Journal of Chromatography, 114 (1975) 369–381

CHROM. 8429

pH CYCLING ZONE SEPARATION OF SUGARS

A PREPARATIVE SEPARATION TECHNIQUE FOR COUNTER-CURRENT DISTRIBUTION AND CHROMATOGRAPHY

MARK E. BUSBICE* and PHILLIP C. WANKAT
School of Chemical Engineering, Purdue University, West Lafayette, Ind. 47907 (U.S.A.)
(First received February 21st, 1975; revised manuscript received May 6th, 1975)

SUMMARY

Cycling zone separation is extended to the use of traveling pH waves. A counter-current distribution type theory was modified for pH waves and extended to Langmuir type isotherms. Experimental results for the separation of fructose and glucose from their aqueous solutions with a dihydroxyborylphenylsuccinamyl derivative of amino-ethyl cellulose were in qualitative agreement with the theory. Very large concentrations of fructose were obtained for continuous feed of the fructose mixture. Glucose was partially separated from fructose. Concentration of fructose was also done by the direct thermal mode and by combining a traveling pH wave with direct heating. Use of the technique for preparative chromatographic separations is discussed.

INTRODUCTION

Several recent papers have discussed cyclic operational techniques such as parametric pumping and cycling zone adsorption, which allow for continuous or semi-continuous feed to chromatographic apparatuses[1–6]. The much larger throughput that can be achieved with continuous feed when compared with pulse feed make these cyclic processes interesting alternatives for preparative chromatography. To force a separation with a continuous feed, the cyclic processes periodically vary some thermodynamic variable which affects the distribution of solute between the stationary and mobile phases.

Pigford *et al.*[1] developed the technique of cycling zone adsorption where the fluid to be separated is pumped in one direction through one or more columns. Operation can be in the "direct" mode, where the columns are heated and cooled periodically, or in the "traveling-wave" mode, where the entering streams are heated and cooled periodically. Pigford *et al.*[1] separated both gases and liquids and noted that other thermodynamic variables besides temperature could be varied. Additional

* Present address: Dow Chemical Co., Freeport, Texas, U.S.A.

investigations, other cyclic techniques, and related chromatographic methods are discussed in a recent review paper[2].

In this paper cycling zone adsorption with pH as the cyclic variable is used to concentrate fructose in water, concentrate glucose in water, and concentrate the fructose in a glucose–fructose–water mixture. In all cases the sugar solution to be separated is fed to the column continuously. The pH of the feed is adjusted periodically to high or low values. The separation occurs because the stationary phase will store solute when the pH is high and release solute when the pH is low. Thus a time-dependent outlet concentration is obtained with low concentrations in the product when the pH is high, and high sugar concentrations when the pH is low. Large separations were obtained in many cases. The counter-current distribution (CCD) theory developed earlier[3] is extended to Langmuir isotherms, adapted to pH waves, and applied to the sugar separations.

pH TRAVELING-WAVE CYCLING-ZONE SYSTEM

The basic apparatus for pH traveling-wave cycling-zone chromatography consists of a chromatographic column with a feed system arranged so that the pH of the entering feed can be varied periodically in some fashion. The material to be separated is fed at all times but with varying pH. A scheme of the system, solute concentration and pH of the feed is shown in Fig. 1. In this figure, the column is represented as a series of equilibrium stages. The feed pH is shown varying as a square wave, but other input pH functions such as sine waves or sawtooths can be used. The cycling-zone system is similar to that used previously[3] except a column is used instead of a CCD apparatus, pH is varied instead of temperature, and the previous study[3] allowed for use of more than one region.

The product from the cycling-zone system is time dependent in that the pH and concentration of the product vary continually. However, the apparatus will eventually reach a repeating state where the product pH and concentration repeat

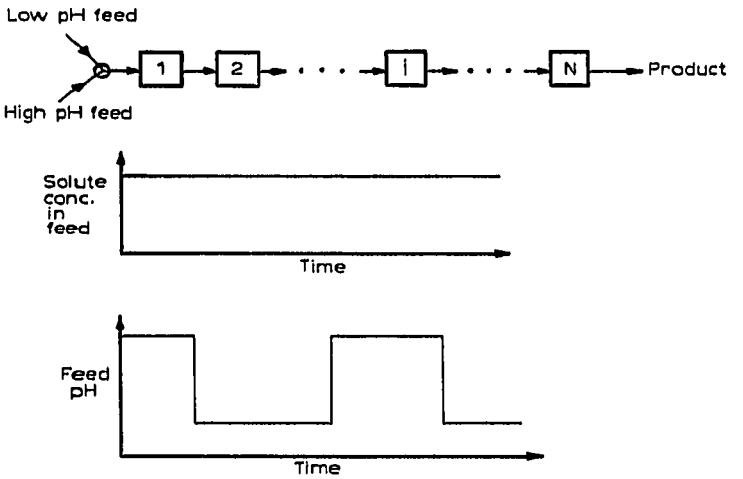


Fig. 1. Schematic diagram of column and feed conditions.

from cycle to cycle. Proper timing of a fraction collector or switching mechanism will result in continuous separation of the inlet feed.

The periodic alternation of the feed pH will cause a pH wave in the system. For the sugar separations studied here adsorption is strong at basic pH and almost negligible under acid conditions. Thus, when a given portion (or stage) of the column is at low pH, the stationary phase will reject solute and the moving phase will become more concentrated. When a given portion of the column is at high pH, the equilibrium distribution coefficient changes and the stationary phase now stores the solute. The larger the pH difference the more the distribution coefficient varies and the greater the separation. The separation occurs for the same reasons when temperature is varied[1-5], and, in fact, any thermodynamic variable that affects the distribution coefficient can be varied to cause separation; in liquid chromatography this would obviously be ionic strength, polarity or concentration of various complexing agents.

THEORY

A CCD model for traveling-wave cycling-zone separation was presented earlier for thermal waves[3]. In this paper the model is extended to pH waves and modified to include Langmuir instead of linear isotherms. Understanding the theoretical calculations presented here will be aided by reference to Wankat[3], but the remainder of the present paper does not require reference to this paper.

In the previous paper each stage was represented as (i,j) where i was the stage number and j was the region. In this paper $j = 1$ since systems with only one region are considered. When the feed has a high pH, the system is in the first half cycle and when the feed pH is low in the second half cycle. The cycle "halves" may be unequal. We will again assume that V_M and V_S, the volumes of the moving and the stationary phase per stage, respectively, are constant. C_M and C_S are the concentrations of solute in the moving and stationary phase, respectively. Let $\mathrm{pH}_{i,j,s}$ and $M_{i,j,s}$ be the pH and mass of solute on stage (i,j) after transfer step s. The solute will be assumed to distribute between the stationary and mobile phases according to a Langmuir adsorption isotherm of the form shown in eqn. 1,

$$C_S = \frac{a\,C_M}{1 + b\,C_M} \tag{1}$$

where a and b both depend upon the pH. Only single solute separation was studied theoretically since this greatly simplifies the calculations and since a simple adsorption isotherm was not obtained when both sugars were present.

The mass balances used to calculate the mass of solute in stage (i,j) after transfer step s are the same as those used in CCD[7], direct mode operation[4], and in the thermal traveling-wave mode[3]. If $i \neq 1$, the mass balance is

$$M_{i,j,s} = f_{i-1,j,s-1}\,M_{i-1,j,s-1} + (1 - f_{i,j,s-1})\,M_{i,j,s-1} \tag{2}$$

If $i = 1$ and $j = 1$

$$M_{1,1,s} = C_{\mathrm{feed}}\,V_M + (1 - f_{1,1,s-1})\,M_{1,1,s-1} \tag{3}$$

where C_{feed} is the concentration of solute in the feed and $f_{i,j,s}$ is the fraction of solute that is in the moving phase in stage (i,j) after transfer step s.

The f values in eqns. 2 and 3 must be calculated for each stage after each transfer step. This requires that the pH for each stage be known and that the distribution of a known mass of solute, $M_{i,j,s}$, between the two phases be calculated using the Langmuir isotherm. This latter step necessitates additional calculations that were not required for the previous theories where a linear isotherm was assumed[3,4,7]. The f value can be calculated as

$$f_{i,j,s} = \frac{C_M V_M}{C_M V_M + C_S \cdot V_S} \tag{4}$$

where C_M and C_S are related by the Langmuir equilibrium (eqn. 1). Once the mass in a stage is known, C_M, C_S and f can be calculated by writing the mass balance for that stage after the equilibrium step:

$$M_{i,j,s} = C_M V_M + C_S V_S \tag{5}$$

removing C_S by substituting eqn. 1 into eqn. 5

$$M_{i,j,s} = C_M V_M + V_S \frac{a C_M}{1 + b C_M} \tag{6}$$

and solving for C_M

$$C_M = \frac{-(V_M + V_S a - b M_{i,j,s}) + \sqrt{(V_M + V_S a - b M_{i,j,s})^2 + 4 M_{i,j,s} V_M b}}{2 V_M b} \tag{7}$$

The plus sign is used in the binomial theorem solution since this gives $C_M = 0$ when $M_{i,j,s} = 0$.

Once C_M has been obtained, C_S is calculated from eqn. 1, $f_{i,j,s}$ from eqn. 4 and eqns. 2 and 3 can be solved for the next transfer step. The use of eqn. 7 requires that the Langmuir "constants" a and b be known, which requires that the pH of each stage be known.

Calculation of the pH in a column with the pH input shown in Fig. 1 is difficult since the adsorbent used in this study has ion-exchange properties, the neutralization reaction must be included, and both buffers may be present simultaneously. Instead of unraveling these complexities, an empirical approach was used to determine pH. The experimental runs showed that pH waves pass through the column with essentially a constant wave velocity. By analogy to the mass balances, which represents a solute wave, and the energy balances[3], which represent a thermal wave, a "pH balance" was written as

$$\mathrm{pH}_{i,j,s} = B\,\mathrm{pH}_{i-1,j,s-1} + (1 - B)\,\mathrm{pH}_{i,j,s-1} \tag{8}$$

where B is the experimentally determined velocity of the pH wave. Use of a constant B in eqn. 8 implies the assumption that the pH wave is not affected by the solute concentration in the column.

The equations are solved by a straightforward iterative solution starting with a known initial condition. To summarize this procedure, first eqn. 8 is solved to obtain the pH on each stage. Then the Langmuir constants a and b are calculated and C_M

is found from eqn. 7. C_S is found from equilibrium eqn. 1, $f_{i,j,s}$ calculated from eqn. 4, and the mass balance eqns. 2 and 3 are used to find the mass of solute on each stage. The calculations for the next time step are started by solving eqn. 8 again. This procedure is easily done on a computer. After several cycles a "repeating state" is reached where each cycle is an exact repeat of the previous cycle. Repeating states were also found in previous work[1-5] and in the experimental work done here. Direct solution for the repeating-state solution as done previously[3,4] would be difficult in this case because of the non-linear isotherm used. An alternative theoretical solution, which treats the column as a continuous contactor, but does not consider zone spreading, was presented by Baker and Pigford[5].

A qualitative understanding of the effect of various variables is easily obtained and will prove helpful in understanding the theoretical and experimental results. The average distance that the solute will travel is $f \times$ (number of transfer steps), and the average distance the pH wave will travel is $B \times$ (number of transfer steps). Both the solute and pH waves will tend to be spread by the zone-spreading phenomena of chromatography, but the solute wave can also be sharpened by the pH changes. At low pH very little solute is held up by the stationary phase, the solute wave tends to become more concentrated since it gains solute from the adsorbent, and the solute wave moves faster (larger f). At high pH the solute wave becomes less concentrated and moves slower. These two effects will tend to cause a separation with concentrated solute exiting when the product pH is low. If the pH wave velocity lies between the solute wave velocities at high and low pH ($f_{\text{low pH}} > B > f_{\text{high pH}}$), a large separating effect can occur. The slowly moving solute wave at high pH will be overtaken by the pH wave and the pH will drop. When this happens, the adsorbent dumps solute into the moving phase. This concentrated solute wave at low pH will tend to move faster than the pH wave and will overtake the pH wave. If it passed the pH wave front, it would go into a region of high pH and be slowed down. As a result the solute wave is "trapped" at the pH wave front and will move at velocity B (equivalent to being at some intermediate pH value). If the column is long enough and the velocities differ by a large amount, a large amount of solute can be trapped at the pH front.

The Langmuir isotherm will tend to limit the separation that can be achieved since at high solute concentrations the adsorbent will be saturated. In addition, if the feed concentration is large compared with the adsorbent capacity, very little separation will be observed. This occurs because the change in concentration resulting from removing solute from the adsorbent will be only a small fraction of the fluid concentration. This incremental change in fluid concentration has a small effect on the overall concentration of the fluid and little separation is obtained. This effect was observed experimentally as well as theoretically and will be discussed later.

EXPERIMENTAL

In the experimental studies the removal of fructose from water, glucose from water and both fructose and glucose from water was measured using a column packed with a dihydroxyborylphenylsuccinamyl derivative of aminoethyl cellulose (DBAE-cellulose). The dihydroxyboryl end of this adsorbent reacts under basic conditions with vicinal *cis* hydroxyl groups of sugars. The reaction is favored for sugars in the furanose form and the complex formed is broken down under acidic conditions. Use

of the adsorbent for sugar separations and for separation of ribonucleotides has been studied by Weith *et al.*[8] and Rosenberg *et al.*[9]. Complete details of the preparation of the adsorbent and the mechanism of adsorption are given by Weith *et al.*[8].

Equilibrium adsorption isotherms at given pH values were obtained by putting known amounts of adsorbent and sugar solution in a test tube in a shaker bath and then analyzing the liquid for sugar concentration. The amount adsorbed was obtained from a mass balance, which in some cases required subtraction of two large numbers with a resulting large error. Complete isotherms, data and data analysis are given by Busbice[10]. The equilibrium data fitted to the Langmuir isotherm (eqn. 1) although the Freundlich isotherm appeared to give a somewhat better fit. The constant b was roughly independent of pH and the constant a fitted to the hydroxyl ion concentration in an equation of Langmuir form:

$$a = \frac{K_1 [OH^-]}{1 + K_2 [OH^-]} (1 - K_3 T) \tag{9}$$

The values of K_1, K_2, K_3 and b are given in Table I. In the Langmuir equation sugar concentration is in mg/ml, amount adsorbed in mg per mg adsorbent, and temperature in °C. The temperature range was from 0° to 40° and the pH ranged from 5.0 to 8.5. As noted by Busbice[10] eqn. 9 is not a good fit for all the data.

TABLE I

EQUILIBRIUM PARAMETERS FOR ADSORPTION OF GLUCOSE AND FRUCTOSE USING 0.05 *M* MORPHOLINE OR 0.05 *M* ACETIC ACID AS BUFFERS

Coefficient	*Glucose*	*Fructose*
K_1	$4.35 \cdot 10^4$	$1.95 \cdot 10^5$
K_2	$1.07 \cdot 10^6$	$1.84 \cdot 10^6$
K_3	$1.24 \cdot 10^{-2}$	$6.09 \cdot 10^{-3}$
b	0.830	1.52

As expected, the equilibrium data showed that fructose is more strongly adsorbed than glucose. At 25° and pH 8.5 the maximum amount of fructose adsorbed was approximately 0.06 mg per mg adsorbent and the maximum glucose adsorption was 0.023 mg per mg adsorbent. The low capacity of the adsorbent limited the feed concentrations that could be separated in the cycling-zone runs. Equilibrium measurements for simultaneous adsorption of fructose and glucose showed that the simultaneous adsorption could not be explained as the simple competition for active sites. Instead, glucose adsorption was greatly inhibited while fructose adsorption was decreased only slightly. In all cases very little sugar was adsorbed at pH 5.0.

Fructose and glucose concentrations for both the equilibrium study and the column runs were analyzed using colorimetric methods. A glucostat procedure was used for glucose and the 2-thiobarbituric acid method for fructose[10]. Standard deviations of 1.8% for glucose and 3.4% for fructose were obtained.

The column system is shown schematically in Fig. 1. A 0.5 × 30 cm glass jacketed Glenco 3050-053 column was packed with 0.6 g of DBAE-cellulose. In early experimental runs studying elution chromatography and breakthrough curves a

gravity feed system was used. For the cycling-zone runs a Harvard syringe pump operating at low pressures was employed. The air space at the top of the column allowed some fluctuation in liquid level above the packing and thus some variation in flow-rate. In addition, the packing tended to compress at the higher flow-rates. When this happened, the column was re-packed before the next run. Product was collected in a fraction collector for later analysis.

In most runs, the pH of the feed was adjusted using 0.05 *M* morpholine at pH 8.5 or pH 8.0 and 0.05 *M* acetic acid at pH 5.0. These buffers were the same as those used in the equilibrium studies. In a few runs 0.05 *M* Tris at pH 8.0 or no buffers with pH adjusted using HCl or NaOH were used. Runs were at 25° unless stated otherwise.

RESULTS

A series of initial elution chromatography runs were made with glucose and water, fructose and water and all three components. From these runs standard methods[7] were used to determine that there were 41 stages for glucose and 36 for fructose. Larger separations were achieved at higher pH, lower temperatures, and with morpholine as buffer instead of Tris or unbuffered systems. The glucose peak appeared first and showed considerable tailing whereas the fructose peak was symmetrical. Both sugars were rapidly eluted by decreasing the pH to 5.0. Breakthrough (frontal development) curves with 0.05 *M* morpholine at pH 8.5 and 25° were also obtained with both sugars present. These experiments showed that glucose exits well before fructose and that the glucose concentration temporarily rose slightly above its feed concentration and then dropped back to the feed concentration. The latter effect occurs because fructose acts as an eluting agent for glucose, and is qualitatively predicted by CCD theory with two solutes competing for adsorbent sites[11]. Complete chromatograms and breakthrough curves are given by Busbice[10].

In order to utilize the staged theory a pH wave velocity must be measured. This was obtained by measuring the midpoint of a pH breakthrough curve and calculating B as the reciprocal of the number of column volumes where the average molecule exits. For breakthrough from pH 8.5 to 5.0 an average of six curves gave $B = 0.400$. For breakthrough from pH 5.0 to 8.5 an average of four values gave $B = 0.387$. A value of $B = 0.394$ was used in the theoretical calculations. The pH wave moves slowly because of stagnant liquid in the column and the ion-exchange properties of the adsorbent. This value of B differs from the value obtained from cycling-zone runs because of dead space in the column.

Since the CCD model developed here is substantially the same as that developed previously[3], except for the use of the Langmuir isotherm, we would expect most of the theoretical results to be qualitatively the same, which appeared to be the case. The one major difference between the two theories occurs when the fluid concentrations are high enough so that the non-linear portion of the equilibrium curve is being used. In this case, the feed concentration becomes a major variable while it is not in the linear theory if the product concentrations are normalized. The effect of feed concentration on the theoretically predicted product concentrations is shown in Fig. 2 for fructose adsorption using the equilibrium expression represented by eqns. 1 and 9 and Table I. Values used in the calculation correspond to the actual column

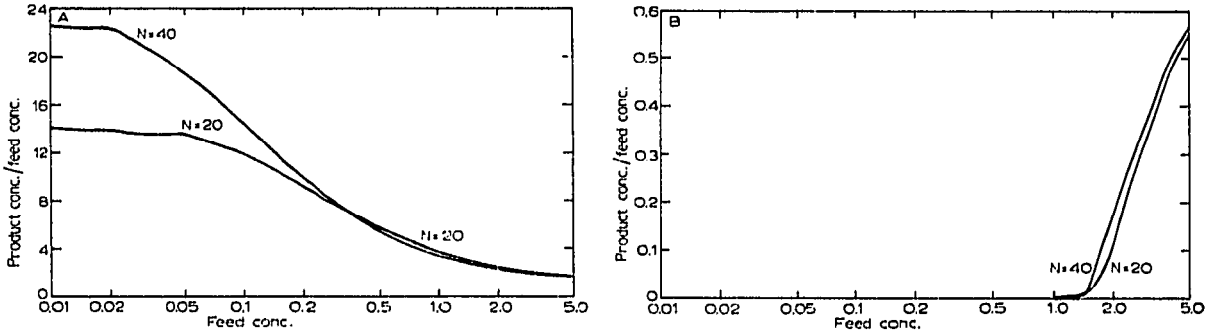


Fig. 2. Theoretical prediction of the effect of feed concentration for fructose. N = number of stages. Equilibrium parameters as given in Table I; $B = 0.394$; $T = 25°$; cycle between pH 8.5 and 5.0; packing, 600 mg; liquid in column, 5.31 ml; 2.2 column void volumes passed through column per half cycle; feed concentration in mg/ml. A, Highest product concentrations; B, lowest product concentrations.

used in the experimental work except in the cycling-zone experiments the column had a much larger HETP than in the pulse experiments. Thus the number of stages, N, used in Fig. 2 is too large to match the experiments.

The theoretical results were in qualitative agreement with the experimental results, but quantitative agreement could not be obtained. The peak-to-peak separation achieved experimentally can be matched with three or four stages. With this small number of stages, the CCD model is no longer a reasonable model for a column. Thus, the remaining portion of the cycle could not be predicted quantitatively. With a larger number of stages the model qualitatively predicted the shapes of the experimental curves and the effects of changing operating conditions. In the two columns plotted in Fig. 2 the same amount of adsorbent is used but they are arranged so that the number of stages is different. As expected for a Langmuir isotherm better separation is achieved with lower feed concentrations. When the feed concentration is high and the adsorbent is operating close to saturation, these results predict that a better separation will be achieved if a short, wide column with few stages is used. It would be interesting to check this conclusion experimentally. At low feed concentrations there is a very slight oscillation in maximum product concentration for the curve where $N = 20$. This occurs because the peak maximum has become very sharp and different values will be obtained if it is centered on one tube (or transfer step) or situated between two tubes. The same phenomenon could occur in a chromatographic column if fractions are collected for later analysis. The normalized dilute product concentration rapidly approaches zero near a feed concentration of 1.25 mg/ml, and then levels out at a small non-zero value ($\approx 10^{-8}$ for $N = 40$ and $\approx 10^{-5}$ for $N = 20$). This occurs because at feed concentrations above this value breakthrough is taking place while after this point almost complete solute removal is predicted. At this feed concentration the transfer step where the minimum concentration exits also shifts. The theory predicts essentially complete removal of solute for the dilute product for $N = 40$ at low feed concentrations. At very low feed concentrations, the normalized product concentrations become independent of feed concentrations as predicted by linear theory[3]. The remainder of the results to be presented are all experimental. As noted above, quantitative simulations were not obtained for the cycling-zone experiments.

The start-up results for the first cycling-zone experiment are shown in Fig. 3. In this and following figures the abscissae represent column void volumes that have been eluted. The system rapidly reaches a repeating state for both outlet pH and concentration. This was checked by doing an experiment for six cycles to show that each cycle was a repeat of the previous cycle. As a first approximation we would expect the solute to peak where the pH is a minimum. This does not occur because at a feed concentration of 4 mg/ml breakthrough occurs before the pH minimum exits from the column and because the solute peak will exit at a pH where its f value equals the pH wave velocity B. The outlet concentration curves also show a shoulder, the outlet concentration being approximately equal to the feed concentration. This is characteristic of systems where the cycle is too long. The same experiment was repeated with glucose as the solute, and with both glucose and fructose. As expected, considerably less separation was obtained for glucose. With both sugars present the individual separations are not additive, but fructose greatly inhibits glucose separation.

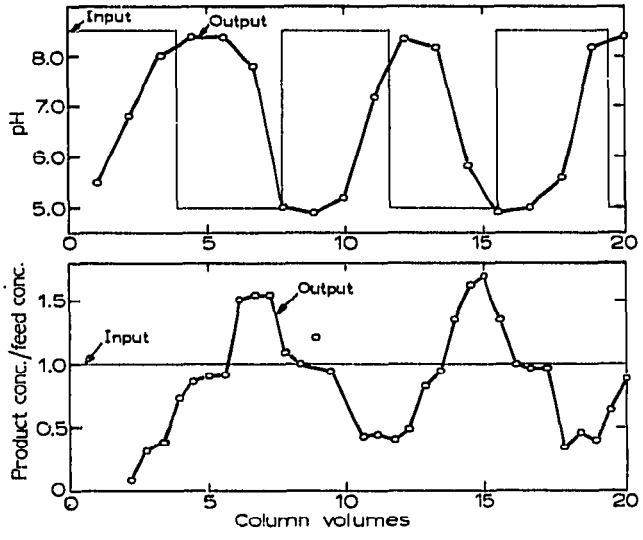


Fig. 3. Experimental start-up results for removal of fructose from water by cycling-zone operation. Feed, 4 mg/ml fructose, with 0.05 M morpholine (pH 8.5) or 0.05 M acetic acid (pH 5.0); T = 25°; 3.9 column void volumes per half cycle; flow-rate, 0.76 ml/min.

Comparison of these preliminary experiments with the theory indicated that better separation could be obtained by shortening the cycle time, decreasing the sugar concentration in the feed, and using a pH greater than 8.5. The last technique was not tried because isomerization of the sugars would be expected at high pH. In Fig. 4 the experiment was repeated but with the fructose feed concentration decreased by a factor of four and a shorter cycle time. With this shorter cycle time the amplitude of the outlet pH curve is attenuated and the maximum and minimum values of 8.5 and 5.0 are not obtained. As predicted by the theory, we see a dramatic increase in the removal of fructose from the dilute product fraction for this lower feed concentration, and a much better average separation since no shoulders are present. Fig. 4 shows that very large separations can be achieved if the column is not operated near satura-

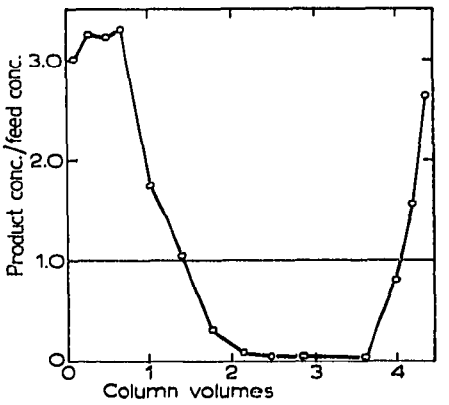


Fig. 4. Separation of a fructose-water mixture. 4th Cycle; 2.2 column void volumes per half cycle; feed, 1 mg/ml. Other conditions as in Fig. 3.

tion, and even larger separations would be expected if lower feed concentrations were used. The separation of fructose–glucose–water mixtures was repeated using the shorter cycle times and lower feed concentrations. These results (Fig. 5) show a much better fructose separation than that obtained previously and a somewhat better glucose separation. Again the fructose inhibits the glucose separation. Since fructose and glucose peak at the same time, the cycling-zone technique utilized here will not separate them although a modification of this technique should be capable of separating multi-component mixtures[6]. The separation obtained here could be used to increase the fructose concentration of invert sugar mixtures and recycle the low fructose fraction to an isomerization reactor.

The effect of flow-rate on the separation of fructose from water was also studied for flow-rates of 0.38, 0.76, and 1.91 ml/min. The lower flow-rates gave slightly larger peak-to-peak separations and longer periods of almost complete re-

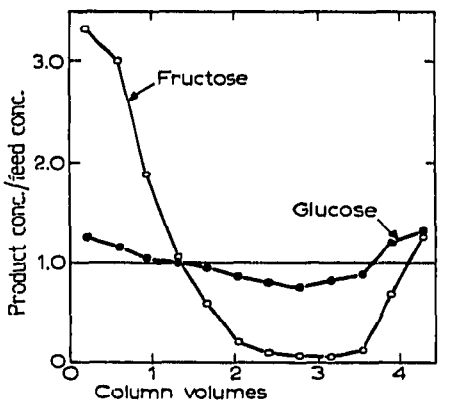


Fig. 5. Separation of a fructose-glucose-water mixture. 4th Cycle; feed, 1 mg/ml fructose and 1 mg/ml glucose; 2.2 column void volumes per half cycle. Other conditions as Fig. 3.

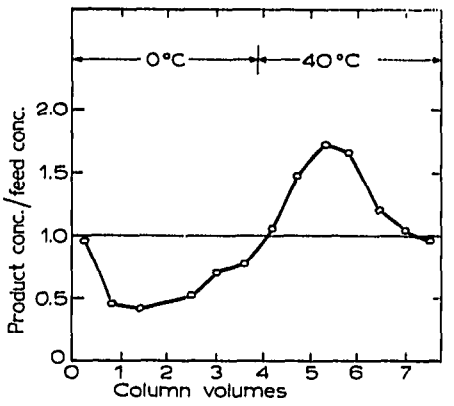
moval of fructose from the dilute product. These results indicate an HETP which increases slightly as the flow-rate increases.

Since the adsorption equilibrium is also temperature dependent, a cycling-zone separation utilizing temperature as the cyclic variable should also remove the sugars from water. The direct mode[1,4,5] of operation was utilized by running hot and cold water at 40° and 0° periodically through the column jacket. These results are shown in Fig. 6 where the pH is maintained at 8.5 throughout the cycle. A good separation is obtained, but considerably less than obtained with the pH traveling-wave mode for the same feed concentration. Since both methods of operation produced a separation, we decided to try using the pH traveling-wave mode and the direct-temperature mode simultaneously to try and improve the separation. The results are shown in Fig. 7. The timing (Table II) used was chosen so that the column was hot when the concentrated fructose mixture was leaving the column. Comparison of Figs. 4 and 7 shows a slightly better removal of fructose from the dilute product and a lower peak concentration for the combined mode of operation. We hypothesize that a large

TABLE II

TIMING OF THE CYCLE FOR COMBINED pH TRAVELING-WAVE AND DIRECT-TEMPERATURE MODE

Column void volume	*pH of feed*	*Jacket temperature (°C)*
0	8.5	40
1.5	8.5	0
2.2	5.0	0
4.1	5.0	40
4.4	8.5	40



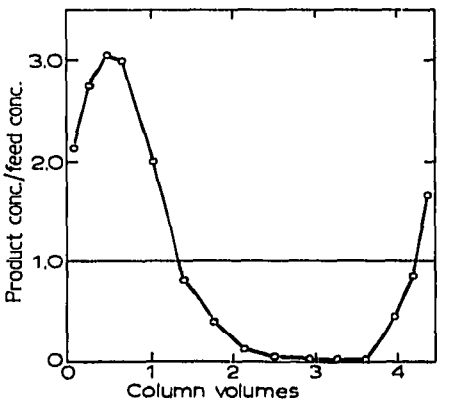


Fig. 6. Separation of fructose from water by thermal direct-mode cycling-zone operation. 4th cycle; feed, 1 mg/ml; 3.9 column void volumes per half cycle; buffer, 0.05 *M* morpholine (pH 8.5); T = 0° or 40° (jacket); flow-rate, 0.76 ml/min.

Fig. 7. Separation of fructose from water by thermal direct-mode and pH traveling-wave mode cycling-zone operation. 4th cycle; feed, 1 mg/ml; feed is either 0.05 *M* morpholine (pH 8.5) or 0.05 *M* acetic acid (pH 5.0); T = 0° or 40° (jacket); flow-rate, 0.76 ml/min. Timing of the cycle: see Table II.

improvement would have been seen if separation were not as complete as that obtained with the pH traveling-wave mode alone.

DISCUSSION

We have shown that partial separations of fructose–glucose–water mixtures can be obtained by elution chromatography, frontal development and cycling-zone adsorption. The cycling-zone separation has a larger throughput than the other techniques and concentrates the solute in a portion of the product, but it sacrifices some of the purity that can be achieved by the other methods and requires the addition of chemicals and/or energy. For relatively easy separations cycling-zone adsorption shows considerable promise as a preparative chromatographic technique.

The DBAE-cellulose used has good selectivity for the sugars but a very low capacity. This low capacity limits the maximum feed concentration that can be easily separated. Since the adsorbent we used has originally been prepared for ribonucleotide separation, the low capacity is not surprising. Higher capacities could easily be achieved by using a heavier loading of dihydroxyboryl groups and by using a support other than cellulose. An example is the adsorbent recently reported on by Barker *et al.*[12]. For commercial use of the technique for separation of fructose from glucose, a higher-capacity adsorbent would be required.

Use of a high-capacity adsorbent with high concentrations in the feed would probably lead to large changes in density and viscosity of the liquid in the different portions of a cycle. These changes might cause operational problems and would probably decrease the separation. These effects need to be checked experimentally.

The theoretical results predicted that more separation would be obtained with a smaller number of theoretical stages if the adsorbent was operating near its saturation limit. With more stages there are more mixing and dispersion effects, and more opportunities for equilibrium between the solid and the fluid. The latter effect usually produces better separation. However, when the adsorbent is grossly overloaded the change in fluid concentration effected by going from total adsorption to zero adsorption is quite small. In this case having more equilibrium stages has little effect and the zone spreading (proportional to $\sqrt{N}$) is more important. Compared with elution chromatography, cycling-zone adsorption can grossly overload the stationary phase and the small N (3 to 4 stages) that was estimated is not extremely surprising. We are now investigating cycling-zone operation in high-pressure liquid chromatography to see what values of N are obtained. Larger values of N and better separation would be expected for very low feed concentrations.

The cycling-zone method used here essentially removes all solutes from a portion of the product. This is the desired result when impurities are being removed from a non-adsorbed solvent. A larger amount of pure solvent can be prepared by use of unsymmetric cycles[5]. When one desires to separate two or more solutes from each other, a modification of the cycling-zone technique can be used[6].

To achieve a large cycling-zone separation a system with both "amplification" and "trapping" is desired. By amplification we mean that a very small change in the thermodynamic variable causes a large change in the amount of solute adsorbed. Amplification is likely to occur with chemadsorption, separation of weak acids or bases, and when a good complexing agent is available. Trapping occurs when the

thermodynamic variable moves through the column at a speed that is greater than the solute velocity when the solute is strongly adsorbed, but less than the solute velocity when it is weakly adsorbed. This causes the solute to be trapped at the front of the wave of the thermodynamic variable and can result in large increases in solute concentration. A similar trapping effect has been observed in gradient elution liquid chromatography by Kirkegaard[13] and Ito *et al.*[14]. Under proper elution conditions large dilute pulses were observed to be separated and concentrated in the chromatographic column. In cycling-zone systems the trapping effect was discussed in somewhat different fashion by Baker and Pigford[5] and Wankat[3]. Naturally, amplification and trapping can reinforce each other.

ACKNOWLEDGEMENTS

A stimulating lecture by Dr. P. T. Gilham first brought the existence of DBAE-cellulose to the authors' attention. The very generous gift of DBAE-cellulose by Drs. P. T. Gilham and R. E. Duncan is gratefully acknowledged. Discussions with Drs. J. Dixon, R. E. Duncan, A. H. Emery and F. Regnier were most helpful. This work was partially supported by NSF Grant Nos. GI-34919A1 and GK-43282.

REFERENCES

1 R. L. Pigford, B. Baker and D. E. Blum, *Ind. Eng. Chem. Fundam.*, 8 (1969) 848.
2 P. C. Wankat, *Separ. Sci.*, 9 (1974) 85.
3 P. C. Wankat, *J. Chromatogr.*, 88 (1974) 211.
4 P. C. Wankat, *Separ. Sci.*, 8 (1973) 473.
5 B. Baker and R. L. Pigford, *Ind. Eng. Chem. Fundam.*, 10 (1971) 283.
6 P. C. Wankat, *Ind. Eng. Chem. Fundam.*, 14 (1975) 96.
7 C. J. King, *Separation Processes*, McGraw-Hill, New York, 1971, pp. 404–415.
8 H. L. Weith, J. L. Weibers and P. T. Gilham, *Biochemistry*, 9 (1970) 4396.
9 M. Rosenberg, J. L. Weibers and P. T. Gilham, *Biochemistry*, 11 (1972) 3623.
10 M. E. Busbice, *M. Sc. Thesis*, Purdue University, West Lafayette, Ind., U.S.A., 1974.
11 P. C. Wankat, *Anal. Chem.*, 46 (1974) 1400.
12 S. A. Barker, B. W. Hatt, P. J. Somers and R. R. Woodbury, *Carbohyd. Res.*, 26 (1973) 55.
13 L. H. Kirkegaard, *Biochemistry*, 12 (1973) 3627.
14 Y. Ito, R. E. Hurst, R. L. Bowman and E. K. Achter, *Sep. Purif. Methods*, 3 (1974) 133.