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Isotachophoretic Analysis of Quaternary Ammonium Ions by Using α-Cyclodextrin as Complex-Forming Agent

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The isotachophoretic behavior of aromatic nucleus-containing quaternary ammonium ions was studied in the presence of a neutral ligand, α-cyclodextrin. Due to the interaction of cyclodextrin with the hydrophobic aromatic nucleus, isomeric ammonium ions containing, e.g., an *o*-, *m*-, or *p*-disubstituted benzene ring, were satisfactorily separated. In the absence of cyclodextrin, the separation of the isomers was poor. A theoretical approach was also used in order to evaluate the binding equilibrium constant (K) between the analyte ion and α-cyclodextrin through an isotachophoretic measurement. The binding constants ($\log K$) with (*o*-, *m*-, and *p*-methylbenzyl)trimethylammonium ions were found to be 0.8, 1.1, and 1.6, respectively.

During the past twenty years, substantial progress has been made concerning isotachophoresis, both in regard to theory and instrumentation, making it an important physicochemical tool for the analysis of ionic species in solution. In this technique, ion separation is based on a difference in the ionic mobilities under an electrical potential gradient. Therefore, the most important task in isotachophoresis is to control or modify the effective mobility of analyte ions in order to expand its analytical applications.

Several approaches have been made to modify the mobility of ionic species: (i) a pH adjustment of the leading solution, (ii) a change in the solvent polarity, and (iii) the use of complex-forming equilibria with counter ions.[1-10] As a new approach, we have recently proposed a system in which an electrically-neutral ligand is used to bind ionic species and to alter their effective ionic mobility.[11] For example, by adding an appropriate amount of crown ethers to the leading solution, some alkali and alkaline earth metal ions (which otherwise did not form electrophoretically resolved zones) could be mutually separated.

On the other hand, cyclodextrins are known to have specific interactions with various aromatic compounds in aqueous solution.[12] This type interaction has recently been successfully used for improving the chromatographic separation of aromatic compounds and amino acids.[13-16] Further, the separation of optical isomers has been attained by taking advantage of the chiral nature of cyclodextrins.[17,18] Such interactions of cyclodextrins with aromatic compounds should be directly applicable to an isotachophoretic system; in fact, we have found that the separation of bulky (lipophilic) inorganic anions can be improved by adding α-cyclodextrin (α-CD).[11]

During the present study, we looked at the effect of α-CD on the isotachophoretic separation of quaternary ammonium ions. These ions, especially those in isomeric forms, are difficult to separate under conventional isotachophoretic conditions. The addition of α-CD greatly improved the separation. The study was also extended to an evaluation of the binding constant between the analyte ammonium ion and α-CD.

Experimental

Apparatus A Shimadzu IP-2A isotachophoretic analyzer was combined and used with a potential gradient detector (model PGD-1). The ion-migration current and the temperature were set at 50 μA and 25 °C, respectively. The length of the analyzing capillary tube (PTFE; i. d. 0.5 mm) was 15 cm.

Reagents. Ten mM (1 M=1 mol dm^{-3}) of hydrochloric acid or 10 mM of an acetic acid–potassium acetate (1:1) buffer solution was used as the leading solution. The solution also contained 0.01 wt% poly (vinyl alcohol) (PVA) and an appropriate amount of α-CD. A terminating solution was prepared by dissolving 5 mM of (*p*-methylbenzyl)trimethylammonium bromide or 5 mM of 1-naphthylamine hydrochloride in water. The quaternary ammonium salts used as analyte ions were synthesized by a conventional reaction between tertiary amines and substituted benzyl halides in methanol or in ethanol. After recrystalization from ethanol–ether, the structure and purity of the product was confirmed by ^{1}H NMR, melting points and an elemental analysis.

Results and Discussion

Mobility of Analyte Ions. In isotachophoresis, each electrophoretic zone migrates at a constant velocity

$$v = \bar{m}_L E_L = \bar{m}_A E_A = \bar{m}_T E_T, \quad (1)$$

where $\bar{m}$ and E are the effective mobility and the (electric) potential gradient, respectively. The suffixes denote the quantities related to leading (L), analyte (A) and terminating (T) zones or ions. In our

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measurements, the potential gradient at each zone was obtained from an isotachopherogram. The mobility of the ion corresponding to each zone was then calculated by using Eq. 1 once the isotachopherogram was calibrated against ions of a known ionic mobility. In doing this, we had to be careful that the potential gradient read on isotachopherogram as a step height (cm) was not an absolute, but rather a relative value. Also, the observed step height could possibly contain a detector-drift term. Therefore, in order to determine the correct mobility of analyte ions from an isotachopherogram, several internal standard ions (calibration ions) of known mobility were required. They were subjected to the same isotachophoresis along with the analyte ions.

In the present study, K^+, Na^+, Li^+, and tetraethylammonium (TEA^+) were used as calibration ions. Figure 1 shows the potential gradient (step height) observed for these calibration ions as well as an analyte ion, (*p*-methylbenzyl)trimethylammonium ion (which is used in this case as a terminating ion). The calibration ions were such that no interaction was reported or could be expected to take place with the α-CD. The observed potential gradient of these ions, however, steadily increased as the α-CD concentration in the leading solution was increased.

In order to analyze the effect of α-CD on the calibration ions, the observed potential gradient (step height) for those ions was plotted against the reciprocals of the mobility of the ions (Fig. 2). Since this depends on their ionic atmosphere or the salt concentration in the solution, an appropriate correction was made to the absolute ionic mobility (the value at infinite dilution) by using the Debye-Hückel-Onsager equation.[19] The ionic concentration in each electrophoretic zone was calculated according to the regulating function derived from isotachophoretic equilibrium.[1]

As shown in Fig. 2, the plots resulted in good straight lines. This could be expected from Eq. 1, except that the observed potential-gradient values contained a drift term as indicated by the intersection of the lines with the ordinate. This is consistent with the experimental observation that when isotachopherogram was recorded from the start of sample-ion electrophoresis, the potential gradient of the leading zone began to drift upward at around the middle of the leading zone and then again became constant (flat) just before the analyte zones appeared. This detector-drift was quite reproducible, but could not be eliminated in spite of the extensive effort made during this study.

Figure 2 further shows that the slopes of the lines increased as the concentration of the α-CD in the leading solution was increased. This α-CD effect is associated with a decrease in the mobility of calibration ions in the presence of α-CD. The complexation of these calibration ions by α-CD might decrease their effective mobilities, but the well-defined straight lines shown in Fig. 2 can hardly be explained by such a bimolecular complexation. On the other hand, since α-CD is a rather large molecule, a change in the physical property of a bulk solution can be expected, even at a relatively low molar α-CD concentration.

According to Stokes' law, the ionic mobility is reciprocally proportional to the viscosity of the medium. This and Eq. 1 predict that if the viscosity of a solution increases with the addition of α-CD, the slopes of the lines in Fig. 2 increase with the increase in the α-CD concentration while retaining their straightness. Therefore, the mobility-lowering effect by α-CD must be entirely due to the change in the viscosity. This conclusion is also in line with the results shown in Fig. 1. Thus, the calibration ions unanimously show only a slight and an almost linear

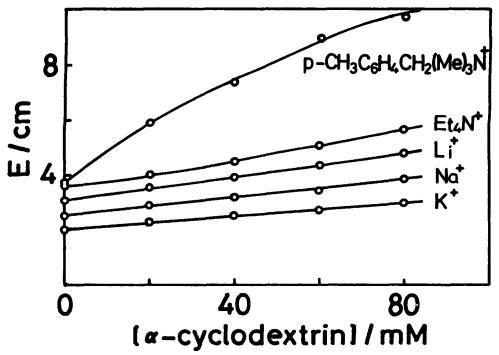


Fig. 1. Isotachophoresis of calibration ion in the presence of α-CD. Relation between potential gradient and α-CD concentration in leading solution. E: potential gradient as measured by step height (cm) in isotachopherogram. Leading solution, 10 mM HCl containing α-CD and 0.01 wt% PVA; terminating solution, 5 mM (*p*-methylbenzyl)trimethylammonium bromide; analyzing capillary tube (PTFE; i.d., 0.5 mm), 15 cm; ion migration current, 50 μA; 25 °C.

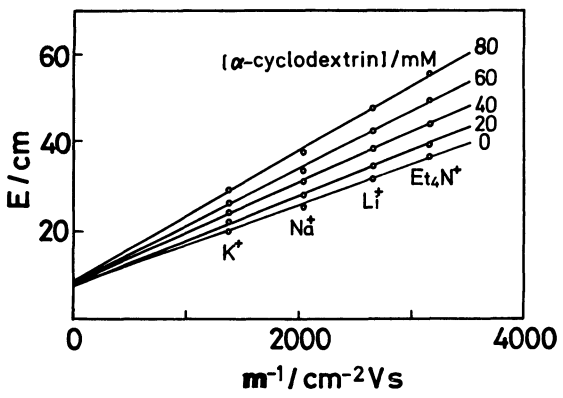


Fig. 2. Relation between potential gradient (E) and reciprocal ionic mobility (m^{-1}) of calibration ions at various α-CD concentrations. Calibration lines. For ionic mobility, see Eq. 2. For E and migrating conditions, see Fig. 1.

increase in potential gradient upon increasing the α-CD concentration. On the other hand, the (*p*-methylbenzyl)trimethylammonium ion, which is expected to make a specific (bimolecular) interaction with α-CD, shows a marked potential-gradient increase. The general behavior, therefore, is quite different.

In order to quantitatively extract the (purely) complexing effect of α-CD from the observed potential-gradient data, it was necessary to either cancel out or to make calibrations for both the detector signal-drift and the viscosity effect of α-CD. For this purpose, a calibration line (the same as that shown in Fig.2) was prepared for each α-CD concentration and was used to correlate the observed potential gradient and the mobility (reciprocal mobility) of the analyte ions. With the aid of such a calibration line, we could obtain the mobility of any analyte ion at any α-CD concentration by simply measuring the corresponding step height on the isotachopherogram.

Another way of looking at this is that the mobility of the analyte ion obtained from such a calibration method is not a real but, rather, an ideal or abstracted one since the contribution from the viscosity effect is artificially eliminated. According to Stokes' law, the above-mentioned mobility m_i and the actual mobility m_i are related by

$$m_i = \frac{\eta_{[CD]>0}}{\eta_{[CD]=0}} \times m_i, \tag{2}$$

where η is the viscocity of the medium.

Isotachophoresis of Quaternary Ammonium Ions and Complexation with α-CD. Figure 3 illustrates an isotachophoretic separation of analyte cations (A^+ and B^+) in the presence of an electrically-neutral ligand (N). L^+ and T^+ indicate the leading and terminating cations, respectively. Q^- represents the counter anion.

The binding constants or association constants of ligand N with A^+ and B^+ are defined by Eqs. 3 and 4. Then, the effective mobilities of A^+ and B^+ ($\bar{m}_A$ and $\bar{m}_B$: mobilities which are observed experimentally) in their sample zones in the presence of ligand N can be expressed by Eqs. 5 and 6. The latter equations indicate that cations A^+ and B^+ can be separated from each other even when they have the same ionic mobilities ($m_A=m_B$) if the binding constants with N are appreciably different ($K_{AN} \neq K_{BN}$):

$$K_{AN} = \frac{[AN^+]_A}{[A^+]_A[N]_A} \tag{3}$$

$$K_{BN} = \frac{[BN^+]_B}{[B^+]_B[N]_B} \tag{4}$$

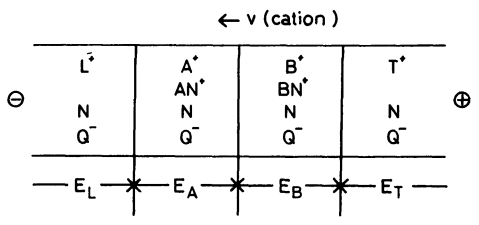


Fig. 3. Isotachophoretic separation of cations in the presence of neutral ligand. E_i: potential gradient in the *i*-th zone, *v*: migration velocity of cation, L^+: leading ion, A^+: B^+: analyte ions, T^+: terminating ion, N: neutral ligand, Q^-: counter ion.

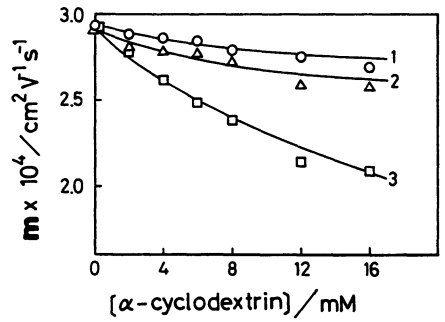
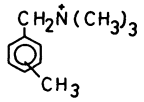


Fig. 4. Complexing effect of α-CD on the mobility of (*o*-, *m*-, and *p*-methylbenzyl)trimethylammonium ions. For ***m***, see Eq. 2. Terminating solution, 5 mM 1-naphthylamine hydrochloride. Other conditions are the same as those in Fig. 1.



1 (*o*-, ○), **2** (*m*-, △), **3** (*p*-, □)

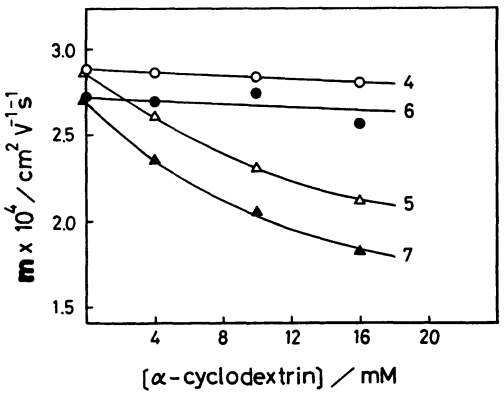
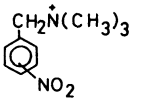
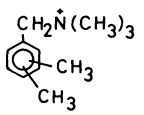


Fig. 5. Complexing effect of α-CD on the mobility of (substituted benzyl)trimethylammonium ions. For ***m***, see Eq. 2. Leading solution, 10 mM acetic acid-potassium acetate (1 : 1) buffer solution containing α-CD and 0.01 wt% PVA; terminating solution, 5 mM 1-naphthylamine hydrochloride; analyzing capillary tube (PTFE; i.d., 0.5 mm), 15 cm; ion migration current, 50 μA; 25 °C.



4 (*o*-, ○), **5** (*p*-, △)



6 (*m*-, *p*-, ●), **7** (*o*-, *p*-, ▲)

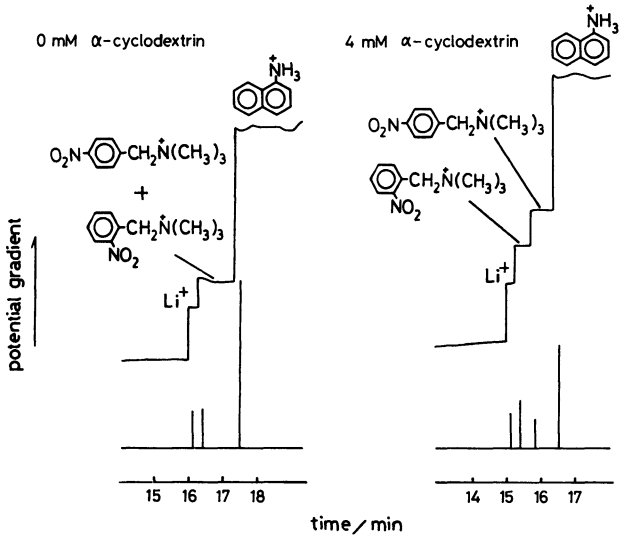


Fig. 6. Isotachophoretic separation of (*o*- and *p*-nitrobenzyl)trimethylammonium ions. See Fig. 5 for conditions.

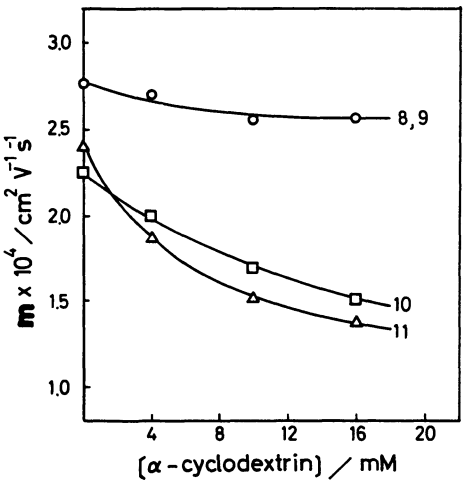
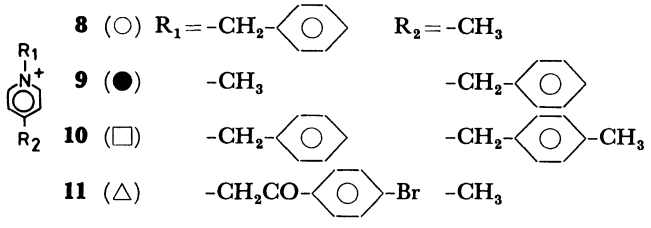


Fig. 7. Complexing effect of α-CD on the mobility of some quaternary pyridinium ions. For ***m***, see Eq. 2. Migration conditions are the same as those in Fig. 5.



$$\bar{m}_A = \frac{m_A[A^+]_A + m_{AN}[AN^+]_A}{[A^+]_A + [AN^+]_A} = \frac{m_A + m_{AN}K_{AN}[N]_A}{1 + K_{AN}[N]_A} \quad (5)$$

$$\bar{m}_B = \frac{m_B[B^+]_B + m_{BN}[BN^+]_B}{[B^+]_B + [BN^+]_B} = \frac{m_B + m_{BN}K_{BN}[N]_B}{1 + K_{BN}[N]_B}. \quad (6)$$

Figures 4 and 5 summarize the effect of α-CD on the mobilities of (substituted benzyl) trimethylammonium ions. Figure 6 shows one of the typical isotachopherograms obtained in these studies. It can be seen that the isomeric ammonium ions showed no indication of a mutual separation in the absence of α-CD. These (quaternary) ammonium ions are not acids nor bases in ordinary pH range (i.e., their electrophoretic behavior is not dependent on pH); therefore, the pH change in the medium can not help their separation. A change of medium from aqueous to organic does not show much promise either. The addition of α-CD brought about a clear separation (indicated in Figs. 4—6). Obviously, the separation was caused by complexation with α-CD (implicated by Eqs. 5 and 6). In fact, it is conceivable that this is the only way to achieve a separation of this type of organic ions by isotachophoresis, since the means are quite scarce for assuring a specific interaction with such isomeric quaternary ammonium ions.

One can see in Figs. 4 and 5 that the complexation characteristics of α-CD is clearly reflected in the mobility change of isomeric ammonium ions. α-CD is a cylinder-like molecule with an internal hydrophobic cavity which fits the size and the polarity of aromatic (benzene) derivatives. When inclusion complexes are formed with disubstituted benzenes, it is generally accepted that o- and m-isomers suffer steric hindrance from the wall of the cavity while p-isomers do not. Among *o*-(**1**), *m*- (**2**), and *p*-(**3**) methyl-substituted benzyl isomers, the p-isomer (**3**) shows the most pronounced mobility decrease upon an addition of α-CD. This indicates that the p-isomer formed the most stable inclusion complex. Because of its high molecular weight, the α-CD complex exhibits a much lower mobility than that of the uncomplexed ammonium ion ($m_A = m_B > m_{AN} = m_{BN}$). If the complex formation (binding) constant of isomer A is larger than that of isomer B ($K_{AN} > K_{BN}$ in Eqs. 3 and 4), the observed (effective) mobility of isomer A becomes smaller than that of isomer B ($\bar{m}_A < \bar{m}_B$ in Eqs. 5 and 6).

A distinct difference in the migration behavior was observed when α-CD was added to isomeric (nitrobenzyl) trimethylammonium ions (**4** and **5**; Fig. 5). The p-substituted isomer indicated a much stronger interaction with α-CD. For trisubstituted aromatic compounds **6** and **7**, however, a simplified consideration can not be made regarding the preference of complexation among the isomers. However, the analytical importance of cyclodextrin complexation is obvious if one looks at the separation achieved by applying only 5 mM α-CD to the leading solution.

Figure 7 shows the effect of α-CD on the mobility of some quaternary pyridinium ions. An inspection of the figure suggests that pyridinium moieties are too hydrophilic to be effectively bound in the hydrophobic cavity of cyclodextrin. It was rather discouraging that the mixture of isomers **8** and **9** could not be resolved. Compounds **10** and **11**, which carry more

lipophilic (larger) substituents on the pyridinium ring, showed a substantial decrease in mobility upon the addition of α-CD.

Estimation of Binding Constant. A theoretical treatment of the present isotachophoretic system is simple since the complexation of analyte ions (cations) only takes place with an electrically neutral ligand (α-CD).

First, a mass balance is taken for counter ions (anion) in the leading (L) and its neighboring analyte (A) zones. This gives

$$-m_Q E_A[Q^-]_A + m_Q E_L[Q^-]_L = v([Q^-]_A - [Q^-]_L), \quad (7)$$

where Q^- stands for a counter anion and the suffixes A, L, and Q denote the quantities related to these ionic species or their zones.

Since the neutral ligand in zone A exists in both free (N) and charged (AN^+) forms, it can move under the potential gradient in the zone. The effective mobility of the ligand in zone A is given by

$$\bar{m}_{N,A} = \frac{m_{AN}[AN^+]_A}{[AN^+]_A + [N]_A}. \quad (8)$$

Then, the mass balance for neutral ligand in the leading and its neighboring analyte zones is given by

$$\bar{m}_{N,A} E_A C_{N,A} = v(C_{N,A} - C_{N,L}), \quad (9)$$

where $C_{N,A}$(=$[AN^+]_A$+$[N]_A$) and $C_{N,L}$ are the concentrations of the neutral ligand in the analyte (A) zone and the leading solution, respectively.

Finally, Eqs. 10 and 11 are obtained from the electrical neutrality of each zone:

$$[A^+]_A + [AN^+]_A = [Q^-]_A \quad (10)$$

and

$$C_L = [Q^-]_L, \quad (11)$$

where C_L is the concentration of leading cation in the leading zone (=concentration of the leading electrolyte).

From these equations, coupled with Eqs. 1 and 3—6, the parameters could be eliminated (except for m_L, m_A, m_{AN}, m_Q, C_L, $C_{N,L}$, $[N]_A$, and K_{AN}). This afforded the following equation, which is a cubic function with respect to $[N]_A$:

$$m^*_{AN}K_{AN}^2[N]_A^3 + (m^*_A + m^*_{AN} - m^*_{AN}K_{AN}C_{N,L})K_{AN}[N]_A^2 + \{m^*_A + (m^*_A - m^*_{AN})m^*_L/m_L K_{AN}C_L - (m^*_A + m^*_{AN})K_{AN}C_{N,L}\} \times [N]_A - m^*_A C_{N,L} = 0 \quad (12)$$

where,

$$m^*_i = m_i + m_Q \ (i=L, A, AN).$$

To solve Eq. 12, the viscosity effect of α-CD must be considered. The mobility of individual ionic species at any α-CD concentration can be expressed as indicated before by Eq. 2, where m_i is the mobility of the ionic species free from the viscosity effect of α-CD and η is the viscosity of a medium containing α-CD at a specific concentration. By using Eq. 2, $\bar{m}_i$ and m_i can be replaced by $\bar{m}_i$ and m_i, respectively, in Eqs. 1 as well as 5-12. The resulting equations, as they appear, indicate only a simple replacement of $\bar{m}_i$ and m_i by $\bar{m}_i$ and m_i, respectively, and a replacement of E_i by $E_i \times (\eta_{[CD]=0}/\eta_{[CD]>0})$. This means that, even if numerical viscosity data is not available, Eq. 12 can be solved for $[N]_A$ by using the mobility data in the presence and the absence of α-CD after calibrating for the viscosity effect of α-CD.

The mobility of uncomplexed analyte ions could be experimentally determined during the present study. On the other hand, the mobility of α-CD-complexed analyte ions (m_{AN} or m_{AN}) could not be experimentally obtained, since complexation never went to completion under our isotachophoresis conditions. Now, a relationship between the relative mobility U (m_{TEA}=1.00) and the molecular weight is known to exist which follows the phenomenological equation:[20]

$$U/Z = 14.7/\sqrt{Mw} - 0.29, \quad (13)$$

where Z is an ionic charge and Mw is a molecular weight. By using this equation we could estimate the absolute mobility of α-CD-bound quaternary ammonium ions (complexed cation) with reasonable accuracy. The value (4.94×10^{-5} $cm^2 V^{-1} s^{-1}$ for (methyl-substituted benzyl)trimethylammonium-α-CD complex; Mw=972.85+164.27=1137.12), thus obtained, was used in Eq. 12. Then, we could numerically calculate the concentration of the free neutral ligand in the analyte zone ($[N]_A$) as a function of the binding constant (K_{AN}), where the concentration of the ligand in the leading zone was set as a parameter. Once $[N]_A$ was known, we could readily obtain $\bar{m}_A$ from Eq. 5; these $\bar{m}_A$ values were compared with the experimentally-obtained $\bar{m}_A$ value in order to check the validity of the value of parametrically-chosen K_{AN} values (see below).

A computer-assisted calculation was performed by using the Newton–Raphson method for solving cubic functions (Eq. 12) and by using an iterative method for approximating the ionic-strength modification to the absolute mobility (m_{0i}) using the Debye–Hückel–Onsager equation. Figure 8 summarizes the result of the theoretical calculation, where the effective mobility of the analyte ion is plotted as a function of the binding constant with α-CD. The effect of the α-CD concentration is also included in the figure.

The profiles in Fig. 8 indicate that as the K_{AN} value decreases or the α-CD concentration becomes lower, the effective mobility of the analyte ion approaches that of the free ion. On the other hand, as the K_{AN} value increases or the α-CD concentration increases, the effective mobility of the analyte ion approaches that of the α-CD-bound (complexed) analyte ion.

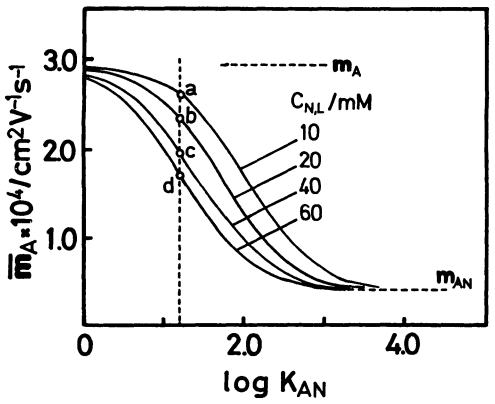


Fig. 8. Mobility change of (methyl-substituted benzyl) trimethylammonium ion (A) as a function of its binding constant (K_{AN}) with α-CD (N). Theoretical calculation. Parameters used are as follows. Absolute mobility m_{0i} ($cm^2\ V^{-1}\ s^{-1}$): $m_{0L}=362.5\times10^{-5}$, $m_{0Q}=79.08\times10^{-5}$, $m_{0A}=31.55\times10^{-5}$, $m_{0AN}=4.94\times10^{-5}$. Concentration (M): $C_L=0.0102$, $C_{N,L}=0.010$, 0.020, 0.040, 0.060.

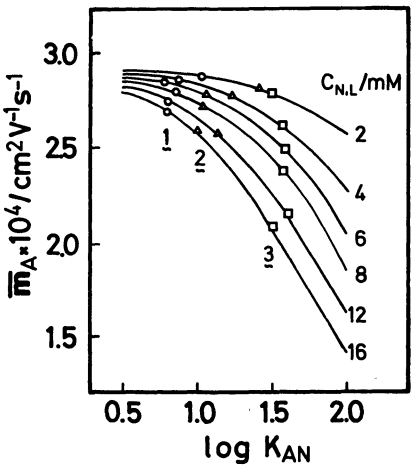


Fig. 9. Comparison of calculated and experimentally-obtained mobility. Determination of binding constant (K_{AN}) of (*o*-, *m*-, and *p*-methylbenzyl)trimethylammonium ions with α-CD. Experimental and calculation conditions are the same as those in Figs. 4 and 8.

Such behavior is in good accordance with our intuitive chemical picture and is quite reasonable.

The series of theoretical lines in Fig. 8 are useful for experimentally evaluating the binding constant between the analyte ions and α-CD. Consider that one carried out isotachophoresis of a certain analyte ion in the presence of 10, 20, 40, and 60 mM α-CD and obtained mobility data at each α-CD concentration. If the assumptions that were made in preparing the theoretical lines in Fig. 8 are correct, the observed mobility data should fall on a single vertical line crossing the four theoretical lines at experimental points a, b, c, and d. The intersect of the vertical line with the abscissa defines the required binding constant K_{AN}.

Figure 9 includes the mobility data obtained for (*o*-, *m*-, and *p*-methylbenzyl)trimethylammonium ions along with some theoretical mobility lines at various α-CD concentrations. Though experimental points are somewhat scattered at low α-CD concentrations, the data define fairly reasonable vertical lines, affording binding constants (log K_{AN}) of 0.8, 1.1, and 1.6 for o-, m-, and p- isomers, respectively. Such low binding constants are usually accessible only by methods which involve multi-stage interactions, such as liquid chromatography. The present method using isotachophoresis is obviously characterized by a similar feature and is considered to be generally useful both for detecting and quantitatively treating weak interactions between an ion and an uncharged ligand.

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