Simultaneous Estimation of the Binding Constant of Saccharide to Sodium Ion and the Swelling Pressure of Cation-exchange Resin

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The apparent distribution coefficients, $K_{app}$, of some mono-, di- and trisaccharides onto cation-exchange resins in the sodium-ion form and having the divinylbenzene (DVB) contents of 4, 6 and 8% were measured at 25°C. A method for simultaneously estimating the swelling pressures of the resins and the binding constants, $B$, of the saccharides to the sodium ion was proposed. By applying the method to the $K_{app}$ values of mono- and disaccharides, the swelling pressures of the resins having the DVB contents of 4, 6 and 8% were estimated to be 18.9, 30.8 and 37.8 MPa, respectively, and the $B$ values of the mono- and disaccharides were also estimated simultaneously. The $B$ values of the trisaccharides were calculated using the estimated swelling pressures. The $B$ values ranged from 0.24 to 2.2 L/mol. The tri-, di- and mono-saccharides had higher $B$ values in this order. There was a tendency that the solute, which can form a tridentate complex with the sodium ion, contains high numbers of axial-equatorial and equatorial-equatorial complexing hydroxyl groups.

Keywords: swelling pressure, distribution coefficient, binding constant, saccharide, ion-exchange resin

Introduction

The liquid chromatographic separation of saccharides has been extensively performed for industrial purposes as well as for analytical ones. The separation can be achieved in some modes. Reversed-phase chromatography has been used for analytical purposes, but it required derivatization of the saccharides prior to the separation (Gomis et al., 2001). High-performance anion-exchange chromatography, which mostly uses pulsed amperometric detection in an alkaline solution as an eluent in order to weakly ionize the solutes, has also been commonly used for analyzing carbohydrates (Catraldi et al., 2000). Another mode for separating saccharides is ligand-exchange chromatography, which uses cation-exchange resins in some ion forms. Because water can be used as an eluent for the chromatography, it has been widely used on an industrial scale as well on an analytical one. Especially, it is suitable for the food industry because of the safety of the eluent, and the separation processes are operated in both batchwise and continuous modes (Hashimoto, 2005). The continuous separation mode is usually realized using a simulated moving-bed (Adachi, 1994).

The separation of solutes during ligand-exchange chromatography is based on the difference in the strength of the interaction between the solute and counter ion. Elution of a solute interacting more strongly with the counter ion is more retarded. The strength of the interaction has been estimated by a capacity factor (Goulding, 1975; Caruel et al., 1991) or an apparent distribution coefficient (Adachi and Matsuno, 2000). Both parameters are estimated from the elution time or volume of the solute, and depend on the degree of cross-linkage or the divinylbenzene content of the resin, even if the counter-ion form is the same. However, the intrinsic strength of the interaction at a specific temperature should be determined only by the combination of solute and counter ion, and it should not depend on the cross-linkage of the resin, which affects the swelling pressure.

In this context, we previously proposed a model for describing the apparent distribution coefficient, which separately dealt with partitioning of a solute from an external solution phase to a resin phase and complex formation between the solute and a counter ion in the resin phase (Adachi and Matsuno 1997; Adachi et al., 1999). The complex formation was estimated by the binding constant of the solute to the

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counter ion, and the binding constants of galactose, mannose and fructose to lithium, sodium, potassium, calcium and strontium ions were evaluated under the assumption that glucose did not form a complex with any ions (Adachi et al., 1999). This assumption was adopted to estimate the swelling pressure of a resin, which could not be evaluated from the chromatographic data.

Based on this study, we propose a method to simultaneously estimate the swelling pressures of resins and the binding constants of solutes to a counter ion from the apparent distribution coefficients of the solutes to the resins having different divinylbenzene contents. The applicability of the method was examined for many pentoses, hexoses, and disaccharides using the resins in the sodium-ion form and having the divinylbenzene contents of 4, 6 and 8%.

**Materials and Methods**

**Materials** Cation-exchange resins of sulfonated poly(styrene-co-divinylbenzene) were supplied by the Mitsubishi Chemical Corporation, Tokyo, Japan, and their divinylbenzene (DVB) contents were 4%, 6% and 8%. They had been conditioned in the sodium-ion form by the supplier, and the particle diameters of the resins with the DVB contents of 4%, 6% and 8% were 320, 220 and 220 μm, respectively, which were the values provided by the supplier.

D-Arabinose, L-arabinose, L-ribose, D-xylene, D-fructose, D-glucose, kojibiose, nigerose, trehalose, lactulose, sucrose, maltotriose and raffinose were purchased from Wako Pure Chemical Industries, Osaka, Japan. L-Lyxose, d-galactose, L-glucose, cellobiose, gentiobiose, isomaltose, maltulose, palatinose, isomaltotriose, melezitose and panose were purchased from Tokyo Chemical Industry, Tokyo, Japan. D-Mannose, maltose and lactose were purchased from Nacalai Tesque, Kyoto, Japan. Neotrehalose and leucrose were purchased from Hayashi-bara, Okayama, Japan and Fulka, Buchs SG, Switzerland, respectively. Dextran T-70, the weight-averaged molecular mass (ca. \(7 \times 10^5\)) and which was used for determining the bed voidage, was purchased from Amersham Pharmacia Biotech, Buckinghamshire, UK.

**Properties of resin** The resin was converted into the hydrogen-ion form according to standard procedures. The exchange capacity of the resin, \(E_{\text{Na}}\), in the units of equiv./g-wet resin, was measured by a titration procedure against 1.0 mol/L sodium hydroxide of a known factor.

A resin was packed into a cylindrical glass column with a water jacket at a bed height, \(Z_n\). The bed voidage, \(\varepsilon_{b,n}\), was determined at 25°C using Dextran T-70 as the solute, which cannot penetrate into the resin due to its large molecular mass, according to the method described below. The resin was converted into the sodium-ion form by supplying a sufficient amount of 4% (w/v) sodium chloride to the bed, and successively washing with distilled water. The bed height, \(Z_{\text{Na}}\), was recorded, and the bed voidage, \(\varepsilon_{b,\text{Na}}\), was determined using Dextran T-70.

The apparent densities of the resins in the hydrogen-ion and sodium-ion forms, \(\rho_{h}\) and \(\rho_{\text{Na}}\), were pycnometrically determined at 25°C, respectively.

The concentration of fixed ions in the resin in the hydrogen-ion form, \(C_{E,h}\), was calculated from the \(E_{h}\) and \(\rho_{h}\) by \(E_{h}/\rho_{h}\). Based on the consistency of the number of fixed ions, the concentration of fixed ions in the resin in the sodium-ion form, \(C_{E,\text{Na}}\), was estimated by Eq. (1).

\[
C_{E,\text{Na}} = \frac{(1-\varepsilon_{b,\text{Na}})Z_{\text{Na}}}{(1-\varepsilon_{b,\text{Na}})Z_{\text{Na}}} C_{E,h}
\]

The wet resin in the sodium-ion form was dried at 105°C. The porosity of the resin, \(\varepsilon_{p}\), was assumed to be equal to the volumetric fraction of water present within the resin, and was estimated by Eq. (2).

\[
\varepsilon_p = \frac{(w_w-w_d)}{w_w/\rho_w}
\]

where \(w_w\) and \(w_d\) are the weights of the wet and dried resins, respectively, and \(\rho_w\) is the density of water at 25°C.

**Molar volume of solute** A solute of an adequate weight, \(w_s\), was put into a 5-mL or 10-mL volumetric flask, the precise volume of which was determined in advance using distilled water, and dissolved in water. The concentration of water, \(C_w\), was calculated from the difference in weight before and after adding the water. The concentration of the solute, \(C_s\), was calculated from the \(w_s\) and the volume of the flask. The measurement was carried out using different solute weights. The molar volume of the solute, \(v_s\), was estimated based on the following equation:

\[
C_w = 1 / v_w - (v_s / v_w) C_s
\]

where \(v_w\) is the molar volume of water.

**Apparent distribution coefficient** A resin was packed into a cylindrical glass column with a water jacket. The inner diameter of the column was 1.58 cm, and the bed height was about 26 cm, which was precisely measured for each experiment. The temperature of the bed was regulated at 25°C by circulating thermostat water through the jacket.

A half milliliter of a 1.0% (w/v) solute solution was applied to the bed and then eluted with distilled water with an MP-3N peristaltic pump (Tokyo Rikakikai, Tokyo, Japan). The column effluent was flowed into a YRU-833 refractometer (Shimamuratech, Tokyo, Japan). The elution profile of
the solute was monitored by the refractometer and recorded every 0.5 s on a Chromatopac C-R8A recorder (Shimadzu, Kyoto, Japan). For each solute, elution curves were observed at, at least, three different flow rates.

The apparent distribution coefficient, \( K_{\text{app}} \), of a solute on the resin was determined by moment analysis of the elution curve (Kucera, 1965). The normalized first-order statistical moment, \( \mu'_n \), which is defined by Eq. (4a), can be related to \( K_{\text{app}} \) by Eq. (4b) (Nakanishi et al., 1977).

\[
\begin{align*}
\mu'_n &= \int_0^t C dt / \int_0^\infty C dt \\
&= (Z_{Na}/u_0)[\varepsilon_{SNa} + (1-\varepsilon_{SNa})K_{\text{app}}] \\
\end{align*}
\]

where \( C \) is the solute concentration of the column effluent, \( t \) is the time, and \( u_0 \) is the superficial velocity. The integration of the right side of Eq. (4a) was numerically done using the detector intensity, which is proportional to \( f \), and that of the right side of Eq. (4b) was numerically done using the Simpson method.

This method was also used to determine the bed voidage, \( \varepsilon_{b, H} \), of the bed packed with the resin in the hydrogen-ion form.

Simultaneous estimation of swelling pressure and binding constant We have proposed an equation for the \( K_{\text{app}} \), taking into consideration the effects of both the resin swelling pressure and the complex formation between a solute and a counter ion in the resin phase (Adachi et al., 1999). Figure 1 schematically illustrates the phenomena which would occur for the distribution of a solute to the resin phase. A solute is partitioned between the external solution phase and the resin phase with the intrinsic distribution coefficient \( K \). The partitioned solute would form a complex with the sodium ion in the resin phase through the ligand-exchange reaction with the binding constant \( B \). The \( K_{\text{app}} \) value is defined as the ratio of the total concentration of free solute and the solute bound to sodium ion in the resin phase to that of the solute in the external solution phase, and is given by Eq. (5).

\[
K_{\text{app}} = K \left( 1 + \frac{BC_{SNa}/\varepsilon_p}{1 + BKC/\varepsilon_p} \right)
\]

The \( K \) value is defined as the ratio of the concentration of free solute in the resin phase and that of the solute in the external solution phase, and is given by Eq. (6) based on the equality of the chemical potentials of the solute in the external and resin phases at equilibrium. It was assumed for the derivation of Eq. (6) that the activity coefficient of the solute in the external solution is the same as that in the resin phase, and that \( v_s \) can be used instead of the partial molar volume.

\[
K = \varepsilon_p \exp(-\Pi v_s / RT)
\]

where \( \Pi \) is the swelling pressure of the resin, \( R \) is the gas constant, and \( T \) is the absolute temperature.

The concentrations of a solute and the sodium ion in the resin are defined based on the total volume of the resin phase, but the solute and the sodium ion can exist only in the pores of the resin. Therefore, their practical concentrations are calculated based on the volume of the pores. The law of mass action is used for defining the \( B \) value as follows:

\[
B = \frac{C_{SNa}/\varepsilon_p}{(C_s/\varepsilon_p)(C_{Na}/\varepsilon_p)}
\]

where \( \overline{C} \) is the concentration in the resin phase, and the subscripts, \( S \), \( Na \) and \( SNa \), indicate the free solute, free sodium ion and the complex of the solute and the sodium ion, respectively.

When the solute concentration in the external solution phase, \( C \), is sufficiently low, Eq. (5) can be approximated by Eq. (8).

\[
K_{\text{app}} = K(1 + B\overline{C}_{SNa}/\varepsilon_p)
\]

In Eq. (8), the swelling pressure, \( \Pi \), and the binding constant, \( B \), are unknown. The other parameters can be evaluated from the experiments mentioned above. The \( \Pi \) value depends only on the DVB content at a specific temperature, while the \( B \) value is intrinsic for a solute and would not be affected by the DVB content of the resin. Therefore, if we assume the \( \Pi \) values for the resins having the DVB contents of 4%, 6% and 8%, the \( B \) values of a solute can be calculated by Eq. (8) for the resins. Although the \( B \) value of the solute

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**Fig. 1.** Schematic illustration of phenomena occurring for the distribution of solute into the resin phase. Solute S partitions between the external solution phase and the resin phase with the intrinsic distribution coefficient \( K \), and forms a complex \( SNa^+ \) with the sodium ion \( Na^+ \) through ligand-exchange reaction with the binding constant \( B \) in the resin phase.
should be the same for the resins as mentioned above, they would be different from each other. The relative standard deviation in the $B$ value was calculated. The relative standard deviation is summarized for all the tested solutes, and the $II$ values, which minimize the sum of the relative standard deviations, were searched using the Solver of MS-Excel (Levie, 1999).

Results and Discussion

Properties of resin  Table 1 summaries the properties of the resins in the sodium-ion form. The particle size of the resin with the DVB content of 4% was larger than those of the resins with the DVB contents of 6 and 8%. The resins with the higher DVB contents possessed a higher apparent density, a higher concentration of fixed ions, and a lower porosity.

Molar volume of solute  Figure 2 shows the water concentrations of the solute solutions having different concentrations for some pentoses and disaccharides. The plots for the pentoses lie on a straight line. From the slope of the line, the molar volume of the pentoses was estimated to be 0.0973 L/mol. The plots for the disaccharides also lie on another straight line, and the molar volume of the disaccharides was estimated to be 0.232 L/mol. The molar volume of the hexoses was cited from our previous study (Adachi and Matsuno, 1997) to be 0.114 L/mol. The molar volume of the trisaccharides was estimated to be 0.332 L/mol from the average value of the molar volumes of maltotriose and raffinose (Adachi and Matsuno, 1997).

Apparent distribution coefficient  According to the above-mentioned procedures, the $K_{app}$ values of all the solutes were estimated for the resins in the sodium-ion form and having the DVB contents of 4%, 6% and 8%, and are listed in Table 2.

In general, the solute with a smaller molecular mass had the higher $K_{app}$ value. The $K_{app}$ value was lower for the resin having the higher DVB content. However, there was a difference in the $K_{app}$ value even among the solutes with the same molecular mass. This difference enables the solutes to be separated during chromatography using the resin.

Among the pentoses, the D- and L-ribose had much higher $K_{app}$ values than the other pentoses. There was no significant difference between the L- and D-enantiomers for all the pentoses. This fact indicates that the separation of the enantiomers cannot be realized by ligand-exchange chromatography. The $K_{app}$ value of fructose was the highest among the tested hexoses, irrespective of the DVB content of the resin. The $K_{app}$ values of the disaccharides depended on both the kind of constituent hexoses and the type of their linkage. Lacturose had the highest $K_{app}$ value among the tested disaccharides, and leucrose followed. The $K_{app}$ values of the trisaccharides were small for all the resins due to their large molecular mass or size. Especially, the values for the resin with the DVB content of 8% were small because of the synergistic effect of the large molecular size of the solutes and the high swelling pressure of the resin.

Estimation of swelling pressure and binding constant  In order to estimate the $B$ and $II$ values according to the method proposed above, the tentative $II$ values are necessary. Among the tested hexoses, the $K_{app}$ values of glucose were the lowest, irrespective of the DVB content of the resin. Therefore, the tentative $II$ values of the resins with the DVB contents of 4%, 6% and 8% were assumed to be 8.8, 17 and 22 MPa, respectively, from the $K_{app}$ values of glucose according to Eq. (6) under the assumption that the $B$ value of glucose was zero. Using the tentative $II$ values, the $B$ values of each solute were calculated for the resins of the different DVB contents. As mentioned above, the $B$ value should be intrinsic to the solute and be free of the DVB content of the resin. Therefore, the $B$ values for the resins having the different

![Fig. 2. Estimation of molar volumes of pentoses and disaccharides at 25°C. Pentoses: (○) D-xylose, (△) D-arabinose, (□) D-ribose, (◇) D-lyxose, (●) L-xylose, (▲) L-arabinose, (■) L-ribose, (◆) L-lyxose; Disaccharides: (○) maltose, (△) trehalose, (□) cellobirose, (◇) isomaltose, (▽) gentiobiose, (●) sucrose, (▲) lactose, (■) palatinose, (◆) leucrose; (▼) turanoose. $C_s$ and $C_w$ are the concentrations of the solute and water, respectively.](image-url)
pressure values. The swelling pressures of the resins in the hydrogen-ion form were evaluated to be ca. 5 and 14 MPa for the resins with the DVB contents of 4% and 8%, respectively (Helfferich, 1962). Based on the relationship between the swelling pressure and an equivalent volume of resin, which was obtained for Dowex 50 × 8 (the DVB content of 8%) (Helfferich, 1962), the swelling pressure of the resin in the sodium-ion form and the DVB content of 8%, which was used in this study, was estimated to be greater than 20 MPa from its equivalent volume. The $P$ values estimated here seem to be slightly high, but are on the same order as the previously estimated ones for the other resins.

There is a tendency that the $B$ values of the mono-, di- and trisaccharides are higher in this order. The tendency would be reasonable because saccharides, having a higher DVB content, were averaged and the relative standard deviation was also calculated. The relative standard deviations of the pentoses, hexoses and disaccharides were summarized. The $P$ values, which minimized the sum of the relative standard deviations, were retrieved using the Solver of Microsoft Excel®. During the retrieval, the most appropriate $B$ value of each solute was simultaneously evaluated.

The $K_{app}$ values of the trisaccharides were not used in the retrieval because the values were so low that the errors would become significant. The $B$ values of the trisaccharides were calculated using the $P$ values that had already been estimated.

The $P$ values were estimated to be 18.9, 30.8 and 37.8 MPa for the resins with the DVB contents of 4%, 6% and 8%, respectively. There are few reports on the swelling pressure values. The swelling pressures of the resins in the hydrogen-ion form were evaluated to be ca. 5 and 14 MPa for the resins with the DVB contents of 4% and 8%, respectively (Helfferich, 1962). Based on the relationship between the swelling pressure and an equivalent volume of resin, which was obtained for Dowex 50 × 8 (the DVB content of 8%) (Helfferich, 1962), the swelling pressure of the resin in the sodium-ion form and the DVB content of 8%, which was used in this study, was estimated to be greater than 20 MPa from its equivalent volume. The $P$ values estimated here seem to be slightly high, but are on the same order as the previously estimated ones for the other resins.

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Table 2. Apparent distribution coefficients $K_{app}$ and intrinsic distributions coefficients $K$ of various saccharides to cation-exchange resins in sodium-ion form, and binding constants $B$ of various saccharides to sodium ion.

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<tr>
<th>Saccharides (molar volume $v_s$ [L/mol])</th>
<th>Apparent distribution coefficient $K_{app}$</th>
<th>Intrinsic distribution coefficient $K$</th>
<th>Binding constant $B$ [L/mol]</th>
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<tr>
<td></td>
<td>DVB 4%</td>
<td>DVB 6%</td>
<td>DVB 8%</td>
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<td>Pentose (0.0973)</td>
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<tr>
<td>$\alpha$-Xylose</td>
<td>0.574</td>
<td>0.362</td>
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<td>$\alpha$-Arabinose</td>
<td>0.606</td>
<td>0.389</td>
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<td>$\alpha$-Ribose</td>
<td>0.571</td>
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* Cited from our previous work (Adachi and Matsuno, 1997).
The degree of polymerization, have more hydroxyl groups that interact with the sodium ion. The small $B$ values of xylose and glucose among the monosaccharides would be ascribed to the fact that the saccharides have a smaller number of complexing sites, consisting of two adjacent axial-equatorial or equatorial-equatorial hydroxyl groups, than other saccharides (Caruel et al., 1991). Because ribose has hydroxyl groups, which can form a tridentate complex with the sodium ion, it would be reasonable that the $B$ values of the $\beta$- and $l$- riboses were the highest among the monosaccharides. Arabinose and galactose have almost the same number of two adjacent axial-equatorial or equatorial-equatorial hydroxyl groups, which is more than that of xylose or glucose. Therefore, it is predicted that the $B$ values of arabinose are very similar to that of galactose, and that the $B$ values of arabinose and galactose are higher than those of xylose and glucose. The prediction is experimentally supported by the values shown in Table 2.

The di- and trisaccharides have many hydroxyl groups, which form the axial or equatorial configuration. Because there are many combinations of the configurations, it is difficult to quantitatively assess their $B$ values. Lactulose, which is a disaccharide consisting of galactose and fructose, had a high $B$ value. It may form a tridentate complex with the sodium ion.

Conclusions

A method for simultaneously estimating the swelling pressures of resins having different DVB contents and the binding constants of a solute with a counter ion was proposed. The apparent distribution coefficients of some mono- and disaccharides onto the resins in the sodium-ion form and having different DVB contents were measured at 25°C. The characteristics of the resins were also measured. From the apparent distribution coefficients and the resin properties, the swelling pressures of the resins and the binding constants of the saccharides with the sodium ion were simultaneously estimated by applying the model. The resins having the higher DVB content had higher swelling pressures. The binding constant was higher for the solute, which has axial-equatorial and equatorial-equatorial complexing hydroxyl groups. These results agree with current empirical knowledge.

Acknowledgement

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