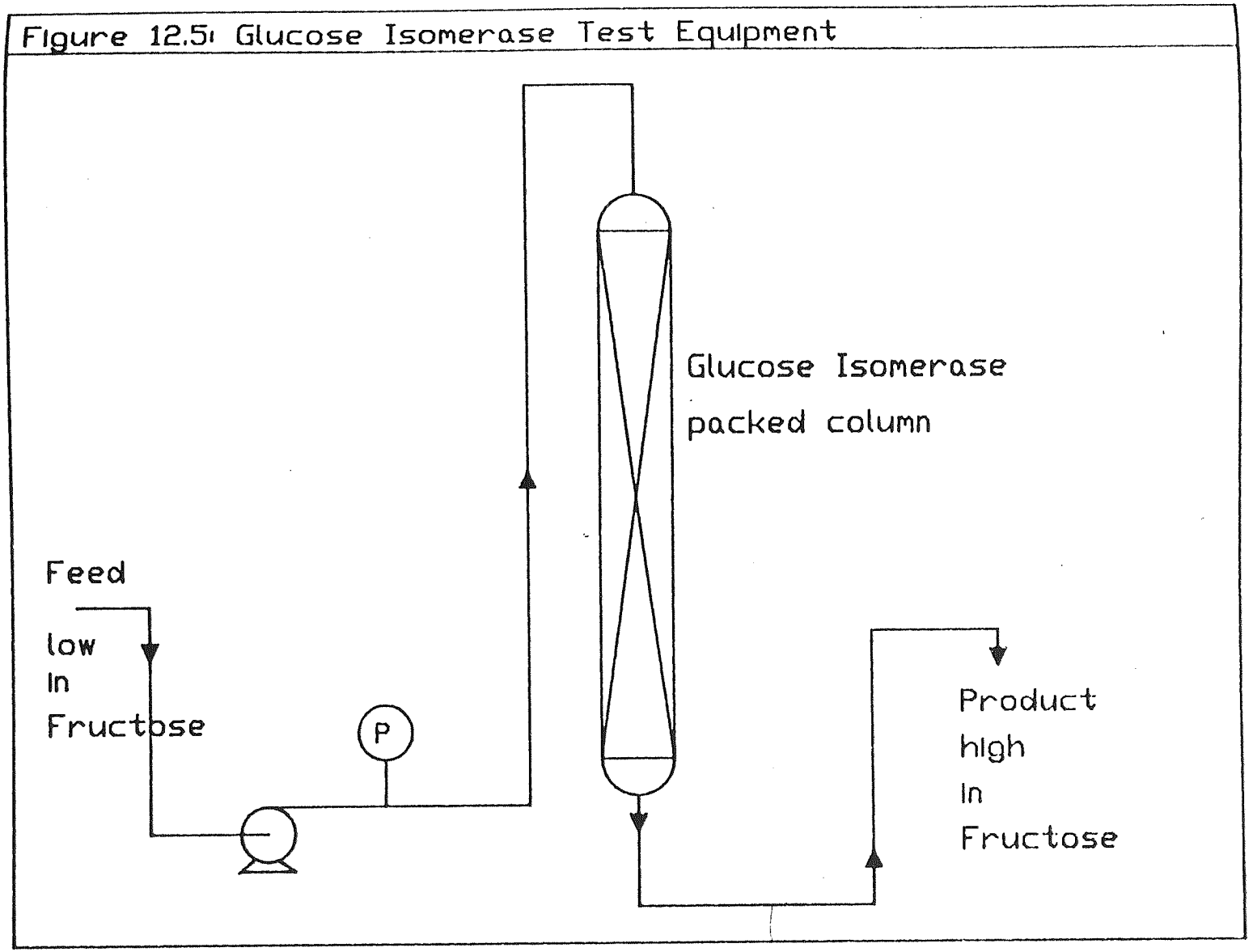


Figure 12.5: Glucose Isomerase Test Equipment
Glucose Isomerase
packed column
Feed
low
in
Fructose
P
Product
high
in
Fructose

CHAPTER THIRTEEN

MATHEMATICAL MODELLING AND COMPUTER SIMULATION OF THE SCCR6

13.1 Introduction

The development of mathematical models to simulate batch chromatographic processes and to predict elution profiles have been developed in the past (76, 77), but most of these models involved statistical approaches which are simplified and applied to special cases.

A mathematical model based on the plate theory was developed by Jonsson (78) which simulates the diffusion and adsorption processes that take place in a chromatographic column. Sciance and Crosser (79) developed a probabilistic model for continuous chromatographic systems relating the degree of separation, system length and operating conditions for a binary feedstock and mid-point feed location.

Al-Madfai (80) developed a model based on the random walk approach to predict the plate height for moving bed continuous counter-current chromatographic gas-liquid system as follows:

$$H = d_p + \frac{2D_m}{u} + \frac{2\mu_1\mu_2}{(\mu_1+\mu_2)^2} \times \frac{(u+u_L)^2}{u\mu_2-u_L\mu_1} \quad \ldots\ 13.1$$

where:

H = plate height

d_p = average particle diameter

D_m = mobile phase diffusivity

u = mobile phase velocity

u_L = stationary phase velocity

μ_1 = rate of transfer of molecules from gas to liquid

μ_2 = rate of transfer of molecules from liquid to gas

Barker and Lloyd (81) employed a transfer unit concept in their modelling of counter-current gas-liquid chromatographic systems and the resulting equations predicting the number of overall gas phase transfer units (N_G) were:

- For the stripping section:

$$(N_G)_S = \frac{1}{(1-V_G/K_oV_L)} \ln\left[\frac{(E_2/K_oV_L)-(C_1(1-V_G/K_oV_L)}{(E_2/K_oV_L)-(C_2(1-V_G/K_oV_L)}\right] \quad \ldots 13.2$$

- and for the rectifying section:

$$(N_G)_R = \frac{1}{V_G/(K_oV_L-1)} \ln\left[\frac{(E_1/K_oV_L)-(C_1(V_G/K_oV_L-1)}{(E_1/K_oV_L)-(C_2(V_G/K_oV_L-1)}\right] \quad \ldots 13.3$$

where:

E_1 and E_2 = mass flowrates of solute leaving products 1 and 2 respectively

C_1 and C_2 = gas phase solute concentrations at points 1 and 2 in the column

K_o = partition coefficient

V_G and V_L = gas and liquid volumetric flowrates

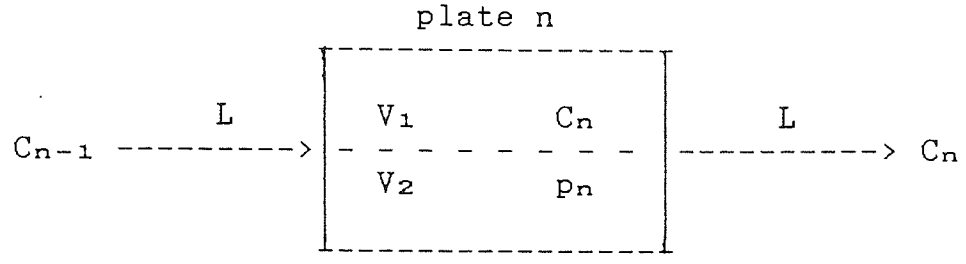
The results indicated that the main resistance to mass

transfer was in the gas phase.

13.2 Approach Used for Simulating the Semi-continuous Operation of the SCCR6 System

A computer program based on plate to plate calculations to describe the operation of a circular counter-current chromatographic system for a gas-liquid system was developed by Sunal (82). Deeble (83) used this approach to simulate the operation of the SCCR1 gas-liquid system and was further developed by Ching (6) for his liquid-liquid chromatographic studies on the SCCR4 system. The computer 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 stationary phase. The mobile phase leaving each stage is assumed to be at equilibrium with the stationary phase in the stage.

Therefore, considering a mobile phase flowrate, L, passing through a plate 'n' having an initial solute concentration of 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:

$$LC_{n-1} = LC_n + V_1 \frac{dC_n}{dt} + V_2 \frac{dp_n}{dt} \qquad \ldots\ldots 13.4$$

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 flowrate

The equilibrium on the plate is represented by the distribution coefficient K_d, which is defined by:

$$K_d = \frac{p_n}{C_n} \qquad \ldots\ldots 13.5$$

Substituting this equation into equation 13.4 gives:

$$LC_{n-1} = LC_n + (V_1 + V_2K_d)\frac{dC_n}{dt} \qquad \ldots\ldots 13.6$$

This equation can be integrated provided δt is sufficiently small to allow C_{n-1} to be considered constant, to give:

$$C_n = C_{n-1}\left[1 - \exp\left[\frac{-L\ \delta t}{V_1+V_2K_d}\right]\right] + C_n^{o}\exp\left[\frac{-L\ \delta t}{V_1+V_2K_d}\right] \qquad ..13.7$$

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 the equation 13.7 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 flowrate F, hence the term C_{n-1} is replaced by the ratio:

$$\frac{LC_{n-1} + FC_f}{L + F} \qquad \ldots\ldots 13.8$$

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.

13.3 Improvements to the Model

The initial simulation work carried out by Ching (6) assumed that the glucose was not retained on the resin and he used the glucose distribution coefficient equal to zero. Chuah (7) and Gould (8) improved Ching's model to account for the glucose retention due to the size exclusion principle and used a finite value for the glucose distribution coefficient. Abusabah (71) used the model to predict the concentration profiles for 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 (9) modified the model developed by Gould to account for the effect of the background concentration on the distribution coefficients. The concentration-distribution coefficient used however, were applicable to the small 0.5cm id x 50 cm long batch column they were derived on, and their contribution to the simulation of the SCCR6 system was limited.

Ganetsos (10) used Thawaits' model as a basis and further modified it to take into account the effect of background concentration and flowrates expressed as linear velocities on the distribution coefficients.

An alternative approach for the modelling of the SCCR systems was used by England (59) where instead of assuming that C_{n-1} remains constant over a small time interval, δt, he obtained n number of simultaneous equations for the n plates, similar in form to equation 13.6, ie.:

$$FC_{fn} + LC_{n-1} = (L+F)C_n - (V_1+K_dV_2)\frac{dC_n}{dt} \quad .... \ 13.9$$

Rearranging and dividing by $(V_1 + K_dV_2)$ gives:

$$\frac{dC_n}{dt} = X_nC_{n-1} - Y_nC_n + Z_nC_{fn} \quad .... \ 13.10$$

where:

$$X_n = \frac{L}{V_1+K_dV_2}$$

$$Y_n = \frac{L + F}{V_1+K_dV_2}$$

$$Z_n = \frac{F}{V_1+K_dV_2}$$

Assuming that a chromatographic system is divided into n stages the resulting set of equations according to Equation 13.10 is as follows:

$$C_1 = XC_o - YC_1 + ZC_{f1}$$

$$C_2 = XC_1 - YC_2 + ZC_{f2}$$

in general:

$$C_n = XC_{n-1} - YC_n + ZC_{fn}$$

or

$$C(t) = AC(t) + BC_f(t) \qquad \ldots\ldots 13.11$$

Equation 13.11 is solved in reference (84) and the solution is:

$$C(t) = \exp(At)\, C_o + \int_0^t \exp[A(t-\tau)]\, BC_f(\tau)\, d\tau \qquad \ldots 13.12$$

If the feed input is not time dependent the solution is:

$$C(t) = \phi(t)\, C_o + \delta(t)\, BC_f$$

where:

$$\phi(t) = \exp(At)$$

and:

$$\delta(t) = A^{-1}[\exp(At)-I] = A^{-1}[\phi(t)-I]$$

Although this approach was theoretically better, it has been found that it required excessive computing time exceeding the maximum time of 10000seconds available on the HARRIS 800 mainframe computer at the University. Therefore the original approach was followed in the simulation work based on equation 13.7.

13.4 Improvements of the Simulation Program and its Application to the SCCR6 System

The two versions of the simulation programs developed by Thawait (9) and Ganetsos (10) were used as a basis. The main section of the programs including the modelling equations was modified to take into account the effect of flowrates, temperature and background concentration on the equilibrium coefficients for glucose, fructose, betaine and potassium chloride. The structures of the programs were modified to suit the molasses feedstocks operating conditions as described later.

The program written was arranged in a general form to improve its compatibility with different SCCR systems of various configurations. All the system related parameters in the main program, ie. dimensions, number 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, flowrates, feed composition, and purging period; and program related parameters such as number of cycles and time increments were replaced as 'dummy' variables which were defined and placed at the beginning of the program.

The program was modified to run as a 'control job point' where it was executed stepwise whenever there was some free computing time available. It was also modified to

run on an IBM PC micro-computer continuously.

The program was rearranged to account for up to four components; the retarded betaine due to size exclusion, the retarded fructose due to the calcium ion complexing, the lesser retarded glucose due to size exclusion, and the fast moving impurities (the non-sugars and ionics). Because of the 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 further modified to account for more than four components by including the required additional mass balance equations in the feed and separating sections.

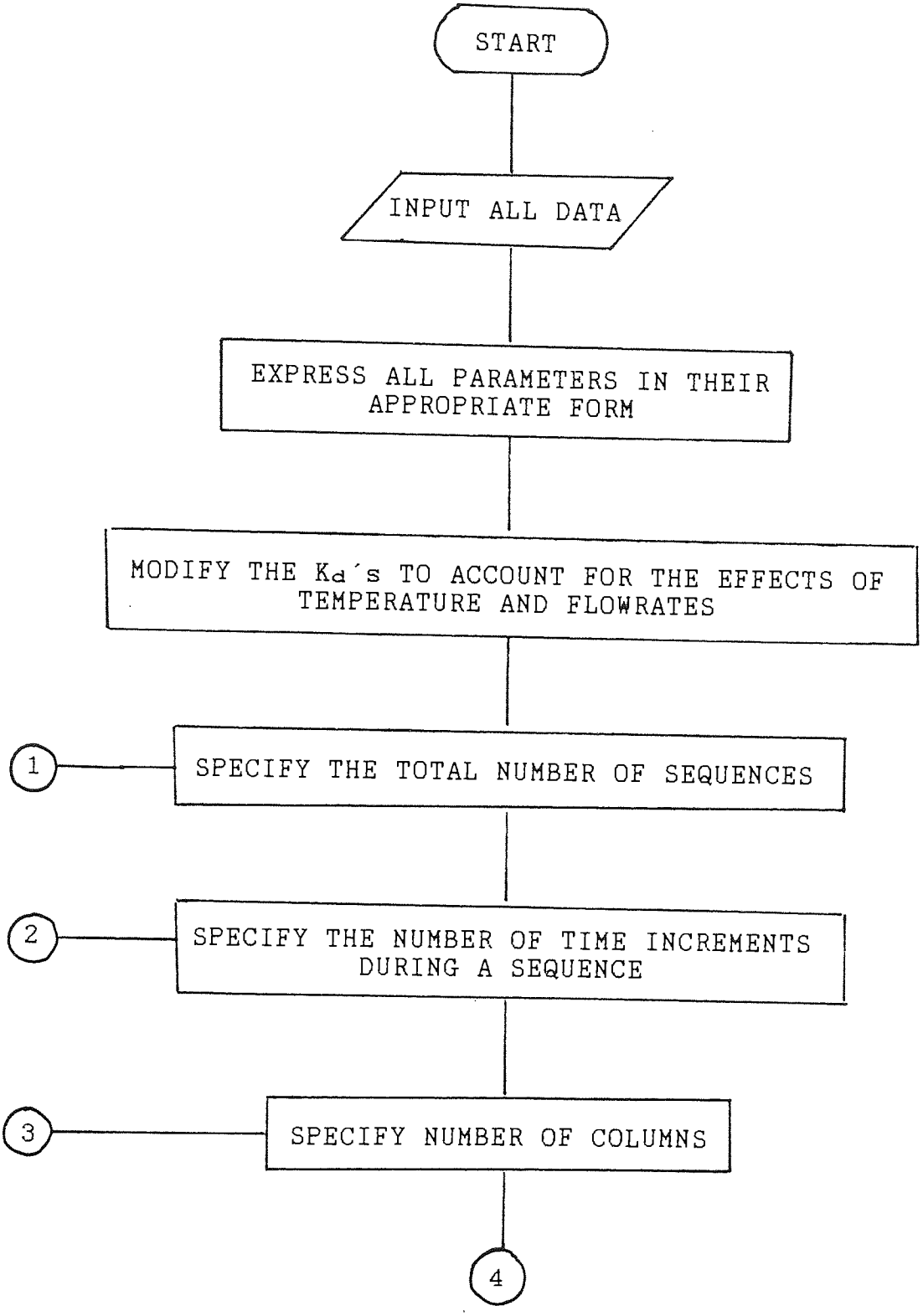
As mentioned earlier, the importance of the distribution coefficients on the simulation was identified by previous workers (6, 7, 8, 71) who used arbitrary values to achieve the best possible agreement between the experimental and simulated profiles. Thawait (9) made a further contribution by identifying the effects of background concentration and temperature on the distribution coefficients, and incorporated a set of concentration vs distribution coefficient 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

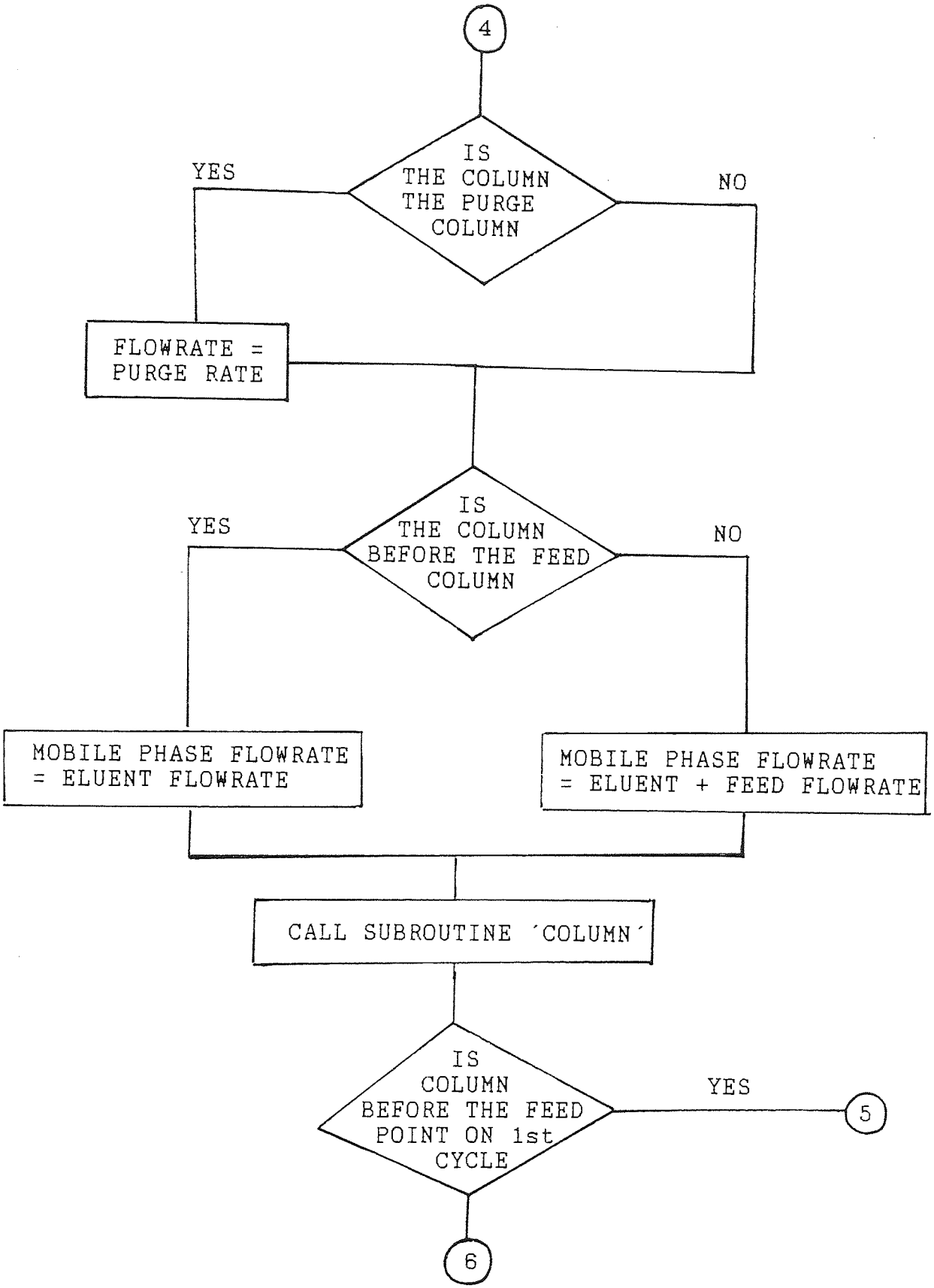
concentration vs K_d relationships.

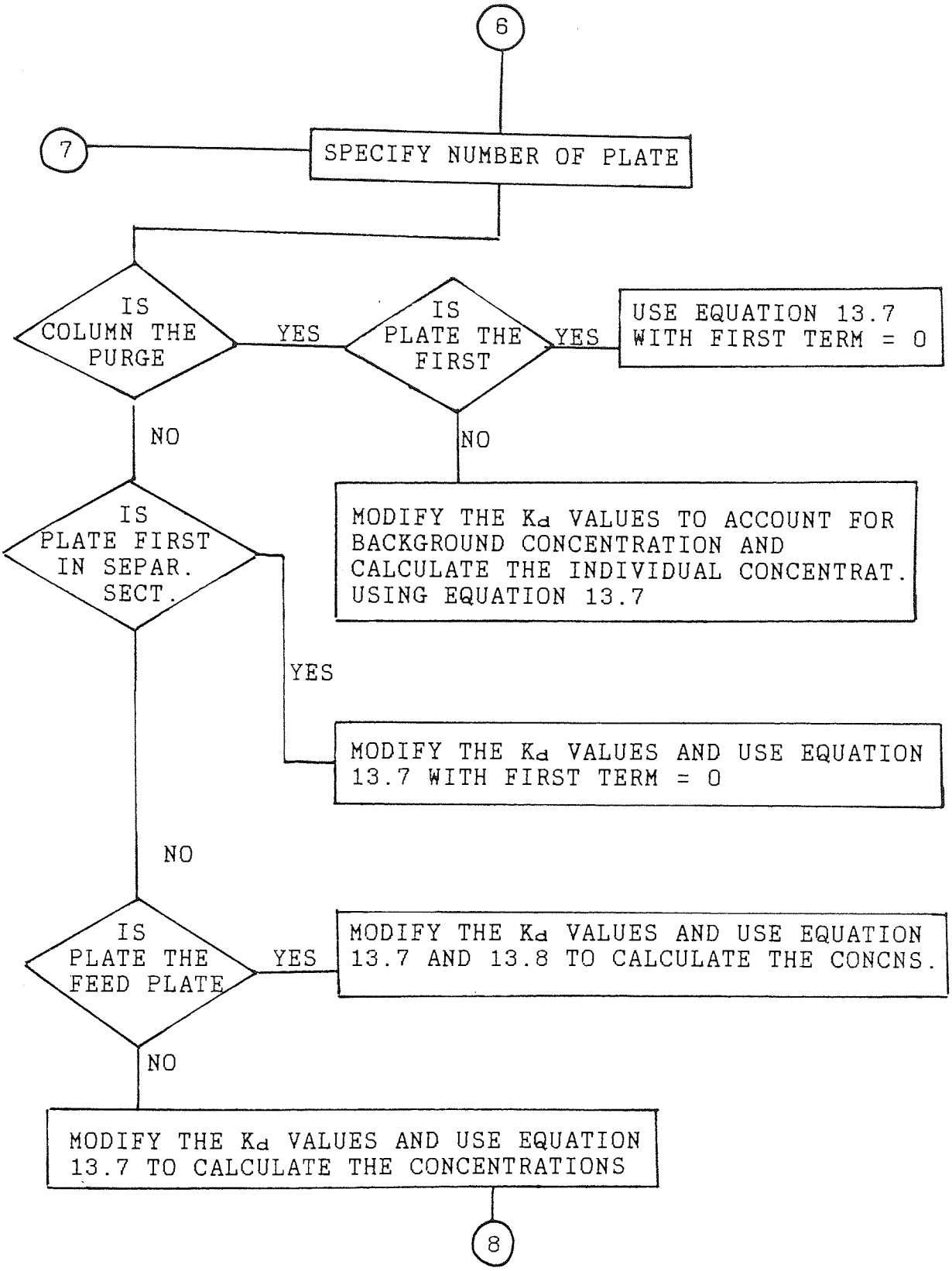
Ganetsos (10) further modified the program by using the infinite dilution coefficients obtained from the column characterisation work of the SCCR7 system and modified these to account for the effects of temperature and liquid flowrates. For the simulation work for this research, the general concentration vs K_d relationships derived in chapter 6 were used to modify the distribution coefficients according to the background concentration on each plate. The actual distribution coefficients for the 'four' components in the plate concentration calculations were the weighted averages of the values obtained using equations 6.15 to 6.26 adjusted according to the composition on each plate.

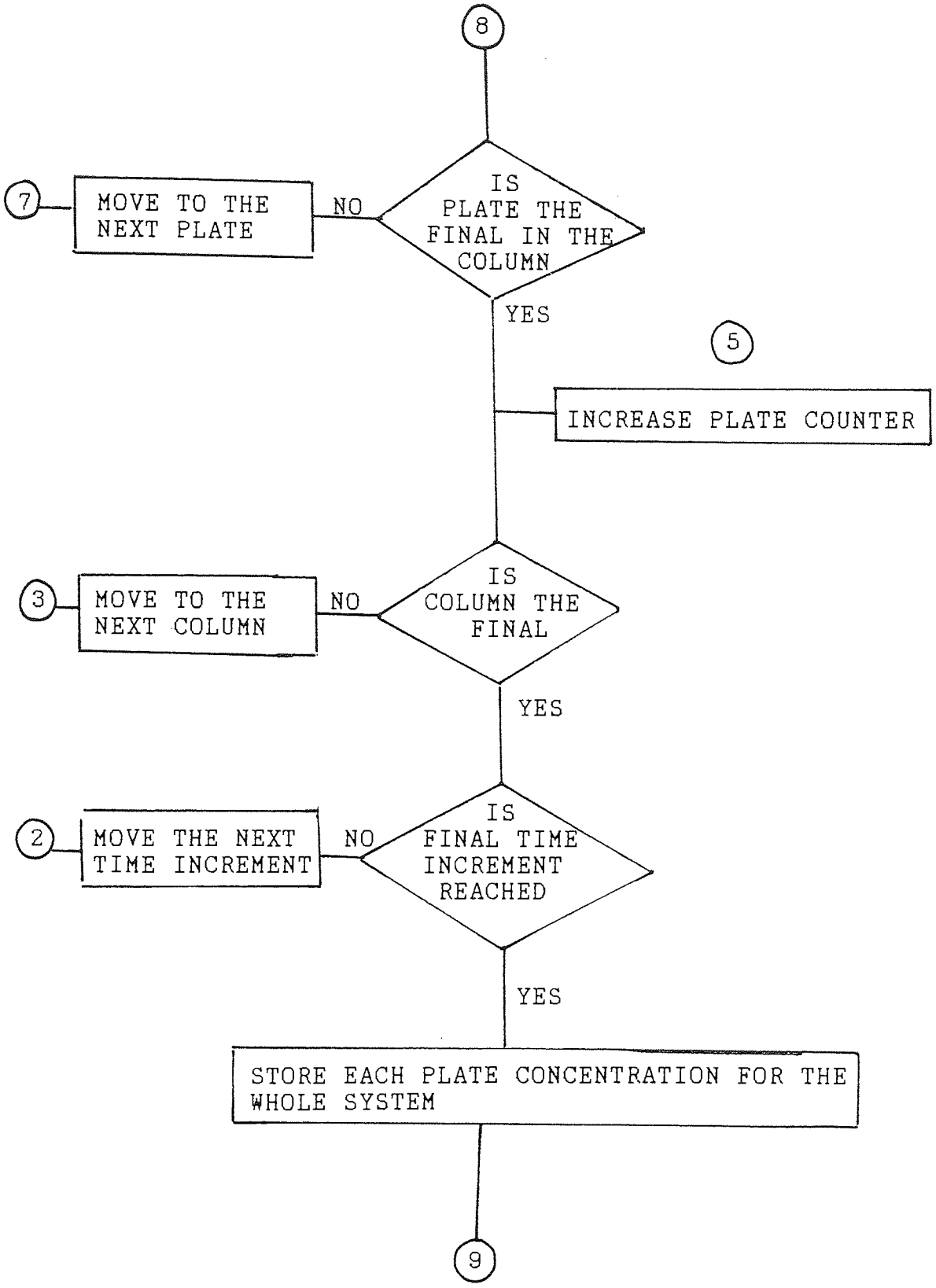
The flow diagram of the simulation program is shown in Figure 13.1. A program listing and a comprehensive list of the parameters used is shown as Figure A1 in Appendix A.

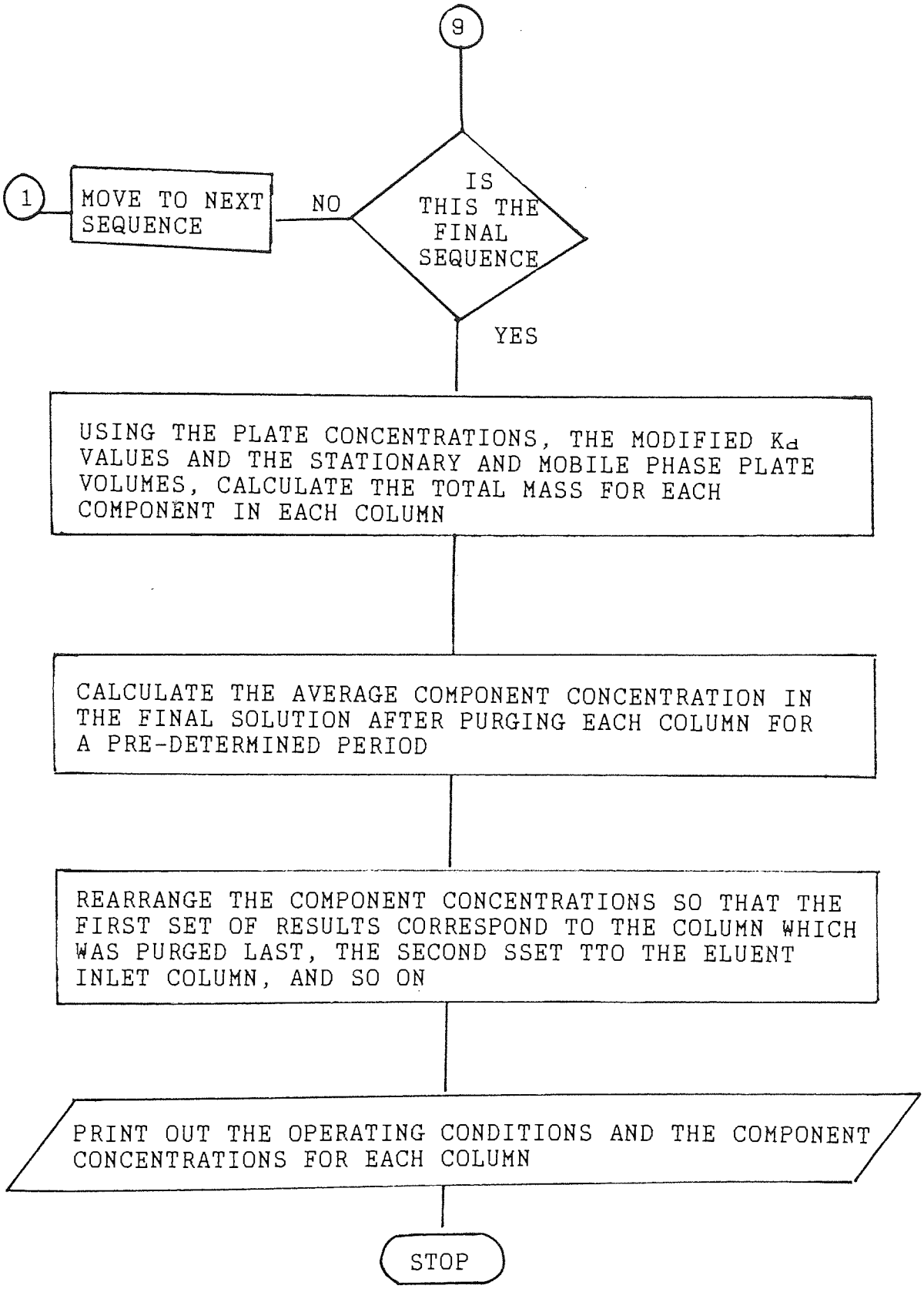
Figure 13.1: Flow Diagram of the Simulation Program




4
IS THE COLUMN THE PURGE COLUMN
YES
NO
FLOWRATE = PURGE RATE
IS THE COLUMN BEFORE THE FEED COLUMN
YES
NO
MOBILE PHASE FLOWRATE = ELUENT FLOWRATE
MOBILE PHASE FLOWRATE = ELUENT + FEED FLOWRATE
CALL SUBROUTINE 'COLUMN'
IS COLUMN BEFORE THE FEED POINT ON 1st CYCLE
YES
5
6


6
7
SPECIFY NUMBER OF PLATE
IS COLUMN THE PURGE
YES
IS PLATE THE FIRST
YES
USE EQUATION 13.7 WITH FIRST TERM = 0
NO
NO
MODIFY THE Kd VALUES TO ACCOUNT FOR BACKGROUND CONCENTRATION AND CALCULATE THE INDIVIDUAL CONCENTRAT. USING EQUATION 13.7
IS PLATE FIRST IN SEPAR. SECT.
YES
MODIFY THE Kd VALUES AND USE EQUATION 13.7 WITH FIRST TERM = 0
NO
IS PLATE THE FEED PLATE
YES
MODIFY THE Kd VALUES AND USE EQUATION 13.7 AND 13.8 TO CALCULATE THE CONCNS.
NO
MODIFY THE Kd VALUES AND USE EQUATION 13.7 TO CALCULATE THE CONCENTRATIONS
8


8
IS PLATE THE FINAL IN THE COLUMN
NO
7
MOVE TO THE NEXT PLATE
YES
5
INCREASE PLATE COUNTER
IS COLUMN THE FINAL
NO
3
MOVE TO THE NEXT COLUMN
YES
IS FINAL TIME INCREMENT REACHED
NO
2
MOVE THE NEXT TIME INCREMENT
YES
STORE EACH PLATE CONCENTRATION FOR THE WHOLE SYSTEM
9


9
IS THIS THE FINAL SEQUENCE
NO
1
MOVE TO NEXT SEQUENCE
YES
USING THE PLATE CONCENTRATIONS, THE MODIFIED Kd 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 CORRESPOND TO THE COLUMN WHICH WAS PURGED LAST, THE SECOND SSET TTO THE ELUENT INLET COLUMN, AND SO ON
PRINT OUT THE OPERATING CONDITIONS AND THE COMPONENT CONCENTRATIONS FOR EACH COLUMN
STOP

13.4 Results and Discussions

The accuracy of the simulation program was examined by using the purge concentration profiles obtained experimentally from runs MOL3,MOL6, MOL13 and MOL16. These experiments were chosen as they were run at optimum conditions and gave the purest products for their set of runs. The experimental and simulated concentration profiles for these runs are plotted in Figures 13.2 to 13.5.

The model seems to describe the four component separation well. An inspection of the respective concentration profiles shows that the 'cross-over' point locations are almost identical. The profiles also show that the individual concentration profiles, especially the fructose profile, are reproduced.

The simulated profiles for betaine and ionics have been included in the figures and they show that the betaine is eluted with the FRP whereas the bulk of the ionics elute with the GRP. This has been confirmed experimentally.

The fructose experimental profiles had slightly higher concentrations in column 3 whereas the simulated profiles were smoother. This could be attributed to the nature of the operation.

The glucose experimental profiles drop slightly towards the GRP column whereas the simulated profiles stable out in the post-feed section. The experimental profiles need further investigation as to why the glucose

concentration is reducing towards the GRP column and have a maximum in the centre section of the system.

Summarising, the simulation program is very useful in presenting a fairly accurate overall picture of the separation expected for any set of operating conditions. The application of the model can minimise the experimental work required and provides a better understanding of the separation.

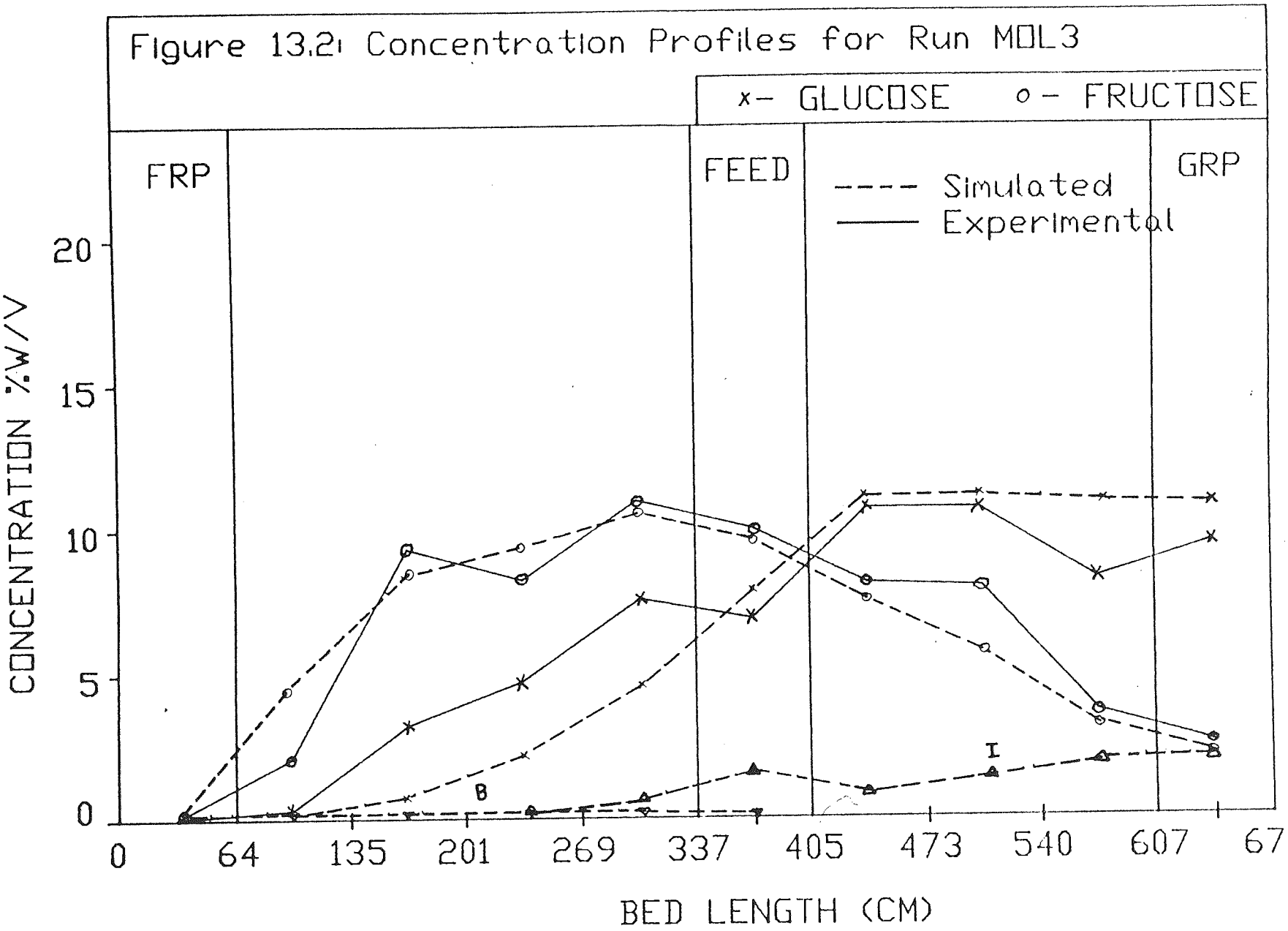


Figure 13.21 Concentration Profiles for Run MOL3
x- GLUCOSE o- FRUCTOSE
FRP
FEED
GRP
---- Simulated
—— Experimental
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
67
BED LENGTH (CM)
B
I

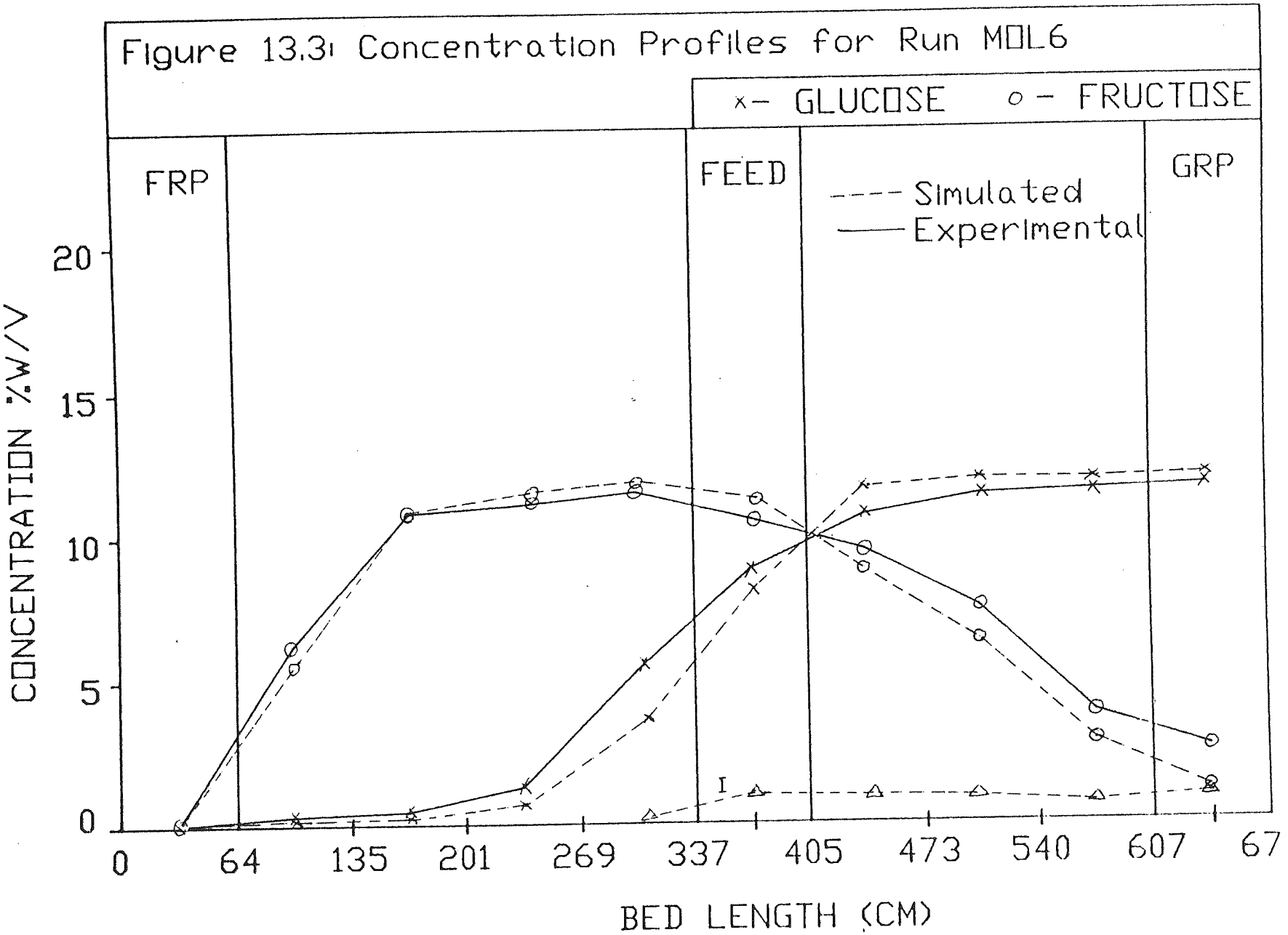


Figure 13.31 Concentration Profiles for Run MOL6
x- GLUCOSE o- FRUCTOSE
FRP
FEED
GRP
---- Simulated
—— Experimental
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
67
BED LENGTH (CM)

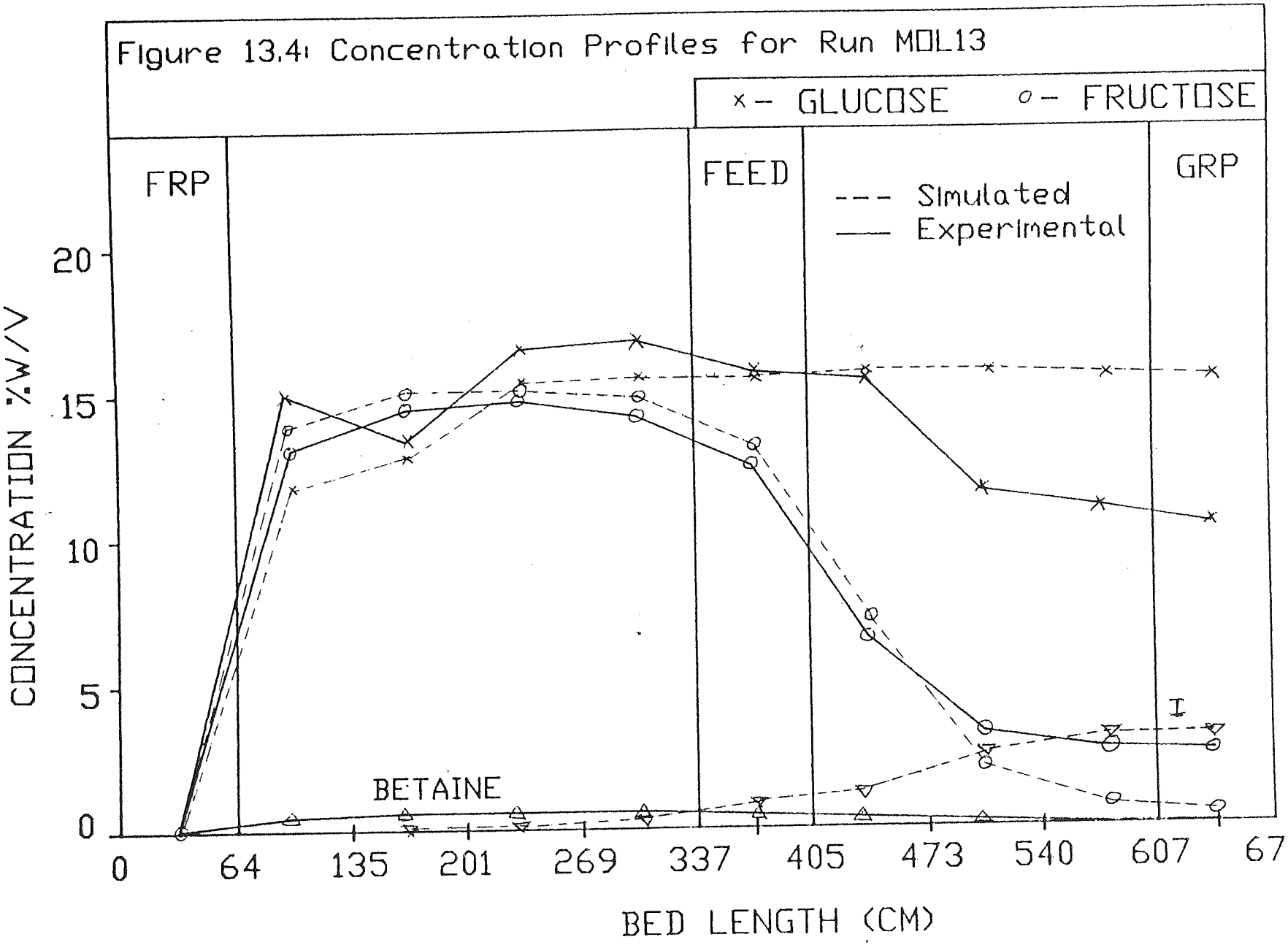


Figure 13.4: Concentration Profiles for Run MOL13
x - GLUCOSE
o - FRUCTOSE
FRP
FEED
GRP
--- Simulated
—— Experimental
CONCENTRATION %W/V
20
15
10
5
0
BETAINE
0
64
135
201
269
337
405
473
540
607
67
BED LENGTH (CM)

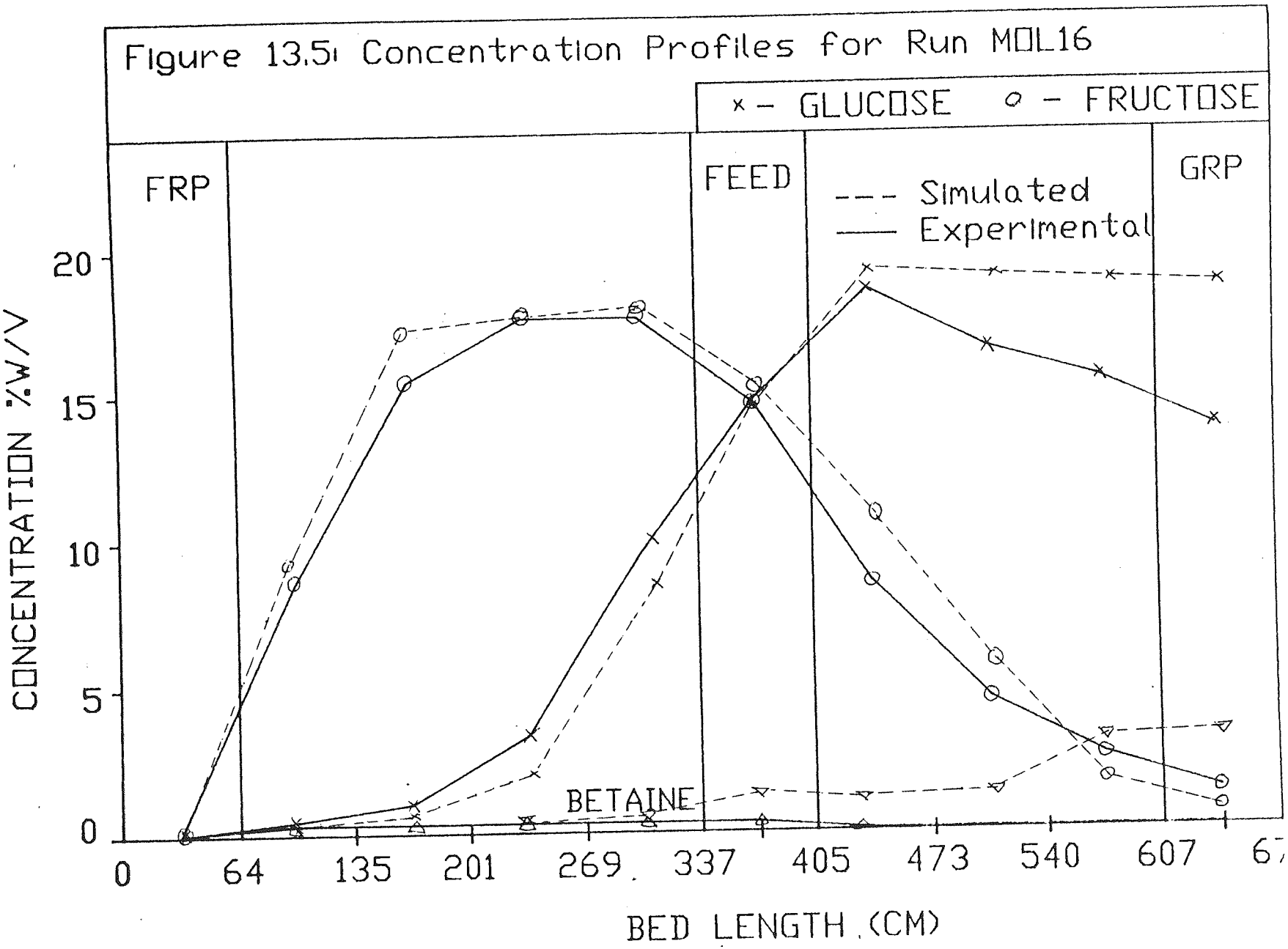


Figure 13.5: Concentration Profiles for Run MOL16
x - GLUCOSE o - FRUCTOSE
FRP
FEED
GRP
--- Simulated
—— Experimental
BETAINE
CONCENTRATION %W/V
0
5
10
15
20
0
64
135
201
269
337
405
473
540
607
BED LENGTH (CM)

CHAPTER FOURTEEN

CONCLUSIONS AND RECOMMENDATIONS

14.1 Conclusions

One of the main aims of this research was to recover a fructose rich product which would be at least 90% rich in fructose when using inverted beet molasses as a feedstock on a semi-continuous chromatographic refiner (SCCR6) system. The operation of the system had to be optimised by identifying and quantifying the effects of various operating parameters. The overall findings and conclusions are summarised as follows in the next section.

14.1.1 The effect of various parameters on the value of the distribution coefficients (K_d)

1 A system was set up using a 73cm length by 10.5cm ID column from the SCCR6 system and a set of runs carried out to establish general relationships between the distribution coefficients, K_d's and eluent flowrate, temperature and background concentration of glucose, fructose, betaine and potassium chloride.

2 It was found that as the eluent flowrate increased, the K_d's for all the components mentioned in 1 above decreased according to the equations 6.10 to 6.13.

3 The distribution coefficients for glucose, betaine and potassium chloride were not affected by a change in temperature between 20°C and 60°C. The K_d value for fructose varied according to equation 6.14 for a change in operating temperature.

4 The effect of background concentration on the distribution coefficients of glucose, fructose, betaine and potassium chloride was studied by carrying out a series of experiments as described in section 6.5.3. It was found that the K_d's for the four components were affected by a background concentration of the other components in the system. The study of K_d's in a potassium chloride background concentration could not be completed due to the displacement of calcium ions from the resin by the potassium ions.

14.1.2 Inversion of sucrose

5 The inversion (hydrolysis) of sucrose was carried out using three possible techniques and complete inversion was achieved using the enzyme Invertase. This technique required the least amount of ancillary equipment and was economically the most favoured. The inversion of sucrose in beet molasses had to be carried out in diluted molasses which have a maximum solids concentration of 51%w/w. This

was due to the high viscosity and the presence of a high concentration of non-sugars in undiluted molasses making it difficult for the enzyme to invert the sucrose.

14.1.3 Separation of inverted sucrose feeds using the SCCR6 system

6 The purities of the glucose rich and fructose rich products were highest for the runs carried out using enzyme inverted feedstocks. It was observed that the free hydrogen ions present in the feedstocks which were prepared using hydrochloric acid or H^+ resin displaced some of the calcium ions from the resin thus reducing its capacity to retard the fructose.

7 During operation, a 'pseudo-equilibrium' was achieved approximately 5 cycles after starting from a completely purged system which was initially filled with water. If however, one or more of the operating conditions are changed during operation, the new 'pseudo-equilibrium' was reached after a further 3 to 4 cycles provided that the system had not been purged.

14.1.4 Separation of inverted sucrose in beet molasses feeds using the SCCR6 system

8 The switch time was found to have a major effect on the separation performance of the SCCR6 system. Small switch time changes 'shifted' the relative positions of the component concentration profiles and were enough to determine whether the final products were going to be within the specified purities or not. It has been suggested by previous workers that for certain operating conditions the respective product purities would be improved by altering the feed point location so that extra system length would be available on one side or the other for the separation. It has been found that the same effect can be achieved by making the appropriate alteration to the switch time.

9 As a rule of thumb a satisfactory separation can be expected when the 'cross-over' point is situated in or very near column 5, the column before the feed inlet column on the FRP side.

10 The separation of glucose and fructose in the inverted molasses was more difficult than when separating inverted sucrose. This was due to the presence of the non-sugar components in the molasses accounting for up to 50% of the

molasses.

11 The product purities decreased as the run progressed. This was due to the sodium and potassium ions in the inverted molasses displacing the calcium ions from the resin. To stop this loss of calcium from the resin, three methods were developed. These are discussed in the next section.

12 The feed concentration was increased and a maximum concentration of 50% total solids was found suitable for separation using the SCCR6 system. It was observed that for feed concentrations higher than 50% total solids, the viscosity of the mobile phase increased exponentially resulting in valve and line failures in the system.

13 There was a significant problem with the control of microbial organisms in the system. The molasses feedstock had to be prepared fresh daily for each cycle and any feed left for more than 6 hours was disposed of. Due to the nature of molasses, it was necessary to run the equipment continuously to prevent the formation of polysaccharide gums during stoppages at night. During these nightly stoppages for the initial runs, it was observed that the microbial organisms caused the degradation of the carbohydrates leading to foul smelling fermented products

being formed.

14.1.5 Calcium addition techniques and product splitting

14 Due to the loss of calcium from the resin, it was necessary to regenerate the resin to replenish the calcium on the resin. Initially the resin in the system was regenerated after completion of a run ie. after 8 or 9 cycles. This method of regeneration was found to be unsuitable as the purity of the products had decreased by cycle 5 resulting in lower purity products. The loss of calcium from the resin was stabilised by the addition of calcium to the feed and eluent streams to maintain a calcium equilibrium in the system. This method worked but resulted in calcium contaminated FRP and GRP. The third method was developed where the system was modified to regenerate the resin one column at a time. A highly dissociated calcium ion solution was passed through the purge column after the FRP had been flushed off in the initial period of the switch. The spent regenerant was flushed off the system before the GRP was collected in the next switch. In this way, the products were not contaminated by the calcium regenerant and the system maintained calcium on the resin.

15 By using the third method of regeneration, the two

products were collected over a shorter time interval and thereby increased the concentration of the products. A product concentration increase of five times was observed for the FRP and two times for the GRP.

14.1.6 Computer simulation

16 The computer simulation of inverted beet molasses separations on an SCCR system has been carried out successfully. The 'plate model' approach was employed and the general relationships correlating the effects of flowrates, temperatures and background concentration on the distribution coefficients were incorporated. The simulated concentration profiles correspond to the purging profiles giving an accurate representation of the average sugar solid concentrations in each column. The simulated profiles show some agreement with the experimental values.

14.2 Recommendations for Future Work

From preliminary economic evaluation carried out by a team of final year BEng Chemical Engineering students for their design project on a 60000 tonnes per annum fructose from inverted beet molasses plant, it was observed that the chromatographic method used in this thesis is an attractive commercial proposition. This needs verification by British Sugar plc who were the sponsors of this research project.

From the work carried out in this research, the following recommendations for future work are suggested:

1 Carry out more separation experiments, operating at intermediate conditions to the ones used in this work to improve the accuracy of the relationships derived to predict the effect of various operating parameters on the switch time.

2 Modify the timers on the product lines to enable the calcium regeneration and product collection to be carried out for different time intervals, ie at the present the timers can only be set to switch on and off half way through a switch period. By using a more sophisticated timer, it would be possible to vary the periods for which the system is being regenerated and the products are being collected and thereby further increasing their concentrations.

3 Carry out a set of experiments in which only the very concentrated part of the FRP is collected and the rest, containing increasing amounts of fructose is recycled in the eluent stream or used to dilute the molasses feed. Employing this technique should further increase the purity and concentration of the FRP.

4 Modify the SCCR6 system so that the whole process could be computer controlled making the process more efficient, less labour intensive and thus less costly.

APPENDIX A

LISTING OF THE COMPUTER PROGRAM, RESULTS AND SYMBOLS USED FOR THE SIMULATION OF THE SCCR6 SYSTEM

```
*****************************************************************
*                                                               *
*       COMPUTER PROGRAM TO SIMULATE THE OPERATION OF THE       *
*           SCCR6 SYSTEM FOR A FOUR COMPONENT SYSTEM            *
*                                                               *
*       The program is written in FORTRAN                       *
*                                                               *
*       KETAN JOSHI                          JANUARY 1988       *
*                                                               *
*****************************************************************
```

```
*****************************************************************
*       The program is divided into two sections:               *
*                                                               *
*       The MAIN program includes all the input data, the       *
*       looping of the columns and plates and the printing of   *
*       results in the file called RESS                         *
*                                                               *
*       The subroutine COLUMN calculates the concentrations     *
*       of the four components for each plate on each column    *
*                                                               *
*****************************************************************
```

```
*****************************************************************
*       SYMBOLS USED IN THE PROGRAM:                            *
*                                                               *
*       DIA      - Diameter of the columns                      *
*       TL       - Total length of the separating section       *
*       CFLOW    - Mobile phase flowrate                        *
*       FFLOW    - Feed flowrate                                *
*       PFLOW    - Purge flowrate                               *
*       XSA      - Column cross-sectional area                  *
*       T        - Temperature                                  *
*       KDC1-4   - Infinite dilution distribution coefficients  *
*                  (Component 1 = glucose component 2 = fructose*
*                  component 3 = betaine  component 4 = ionics) *
*       CFEED(i)- Feed concentration of component i             *
*       NP       - Number of apparant plates in each column     *
*                  based of fructose                            *
*       V1       - Mobile phase plate volume                    *
*       V2       - Stationary phase plate volume                *
*       TC       - Total feed concentration                     *
*       F        - Average linear velocity of the mobile phase  *
*       TKDC1-4  - Modified distribution coefficients to take   *
*                  temperature into account                     *
*       FKDC1-4  - Modified distribution coefficients to take   *
*                  flowrate into account                        *
*       CKD11-4  - Modified distribution coefficients to account*
*                  for background concentration of glucose      *
*       CKD21-4  - Modified distribution coefficients to account*
*                  for background concentration of fructose     *
*       CKD31-4  - Modified distribution coefficient to account *
*                  for background concentration of betaine      *
*       CKD41-4  - Modified distribution coefficient to account *
*                  for background concentration of KCl          *
*       NCOL     - Total number of columns                      *
*       DT       - Time increment based on initial approximations*
*       NSEQ     - Total number of sequences                    *
*       NTIME    - Last time increment in a sequence            *
*       NCOM     - Number of column for which the concentration *
*                  is being evaluated                           *
*       NBLOCK   - Counter                                      *
*       CINI(i)  - Concentrations in the first plate of a column*
*       CLAST(i)- Concentrations in the last plate of a column  *
*       NCONTR1  - Counter                                      *
*****************************************************************
```

```
C       MAIN PROGRAM

        DIMENSION CINI(4),CLAST(4),CONCEN(4,10,25),SUMCON(4)
        COMMON /DATA1/ V1,V2,DT,NP
        COMMON /DATA2/ FKDC1,FKDC2,FKDC3,FKDC4
        COMMON /DATA3/ TC,CFLOWC,CFLOW,FFLOW,CFEED(4)
        REAL KDC1,KDC2,KDC3,KDC4

C       OPEN RESULT FILE 'RESS'

        OPEN(UNIT=3,FILE='RESS')

C       INPUT ALL DATA

        DT=1
        NSEQ=90
        NTIME=10

        DIA=10.8
        TL=650.0
        CFLOW=105.0
        FFLOW=35.0
        PFLOW=380.0

        XSA=(3.1416*(DIA**2))/4.0
        T=60.0

        KDC1=0.2
        KDC2=0.7
        KDC3=0.6
        KDC4=0.10

        CFEED(1)=0.2531
        CFEED(2)=0.2574
        CFEED(3)=0.15
        CFEED(4)=0.20

        NP=25
        V1=2800.0/NP
        V2=3200.0/NP

        TC=CFEED(1)+CFEED(2)+CFEED(3)+CFEED(4)
        F=((CFLOW*2.0)+FFLOW)/(XSA*2.0)

C       INPUT DISTRIBUTION COEFFICIENTT RELATIONSHIPS FOR TEMPERATURES
C       AND FLOWRATES

        TKDC1=KDC1
        TKDC2=(114.55-(0.563*T))*KDC2/100.0
        TKDC3=KDC3
        TKDC4=KDC4

        FKDC1=(131.70-(28.4*F))*TKDC1/100.0
```

```
      FKDC2=(118.20-(15.4*F))*TKDC2/100.0
      FKDC3=(153.00-(43.4*F))*TKDC3/100.0
      FKDC4=(183.10-(69.6*F))*TKDC4/100.0

C     CALCULATION OVER TOTAL NUMBER OF SEQUENCES

      NCNUM=5
      NBLOCK=0

      DO 100 K=1,NSEQ

C     CALCULATION OF SWITCHING TIME INCREMENT (9 X 10 TIMES)
C     DURING A SEQUENCE

      NCONTR0=1
      NCONTR1=NCONTR0

      DO 200 KK=1,NTIME

      NCNUM=NCNUM+1
      NBLOCK=NBLOCK+1

C     SET INITIAL FEED PLATE :- IN COLUMN NUMBER 6
CC    NCNUM=COLUMN NUMBER (DIFFERENT FROM NCOL WHICH IS THE COUNTER
CC    OF THE AMOUNT OF COLUMN (1-10)
C     COLUMN FUNCTIONING MODE

      DO 300 NCOL=1,10

      IF(NCOL.EQ.1) THEN
         CFLOWC=PFLOW
         DO 111 NCOM=1,4
         CINI(NCOM)=CLAST(NCOM)
  111    CONTINUE
      ELSE IF(NCOL.LE.5.AND.NCOL.NE.1) THEN
         CFLOWC=CFLOW
         DO 222 NCOM=1,4
         CINI(NCOM)=CLAST(NCOM)
  222    CONTINUE
      ELSE IF(NCOL.EQ.6) THEN
         CFLOWC=CFLOW+FFLOW
         DO 333 NCOM=1,4
         CINI(NCOM)=CINI(NCOM)+CFEED(NCOM)
  333    CONTINUE
      ELSE
         CFLOWC=CFLOW+FFLOW
         DO 444 NCOM=1,4
         CINI(NCOM)=CLAST(NCOM)
  444    CONTINUE
      ENDIF

C     CONVERTS LAST CONC. OF PREVIOUS COLUMN TO THE FIST OF NEXT COLUMN
CC    i.e.  CHANGE CLAST(I) TO CINI(I)
CC    CALLS SUBROUTINE 'COLUMN' TO CALCULATE THE CONCENTRATIONS OF THE
C     COMPONENTS ON A PARTICULAR PLATE
```

```
      CALL COLUMN(NCONTR1,NCNUM,CINI,CLAST,CONCEN)

      DO 666 INP=1,25
      WRITE(*,123) NCNUM,NCONTR1,(CONCEN(NN,NCONTR1,INP),NN=1,4)
  666 CONTINUE
  123 FORMAT(I5,I5,4(2X,F10.4))
      NCONTR1=NCONTR1+1
      IF(NCONTR1.GT.10)NCONTR1=1

  300 CONTINUE

      NCONTR0=NCONTR0+1
      IF(NCONTR0.GT.10)NCONTR0=1
      NCONTR1=NCONTR0

      IF(NBLOCK.GE.10) THEN
         NBLOCK=0
      ENDIF
      IF(NCNUM.GE.10) THEN
         NCNUM=0
      ENDIF

  200 CONTINUE

  100 CONTINUE

C     CALCULATES THE AVERAGE INDIVIDUAL COMPONENT CONCENTRATION
C     IN EACH COLUMN DURING THE FINAL CYCCLE

C     PRINTS OUT THE RESULTS
C     COLUMN ONE REPRESENTS THE PURGED COLUMN

      DO 777 ICOL=1,10
      SUMCON(1)=0.0
      SUMCON(2)=0.0
      SUMCON(3)=0.0
      SUMCON(4)=0.0
      DO 888 N=1,4
      DO 999 IPLATE=1,25
      SUMCON(N)=SUMCON(N)+CONCEN(N,ICOL,IPLATE)
  999 CONTINUE
      SUMCON(N)=SUMCON(N)/25.0
  888 CONTINUE
      SUMCON(N)=SUMCON(N)*100
      WRITE(3,234)ICOL,(SUMCON(I),I=1,4)
  234 FORMAT(I5,4F10.4)
  777 CONTINUE

      CLOSE(UNIT=3)

      STOP
      END
```

```
**********************************************************************
*                                                                    *
*    SUBROUTINE "COLUMN"                                             *
*                                                                    *
*                                                                    *
*    THIS SUBROUTINE CALCULATES THE CONCENTRATIONS OF THE FOUR       *
*    COMPONENTS ON A PARTICULAR PLATE IN THE COLUMN.                 *
*    THE EFFECT OF THE BACKGROUND CONCENTRATIONS ON THE              *
*    DISTRIBUTION COEFFICIENTS FOR THE FOUR COMPONENTS IS            *
*    TAKEN INTO ACCOUNT.                                             *
*                                                                    *
**********************************************************************

C  CI=INITIAL CONCENTRATION, CL=CONC. AT PLATE 25 (BOTH IN ARRAY)
C  CONP(N,NCOL,I)=CONCENTRATION OF EACH COMPONENT IN EACH PLATE

      SUBROUTINE COLUMN(NCOL,NCNUM,CI,CL,CONP)

      DIMENSION CI(4),CL(4),CONP(4,10,25),CLST(4,25),ACKD(4,25)
      DIMENSION CFST(4,25),RR(4,25)
      COMMON /DATA1/ V1,V2,DT,NP
      COMMON /DATA2/ FKDC1,FKDC2,FKDC3,FKDC4
      COMMON /DATA3/ TC,CFLOWC,CFLOW,FFLOW,CFEED(4)

      AA=CFLOWC*DT
      NFEED=NCNUM
      IF(NFEED.GT.10) THEN
        NFEED=10
      ENDIF

      DO 400 I=1,NP

      IF(I.EQ.1) THEN
          DO 10 N=1,4
          CFST(N,I)=CI(N)
   10     CONTINUE
      ELSE
          DO 20 N=1,4
          CFST(N,I)=CL(N)
   20     CONTINUE
      ENDIF

C     CALCULATION OF THE DISTRIBUTION COEFFICIENTS FOR THE
C     PARTICULAR BACKGROUND CONCENTRATIONS

      CKD11=((320.9*CONP(1,NCOL,I))+102.9)*FKDC1/100.0
      CKD12=((198.3*CONP(2,NCOL,I))+107.4)*FKDC1/100.0
      CKD13=((366.9*CONP(3,NCOL,I))+89.71)*FKDC1/100.0
```

```
      CKD14=FKDC1
      ACKD(1,I)=(CKD11*CFEED(1)/TC)+(CKD12*CFEED(2)/TC)
     *+(CKD13*CFEED(3)/TC)+(CKD14*CFEED(4)/TC)

      CKD21=((173.1*CONP(1,NCOL,I))+95.71)*FKDC2/100.0
      CKD22=((194.5*CONP(2,NCOL,I))+101.6)*FKDC2/100.0
      CKD23=((89.99*CONP(3,NCOL,I))+99.54)*FKDC2/100.0
      CKD24=FKDC2
      ACKD(2,I)=(CKD21*CFEED(1)/TC)+(CKD22*CFEED(2)/TC)
     *+(CKD23*CFEED(3)/TC)+(CKD24*CFEED(4)/TC)

      CKD31=((139.7*CONP(1,NCOL,I))+96.62)*FKDC3/100.0
      CKD32=((102.4*CONP(2,NCOL,I))+68.34)*FKDC3/100.0
      CKD33=((159.9*CONP(3,NCOL,I))+100.1)*FKDC3/100.0
      CKD34=FKDC3
      ACKD(3,I)=(CKD31*CFEED(1)/TC)+(CKD32*CFEED(2)/TC)
     *+(CKD33*CFEED(3)/TC)+(CKD34*CFEED(4)/TC)

      CKD41=((759.9*CONP(1,NCOL,I))+138.9)*FKDC4/100.0
      CKD42=((453.4*CONP(2,NCOL,I))+112.5)*FKDC4/100.0
      CKD43=((976.9*CONP(3,NCOL,I))+108.7)*FKDC4/100.0
      CKD44=FKDC4
      ACKD(3,I)=(CKD41*CFEED(1)/TC)+(CKD42*CFEED(2)/TC)
     *+(CKD43*CFEED(3)/TC)+(CKD44*CFEED(4)/TC)

      CFLOWS=CFLOW/60.0
      FFLOWS=FFLOW/60.0

      DO 30 N=1,4
      RR(N,I)=EXP(-AA/(V1+(V2*ACKD(N,I))))

C     CALCULATES THE CONCENTRATIONS OF THE COMPONENTS ON THE PLATE

      IF (NCOL.EQ.NCNUM.AND.I.EQ.1) THEN
      CLST(N,I)=((1-RR(N,I))*((CFLOWS*CFST(N,I))+(FFLOWS*CFEED(N)))
     */CFLOWC)+(RR(N,I)*CONP(N,NCOL,I))
      ELSE
      CLST(N,I)=((1-RR(N,I))*CFST(N,I))+(CONP(N,NCOL,I)*RR(N,I))
      ENDIF
   30 CONTINUE

      DO 40 N=1,4
       CONP(N,NCOL,I)=CLST(N,I)
   40 CONTINUE

      DO 50 N=1,4
      CL(N)=CLST(N,I)
   50 CONTINUE

  400 CONTINUE

      RETURN
      END
*****************************************************************
```

Results obtained using the simulation program

COLUMN NUMBER	GLUCOSE CONCN.	FRUCTOSE CONCN.	BETAINE CONCN.	IONIC CONCN.
1	0.00	0.00	0.00	0.00
2	0.20	9.32	0.13	0.03
3	1.46	17.50	0.15	0.08
4	4.08	18.33	0.24	0.31
5	8.06	17.77	0.26	0.54
6	14.60	14.92	0.31	1.97
7	18.69	10.81	0.17	1.93
8	18.72	5.15	0.00	2.42
9	18.43	3.05	0.00	3.98
10	18.07	1.03	0.00	4.19

These values are in %w/w and correspond to a run similar to run MOL16 in terms of feed concentration used.

NOMENCLATURE

A	Eddy diffusion term in the Van Deemter equation
A´	Cross-sectional area
B	Longitudinal diffusion term in the Van Deemter equation
B	Betaine
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 extraparticle, stagnant mobile phase and stationary phase respectively
D_r	Solute radial diffusivity
e	Natural logarithm
F	Fructose
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
I	Ionic components present in molasses
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 105$cm^3.min^{-1}$ flowrate
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
L	System's length
L_e	Pre-feed mobile phase flowrate
L_i	Mobile phase flowrate
L_m	Average mobile phase flowrate
L_1	Eluent phase flowrate
L_2	Feed flowrate (L_f)
L_3	Purge flowrate
N	'True' number of theoretical plates
N_i	Number of theoretical plates for component i
NTP	Number of theoretical plates for the semi-continuous phase

P	Stationary phase effective flowrate
P_i	Concentration of component i in the stationary phase
r	Column radius
R_s	Resolution
s	Switch period
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_i	Concentration of ion i in the solute
x_i	Stationary phase concentration of component i
XSA	Cross-sectional area
Y_i	Concentration of ion i in the resin
y_i	Mobile phase concentration of component i

Greek Letters

α	Separation factor
β	Affinity coefficient
σ^2	Peak variance in units of volume
μ	Fluid viscosity
τ	Radial Labyrinth factor

τ_1	Rate of transfer of molecules from gas to liquid
τ_2	Rate of transfer of molecules from liquid to gas
δ_{1i}	Fractional change due to the effect of concentration on the distribution coefficient of component i
δ_{2i}	Fractional change due to the effect of system characteristics on the distribution coefficient of component i
ϵ	Column voidage

Superscripts

D	Dextran
F	Fructose
G	Glucose
B	Betaine
I	Ionic components in molasses

Subscripts

f	Fructose
g	Glucose
b	Betaine
p	Ionic components in molasses

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