CHROM. 20 639

EFFECT OF THE CONTENT OF DIVINYLBENZENE IN ION-EXCHANGE RESINS ON THE CHROMATOGRAPHIC SEPARATION OF α-CYCLODEXTRIN AND GLUCOSE

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(First received February 19th, 1988; revised manuscript received May 2nd, 1988)

SUMMARY

In the chromatographic separation of α-cyclodextrin and glucose, the parameters relating to the adsorption equilibria and rate processes of the solutes were evaluated for the sodium forms of cation-exchange resins with contents of divinylbenzene (DVB) of 2, 4, 6 and 8%. By using these parameters, the chromatograms of the solutes were then calculated. It is concluded that the resin with 6% DVB is most suitable for the chromatographic separation of the solutes on a preparative scale.

INTRODUCTION

Cyclodextrins (CDs) are cyclic and non-reducing maltooligosaccharides produced from starch by cyclodextrin glucosyltransferases. They have an ability to form complexes with guest compounds, and have been utilized in food and pharmaceutical industries. The enzymes, regardless of their origins, produce a mixture of α-, β- and γ-CDs and maltooligosaccharides[1]. In order to produce a specific CD, a separation process is indispensable.

β-CD can easily be separated from other saccharides due to its low solubility. The co-operative action of taka-amylase and glucoamylase hydrolyzes γ-CD and maltooligosaccharides to glucose, but the enzymes do not affect α-CD[2]. Therefore, production of α-CD with high purity requires the separation of α-CD and glucose.

Among separation procedures, chromatography may be promising because of the non-use of additives. Lammers[3] demonstrated the separation by adsorption

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chromatography using gradient elution on charcoal, and Carter and Lee[4] reported the adsorption chromatography of CDs on Sephadex G-15. These methods are, however, tedious and time-consuming. Chromatography on polyacrylamide gels has been applied to the separation of CDs and maltooligosaccharides[5,6]. Since the gels are relatively expensive and give a large pressure drop in the packed bed because of their small diameter and softness, they are not suitable for industrial-scale application. Okada *et al.*[7] showed that the sodium or calcium form of strongly acidic cation-exchange resins, comprised of a co-polymer of styrene and divinylbenzene (DVB), could be used to separate α-CD and glucose. However, they did not present details of the adsorption isotherms and rate processes.

In this study we have evaluated the parameters relating to adsorption equilibria and rate processes of α-CD and glucose for the sodium forms of cation-exchange resins with various contents of DVB. Since the separability of the solutes is a complex fraction of the parameters, it is difficult to conclude which ion-exchange resin is most suitable for the chromatographic separation through a simple experiment. Therefore, the optimum DVB content of the ion-exchange resin was determined through simulation of the chromatograms of the solutes on columns packed with each ion-exchange resin, using the parameters estimated.

EXPERIMENTAL

Materials

Four kinds of strongly acidic cation-exchange resins, possessing sulphonate groups, and different DVB contents were used. The DVB contents were 2, 4, 6 and 8%, and the corresponding exchange capacities were 0.7, 1.2, 1.6 and 1.7 mequiv./ml bed, respectively, in the hydrogen forms. Each of the ion-exchange resins (Amberlites HFS-471X, Japan Organo) was conditioned by 1 M sodium chloride, and then washed with a large quantity of distilled water. α-Cyclodextrin was obtained from Ensuiko Seito (Japan), glucose from Kanto Chemicals (Japan).

Physical properties of ion-exchange resins

The apparent density, ρ_p, of each ion-exchange resin was measured pycnometrically. The water regain, W_r, which is defined as the weight of water held in the wet resin per unit weight of dry resin, was evaluated from the difference in weights between the wet and dry resins. The diameter was measured by a microphotograph for at least 250 particles. The mean value was calculated by the following equation[8]:

$$\overline{d_p} = (\Sigma d_p^5 / \Sigma d_p^3)^{1/2} \tag{1}$$

Chromatography

A cation-exchange resin (Na$^+$) was carefully packed under vibration in a cylindrical glass column equipped with a water jacket. The inner diameter of the column was $1.5 \cdot 10^{-2}$ m and the bed length was *ca.* 0.45 m (measured exactly in each experiment). The bed was kept at 333 K by circulating thermostatted water through the jacket. A small amount (usually 0.5 ml) of a solute solution was carefully loaded on top of the bed, and the flow was started. When all the sample solution had been sucked into the bed, an eluent, degassed distilled water, was fed by use of a constant-delivery

pump (MP-101; Tokyo Rikakiki, Japan). At appropriate intervals, the effluent was collected at the bottom of the bed by a fraction collector (SF-100G; Toyo Roshi, Japan). The concentration of α-CD in the sample was in the range of 3–11% (w/v), while that of glucose was 10–30% (w/v). The bed voidage, ε_b, was estimated from the response curve of an impulse of 1% (w/v) soluble starch, whose molecular weight was more than $1 \cdot 10^4$.

Analysis

α-CD in the effluent was assayed by the following method. The effluent was appropriately diluted in distilled water. A 0.5-ml volume was mixed with 2.5 ml of iodine–potassium iodide solution ($1.25 \cdot 10^{-4}$ *M*), and then the increase in absorbance at 352.5 nm was recorded. The increase was proportional to the α-CD concentration below 0.02% (w/v), without any interference from glucose. The concentration of glucose in the effluent was determined by the glucose oxidase–peroxidase method (New Glucostat, Washington Comp.). A high-performance liquid chromatograph (L-5000; Yanagimoto Seisakusho, Japan) equipped with a separation column (TSKgel SCX; Tosoh, Japan) and a differential refractometer (SE-11; Showa Denko, Japan) was also used to determine the concentrations of α-CD and glucose.

The soluble starch in the effluent was completely hydrolyzed to glucose by addition of glucoamylase (pure grade, Seikagaku Kogyo, Japan) dissolved in a 0.05 *M* acetate buffer (pH 4.5). The glucose produced was analyzed by the glucose oxidase–peroxidase method.

Determination of distribution, intraparticle diffusion and axial dispersion coefficients

Preliminary experiments showed that the adsorption isotherms of α-CD and glucose on the ion-exchange resin, regardless of the DVB content, were linear and independent of each other. Therefore, the moment analysis of the response curve of an impulse of the solute was adopted to estimate the values of the distribution coefficient, m, the axial dispersion coefficient, D_z, and the intraparticle diffusion coefficient, D_s. The first-order normalized statistical moment, μ_1, and the second-order normalized central moment, μ_2, of a chromatogram are related to the values of m, D_z, D_s and the bed voidage, ε_b, by the following equations assuming that the film mass-transfer resistance is insignificant[9]

$$\mu_1 = \int_0^\infty tC(Z, t)\, dt \Big/ \int_0^\infty C(Z, t)\, dt$$

$$= (Z/u_0)[\varepsilon_b + (1 - \varepsilon_b)m] \tag{2}$$

$$\mu_2 = \int_0^\infty (t - \mu_1)^2 C(Z, t)\, dt \Big/ \int_0^\infty C(Z, t)\, dt \tag{3}$$

$$\mu_2/(2Z/u) = (D_z/u)(1 + Hm)^2 (1/u) + HmR^2/(15D_s) \tag{4}$$

where C is the concentration of a solute, Z the height of the bed, u_0 the superficial velocity, u the interstitial velocity ($= u_0/\varepsilon_b$), R the particle radius and $H = (1 - \varepsilon_b)/\varepsilon_b$. Eqn. 4 was used in this study since the corresponding mass balance equations included all the parameters related to the spreading of the chromatogram. Eqns. 2 and 4 have been successfully utilized to estimate the parameters in gel chromatography[9]. A plot of $\mu_2/(2Z/u)$ *versus* $1/u$ gives a straight line since D_z/u is independent of the flow-rate in a range of Reynolds numbers[10] in these experiments. The value of the bed voidage was determined from a plot of μ_1 *versus* Z/u_0 for soluble starch, which is too large to penetrate the resins.

Calculation of elution curve

The elution curve, $C(Z, t)$, was calculated numerically through an inverse transformation of its solution in the Laplace domain[11].

RESULTS AND DISCUSSION

Table I lists the apparent densities, the water regains and the mean diameters of the ion-exchange resins. The water regain, which may reflect the porosity of the resins, changes considerably between the DVB contents of 4 and 6%. Since the physical properties of the resins are influenced by the DVB content, the equilibrium and mass transfer parameters of α-CD and glucose may depend largely on the DVB content of the resin.

The equilibrium and mass transfer parameters were evaluated from moment analysis of the elution curves for each component. Fig. 1a shows a plot of μ_1 *versus* Z/u_0 for the resin with 2% DVB. The slope of the line for soluble starch gives the bed voidage. The distribution coefficients of α-CD and glucose were also estimated from the slope of the line for each component. The values are listed in Table II. Fig. 1b shows a plot of $\mu_2/(2Z/u)$ *versus* $1/u$. The values of D_z/u and D_s were calculated from the slope and intercept of the line, respectively, according to eqn. 4. The results shown in the figure were also obtained for the resin with 2% DVB. The same kinds of plots were drawn for other resins with different DVB contents. The values of m, D_z/u and D_s of the components are listed in Table II together with the bed voidages. The m and D_s values for α-CD and glucose depend on the DVB content. Large changes in the values result upon varying the DVB content between 4 and 6%, as predicted from the water regains. It is known that D_z/u is one to two times the particle diameter[9,10]. The D_z/u values obtained in this study were also within this range.

TABLE I

PHYSICAL PROPERTIES OF THE SODIUM FORMS OF THE CATION EXCHANGERS USED (AMBERLITE HFS-471X)

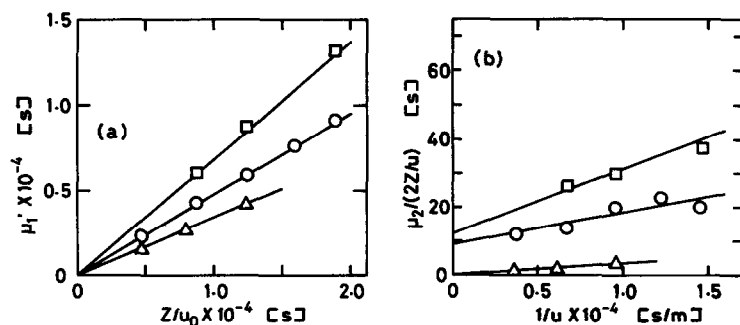|	DVB content (%)			
	2	4	6	8
Mean diameter, $\overline{d_p} \cdot 10^4$ (m)	3.43	3.73	3.24	3.38
Water regain, W_r (kg/kg)	2.449	2.071	1.214	1.092
Apparent density, ρ_p (kg/m³)	1139	1159	1229	1251

Fig. 1. Estimation of the bed voidage by soluble starch ($\triangle$) and the distribution, the axial dispersion and the intraparticle diffusion coefficients for glucose ($\square$) and α-CD ($\bigcirc$) for the resin with 2% DVB.

The distribution coefficients of both α-CD and glucose become larger as the water regain increases. The difference between them, however, is not greatly dependent on the water regain. A lower water regain means that the resin is harder. The use of hard ion-exchange resins in chromatography has some advantages such as a small bed shrinkage at high concentrations of solutes and a low pressure drop.

The D_s values of α-CD and glucose also depend on the water regain. The D_s value of glucose is about one tenth of the molecular diffusivity, $ca.$ $1.4 \cdot 10^{-9}$ m^2/s at 60°C, estimated from the data of Gladden and Dole[12]. The D_s values of α-CD for resins with DVB contents of 6 and 8% are less than those for resins with DVB contents of 2 and 4%. Although it is supposed that these lower values may result in spreading of the elution curve, this may not always be correct because the extent of spreading depends on the ratio of m to D_s.

From these results it may be supposed that the use of resins having a DVB content of 6 and 8% is advantageous in the chromatographic separation of α-CD and glucose. The selectivity, which is defined as the ratio of m_G to m_α, also supposes the predominance of the resins with DVB contents of 6 and 8%. This supposition was examined in detail.

By varying the sample volume loaded on the chromatographic column, the elution curves of α-CD and glucose were calculated under the same conditions for each of the resins. The column was 0.5 × 0.02 m I.D., the volumetric flow-rate was 0.25 ml/min and the concentrations of α-CD and glucose loaded were 10 and 30% (w/v), respectively. By using the values of the parameters listed in Table II, the elution curves of α-CD and glucose were calculated. Fig. 2 shows the elution curves calculated for the resin with 6% DVB; X_0 is the sample volume normalized by the void volume of the bed. Although the figure shows the curves in intervals of 0.1 in X_0, the actual calculations were carried out in more detail. The elution curves were also calculated for other resins with different DVB contents. From these elution curves the recovery, Y, the purity, P, and the average concentration, C_{av}, of α-CD in its fraction were calculated by

$$Y = Q \int_{t_{1\alpha}}^{t_{2\alpha}} C_\alpha \, dt / (C_{\alpha 0} V_0) \qquad (5)$$

TABLE II

THE DISTRIBUTION, m, AXIAL DISPERSION, D_z, AND INTRAPARTICLE DIFFUSION, D_s, COEFFICIENTS OF α-CD AND GLUCOSE FOR THE SODIUM FORMS OF THE CATION EXCHANGER (AMBERLITE HFS-471X) WITH VARIOUS DVB CONTENTS, AND THE BED VOIDAGE, ε_b (60°C)

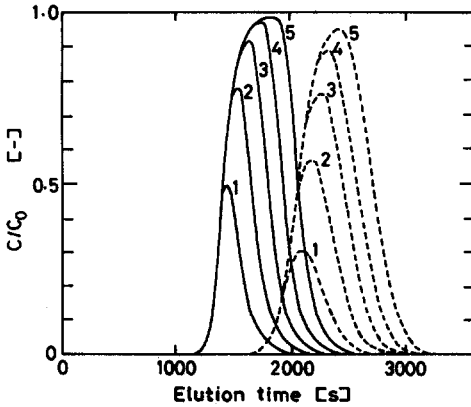|	2% DVB			4% DVB			6% DVB			8% DVB		
	Starch	*α-CD*	*Glucose*	*Starch*	*α-CD*	*Glucose*	*Starch*	*α-CD*	*Glucose*	*Starch*	*α-CD*	*Glucose*
ε_b	0.341	–	–	0.340	–	–	0.351	–	–	0.343	–	–
m	–	0.204	0.508	–	0.152	0.503	–	0.042	0.304	–	0.035	0.239
$D_z/u \cdot 10^4$ (m)	3.33	4.87	4.90	3.00	3.98	5.80	3.13	5.97	3.17	3.06	3.45	4.48
$D_s \cdot 10^{10}$ (m²/s)	–	0.86	1.59	–	0.76	1.33	–	0.33	1.09	–	0.28	0.93

Fig. 2. Chromatograms of α-CD (——) and glucose (---) calculated for the column packed with the cation-exchange resin having 6% DVB at various volumes of sample solution loaded. $X_0 = 0.1$ (1), 0.2 (2), 0.3 (3), 0.4 (4) and 0.5 (5). The details of the conditions are given in the text.

$$P = \int_{t_{1\alpha}}^{t_{2\alpha}} C_\alpha \, dt \Big/ \Big(\int_{t_{1\alpha}}^{t_{2\alpha}} C_\alpha \, dt + \int_{t_{1\alpha}}^{t_{2\alpha}} C_G \, dt\Big) \qquad (6)$$

$$C_{av} = \int_{t_{1\alpha}}^{t_{2\alpha}} C_\alpha \, dt / (t_{2\alpha} - t_{1\alpha}) \qquad (7)$$

where Q is the volumetric flow-rate, $C_{\alpha 0}$ the feed concentration of α-CD and V_0 the sample volume loaded. The subscripts α and G denote α-CD and glucose. t_{1i} and t_{2i} ($i = \alpha$ and G) are the times when $C_i/C_{i0} = 0.1$ in the leading and tailing parts of the chromatogram, respectively. When the chromatograms of α-CD and glucose cross
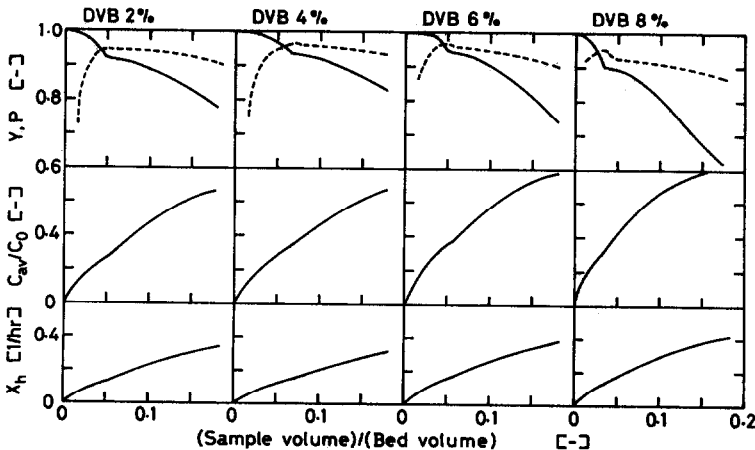

Fig. 3. The purity, P (——), the yield, Y (---), the average concentration, C_{av}, of α-CD in its fraction and the sample volume capable of being treated per hour, X_h, normalized by the whole volume of the bed volume, at various volumes of sample solution loaded.
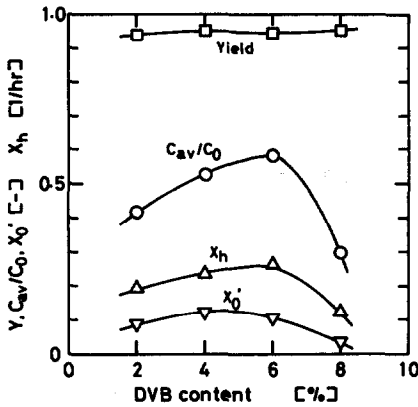
Fig. 4. Comparison of the efficiency of columns packed with cation-exchange resins with various DVB contents to obtain the α-CD solution with a purity of 90%. Y is the yield of α-CD; C_{av} is the average concentration of α-CD in its fraction and C_0 its concentration in the feed; X_h is the sample volume capable of being treated per hour, normalized by the bed volume; X_0 is the sample volume normalized by the bed volume.

over 10% of the feed concentrations, $t_{2\alpha}$ and t_{1G} are regarded as identical. In preparative chromatography the sample injection and the supply of an eluent are alternated. Since the time necessary for an elution is $t_{2G} - t_{1\alpha}$, the sample volume capable of being treated per hour, X_h, which is normalized by the whole volume of the bed, V_t, is given by

$$X_h = [60V_0/(t_{2G} - t_{1\alpha})]/V_t \tag{8}$$

where t_{2G} and $t_{1\alpha}$ are expressed in minutes.

Fig. 3 illustrates Y, P, C_{av} and X_h at various sample volumes for each resin with different DVB contents. The abscissa shows the sample volume normalized by the whole volume of the bed.

The yield, the average concentration and the sample volume at the purity = 0.9 are plotted against the DVB content of the resin in Fig. 4. This reveals that the resin with 6% DVB is most suitable for the preparative chromatographic separation of α-CD and glucose. The mechanical strength of the resin also seems to be good, judging from its water regain and apparent density.

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