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Effect of the Net Surface Charge Density of Heptakis-6-bromo-6-deoxy-β-cyclodextrin Bonded Silica Gels on the Retention Behaviors of Neutral Cresol Isomers in HPLC

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The effect of the surface charge density of heptakis-6-bromo-6-deoxy-β-cyclodextrin (β-CD-BR) bonded silica gels, which was used as the stationary phase of a packed capillary column for HPLC, was investigated concerning the retention behaviors of neutral cresol isomers. On the whole, the retention factors of the cresol isomers increased with an increase in the pH values of the mobile phase, although they were slightly smaller at pH 6.1 than at pH 4.7. An investigation on the retention variation using a van't Hoff plot revealed that the increase in the retention factor (k) at a higher pH region could be mainly attributed to the increase in ΔS, while a partial decrease in k around pH 5 – 6 was caused by a decrease in the $-\Delta H/T$ value. On the other hand, a measurement of the electroosmotic flow velocity under various pH of the mobile phase solutions revealed that the retention variations of the neutral cresol isomers were strongly correlated with the surface charge on the packing materials. The positive charge of secondary ammonium functional groups to bind β-CD-BR inhibit the insertion of the cresol isomers into the cavity of β-CD-BR while reducing the retention factor, whereas the negative charge of silanol group enhanced it through a local change in the mobile phase composition.

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Introduction

Cyclodextrins (CDs), which are torus-shaped cyclic oligosaccharides, are among the well-known host molecules that form an inclusion complex with guest molecules. The CDs and their derivatives are widely used to modify the packing materials as the stationary phase, or are added to the mobile phase to achieve a separation of the structural isomers and chiral compounds in high-performance liquid chromatography (HPLC), capillary electrochromatography (CEC), and capillary electrophoresis (CE).[1-18] In particular, β-CD, which is composed of seven α-(1,4)-linked D-glycopyranose units, has been frequently used, and the chromatographic behavior using β-CD and its derivatives as the stationary phase or additive to the mobile phase has been investigated.

Crini *et al*. reported on the separations of various *o*-, *m*-, and *p*-isomers of disubstituted benzenes by HPLC using the β-CD stationary phase.[6] They investigated the effects of the amount of bonded β-CD on the surface of packing materials, the organic modifier content in the mobile phase, and the pH of the mobile phase on their retention factors. They also demonstrated that the retention factors of nitrophenol and nitrobenzoic acid isomers were definitely varied by the pH of the mobile phase (pH 5.0 to 7.0). Meanwhile, Morin *et al*. studied the temperature effect on the retention factor for imidazole compounds in a β-CD modified stationary phase using a van't Hoff plot with the mobile phase of various pH.[4] They concluded that the dependency of the retention on the column temperature was significantly affected by the pH of the mobile phase. Moreover, Zarzycki *et al*. investigated the effect of temperature on the retention of steroids using the C18 stationary phase and the mobile phase, including β-CD. They reported that the addition of β-CD provided a curved van't Hoff plot for the steroids in the range from 0 to 80°C.[13] Though such effects in the β-CD modified stationary phase and the mobile phase modifier have often been reported, the detailed mechanism has not been sufficiently clarified. In particular, the relationship between the variation in the retention factor and the surface charge density of the β-CD modified stationary phase has not been investigated, even though a significant surface charge variation on the pH of mobile phase was easily predictable.

In this work, using a column packed with silica gel particles modified with heptakis-6-bromo-6-deoxy-β-cyclodextrin (β-CD-BR), we studied the effect of the surface charge density of the β-CD-BR modified stationary phase on the variation in the retention factors of neutral cresol isomers, mainly by focusing on the pH of the mobile phase in HPLC. A schematic diagram of the surface of β-CD-BR modified silica gels is shown in Fig. 1. The β-CD-BR is bonded to the silica gel surface *via* the secondary ammonium group, which can provide positive charge by protonation. On the other hand, the dissociation of residual silanol groups on the surface can cause a negative charge. The combination of these cationic and anionic functional groups allows variously charged surfaces, such as positively charged, neutral, and negatively charged surface, as shown in Fig. 1. Meanwhile, the retention behavior of analyte in HPLC is dominated by the thermodynamic parameters, such as the enthalpy and entropy, which control the variation in the retention factor on the pH of the mobile phase. The values of the enthalpy and entropy can be estimated from the relationship

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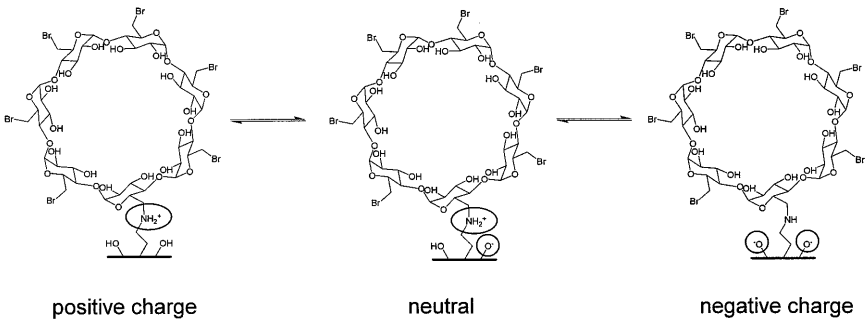

Fig. 1 Variation in the surface charge of silica gels modified with heptakis-6-bromo-6-deoxy-β-cyclodextrin (β-CD-BR).

between the column temperature and the retention factor of the analyte using van't Hoff's equation. In this paper, first, the retention factors of neutral cresol isomers, which have no electrostatic interaction with the ionized functional group on the packing material, were measured with various eluents of different pH. Then, their variations were discussed with regard to the thermodynamic parameters and the net surface charge density.

Theory

Effect of temperature on the retention and separation factors

In chromatographic separations, the relationship between the column temperature (T) and the retention factor of sample solute (k) can be described by van't Hoff's equation,

$$\ln k = \frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \phi, \quad (1)$$

where ΔH°, ΔS°, R, and ϕ are the enthalpy of transfer of the solute from the mobile phase to the stationary phase, the entropy of transfer of the solute from the mobile phase to the stationary phase, the gas constant, and the phase ratio of a column, respectively. By the derivation from Eq. (1), the relationship between the separation factor ($\alpha = k_2/k_1$) and the column temperature can be induced as

$$\ln \alpha = \frac{\Delta(\Delta H^\circ_{2,1})}{RT} + \frac{\Delta(\Delta S^\circ_{2,1})}{R}, \quad (2)$$

where $\Delta(\Delta H^\circ_{2,1}) = \Delta H^\circ_2 - \Delta H^\circ_1$, and $\Delta(\Delta S^\circ_{2,1}) = \Delta S^\circ_2 - \Delta S^\circ_1$. Namely, when both $\ln k$ and $\ln \alpha$ are a linear function of $1/T$, respectively, ΔH°, $\Delta S^\circ/R + \ln \phi$, $\Delta\Delta H^\circ$, and $\Delta\Delta S^\circ$ can be calculated using Eqs. (1) and (2). Furthermore, the $\Delta\Delta H^\circ$, and $\Delta\Delta S^\circ$ values can also be estimated from the values of ΔH° and $\Delta S/R + \ln \phi$ for each compound.

Effect of surface charges on electroosmotic flow

The electroosmotic flow (EOF) is strongly correlated with the net surface charge density of the packing materials, which are allowed to vary by the pH of the eluent.[19–21] Since the β-CD-BR packing material has both cationic and anionic functional groups, as shown in Fig. 1, the relationship between the EOF velocity (v_{eof}) and the activity of hydrogen ion (a_H) can be described by the following equation in this experiment:[20]

$$v_{eof} = \sin h^{-1}\left(\frac{\Delta\sigma_n}{C_1} + \frac{e\gamma_{SiOH}K_{a,SiOH}}{C_2(K_{a,SiOH} + a_H)} - \frac{e\gamma_{NH}a_H}{C_3(K_{a,NH} + a_H)} \right), \quad (3)$$

where σ_n, e, γ, and K_a are the surface charge density independent of the mobile phase pH, the elementary charge, the density of functional group, and the equilibrium constant. The subscripts of SiOH and NH correspond to the silanol groups and the secondary ammonium groups, respectively. The parameters of C_1, C_2, and C_3 are constants, depending on the experimental conditions, such as the dielectric constant, viscosity, and salt concentration.

Experimental

Capillary liquid chromatography

The apparatus comprised an LC pump (LC-6A, Shimadzu, Kyoto, Japan), an ultra violet detector (UV 970, Jasco, Tokyo, Japan), an injector (Model 7410, Rheodyne, USA), a laboratory-made packed capillary column, and a laboratory-made splitter (using for split injection method). Silica gel particles modified with heptakis-6-bromo-6-deoxy-β-cyclodextrin (dp., 5 μm; pore size, 12 nm; pore volume, approximately 1.1 ml/g, CHIRAL β-CD-BR, kindly donated by YMC, Kyoto) were packed into fused-silica capillaries (i.d. 0.075 mm and 0.15 mm, GL Science, Tokyo, Japan) using a slurry packing method (packed length, 127 mm and 158 mm, respectively). The wider-bore capillary column (i.d. 0.15 mm) was used to study the effect of the pH on the retention factor at room temperature, whereas the narrow one (i.d. 0.075 mm) was used to calculate the thermodynamic parameters. With the latter column, it is easier to control the temperature, although it has disadvantage concerning the sensitivity compared with the former one with a wider bore. The narrow capillary column was covered with a laboratory-made water jacket to control the column temperature (283 to 333 K). The water used to control the column temperature was supplied at a flow rate of 4 ml/min by a micro-tube peristaltic pump (Micro Tube Pump MP-3, Tokyo Rikakikai, Tokyo, Japan), and the temperature-controlled water flowed through the water jacket for approximately 30 s. Since the walls of the water jacket are sufficiently thin, the surface temperature of the jacket would be approximately identical to the water temperature (and also regarded as the internal temperature in the column). The column temperature (the surface temperature of the water jacket) was measured by a thermocouple during separation.

A mixture of 10 mM phosphate buffer and methanol (80/20, v/v) was used as the eluent. The pH of the phosphate buffer was controlled at between 3.1 and 7.8. In this experiment, we represent the pH value of the aqueous buffer as that of the eluent containing methanol, although the actual pH for the mobile phase containing methanol was not certifiable. Cresol isomers (5 mM each) were dissolved in a mixture of distilled water and methanol (80/20, v/v) and used as the sample. In this system, thiourea was eluted faster than $NaNO_2$, which is a typical t_0 marker, probably

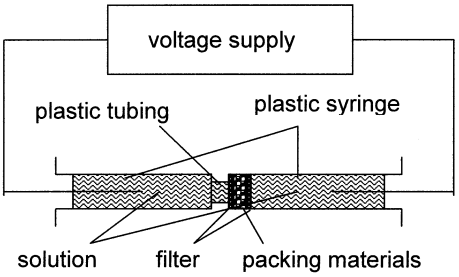

Fig. 2 Schematic diagram of the apparatus for measuring the EOF rate.

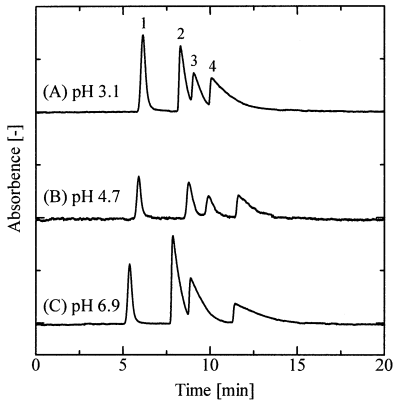

Fig. 3 Separation of cresol isomers with various eluent pH (phosphate buffer) at (A) 3.1, (B) 4.7, and (C) 6.9. Peaks of 1, 2, 3, and 4 correspond to thiourea (t_0 marker), *o*-, *m*-, and *p*-cresol, respectively. Column: i.d., 0.15 mm; packed length, 158 mm; packed materials, CHIRAL β-CD-BR; eluent, mixture of 10 mM phosphate buffer and methanol (80/20, v/v); flow, constant-pressure mode of 10 MPa; detection, UV 210 nm; column temperature, room temperature.

because the positive charge of the secondary ammonium groups in β-CD-BR may attract NO_2^- by the electrostatic interaction. In this experiment, therefore, thiourea was added to the sample solution as the t_0 marker. All chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Measurement of the electroosmotic flow rate

The electroosmotic flow rate under various pH solutions was measured to estimate the variation in the net surface charge density using an apparatus reported previously.[20] Figure 2 shows the apparatus used to measure the EOF rate, which was composed of a voltage power supply (NC-1010, Nihon Eido, Tokyo, Japan), 2 plastic syringes (1 ml, Terumo, Tokyo, Japan), a syringe filter (Millex-LG, Millipore, MA, USA), a filter paper (Type 5B, Advantec, Tokyo, Japan), Pt wire electrodes (Nilaco, Tokyo, Japan), and flexible plastic tubing (Tygon, Saint-gobain, Tokyo). The β-CD-BR modified silica gels were packed between filters with approximately 1 cm in length. The volumetric variation of the solution in the partially packed syringe (right side syringe in Fig. 2) was measured after applying 200 V for 10 min using several sample solutions of different pH, which was a mixture of 10 mM phosphate buffer and methanol (80/20, v/v). The measurement of the EOF rate was performed at room temperature.

Results and Discussion

Effect of the pH on the retention factor, enthalpy, and entropy

The effects of the buffer pH on the retention and separation factors of neutral cresol isomers in a β-CD-BR packed column (0.15 mm i.d.) were investigated. Figure 3 shows the separations of the cresol isomers at room temperature using mixtures of 20% methanol and 80% phosphate buffer of various pH at (A) 3.1, (B) 4.7, and (C) 6.9 as the mobile phases. In all of the chromatograms, the elution order of the isomers is *o*-, *m*-, then *p*-cresol (peaks 2, 3, and 4, respectively, and peak 1 corresponds to thiourea as a t_0 marker). The retention times of the isomers more or less varied depending on the pH of the mobile phase. In particular, the retention variation of *p*-cresol (peak 4) is larger than that of *o*- and *m*-cresols (peaks 2 and 3). The relationships between the pH of the phosphate buffer in the mobile phase and (A) the retention factors of the cresol isomers and (B) their separation factor are shown in Fig. 4. Both the retention and separation factors were raised with an increase in the pH of the mobile phase, on the whole. However, the retention factors of all the isomers slightly decreased with a change of the pH from 4.7 to 6.1.

To reveal these phenomena, a further investigation concerning the thermodynamic parameters was performed. The cresol isomers in the mobile phase of pH 5.0 (phosphate buffer) were separated using a temperature-controllable narrow-bore column under several column temperatures between 283 and 333 K. The chromatograms observed at various column temperatures are shown in Fig. 5. Since the pump was operated in the constant-pressure mode, t_0 was significantly reduced along with the elution times of the cresol isomers due to a decrease in the viscosity of the eluent at higher temperature. The variation of the flow rate sometimes affects the retention behavior of the analyte, particularly when an asymmetric peak is obtained. However, the retention factors of cresol isomers were approximately constant under various pressurized flow velocities, ranging from 0.25 to 1.48 mm/s at room temperature. Therefore, the effect of the temperature-induced flow-rate variation on the retention factor would also be negligible in this experiment. Figure 6 shows the effect of the column temperature on the retention and separation factors of the cresol isomers. Under this experimental condition, a high linearity in the relationship between $1/T$ and $\ln k$, that is the van't Hoff plot, was obtained as shown in Fig. 6(A) ($R^2 > 0.99$), which suggested that no phase transition took place for each cresol isomer in this temperature range. On the other hand, the separation factor of *m*-cresol to *o*-cresol ($\alpha_{m/o\text{-cresol}}$) was significantly enhanced by increasing the column temperature, as shown in Fig. 6(B), though that of *p*-cresol to *m*-cresol ($\alpha_{p/m\text{-cresol}}$) was approximately constant between 283 and 333 K.

The ΔH° and $\Delta S^\circ/R + \ln \phi$ for *o*-, *m*-, and *p*-cresols, calculated from the relationships in Fig. 6(A) using Eq. (1) are listed in Table 1. In addition, the $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ for $\alpha_{m/o\text{-cresol}}$ and $\alpha_{p/m\text{-cresol}}$, calculated from the relationships in Fig. 6(B) using Eq. (2), are summarized in Table 2. In both tables, the ΔH°, $\Delta S^\circ/R + \ln \phi$, $\Delta\Delta H^\circ$, and $\Delta\Delta S^\circ$ measured at pH 5.9 and 6.9 (in buffer) are also listed as well as those at pH 5.0. The elution order of the cresol isomers was *o*-, *m*-, then *p*-cresol in our experimental condition, as shown in Fig. 3. As listed in Table 1, the separation between *o*-cresol and the other isomers was mainly attributed to the difference in the ΔH° value. Meanwhile, since the ΔH° values of *m*- and *p*-cresol were approximately the same, the difference in their retentions might be mostly caused by $\Delta S^\circ/R + \ln \phi$. As for $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$, in addition to the values given in Table 2, they can be also calculated from the ΔH° and

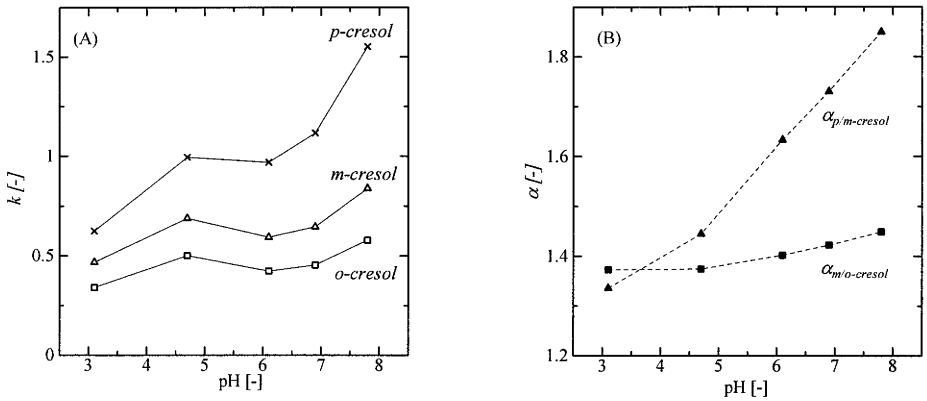

Fig. 4 Relationships between the pH of phosphate buffer in the mobile phase and (A) retention factors of *o*-cresol (square), *m*-cresol (triangle), *p*-cresol (cross), and (B) separation factors of *m*/*o*-cresol (closed square) and *p*/*m*-cresol (closed triangle). Other conditions were the same as in Fig. 3.

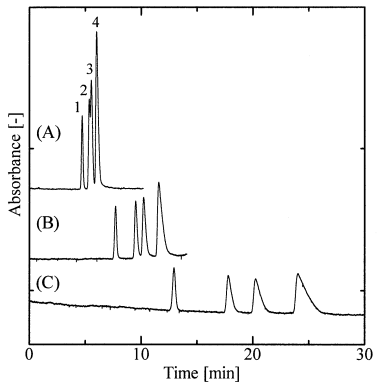

Fig. 5 Separation of cresol isomers under various column temperatures of (A) 321 K, (B) 301 K, and (C) 285 K. Peaks of 1, 2, 3, and 4 correspond to thiourea (t_0 marker), *o*-, *m*-, and *p*-cresol, respectively. Column: i.d., 0.075 mm; packed length, 127 mm; packed materials, CHIRAL β-CD-BR; eluent, mixture of 10 mM phosphate buffer (pH 5.0) and methanol (80/20, v/v); flow, constant-pressure mode of 10 MPa; detection, UV 210 nm.

Table 1 Variations in the ΔH° and $\Delta S^\circ/R + \ln \phi$ values for cresol isomers

pH	ΔH° (kJ/mol)			$\Delta S^\circ/R + \ln \phi$ (–)		
	o-cresol	*m*-cresol	*p*-cresol	*o*-cresol	*m*-cresol	*p*-cresol
5.0	–21.7	–25.0	–24.7	–10.1	–11.1	–10.6
5.9	–18.9	–22.3	–21.7	–9.17	–10.2	–9.43
6.9	–17.5	–20.6	–20.5	–8.20	–9.10	–8.51

Eluent: mixture of 10 mM phosphate buffer (pH 5.0, 5.9, 6.9) and methanol (80/20, v/v). Other conditions were the same as in Fig. 5.

$\Delta S^\circ/R + \ln \phi$ values in Table 1, as descried in Theory section. The values of $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ for corresponding isomers estimated by both methods were approximately the same. The less dependency of $\alpha_{p/m\text{-cresol}}$ on the column temperature, shown in Fig. 6(B), was derived from the small $\Delta\Delta H^\circ$ value, *i.e.*, the ΔH° values for *m*- and *p*-cresol were the approximately the same.

As listed in Table 1, both the ΔH° and $\Delta S^\circ/R + \ln \phi$ values for the cresol isomers were increased (negative values raised up to zero) along with an increase in the pH of the mobile phase. An increase in ΔH° value generally causes an decrease in the retention factor (k) as described in Eq. (1). Meanwhile, the k values of the cresol isomers clearly increased at higher pH of the eluent, as shown in Fig. 4(A). Hence, this increase in k should be chiefly attributed to the increases in ΔS with pH. In addition, Fig. 4(A) shows a slight decrease in the retention factors of all the isomers with a change in the pH from 4.7 to 6.1, though the ΔS values increase from pH 5.0 to 5.9 in Table 1. Namely, these partial decrease in the retention factors of the cresol isomers at around pH 5 – 6 were thought to be caused by an increase in ΔH°, *i.e.*, decrease in $-\Delta H^\circ/T$ value. Since the $-\Delta H^\circ/T$ value depends on the temperature, partial decrease in the k values might not be observed at a higher column temperature, where the contribution of $-\Delta H^\circ/T$ becomes quite small.

Concerning the relationship between the separation factors and the pH of the mobile phase, the $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ values given in Table 2 do not show any clear correlation with the pH of the eluent compared to ΔH° and $\Delta S^\circ/R + \ln \phi$. Namely, the behaviors of both $\alpha_{p/m\text{-cersol}}$ and $\alpha_{m/o\text{-cersol}}$ on the mobile phase pH were not caused by ether $\Delta\Delta H^\circ$ or $\Delta\Delta S^\circ$ alone, but their combination and the column temperature.

Relationship between the retention variation and the net surface charge density

The retention of neutral compounds in the mixed-mode stationary phase of ODS and anion-exchangers (ODS/SAX) was affected by the electric charge in the stationary phase.[21] Therefore, the retention variations of the cresol isomers on the eluent pH in a β-CD-BR packed column may also have some relation with the surface charge of the stationary phase. Figure 7 shows the relationship between the pH of the buffer in the mobile phase and the electroosmotic flow (EOF) rate generated by voltage application using the apparatus shown in Fig. 2, which can assess the net surface charge on the β-CD-BR-modified packing materials. The EOF rate significantly depended on the pH of the mobile phase, and varied while forming 2 steps, which were caused by both the silanol and secondary ammonium groups. The EOF direction altered around pH 4. This fact indicated that the sign of the net surface charge density changed in this pH region. The solid line in Fig. 7 was produced by a curve fitting with Eq. (3); the $pK_{a,SiOH}$ and $pK_{a,NH}$ values were calculated as 3.8 and 6.5, respectively. Since the actual pH for the sample solution containing 20% methanol was not certifiable, the pH value in Fig. 7 was for an aqueous buffer in the sample solution. Furthermore, the pK_a values of

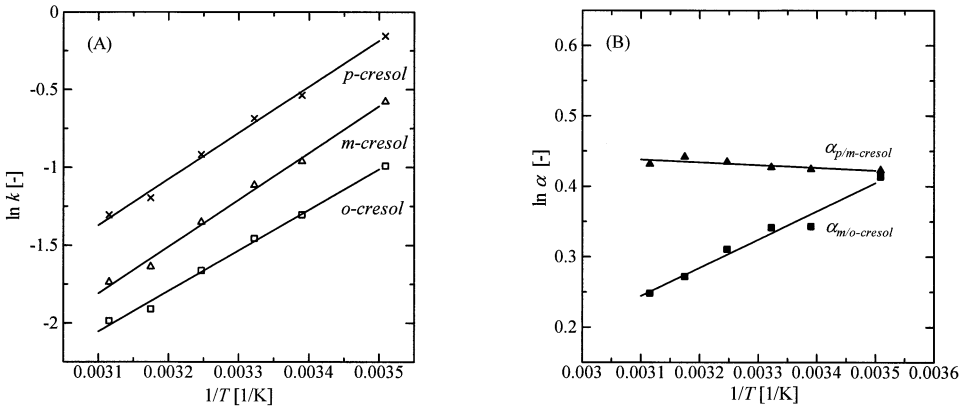

Fig. 6 Relationship between 1/T and (A) ln k of *o*-cresol (square), *m*-cresol (triangle), *p*-cresol (cross), and (B) ln α of *m*/*o*-cresol (closed square), *p*/*m*-cresol (closed triangle). Other conditions were the same as in Fig. 5.

Table 2 Variations in $\Delta\Delta H^{o}$ and $\Delta\Delta S^{o}$ between cresol isomers

pH	$\Delta\Delta H^{o}$ (kJ/mol)		$\Delta\Delta S^{o}$ (kJ/K mol)	
	o- and *m*-cresol	*m*- and *p*-cresol	*o*- and *m*-cresol	*m*- and *p*-cresol
5.0	–3.33	0.33	–0.0083	0.0047
5.9	–3.46	0.66	–0.0088	0.0066
6.9	–3.12	0.07	–0.0075	0.0050

Conditions were the same as in Table 1.

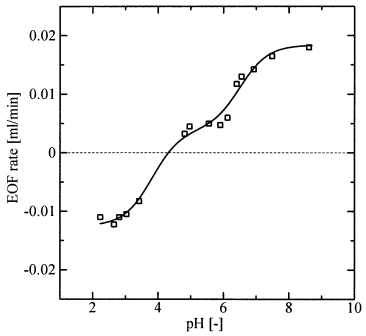

Fig. 7 Relationship between the pH of the buffer and the EOF rate. EOF generator, β-CD-BR; packed length, 1 cm; applied voltage, 200 V for 10 min; sample solution, mixture of 10 mM phosphate buffer and methanol (80/20, v/v).

both the silanol and secondary ammonium groups would be affected by the methanol contained in the sample solution. Therefore, the estimated pK_a values should be different from those in the aqueous solution. However, Fig. 7 directly reflects the variation of the surface charge under chromatographic separation because the composition of the sample solution was the same as that of the chromatographic eluent.

The variation pattern of the EOF velocity, shown in Fig. 7, is quite similar to those in the retention factors of the cresol isomers shown in Fig. 4(A). With a pH change from 3.1 to 4.7, the net surface charge density approached neutral by the dissociation of silanol groups, and the retention factors of the cresol isomers increased. Furthermore, the retention factors were slightly reduced with the pH change from 4.7 to 6.1, as shown in Fig. 4(A). Judging from Fig. 7, the negative net charge might be quite small in the corresponding pH region. In the range between pH 6.1 and 7.8, both the negative net surface charge and the retention factors were significantly increased with an increase in the pH of the mobile phase. As a whole, the retentions of the cresol isomers in the β-CD-BR packed column show a close correlation with the net surface charge on the packing materials.

In the region from pH 6.1 to 7.8, the variation in the net surface charge was principally dominated by the secondary ammonium group, and the insertion of the cresol isomers to the cavity of β-CD-BR may be inhibited by the positive charge of the secondary ammonium group. Such a charge-induced decrease in the retention factor can also be observed in the ODS/SAX column.[21] In contrast to the secondary ammonium group, the negative charge of the silanol group enhanced the insertion of the cresol isomers to β-CD-BR in the pH range between 3.1 and 4.7 to cause an increase in the retention. Though the mechanism of this increase in the retention can not be satisfactory revealed, the negative charge may strongly attract the water in the eluent compared to methanol. The partial enhancement of the water content in the mobile phase around the surface may induce an increase in the retention factors.

Figure 4(B) shows the dependency of the separation factor on the pH of the mobile phase. The separation factors for both *m*/*o*-cresol and *p*/*m*-cresol increased with an increase in the pH value of the mobile phase. Namely, the magnitude of the k variation for the cresol isomers on the pH was in the rank order of *p*-, *m*-, and *o*-cresol. The highly retained analyte (*p*-cresol) is the most sensitive to the pH variation. The higher retention of *p*-cresol compared to the other isomers may be given by a deeper insertion of the methyl group of *p*-cresol to the cavity of β-CD-BR, mainly under the higher pH conditions. The positive charge of the secondary ammonium group may inhibit insertion of the cresol isomers to the cavity, and attribute the largest inhibition to the *p*-cresol. In the acidic condition (pH 3 – 5), the $\alpha_{m/o\text{-cresol}}$ and $\alpha_{p/m\text{-cresol}}$ crossed, as shown in Fig. 4(B); $\alpha_{p/m\text{-cresol}}$ increased significantly with a pH change from 3.1 to 4.7, though $\alpha_{m/o\text{-cresol}}$ was approximately constant. In this region, the variation in the retention factor may be caused by a partial enhancement of the water content in the mobile phase around the surface of the packing materials. Since the variation in the water content strongly affects the highly retained analyte, the increase in the retention factor of *p*-cresol was larger than that of *m*-cresol, and the increase in $\alpha_{p/m\text{-cresol}}$ became significant.

Conclusion

The pH of the mobile phase in HPLC affected the retention of neutral cresol isomers in a β-CD-BR packed column. An investigation of the thermodynamic parameter revealed that the variation in the retention factor on the pH was mainly dominated by the enthalpy variation. Though the mechanism of the retention variation has not been completely revealed, the surface charge originating from both the secondary ammonium and silanol groups on β-CD-BR modified silica gels may have important roles. The positive charge of the secondary ammonium group might directly affect the interaction between β-CD-BR and the analyte, and the silanol groups on the surface might partially alter the mobile phase composition around the surface of the packing materials. The difference in the role of these two ionized functional groups has not been sufficiently clarified, but the distance from the charge to the cavity of the cyclodextrin may be an important factor. A further study in this line is currently in progress.

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