

## Chromatographic Separation Property of Disaccharides with a Cation-Exchange Resin

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### Synopsis

It was attempted to estimate the binding constant,  $B$ , of a disaccharide, to a counter-ion from the apparent distribution coefficient,  $K_{app}$ , of the solute to the resin with DVB content of 8% in cation-exchange chromatography. The  $K_{app}$  values for heterogeneous disaccharides were higher than those for homogeneous disaccharides except maltose. The  $B$  values for disaccharides were estimated under the assumption that the binding constant of gentiobiose, which has the lowest  $K_{app}$  value in disaccharides used, is zero; that is, no complex is formed between gentiobiose and  $\text{Na}^+$ . It was indicated that the ability of heterogeneous disaccharide for the complex formation would be high in the resin phase because of its high  $B$  value.

**Key Word:** Disaccharide, Chromatographic separation, Cation-exchange resin, Apparent distribution coefficient, Binding constant.

### 1. Introduction

Cation-exchange resins have been widely used for chromatographic separation of saccharides on analytical and preparative scales. Separation with a cation-exchange resin can be realized using water as an eluent and is adequate for preparative purposes. As a mechanism where electrically neutral saccharides (solute) can be separated with a cation-exchange resin, exchange of the solute molecule with the water molecules held in the hydration sphere of a counterion has been proposed<sup>1)</sup>. In designing a chromatographic separation process, the distribution coefficient of a solute to a resin or gel has usually been used to predict the elution time of the solute. An equation for the apparent distribution coefficient which includes the effects of the solute such as saccharide and counter-ion has been proposed<sup>2, 3)</sup>. In this study, it was attempted to estimate the binding constant of a solute, such as disaccharide, to a counter-ion from the apparent distribution coefficient of the solute to the resin with DVB content of 8%. The method can be applied for estimation of the binding constants of some disaccharides to the sodium ion.

### 2. Materials and methods

#### 2.1 Materials

The cation-exchange resins, with sulfonate groups and a divinylbenzene (DVB) content of 8% (DIAION®UBK550) was supplied by Mitsubishi

Chemical Co. (Tokyo, Japan). Dextran T-70, the weight-averaged molecular weight of which was ca.  $7 \times 10^4$  and which was used to estimate the bed voidage, and isomaltose were purchased from Tokyo Chemical Industry (Tokyo). Maltose, cellobiose, sucrose and palatinose were purchased from Wako Pure Chemical Industries (Osaka, Japan). Trehalose and lactose were obtained from Kanto Chemical Co. (Tokyo) and Yoneyama Yakuhin Kogyo (Osaka), respectively.  $\beta$ -Gentiobiose, leucrose and turanose were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA).

#### 2.2 Measurement of resin property

The exchange capacity of the resin in its hydrogen form  $E_H$  was measured by a standard titration procedure<sup>4)</sup> against ca. 0.2 N NaOH, the normality of which was precisely determined with 0.1 N HCl of known factor. The apparent density of the resin  $\rho_H$  was pycnometrically determined. The equivalent volume of the resin in the sodium form  $V_{e,H}$  was then estimated by  $1/(E_H \rho_H)$ .

The equivalent volume of the resin in the sodium form  $V_{e,Na}$  can be estimated by Eqn. 1, because the number of fixed ions remains constant for a given amount of the resin even in any of its form.

$$V_{e,Na} = \frac{Z_{Na}(1 - \epsilon_{b,Na})}{Z_H(1 - \epsilon_{b,H})} V_{e,H} \quad \text{Eqn. 1}$$

where subscripts H and Na indicate the resins in

the hydrogen and sodium forms, respectively. The wet resin in the sodium form was weighed ( $w_{\text{wet}}$ ) and dried at 105°C to reach constant weight ( $w_{\text{dry}}$ ). The apparent density of the resin  $\rho_{\text{Na}}$  was determined pycnometrically at 25°C. The volumetric fraction of water present within the resin  $f_w$  is calculated by Eqn. 2, and it is approximately regarded as the porosity of the resin  $\epsilon_p$ .

$$f_w = \frac{\rho_{\text{Na}} w_{\text{wet}} - w_{\text{dry}}}{\rho_w w_{\text{wet}}} \approx \epsilon_p \quad \text{Eqn. 2}$$

where  $\rho_w$  is the density of water.

All measurements were done in triplicate, and the values were averaged.

### 2.3 Estimation of molar volume of disaccharide

The molar volume of each solute,  $v_s$ , was pycnometrically determined at 25°C under the assumptions that the volumes of a solute and water are independent and that additivity holds.

### 2.4 Estimation of apparent distribution coefficient

Figure 1 illustrates the scheme of chromatographic separation equipment. A cation-exchange resin converted into the hydrogen form was packed into a cylindrical plastic column of 1.79 cm I.D.. The bed height was about 30 cm, which was precisely measured for each experiment. The bed voidage and the apparent distribution coefficient of a solute onto the resin were determined by means of the pulse response. A half milliliter of 1.0 % (w/v) Dextran T-70 was applied to the bed, then elute with distilled water supplied by a peristaltic pump (PST-110, Iwaki Pumps, Tokyo). The elution profile was monitored with a refractometer (YRU-833, Shimamura Keiki Seisakusyo, Tokyo) and recorded on a strip recorder (EPR-10B, DKK-Toa Co., Tokyo). Because the elution profile of Dextran T-70 was symmetrical, the bed voidage was evaluated from the elution time at the peak top. The resin was converted into the sodium form, without taking it out of the column, by supplying 1 mol/L NaCl and successively washing with distilled water. The bed voidage for the resin in the sodium form was measured according to the above-mentioned method. A half milliliter of a 1.0 % (w/v) solute solution was then applied and eluted with distilled water. Homogeneous disaccharides such as maltose, trehalose, cellobiose, isomaltose and gentiobiose, and heterogeneous disaccharides such as sucrose, lactose, palatinose, leucrose and turanose were used as solutes. Glucose and maltotriose also were used to be compared. The apparent distribution coefficient  $K_{\text{app}}$  of the solute onto the resin in the sodium form was determined by moment analysis of the elution curve<sup>5)</sup>. The normalized first order statistical moment  $\mu_1^*$ , which is defined by Eqn. 3a, can be related to  $K_{\text{app}}$  by Eqn. 3b.

$$\mu_1^* = \frac{\int_0^{\infty} t C(z,t) dt}{\int_0^{\infty} C(z,t) dt} \quad \text{Eqn. 3a}$$

$$= (Z / u_0) [\epsilon_b + (1 - \epsilon_b) K_{\text{app}}] \quad \text{Eqn. 3b}$$

where  $C$  is the concentration of the solute,  $t$  is the time,  $u_0$  is the superficial velocity,  $Z$  is the bed height and  $\epsilon_b$  is the bed voidage.

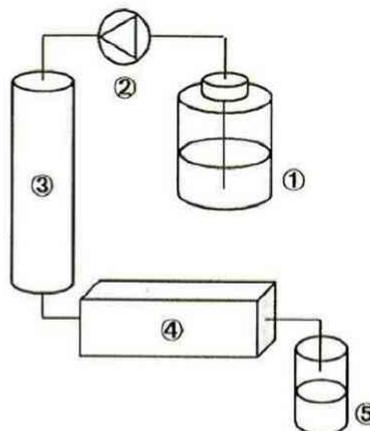


Fig. 1. Scheme of chromatographic separation equipment: (1) feed reservoir, (2) pump, (3) column packed with a cation-exchange resin, (4) refractometer, and (5) effluent reservoir.

## 3. Results and discussion

### 3.1 Property of resin

Table 1 shows the exchange capacity  $E_H$ , the apparent density  $\rho_{\text{app,H}}$  and the equivalent volume  $V_{e,H}$  of the resins in the hydrogen form, and the equivalent volume  $V_{e,\text{Na}}$ , the concentration of fixed ions  $C_{E,\text{Na}}$ , the apparent density  $\rho_{\text{app,Na}}$  and the porosity  $\epsilon_p$  of the resins in the sodium form. The volumetric fraction of water within the wet resin, which was measured from the difference in weight between the wet and dry resins, is regarded as the porosity of the resin. The concentration of fixed ions is calculated from the reciprocal of the equivalent volume  $V_{e,\text{Na}}$ .

Table 1. Property of resin in sodium form.

Resin	DIAION <sup>®</sup> UBK550
DVB content [%]	8
$E_H$ [mequiv./g]	1.78
$\rho_{\text{app,H}}$ [g/mL]	1.24
$\rho_{\text{app,Na}}$ [g/mL]	1.23
$V_{e,H}$ [mL/mequiv.]	0.453
$V_{e,\text{Na}}$ [mL/mequiv.]	0.501
$C_{E,\text{Na}}$ [equiv./L]	2.00
$\epsilon_p$ [-]	0.690

### 3.2 Apparent distribution coefficients of disaccharides

Figure 2 illustrates examples of elution curves, which were observed for sucrose for the column packed with UBK550 in the sodium form at 25°C.

The ordinate is the response of the refractometer. The elution time and the height  $H$  from the baseline at the time were read at many points. The integration in the right side of Eqn. 3a was numerically done using the  $H$  instead of  $C$ , because  $H$  is proportional to  $C$ , and the  $\mu'_1$  was evaluated.

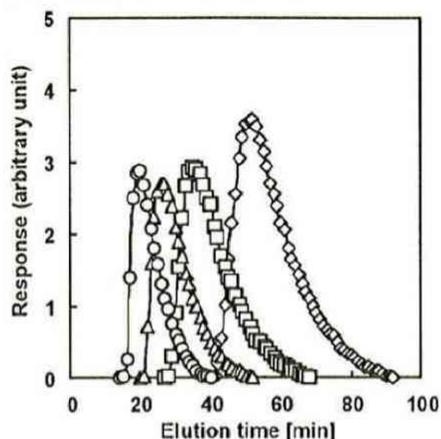


Fig. 2 Response curves for rectangular inputs of sucrose solution fed to the resin with a DVB content 8% at the flow rate of ( $\diamond$ ) 0.84, ( $\square$ ) 1.21, ( $\Delta$ ) 1.58 and ( $\circ$ ) 2.08 mL/min at 25°C.

Figure 3 shows the plots of  $\mu'_1$  against  $Z/u_0$  for disaccharide solution. The apparent distribution coefficient,  $K_{app}$ , was then estimated according to Eqn. 3b using the slope in the plots of  $\mu'_1$  against  $Z/u_0$ , and the bed voidage,  $\varepsilon_b$ . The elution curves for each solute were observed at two to four different flow rates. The estimated  $K_{app}$  values were averaged and shown in Table 2. The  $K_{app}$  value for maltooligosaccharide largely depended on a degree of polymerization of 1 to 3 and was higher for higher the degree. The  $K_{app}$  values for heterogeneous disaccharides were higher than those for homogeneous disaccharides except maltose.

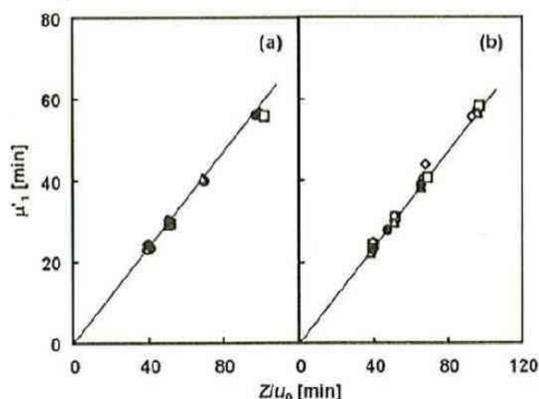


Fig. 3 Plots of normalized first-order moment,  $\mu'_1$ , against  $Z/u_0$  for the elution of (a) homogeneous disaccharides: ( $\circ$ ) maltose, ( $\square$ ) trehalose, ( $\Delta$ ) cellobiose, ( $\diamond$ ) isomaltose and ( $\bullet$ ) gentiobiose, and (b) heterogeneous disaccharides: ( $\circ$ ) sucrose, ( $\square$ ) lactose, ( $\Delta$ ) palatinose, ( $\diamond$ ) leucrose and ( $\bullet$ ) turanose solution.  $Z$  and  $u_0$  are respectively the bed height and the superficial velocity of the eluent.

Table 2. The apparent distribution coefficient,  $K_{app}$ , of saccharides onto cation-exchange resins, UBK 550, with a DVB content 8%, and the binding constant,  $B$ , of disaccharide to the sodium ion assuming that the binding constant of gentiobiose is zero.

Solute	$K_{app}$	$B$ [L/mol]
Monosaccharide		
glucose	0.259	—
Homogeneous disaccharide		
maltose	0.162	0.0967
trehalose	0.128	0.00426
cellobiose	0.128	0.00404
isomaltose	0.130	0.0104
gentiobiose	0.126	—
Heterogeneous disaccharide		
sucrose	0.150	0.0658
lactose	0.148	0.0588
palatinose	0.130	0.0104
leucrose	0.197	0.192
turanose	0.145	0.0508
Trisaccharide		
maltotriose	0.0570	—

The intrinsic distribution coefficient of a solute,  $K$ , with partial molar volume,  $\bar{v}_s$ , and the swelling pressure of the resin,  $\Pi$ , onto a resin can be represented as follows<sup>6)</sup>.

$$K = \gamma_0 \exp\left(-\frac{\Pi}{RT} \bar{v}_s\right) \quad \text{Eqn. 4}$$

where  $R$  is the gas constant,  $T$  is the absolute temperature, and  $\gamma_0$  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. Although swelling of an ionic gel is a complicated phenomenon, a model has been proposed in which the matrix of an ion-exchange resin is a network of elastic springs, and the equivalent volume of the resin is a linear function of the swelling pressure<sup>7)</sup>:

$$V_e = a\Pi + b \quad \text{Eqn. 5}$$

where the empirical constants,  $a$  and  $b$ , are characteristic of the resin and independent of the ionic form and temperature, constant  $b$  being the equivalent volume of the unstrained resin. As shown in Eqn. 1 and 5, the swelling pressure is evaluated by the bed height, but the  $Z$  values were close for all disaccharides used. Thus, the molar volume of each disaccharide,  $\bar{v}_s$ , was measured for more detail investigation about the influence of the size of the solute on the distribution instead of the partial molar volume. Figure 4 shows the

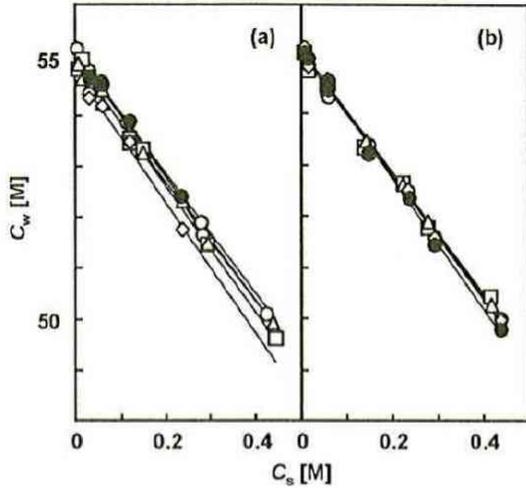


Fig.4 Relationship between the concentration of a solute,  $C_s$ , and that of water,  $C_w$ , at 25°C for (a) homogeneous disaccharides: (O) maltose, (□) trehalose, (Δ) cellobiose, (◇) isomaltose and (●) gentiobiose, and (c) heterogeneous disaccharides: (O) sucrose, (□) lactose, (Δ) palatinose, (◇) leucrose and (●) turanose.

relationship between the concentration of a solute,  $C_s$ , and that of water,  $C_w$ , at 25°C for disaccharides. The  $v_s$  values of the solutes and the molar volume of water,  $v_w$ , were obtained from the slope and the intercept, respectively. Figure 5 shows dependency of the  $K_{app}$  values of disaccharides onto the resin on their  $v_s$  values. Assuming that parameter  $\gamma_0$  is common to all solutes and the molar volume of each solute can be used instead of its partial molar volume, the result shown in Fig. 5 can not be explained by Eqn. 4. This would imply that the formation of the complex between the counter ion,  $Na^+$ , and the solute affects the  $K_{app}$  values estimated.

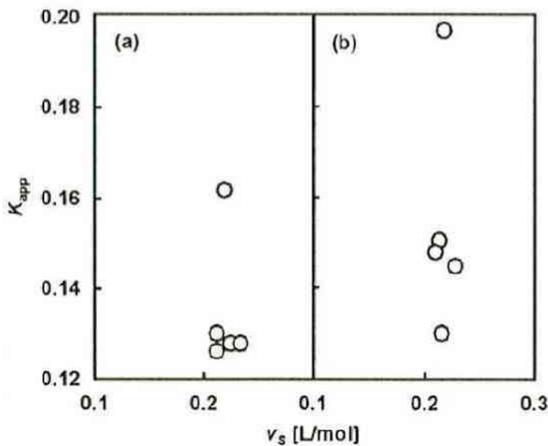


Fig.5 Dependency of apparent distribution coefficient,  $K_{app}$ , for (a) homogeneous and (b) heterogeneous disaccharides onto the bed packed with the resin with a DVB content 8% on the molar volume of each disaccharide.

### 3.3 Binding constant for $Na^+$ -solute complex formation

The binding constant for complex formation,  $B$ , is given by

$$B = \frac{\overline{C_{Na,j}}/\epsilon_p}{(\overline{C_i}/\epsilon_p)(\overline{C_{Na,i}}/\epsilon_p)} \quad \text{Eqn. 6}$$

where  $\overline{C_i}$ ,  $\overline{C_{Na}}$  and  $\overline{C_{Na,i}}$  indicate the concentrations of a solute  $i$ ,  $Na^+$  ion and  $Na^+$ -solute complex in the resin phase, respectively. Based on electroneutrality in the resin phase, the following equation can be obtained if the concentrations of hydrogen and hydroxyl ions in the resin are negligibly low:

$$\overline{C_{Na}} + \overline{C_{Na,i}} = \overline{C_E} \quad \text{Eqn. 7}$$

where  $\overline{C_E}$  is the concentration of fixed ions in the resin phase. If  $C_i$  is sufficiently low, that is  $B_i K_i C_i / \epsilon_p \ll 1$ , the apparent distribution coefficient,  $K_{app}$ , is given from Eqn. 4, 6 and 7 by

$$K_{app,i} = K_i (1 + B_i \overline{C_E} / \epsilon_p) \quad \text{Eqn. 8}$$

The ratio  $R_{i,j}$  of the apparent distribution coefficient of solute  $i$  onto the resin,  $K_{app,i}$ , to that of solute  $j$  onto the resin,  $K_{app,j}$ , is given by Eqn. 9 from Eqn. 8, as the intrinsic distribution coefficient of a solute,  $K_i$ , will be common for the solutes, such as disaccharides, having the same  $v_s$  value.

$$R_{i,j} = \frac{K_{app,i}}{K_{app,j}} = \frac{1 + B_i \overline{C_E} / \epsilon_p}{1 + B_j \overline{C_E} / \epsilon_p} \quad \text{Eqn. 9}$$

The  $B$  values for disaccharides were estimated under the assumption that the binding constant of gentiobiose as  $j$  component, which has the lowest  $K_{app}$  value in disaccharides used, is zero; that is, no complex is formed between gentiobiose and  $Na^+$  and shown in Table 2. The higher  $B$  value indicates the more electronegativity of a disaccharide relative to that of gentiobiose. The ability of heterogeneous disaccharide for the complex formation would be high in the resin phase because of its high  $B$  value.

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