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Estimation of the Binding Constants of Hexoses to the Counter-ion of a Cation-exchange Resin Based on Their Apparent Distribution Coefficients to the Resin

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The apparent distribution coefficients K_{app} of glucose, galactose, mannose and fructose onto the resins with different divinylbenzene contents in Li^+ , Na^+ , K^+ , Ca^{2+} and Sr^{2+} forms were determined in a dilute system by the moment analysis of the elution profile. The binding constant B of a solute to a counter-ion, which was free from the effect of swelling pressure of the resin, was estimated from the K_{app} values based on our previous model where glucose was assumed to form no complex with any counter-ion. The B value for each solute, except for glucose, could be correlated with the dynamic hydration number of the counter-ion.

Key words: distribution coefficient, cation-exchange resin, binding constant, saccharide

1. Introduction

Chromatographic separation of saccharides using a cation-exchange resin has been widely used in the food industry. Exchange of a solute molecule with the water molecules held in the hydration sphere of a counter-ion has been proposed as a mechanism where electrically neutral saccharides (solutes) can be separated with a cation-exchange resin, and the binding affinity of a solute to the counter-ion has been estimated by a parameter called the stability constant [1]. Based on the mechanism, separation of saccharides with a cation-exchange resin is often called ligand-exchange chromatography [2].

Knowledge of the parameters concerning both equilibrium and rate process is required to design the separation process reasonably. A distribution coefficient, which is defined as the ratio of a solute concentration in the resin phase to that in the external solution phase, is an important parameter for predicting the elution time of the solute.

We have proposed a model for the distribution coefficient of a solute onto a cation-exchange resin where both the swelling pressure of the resin and the complex formation between the solute and a counter-ion are taken into consideration [3,4] and have shown experimentally that the coefficient depends on the solute concentration [3,5] although it has been treated as a constant. Based on the model, methods to estimate the binding constant B for the complex formation, which is free from the effect of the

swelling pressure, have also been proposed [5,6]. The method proposed in our previous paper [6] was applied to estimate the B values of some hexoses to Na^+ ion. In this paper, the B values of galactose, mannose and fructose to some alkali metal ions and alkaline earth metal ions were evaluated by the method.

2. Materials and Methods

2.1 Materials

Cation-exchange resins with sulphonate groups and different divinylbenzene (DVB) contents, Amberlite FX-1020 (DVB 2%), FX-1040 (4%), CR-1310 (6%) and FX-1080 (8%), were supplied by Japan Organo, Tokyo, Japan. The resins were converted into the H^+ , Li^+ , Na^+ , K^+ , Ca^{2+} or Sr^{2+} form according to standard procedures [7].

Glucose, galactose, mannose and fructose were purchased from Wako Pure Chemical Industries, Osaka, Japan. Dextran T-70, the weight-averaged molecular weight of which was *ca.* 7×10^4 and which was used to estimate the bed voidage because of its large molecular size, was obtained from Amersham Pharmacia Biotech, Buckinghamshire, UK.

2.2 Apparent distribution coefficient

A cation-exchange resin in an ionic form was packed into a cylindrical glass column of 1.6 mm I.D. The bed height was about 18 cm for the resins with DVB contents of 2% and 4% or 35 cm for the resins with DVB contents of 6% and 8% and was precisely measured for each experiment. A half milliliter of 1.0% (w/v) solute solution was applied to the

bed, then eluted with distilled water by a TMP-6L peristaltic pump (Taitec, Tokyo) and recorded on a 201-type strip recorder (Asahi Kagaku, Tokyo). The measurement of elution profile was carried out at room temperature ($25 \pm 2^\circ\text{C}$) and at three different flow rates, 0.7–1.0 mL/min, for a solute. The normalized first-order statistical moment μ_1' , which is defined by Eq. (1a), was calculated by numerical integration of an elution profile. The μ_1' can be related to the apparent distribution coefficient K_{app} by Eq. (1b) [8,9].

$$\mu_1' = \int_0^\infty tC(Z, t)dt / \int_0^\infty C(Z, t)dt \quad (1a)$$

$$= (Z/u_0)[\varepsilon_b + (1 - \varepsilon_b)K_{app}] \quad (1b)$$

where C is the concentration of the solute, t is the time, u_0 is the superficial velocity, Z is the bed height and ε_b is the bed voidage. The K_{app} value of Dextran T-70 is zero because of its large molecular size; therefore, the ε_b value was estimated from the slope of the line connecting the plots of μ_1' versus Z/u_0 . The K_{app} of a solute was then determined in a similar manner.

2.3 Properties of resins

The equivalent volume of the resin in an ionic form V_e , the apparent density of the resin ρ , and the porosity of the resin ε_p were determined by the same methods as in a previous study [6]. The concentration of fixed ions, $\bar{C}_E$, was given by the reciprocal of V_e .

3. Results and Discussion

3.1 Apparent distribution coefficients K_{app}

Figure 1 shows examples of elution profiles of the hexoses, which were observed at a flow rate of *ca.* 1 mL/min, which was precisely measured in each experiment, for Amberlite FX-1040 with a DVB content of 4% in K^+ and Sr^{2+} forms. Glucose eluted fastest among the solutes for all the resins in any ionic form, and galactose, mannose and fructose followed in this order for most of the resins. The μ_1' value of each elution profile was calculated by numerically integrating the elution profile by Simpson's method.

To estimate the ε_b and K_{app} values according to Eq. (1b), the μ_1' values were plotted versus the superficial residence time Z/u_0 , as shown in Fig. 2, and the plots were connected by a line passing through the origin.

Figures 3 (a)–(e) show the dependencies of the K_{app} values of glucose, galactose, mannose and fructose onto the resins in Li^+ , Na^+ , K^+ , Ca^{2+} and Sr^{2+} forms, respectively, on the DVB content of the resin. The data for the resins in Na^+ form were cited from our previous paper [6]. The K_{app} values were smaller for the resins with higher DVB content for all the solutes. The K_{app} of glucose was the smallest and that of fructose was the largest among the solutes for the

Fig. 1. Elution profiles of glucose (Glc), galactose (Gal), mannose (Man) and fructose (Fru) for the columns packed with Amberlite FX-1040 in the K^+ (solid curves) and Sr^{2+} (broken curves) forms. The dimensions of the columns were 1.6 cm $\times$ *ca.* 18 cm. The abscissa is the elution time normalized by the superficial residence time Z/u_0 , and the ordinate represents the solute concentration in the effluent divided by C_0X_0 where C_0 is the concentration of solute injected and X_0 is the sample injection time normalized by the residence time. The flow rate was *ca.* 1.0 mL/min for each experiment.

Fig. 2. Estimation of the bed voidage ε_b and the apparent distribution coefficients K_{app} of (Δ) glucose, ($\square$) galactose, ($\diamond$) mannose and ($\circ$) fructose for Amberlite FX-1040 in the Sr^{2+} form according to Eq. (1). The symbol ∇ represents Dextran T-70 for estimation of the bed voidage.

resins in any ionic form and with any DVB content. The difference in K_{app} among the solutes was larger for the resin in alkaline earth metal ion form (Ca^{2+} or Sr^{2+}) than for the resin in alkali metal ion form (Li^+ , Na^+ or K^+).

3.2 Estimation of the binding constant B

Based on a model where the effects of swelling pressure and the 1:1 complex formation between a solute and a counter-ion of monovalency on the K_{app} are taken into con-

Fig. 3. Dependencies of the apparent distribution coefficients K_{app} of glucose, galactose, mannose and fructose on the DVB content of resins. The resins were in (a) Li^+ , (b) Na^+ , (c) K^+ , (d) Ca^{2+} and (e) Sr^{2+} forms. The keys are the same as in Fig. 2. The data for the resins in Na^+ form were cited from our previous paper [6].

sideration, we have proposed an equation for the K_{app} [6]. The model can easily be applied for the $K_{app, i}$ of solute i onto a resin with a counter-ion of any valency z_M :

$$K_{app, i} = K_i \left(1 + \frac{B_i \bar{C}_E / (z_M \epsilon_p)}{1 + B_i K_i \bar{C}_i / \epsilon_p} \right) \quad (2)$$

where B_i is the binding constant of solute i to a counter-ion and $\bar{C}_i$ is the solute concentration in the external solution phase. In the derivation of Eq. (2), a solute was assumed to form a 1:1 complex with a counter-ion of any valency. K_i is the hypothetical distribution coefficient of solute i when the solute forms no complex with the counter-ion and is given by Eq. (3) based on the chemical potentials of the solute in the resin and external solution phases [3]:

$$K_i = \gamma_0 \exp \left(- \frac{\Pi \bar{v}_i}{RT} \right) \quad (3)$$

where R is the gas constant, T is the absolute temperature, $\bar{v}_i$ is the partial molar volume of solute i , Π is the swelling pressure of the resin and 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.

If the molar volume of a solute v_i is assumed to be used instead of $\bar{v}_i$, the v_i values of the hexoses used in this study are practically the same ($1.14 \times 10^{-4} \text{ m}^3/\text{mol}$) [4]. Therefore, the K_i values of the hexoses onto a resin would be the same. As shown in Fig. 3, the K_{app} of glucose was the smallest for any resin. Let us assume that the B value of glucose to any counter-ion is zero. Taking the ratio $R_{i, \text{Glc}}$ of the $K_{app, i}$ of solute i to that of glucose, $K_{app, \text{Glc}}$, at sufficiently low $\bar{C}_i$, we can obtain the following equation [6]:

$$R_{i, \text{Glc}} - 1 = B_i \bar{C}_E / (z_M \epsilon_p) \quad (4)$$

If the above assumptions are held, the plots of $R_{i, \text{Glc}} - 1$ versus $\bar{C}_E / (z_M \epsilon_p)$ give a straight line passing through the origin with the slope of B_i .

Figures 4 (a), (b) and (c) show the plots of $R_{i, \text{Glc}} - 1$ versus $\bar{C}_E / (z_M \epsilon_p)$ for galactose, mannose and fructose, respectively. For every solute, the plots for each counter-ion form lay on a line passing through the origin,

Fig. 4. Estimation of binding constants of (a) galactose, (b) mannose and (c) fructose to (∇) Li^+ , ($\diamond$) Na^+ , ($\triangle$) K^+ , ($\circ$) Ca^{2+} and ($\square$) Sr^{2+} according to Eq. (4) under the assumption that the binding constant of glucose to any counter-ion is zero.

although the deviation was fairly large for the Li^+ form because of low K_{app} values. The B_i value of the solute for each counter-ion was evaluated from the slope of the line.

As mentioned above, the exchange of a solute molecule with the water molecules held in the hydration sphere of a counter-ion is thought to be the mechanism for the separation of saccharides with a cation-exchange resin [1]. It seems that the hydroxyl group of the solute would participate in the interaction between the solute and counter-ion [10], that is, the hydroxyl group of the solute would be replaced with a water molecule in the hydration sphere. It is expected from the supposition that the B value correlates to the dynamic hydration number of the counter-ion n_{DHN} . As shown in Fig. 5, the plots of the B value versus the n_{DHN} value [10] on a semi-logarithmic scale gave a straight line for each solute, and a counter-ion with the higher n_{DHN} more strongly formed the complex with a solute. The line for fructose was located the most upward, and the lines for mannose and galactose followed. That is, a counter-ion easily forms a complex with fructose, mannose and galactose in this order. The hydroxyl groups of a hexose are in either axial or equatorial direction. It has been pointed out [11] that the complex formation between a hexose and a counter-ion depends on the configuration of the hydroxyl groups. The order described above would reflect the configuration.

3.3 Separability of two solutes

The difference in the K_{app} between two solutes i and j , $\Delta K_{\text{app}, i/j}$, is a crucial parameter for the separability of the solutes. The $\Delta K_{\text{app}, i/j}$ for the solute with the same partial molar volume $\bar{v}$ can be given by Eq. (5) from Eq. (2) when the concentrations of the solutes are sufficiently low [6]:

Fig. 5. Correlation of the binding constant B with the dynamic hydration number n_{DHN} of counter-ion for each solute. The n_{DHN} values of Li^+ , Na^+ , K^+ , Ca^{2+} and Sr^{2+} are 7.77, 3.33, -0.56, 15.0 and 12.8, respectively [10]. The keys are the same as in Fig. 2.

Fig. 6. Dependency of the $K_{\text{app}, \text{Glc}} \bar{C}_E / (z_M E_p)$ on the DVB content of the resin. The keys are the same as in Fig. 4.

$$\Delta K_{\text{app}, i/j} = \gamma_0 \exp\left(-\frac{\Pi \bar{v}}{RT}\right) (B_i - B_j) \frac{\bar{C}_E}{z_M E_p} \quad (5)$$

Both the terms $\gamma_0 \exp(-\Pi \bar{v}/RT)$ and $\bar{C}_E/(z_M E_p)$ depend on the DVB content of the resin, while the B_i and B_j values are dependent only on the form of the counter-ion. When the B value of glucose is assumed to be zero, the $K_{\text{app}, \text{Glc}}$ is equal to the term $\gamma_0 \exp(-\Pi \bar{v}/RT)$, which is common for all the hexoses because their molar volumes are practically the same. Therefore, the separability of two hexoses can be estimated by the product of $K_{\text{app}, \text{Glc}}$ and $\bar{C}_E/(z_M E_p)$ [6].

Figure 6 shows the dependence of the product on the DVB content of the resin. When the counter-ion is an alkali metal ion (Li^+ , Na^+ or K^+), the resin with a DVB content of 6% is the most adequate for the separation of two hexoses. For the resins in Ca^{2+} or Sr^{2+} form, the value of the product scarcely depended on the DVB content. The values of the product for the alkali metal ions were larger than those for alkaline earth metal ions. However, because the difference in the B value between any two hexoses was much larger for the resins with alkaline earth metal ions than for the resins in alkali metal ions, the value of $\Delta K_{\text{app}, i/j}$ was larger for the resins with alkaline earth metal ions. Therefore, the resin in alkaline earth metal ion form would be suitable for the separation of hexoses.

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*** 和文要約 ***

陽イオン交換樹脂に対するヘキソースの見掛けの分配係数からの 対イオンとの結合定数の推定

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陽イオン交換樹脂を用いた糖の分離は約半世紀前から知られた分離法であり、工業的規模で広く実施されている。溶離液として水が使用できるため、食品工業には適した分離法である。溶質の樹脂に対する見掛けの分配係数 K_{app} は、その溶質の溶出時間を決める重要なパラメータであり、クロマト分離プロセスの成否を支配する。一般にこの K_{app} は、糖と樹脂の組み合わせによって決まる定数であり、溶質濃度には依存しないとして取り扱われることが多い。

非電解質である糖が陽イオン交換樹脂を用いて分離できる理由は、樹脂内の対イオンとの複合体形成の強さが糖（溶質）によって異なるためと考えられ[1]、その分離機構から本分離法は ligand-exchange chromatography と呼ばれている[2]。このような複合体形成を考えると、 K_{app} を溶質濃度に依存しない定数として取り扱うことには疑念が生じる。一方、イオン交換樹脂充填層に糖液を供給すると、その濃度に応じて層の収縮または膨張が起こる。すなわち、樹脂の膨潤圧が変化する。樹脂の膨潤圧は糖の分配平衡に影響を及ぼすと考えられるが、定量的な議論はほとんどなされてこなかった。著者らは、 Na^+ 形陽イオン交換樹脂に対する糖の K_{app} が溶質濃度に依存することを実験的に示す[3,5] とともに、樹脂の膨潤圧と対イオンとの複合体形成の両方を考慮して、糖の陽イオン交換樹脂に対する見掛けの分配係数 K_{app} に及ぼす諸因子の影響を定量的に表現するモデルを提出した[4,6]。また、そのモデルに基づいて、 K_{app} の糖濃度依存性[4]あるいは希薄な系での架橋度の異なる樹脂に対する K_{app} [6] から対イオンと糖との複合体形成の結合定数 B を推定する方法を提出した。

本報では、溶質濃度が希薄な系で、架橋度の異なるアルカリ金属イオン (Li^+ , Na^+ , K^+) またはアルカリ土類金属イオン (Ca^{2+} , Sr^{2+}) 形陽イオン交換樹脂に対するグルコース、ガラクトース、マンノースおよびフラクトースの K_{app} を測定した。Fig. 3 に示すように、いずれの対イオン形においても樹脂の架橋度 (DVB 含有率) が高くなるほど、 K_{app} は小さくなった。また、2種の糖の K_{app} の差は、アルカリ金属イオン形よりアルカリ土類金属イオン形の樹脂の方が大きかった。

グルコースは対イオンと複合体を形成しないと仮定して、以前に提出した方法[6] を対イオンが任意の価数の場合に適用できるように拡張し、ここで得られた K_{app} に適用し

て、各ヘキソースの各種対イオンに対する結合定数 B を算出した (Fig. 4)。本法では膨潤圧の影響を含む項は相殺されているので、ここで得られた B 値は樹脂の膨潤圧の影響を受けない真の値と考えられる。分離機構から対イオンに補足された水分子が糖の水酸基と置き換わる程度は、対イオンの動的水和数 n_{DHN} と関連し、それが B 値に反映されると考えられる。そこで、上記で得られた B 値を対イオンの n_{DHN} と片対数プロットする (Fig. 5) と、各溶質について直線が得られた。フラクトースに対する直線がもっとも上方に位置し、ついでマンノース、ガラクトースの順であった。糖の水酸基は axial または equatorial のいずれかの位置をとり、それらの数と配置が対イオンとの複合体形成のし易さに関係すると指摘されており[11]、これらの順序はそのことを反映しているものと考えられる。

イオン交換樹脂により糖を分離する場合、対イオン形とともに樹脂の架橋度の選択が問題となる。そこで、部分モル体積の等しい2種の溶質を分離する場合の樹脂架橋度の選択規準について考察した (Eq. (5))。Fig. 6 に示すように、アルカリ金属イオンを対イオンとする場合には、DVB が6%の樹脂がもっとも適しているが、対イオンがアルカリ土類金属イオンの場合には樹脂の架橋度は分離度に顕著な影響を及ぼさないことが示唆された。なお、2種のヘキソースに対する B 値の差は、アルカリ金属イオンよりアルカリ土類金属イオンの方が大きいので、ヘキソースの分離にはアルカリ土類金属イオン形樹脂が適していると考えられる。なお、ここでは希薄な系での分離度のみに着目しているが、高濃度の糖液を対象とする実際の系では、樹脂の膨潤・収縮が K_{app} に影響するので、この点も考慮して樹脂の選択を行う必要がある。

また、従来 K_{app} が糖濃度に依存しない定数として取り扱われてきた理由は Eq. (1) と Eq. (2) から推測できる。溶質濃度 C_i が大きくなると、Eq. (1) 右辺括弧内の分母の寄与により K_{app} は小さくなり、一方、溶質濃度が高くなると樹脂充填層は収縮する。すなわち、樹脂の膨潤圧 Π が低下し、複合体形成が起こらないと考えたときの分配係数 K_i が大きくなる (Eq. (2))。 K_{app} はそれらの積として与えられるので、両効果が相殺されて K_{app} の溶質濃度依存性は小さくなるため、定数として扱われてきたものと思われる。しかし、詳細な検討を行うと、 K_{app} は溶質濃度に依存する[5] ので、分離プロセスを設計する場合には、この点を考慮する必要がある。