

Apparent Distribution Coefficients of Glucose and Fructose onto Cation-Exchange Resins in Calcium-Ion Form with Different Divinylbenzene Contents

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The apparent distribution coefficients, K_{app} , of glucose and fructose onto cation-exchange resins in calcium-ion form with different divinylbenzene (DVB) contents were observed over a wide concentration ranges. Irrespective of the DVB content of the resin, the K_{app} value of glucose was larger with an increase in the glucose concentration, while the K_{app} value of fructose was smaller at higher fructose concentrations. A method for estimating the binding constant of fructose to the calcium-ion, B_{Fru} , was proposed under the assumption that the constant of glucose could be approximated as zero, and the B_{Fru} value was evaluated as 0.66 l/mol. The K_{app} values of mannose onto a resin with a DVB content of 4% were also measured at various solute concentrations, and the B_{Man} value was estimated to be 0.31 l/mol.

Keywords: distribution coefficient, chromatography, cation-exchange resin, glucose, fructose

Cation-exchange resins in calcium-ion form have been widely used to separate saccharides for analytical and industrial purposes. Especially, the production of high fructose syrup is achieved in a batch or continuous mode using the resin in the counter-ion form on an industrial scale (Sakiyama, 1997). In this production, water, the safest solvent for food processing, is used as the eluent. The apparent distribution coefficient, K_{app} , is a crucial parameter governing the separability of solutes. The coefficient is usually treated as a constant independent of the solute concentration (Viard & Lameloise, 1992). However, we demonstrated that the K_{app} values of maltooligosaccharides (Adachi *et al.*, 1995), glucose and fructose (Adachi & Matsuno, 1999) onto a resin in Na^+ form depended on the solute concentrations. Ito *et al.* (1992) also reported that the K_{app} value of nistose onto a resin in Na^+ form depended on its concentration. We proposed a model for explaining the concentration dependence of K_{app} taking the effect of swelling pressure of the resin on the distribution of solute and the complex formation between the solute and the counter-ion into consideration (Adachi & Matsuno, 1997). We showed some methods for estimating the binding constant B of a solute to the counter-ion (Adachi & Matsuno, 1999; Adachi *et al.*, 1999) based on the model.

In this study, the K_{app} values of glucose and fructose onto resins in Ca^{2+} form with different divinylbenzene (DVB) contents were measured at various solute concentrations. The K_{app} value of mannose onto the resin with a DVB content of 4% was also observed at various concentrations because it was intermediate between those of glucose and fructose. Furthermore, another method for estimating the B value was proposed.

Materials and Methods

Materials Cation-exchange resins with sulfonate groups and with different DVB contents were supplied by Japan Organo, Tokyo, (Amberlite resins) or purchased from Muromachi

Chemicals, Tokyo (Dowex resins). The resins were converted to the H^+ or Ca^{2+} form according to standard procedures (Kakihana & Mori, 1969).

Glucose, fructose and mannose were purchased from Wako Pure Chemical Industries, Osaka. Dextran T-70, the weight-averaged molecular weight of which was *ca.* 7×10^4 and which was used to estimate the bed voidage, was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden.

Apparent distribution coefficient A resin in Ca^{2+} form was packed into a cylindrical glass column having an inner diameter of 16 mm. The bed height was *ca.* 35 cm and was precisely measured for each experiment. Dextran T-70 solution, which was dissolved with distilled water at a concentration of 0.5% (w/v), was continuously fed to the bed at a flow rate of *ca.* 1 ml/min by a peristaltic pump (TMP-GL, Toyo Kagaku Sangyo, Tokyo). The flow rate was also precisely measured for each experiment. The effluent was fractionated every 1 min, and the concentration of Dextran T-70 was determined to obtain its breakthrough curve. Dextran T-70 could not penetrate into the resin because of its large molecular size; therefore, the bed voidage ϵ_b was evaluated by the following equation:

$$\epsilon_b = \frac{Q}{V_t} \left[t_E - \sum_{i=0}^N \left(\frac{C_{Si}}{C_{S0}} \right) \Delta t \right], \quad (1)$$

where C_{Si} is the solute concentration in the i th fraction, C_{S0} is the solute concentration in the feed, Q is the volumetric flow rate, t_E is the elution time at the endpoint, V_t is the bed volume, N is the number of fractions, and Δt is the interval of fractionation.

After the solute existing in the previous experiment had been washed out with a large amount of distilled water, a solute (hexose) solution of a specified concentration C_{S0} was continuously fed to the bed. The effluent was fractionated at appropriate intervals (1 to 3 min), and the solute concentration of each fraction was determined. The amount of solute adsorbed q was calculated by Eq. (2) from the mass balance of the solute.

$$(1-\varepsilon_b)V_t q = Q C_{S0} t_E - \varepsilon_b V_t C_{S0} - Q \sum_{i=1}^N C_{Si} \Delta t \quad (2)$$

The apparent distribution coefficient K_{app} was defined as q/C_{S0} . The solute concentration in the feed was in a range of 5.6×10^{-3} to 3.33 mol/l (0.1 to 60 % (w/v)) for each solute.

The voidage of the bed filled with a solute of concentration C_{S0} was also measured as follows: after the bed was filled with the solute solution, 0.5% Dextran T-70 solution dissolved in the solute solution was fed to the bed to observe the breakthrough curve for Dextran T-70. The bed voidage was evaluated according to Eq. (1).

Response curves for rectangular input Dowex 50W X 4 resin in Ca^{2+} form was packed into a cylindrical column of the same dimensions as the column used in the breakthrough curve measurements. A mixture (3.4 ml) of glucose and fructose, each concentration of which was $5.6 \times 10^{-2} \text{ mol/l}$ (1% (w/v)) or 2.22 mol/l (40%), was applied to the bed and was eluted with water at a flow rate of 1.02 ml/min. The effluent was fractionated at intervals of 2 min. The concentrations of the solutes in each fraction were determined by HPLC after appropriate dilution.

Properties of resin A resin was converted to the H^+ form, and its exchange capacity, E_{H^+} , was measured by a standard titration procedure (Kakihana & Mori, 1969). The apparent density of the resin ρ_{H^+} was determined pycnometrically. The measurements of E_{H^+} and ρ_{H^+} were repeated at least three times and were averaged for each property. The equivalent volume, V_{e,H^+} , which is defined as the volume of resin per unit equivalent of fixed ion, was calculated by $1/(\rho_{\text{H}^+} E_{\text{H}^+})$.

The resin in H^+ form was packed into a glass column, and the bed voidage $\varepsilon_{b,\text{H}^+}$ was measured according to the above-mentioned procedures. The resin was converted to the Ca^{2+} form by supplying 1.0 mol/l CaCl_2 solution and then washing with distilled water without taking the resin out of the column. The bed voidage for the resin in Ca^{2+} form, $\varepsilon_{b,\text{Ca}^{2+}}$, was then measured using Dextran T-70 as a solute. The equivalent volume of the resin in Ca^{2+} , $V_{e,\text{Ca}^{2+}}$, was calculated by Eq. (3),

$$V_{e,\text{Ca}^{2+}} = \frac{Z_{\text{Ca}^{2+}}}{Z_{\text{H}^+}} \frac{1 - \varepsilon_{b,\text{Ca}^{2+}}}{1 - \varepsilon_{b,\text{H}^+}} V_{e,\text{H}^+} \quad (3)$$

where Z_{H^+} and $Z_{\text{Ca}^{2+}}$ are the heights for the bed packed with the resins in H^+ and Ca^{2+} forms, respectively. The reciprocal of $V_{e,\text{Ca}^{2+}}$ represents the concentration of fixed-ions \bar{C}_E for the resin in Ca^{2+} form.

The wet resin in Ca^{2+} form was weighed (w_{wet}) and dried at 105°C to reach a constant weight (w_{dry}). The porosity of the resin ε_p was approximately estimated from the volumetric fraction of water present within the resin by Eq. (4) (Adachi *et al.*, 1999),

$$\varepsilon_p \approx \frac{\rho_{\text{Ca}^{2+}}}{\rho_w} \frac{w_{\text{wet}} - w_{\text{dry}}}{w_{\text{wet}}} \quad (4)$$

where ρ_w is the density of water and $\rho_{\text{Ca}^{2+}}$ is the apparent density

of wet resin.

Analysis When each fraction contained only a solute (Dextran T-70 or a hexose), the solution concentration was determined by the flow-injection method as follows: the solution of each fraction was approximately diluted and then 0.5 ml of the solution was loaded into a sample injector. The loaded sample was caused to flow into a YRU-883 refractometer (Shimamura Seisakusho, Tokyo) using a peristaltic pump, and the response of the refractometer was recorded on a strip recorder (R-50, Rika Denki, Tokyo). The concentration was determined from the height of the response. A calibration curve was prepared using the solute solutions of known concentrations.

The Dextran T-70 concentration in the fraction where hexose coexisted was measured by an LC-6A HPLC (Shimadzu Seisakusho, Kyoto) with a separation column and a refractometer (RID-6A, Shimadzu Seisakusho). A St/6DVB-15(Ca) column (Japan Organo) in which a cation-exchange resin in Ca^{2+} form was packed ($4.6 \text{ mm}\phi \times 25 \text{ cm} \times 2$) was used as the separation column. The eluent used was distilled water, and the flow rate was 1.0 ml/min.

The concentrations of glucose and fructose in the fractions of the response experiments for rectangular inputs were determined by HPLC with a Cosmosil 5NH₂-MS column ($4.6 \text{ mm}\phi \times 250 \text{ mm}$, Nacalai Tesque, Kyoto) and an RID-6A refractometer. The eluent was a mixture of acetonitrile and water (80:20 in volume), and the flow rate was 1.5 ml/min.

Results and Discussion

Properties of resins Table 1 summarizes the concentration of fixed ions \bar{C}_E and the porosity ε_p for the resins in Ca^{2+} form with various DVB contents. The resin with higher DVB content had a higher \bar{C}_E and lower ε_p . The concentration of Ca^{2+} within the resin should be higher for the resin with a higher \bar{C}_E to maintain electroneutrality in the resin phase.

Apparent distribution coefficients Figure 1 shows examples of breakthrough curves of Dextran T-70, glucose and fructose for the bed packed with Dowex 50W X 4 (DVB 4%) in Ca^{2+} form. The curve for Dextran T-70 shown in the figure was observed under the condition where 0.28 mol/l glucose coexisted. From the curve of Dextran T-70, we could estimate the bed voidage ε_b according to Eq. (1). The amount of glucose and fructose adsorbed q was calculated according to Eq. (2); then the apparent distribution coefficient K_{app} was obtained by dividing q by the feed concentration C_{S0} . Glucose was eluted earlier than fructose, indicating that the K_{app} value of glucose was smaller than that of fructose. The breakthrough curve for glucose observed when C_{S0} was 2.22 mol/l was behind that at $C_{S0}=0.28 \text{ mol/l}$. This indicates that the K_{app} at $C_{S0}=0.28 \text{ mol/l}$ is smaller than that at $C_{S0}=2.22 \text{ mol/l}$. On the other hand, because fructose was eluted earlier when $C_{S0}=2.22 \text{ mol/l}$ than when $C_{S0}=0.28 \text{ mol/l}$, the K_{app} at $C_{S0}=2.22 \text{ mol/l}$ should be smaller than that at

Table 1. The concentration of fixed ions \bar{C}_E and the porosity ε_p of resins in Ca^{2+} form with various DVB contents.

Resin DVB [%]	Amberlite FX-1080 8	Dowex 50W X 8 8	Amberlite CR-1310 6	Dowex 50W X 4 4	Dowex 50W X 2 2
\bar{C}_E [equiv./l]	2.82	2.74	2.47	1.77	1.34
ε_p	0.620	0.606	0.676	0.723	0.781

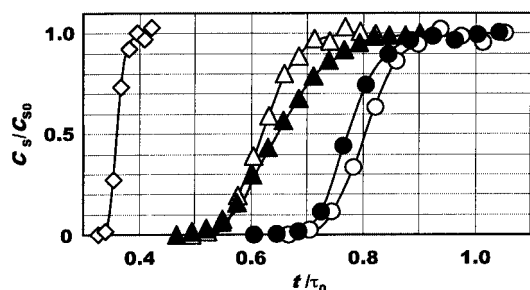


Fig. 1. Breakthrough curves of (\diamond) Dextran T-70, (Δ , \blacktriangle) glucose and (\circ , \bullet) fructose in the bed packed with Dowex 50W X 4 in Ca^{2+} form. The curves were separately measured for a column with dimensions of 1.6 cm I.D. and ca. 35 cm height. Dextran T-70 was dissolved at a concentration of 0.5% (w/v) with 0.28 mol/l glucose solution. Open and closed symbols for glucose and fructose indicate that the concentrations of the solutes were 0.28 and 2.22 mol/l, respectively. The ordinate is the solute concentration in the effluent C_s relative to its feed concentration C_{S0} , and the abscissa represents the elution time t normalized by the superficial residence time τ_0 . The τ_0 value was calculated based on the bed height when the feed was stopped.

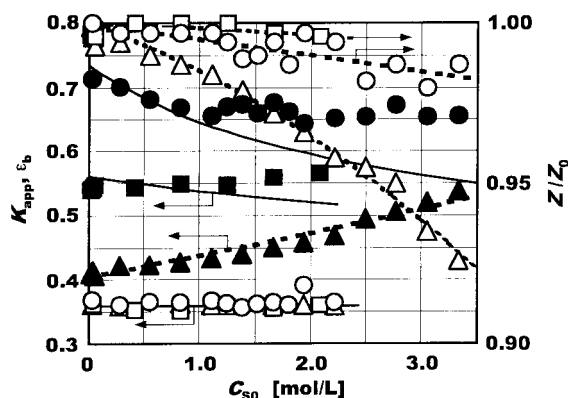


Fig. 2. Apparent distribution coefficients of glucose, mannose and fructose, bed voidage, and relative bed height at various solute concentrations for Dowex 50W X 4 in the Ca^{2+} form. Triangle, square and circle indicate glucose, mannose and fructose, respectively. The closed symbols represent the apparent distribution coefficient, K_{app} . The open symbols represent the bed voidage, ϵ_b , or the relative bed height, Z/Z_0 . The broken curve for the K_{app} of glucose shows the correlation of the K_{app} and C_s by Eq. (7). The solid curve represents the K_{app} values of fructose and mannose calculated by substituting the K and B values estimated in this study into Eq. (5). The broken curves for the Z/Z_0 values were drawn by empirically fitting the points by a quadratic equation passing 1.0 on the ordinate. The solid line for the ϵ_b indicates the averaged value.

$C_{S0} = 0.28$ mol/l.

The breakthrough curves for Dextran T-70, glucose, mannose and fructose were separately measured at various concentrations. Figure 2 shows the K_{app} values of glucose, mannose and fructose, the bed voidage ϵ_b and relative bed height Z/Z_0 at various solute concentrations. The relative bed height indicates the bed height at the endpoint divided by the initial height. For glucose, the bed largely shrank, and the K_{app} became large at higher C_s . The bed shrinkage was not remarkable for fructose and mannose even at high C_s . The concentration dependence of the K_{app} for fructose and mannose was different from that for glucose. The K_{app} of fructose decreased with an increase in its concentration and then was almost constant at concentrations higher than 1.0 mol/l. The K_{app} of mannose was almost constant at low concentrations and slightly increased at concentrations higher than 1.5 mol/l. The bed voidage scarcely depended on the solute concentration. Therefore, for the bed packed with other resins, the ϵ_b value esti-

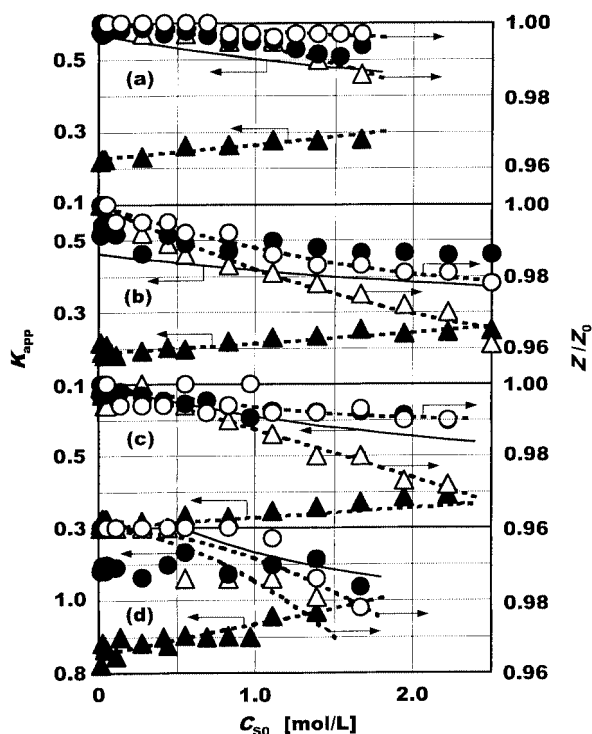


Fig. 3. Apparent distribution coefficients, K_{app} , of glucose and fructose, and relative bed height, Z/Z_0 , at various solute concentrations for (a) Amberlite FX-1080 (DVB content of 8%), (b) Dowex 50W X 8 (8%), (c) Amberlite CR-1310 (6%) and (d) Dowex 50W X 2 (2%). The symbols and curves are the same as in Fig. 2.

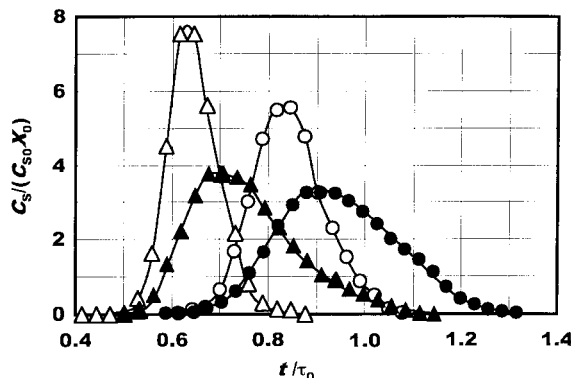


Fig. 4. Response curves for rectangular inputs of (Δ , \blacktriangle) glucose and (\circ , \bullet) fructose. The open and closed symbols indicate that the respective concentrations of the solutes in the samples C_{S0} were 5.6×10^{-2} and 2.22 mol/l, respectively. The ordinate represents the normalized solute concentration, $C_s/(C_{S0}X_0)$, where X_0 is the sample injection time normalized by the superficial residence time τ_0 . The residence time was, in this case, calculated based on the initial bed height.

ated in the absence of hexose was used for calculation of the q .

The K_{app} values of glucose and fructose were observed at various concentrations for the resins in Ca^{2+} form with various DVB contents. Figures 3 (a), (b), (c) and (d) show the plots of the K_{app} and Z/Z_0 values versus the solute concentration C_{S0} for Amberlite FX-1080 (DVB of 8%), Dowex 50W X 8 (8%), Amberlite CR-1310 (6%) and Dowex 50W X 2 (2%), respectively. For each resin, the concentration dependence of the K_{app} values of glucose and fructose was similar to that for Dowex 50W X 4 shown in Fig. 2.

Response curves for rectangular input As shown in Figs.

2 and 3, the difference in the K_{app} between glucose and fructose became smaller at their higher concentrations. The separability of the solutes would be low when the solutes of high concentrations were applied to a bed packed with resin in the Ca^{2+} form. The response curves were measured for a rectangular input of the mixture of glucose and fructose, each concentration of which was 5.6×10^{-2} mol/l or 2.22 mol/l (Fig. 4). The feed concentrations substantially affected the response curves. When the solute concentrations were low, the response curves of the solutes were sharp and the separability was fairly good. The curves were broad and the separability was low, however, when the mixture of glucose and fructose of 2.22 mol/l each was used.

Estimation of the binding constant of fructose to Ca^{2+} As shown in Figs. 2 and 3, the bed scarcely shrank when fructose was fed to the bed at any C_s , indicating that the change in the swelling pressure of the resin was small over the C_s range tested. Furthermore, the K_{app} of glucose was much smaller than that of fructose. Based on these observations, we devised a method for estimating the B value of fructose.

We proposed an equation for the K_{app} value of solute i at concentration C_i (Adachi *et al.*, 1999),

$$K_{app,i} = K_i \left(1 + \frac{B_i \bar{C}_E / z_M \epsilon_p}{1 + K_i B_i C_i / \epsilon_p} \right), \quad (5)$$

where B_i is the binding constant of the solute i to the counter-ion with a valency of z_M . K_i is the intrinsic distribution coefficient of solute i and is given by

$$K_i = \gamma_0 \exp\left(-\frac{\Pi \bar{v}_i}{RT}\right), \quad (6)$$

where γ_0 is a parameter reflecting both the ratio of the activity coefficient of the solute in the external solution phase to that in the resin phase and the steric effect of the network of the resin frame on the distribution, Π is the swelling pressure of the resin, \bar{v}_i is the partial molar volume of the solute, R is the gas constant, and T is the absolute temperature. The \bar{v}_i values of glucose and fructose were the same if the molar volumes, which were 0.114 l/mol (Adachi & Matsuno, 1997), were usable instead of \bar{v}_i . Therefore, the K_i value would be common for both the solutes at a Π value. Thereafter, K_i will be simply denoted by K .

Let us assume that the binding constant of glucose B_{Glc} is zero; that is, no complex is formed between glucose and Ca^{2+} , and the bed does not shrink when fructose is fed, so that the K value is independent of C_s . Under these assumptions, the concentration dependence of the K_{app} value of glucose is principally ascribed to the swelling pressure of the resin, and its value at $C_s \rightarrow 0$, $K_{app,Glc,0}$, can be approximately equal to the K value. To estimate the $K_{app,Glc,0}$ value, the $K_{app,Glc}$ was empirically correlated with C_s by Eq. (7),

$$K_{app,Glc} = K_0 \exp(aC_s), \quad (7)$$

where K_0 and a are the positive constants and $K_0 = K_{app,Glc,0} = K$. The broken curves in Figs. 2 and 3 show the correlation by Eq. (7). Defining the ratio of $K_{app,Fruc}$ to K as R , and rearranging Eq. (5), we can obtain the following equation:

$$\frac{\bar{C}_E / z_M}{(R-1)C_s} - K = \frac{1}{B_{Fruc}} \frac{1}{C_s / \epsilon_p}. \quad (8)$$

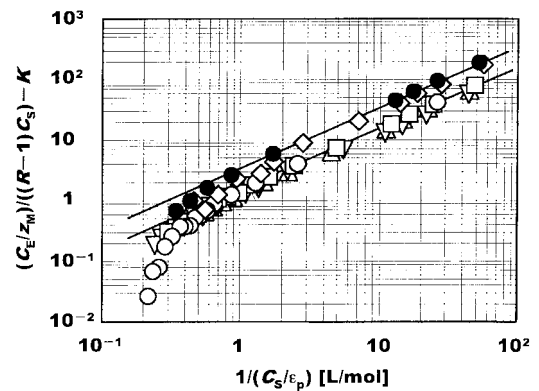


Fig. 5. Estimation of the binding constant of fructose or mannose, B_{Fruc} or B_{Man} , to the Ca^{2+} according to Eq. (8). The open and closed symbols represent the plots for estimation of B_{Fruc} and B_{Man} , respectively. The resins used were (Δ) Amberlite FX-1080 (DVB content of 8%), (∇) Dowex 50W X 8 (8%), (\square) Amberlite CR-1310 (6%), (\circ , \bullet) Dowex 50W X 4 (4) and (\diamond) Dowex 50W X 2 (2%). The valency of the counter-ion, z_M , was 2 for Ca^{2+} .

Equation (8) indicates that, irrespective of the DVB content of resin, the plots of the left-side term versus $1/(C_s/\epsilon_p)$ should give a straight line passing through the origin, and the B_{Fruc} value can be evaluated from the slope of the line.

Figure 5 shows the plots for the data obtained for all the resins on both logarithmic scales. Except for the resin with a DVB content of 2%, the plots at larger values on the abscissa, that is, at the lower C_s values, lay on a straight line with a slope of unity, and the B_{Fruc} value was calculated to be 0.66 l/mol. The plots at smaller values on the abscissa corresponding to the higher C_s values deviated from the line because the assumption of no shrinkage of the bed did not hold. The figure also shows the plots for mannose, the K_{app} values of which were measured only for Dowex 50W X 4 with a DVB content of 4%. The plots for the lower C_s values could be connected by a line with a slope of unity, and the binding constant of mannose to Ca^{2+} , B_{Man} , was estimated to be 0.31 l/mol.

The $K_{app,Fruc}$ or $K_{app,Man}$ values were calculated by substituting the $K = K_{app,Glc,0}$ and the B_{Fruc} or B_{Man} value into Eq. (5) at various C_s values. The calculated results are shown in Figs. 2 and 3 by solid curves. The curves fitted well with the experimental results at low C_s values, except for Dowex 50W X 2, but deviated from the experimental K_{app} values at high C_s values. In the calculation, no change in the swelling pressure was assumed over the whole range of C_s . However, the bed gradually shrank with an increase in C_s because of the decrease in the swelling pressure Π , which resulted in an increase in the K value as understood from Eq. (6). These factors would be the cause of the deviation.

As mentioned above, the apparent distribution coefficients of glucose and fructose onto a cation-exchange resin in the Ca^{2+} form were not constant but depended on the solute concentrations. As for the concentration dependence of the K_{app} of glucose, the swelling pressure of the resin played an important role. The K_{app} value of fructose was affected by the complex formation with the counter-ion as well as by the swelling pressure of the resin.

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