Journal of Chromatography A, 653 (1993) 313–319
Elsevier Science Publishers B.V., Amsterdam

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Effect of cetyltrimethylammonium chloride on electroosmotic and electrophoretic mobilities in capillary zone electrophoresis

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(First received March 30th, 1993; revised manuscript received July 21st, 1993)

ABSTRACT

The effect of a cationic surfactant, cetyltrimethylammonium chloride (CTAC), on the electroosmotic mobility and the electrophoretic mobility of organic anions in capillary zone electrophoresis was investigated. The electroosmotic mobility showed four stepwise changes, including a reversal, with increasing CTAC concentration. The behaviour, especially the reversal of the electroosmotic mobility, was explained by assuming the formation of hemimicelles on the capillary wall. That is, CTAC first adsorbs individually by electrostatic interactions and then begins to associate into hemimicelles by Van der Waals attraction. The formation of hemimicelles changes the surface charge of the capillary wall from negative to positive and causes the reversal of the electroosmotic mobility. The effective electrophoretic mobilities of organic anions such as benzoic acid analogues were also influenced by the CTAC concentration. It was concluded that the behaviour was due to the interaction with hemimicelles on the capillary wall and also ion association with the monomer of CTAC and the interaction of micelles in bulk solution.

INTRODUCTION

Capillary zone electrophoresis (CZE) is one of the most powerful separation techniques and many excellent separations by CZE have been reported in the last decade [1–4]. The separation in CZE is performed in a narrow fused-silica capillary tube filled with an electrolyte solution. On applying a high electric field to both ends of the capillary, an electroosmotic flow from the anode to cathode is generated that carries sample solutes to a detector. The apparent electrophoretic mobilities of cationic species increase because the electroosmotic mobility is added to the electrophoretic mobility. In contrast, those of anionic species decrease because these species migrate electrophoretically in the opposite direction to the electroosmotic flow. If the electrophoretic mobilities of the anionic species are close to or larger than the electroosmotic mobility, it takes a long time to detect them or sometimes it is difficult to detect them under ordinary CZE conditions. The control of the electroosmotic mobility is very important for improving the separation and shortening the analysis time in CZE. The electroosmotic mobility can be varied by altering the composition of the background electrolyte. Especially cationic surfactants have a large effect on electroosmotic mobility [5,6]. On increasing the concentration of a cationic surfactant, the electroosmotic mobility decreases and then the direction of the flow is reversed. This reversed electroosmotic flow is advantageous in the separation of anions [7,8].

On the other hand, it is essential for optimization of the separation selectivity in micellar

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0021-9673/93/$06.00

electrokinetic capillary chromatography (MECC) to investigate the ability of various kinds of surfactants, because a selectivity different from that in MECC using sodium dodecyl sulphate, which is the most popular surfactant in MECC, is to be expected when using cationic surfactants. However, there are few reports on the utility of cationic surfactants in MECC [9,10]. The effect of cationic surfactants on electroosmotic and electrophoretic mobilities has been hardly studied in detail.

We have previously reported the migration behaviour of inorganic anions in MECC using cetyltrimethylammonium chloride (CTAC) as a cationic surfactant [11]. The separation selectivity for these inorganic anions could be controlled by adding CTAC to the migrating electrolyte solution. We have also evaluated the interaction between inorganic anions and CTAC by using the effective electrophoretic mobility. In this work, the dependence of the electroosmotic mobility on the concentration of CTAC was investigated in detail. With increasing CTAC concentration, the electroosmotic mobility showed four stepwise changes including a reversal. These stepwise changes and the reversal cannot be explained only the electrostatic interaction between CTAC and the capillary wall. The formation of hemimicelles has been discussed by Emmer *et al.* [12], but they did not show experimental evidence for hemimicelle formation [12]. The hemimicelle theory proposed by Fuerstenau and co-workers [13,14] was introduced to explain the behaviour of the electroosmotic mobility. The effective electrophoretic mobilities of some organic anions as test solutes were also investigated in the presence of CTAC. It was demonstrated that the electrophoretic mobilities of these anions were controlled by the interaction with (1) the monomer of CTAC based on ion-pairing equilibria, (2) the hemimicelles formed on the capillary wall and (3) the micelles in the bulk solution.

EXPERIMENTAL

Apparatus

Fused-silica capillary tubes (500 mm × 50 μm I.D.) were obtained from GL Sciences (Tokyo, Japan). An HCZE-30PN0.25 high-voltage power supply (Matsusada Precision Devices, Shiga, Japan) was used. A Model CV^4 variable-wavelength absorbance detector (ISCO, Lincoln, NE, USA) was used to measure the absorbance at 210 nm. Detection was carried out by measuring the absorbance on the column at a position 20 cm from the positive or negative end of the capillary tube. The signal was recorded with an FBR-251A strip-chart recorder (Toa Electronic). The pretreatment of the capillary and the method of sample injection were described in a previous paper [11]. The electroosmotic mobility of the bulk solution was evaluated from the methanol peak. The signs of the electroosmotic and electrophoretic mobilities were defined such that the direction of migration from the cathode to the anode was positive. The migration voltage was kept constant at 15 kV and the current was varied from 8 to 14 μA with increasing surfactant concentration. All experiments were performed at room temperature (*ca.* 22°C).

Materials

All reagents were of analytical-reagent grade and were used without further purification. Cetyltrimethylammonium chloride (CTAC), benzoic acid and tropic acid were obtained from Wako (Osaka, Japan) and cinnamic acid, hydrocinnamic acid and 2-phenylpropanoic acid from Tokyo Kasei Kogyo (Tokyo, Japan). All organic acids were dissolved in methanol and the concentrations of the standard solutions were 10 m*M*. Sample solutions were prepared by diluting each standard solution to 0.5 m*M* with methanol. The background electrolyte solution was prepared as follows: the required amounts of CTAC were dissolved with water and then 0.5 ml of 1 *M* potassium dihydrogenphosphate and 1.0 ml of 1 *M* tris(hydroxymethyl)aminomethane (Tris) were added to the solution. After the pH of the solution had been adjusted by adding 1 *M* citric acid, the solution was diluted to 50 ml. The final concentrations of phosphate and Tris were 10 and 20 m*M*, respectively. For an experiment under acidic conditions, 4 m*M* phosphoric acid solution (pH 2.7) was used as the electrolyte solution and the solution had the same conductivity as the other buffer solutions.

RESULTS AND DISCUSSION

We first studied the dependence of the electroosmotic mobility on CTAC concentration. The relationship between CTAC concentration and electroosmotic mobility is shown in Fig. 1. These curves can be divided into the four sections, where the electroosmotic mobility decreases gently (I), changes considerably and turns in the opposite direction (II), hardly changes at concentrations from $1 \cdot 10^{-4}$ to $5 \cdot 10^{-3}$ *M* (III) and then changes considerably again (IV). The electroosmotic mobility depends on the pH of the background electrolyte until the CTAC concentration is $1 \cdot 10^{-4}$ *M* (sections I and II), but not above that concentration (sections III and IV).

This behaviour of the electroosmotic mobility is similar to the dependence of the zeta potential of quartz on the concentration of primary alkylammonium acetates as reported by Fuerstenau [13]. According to his explanation, the surfactants adsorb as individual ions in section I. In section II, the surfactant ions in the Stern layer next to the surface increase and once these ions are close enough together Van der Waals attraction acts between their hydrocarbon chains to associate into hemimicelles. Section III appears to be the onset of multi-layer adsorption. However, as the second layer would not be held strongly on the capillary wall by coulombic force and these ions in the second layer are stripped off by the streaming liquid, the zeta potential does not change much in this section. Although Fuerstenau did not find section IV, we can interpret it as follows. The surfactant ions in the second layer would not be stripped off in section IV because the interaction between CTAC should be stronger beyond the critical micellar concentration (cmc) in the bulk solution, which is about 1 m*M* [15].

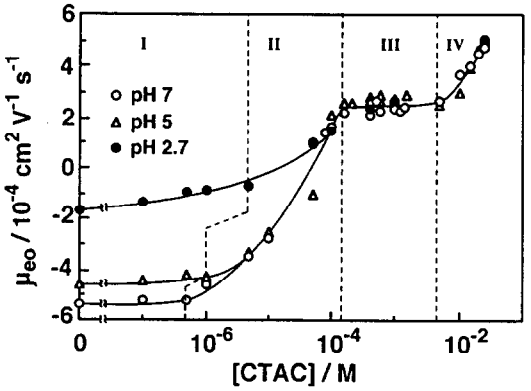

Fig. 1. Effect of CTAC concentration on electroosmotic mobility. Buffers: 10 m*M* phosphoric acid–20 m*M* Tris–citric acid (pH 7.0 and 5.0) and 4 m*M* phosphoric acid (pH 2.7). Migration voltage: 15 kV (9–14 μA). Room temperature (22°C). ○ = pH 7; △ = pH 5; ● = pH 2.7.

In the range of lower CTAC concentrations (section I), the electroosmotic mobility depends on the pH of the background electrolyte solution because the electroosmotic mobility is mainly governed by the degree of ionization of the silanol groups on the capillary wall. As the dissociation constant of the silanol groups is about $10^{-5.3}$ in an aqueous solution [16], the degree of dissociation of the silanol groups should vary widely in the pH range 3–7. It is considered that the adsorption of the surfactants on the capillary wall can be attributed to the electrostatic interaction between CTA^+ and the dissociated silanol groups at lower CTAC concentrations [17].

In section II, the electroosmotic mobility changes considerably and reversed electroosmotic flow is observed. The electroosmotic mobility in this region still shows a dependence on pH. This region corresponds to the portion of hemimicelle formation reported by Fuerstenau and both the electrostatic interaction and Van der Waals attraction contribute to the adsorption of CTAC. The excess positive charges of the adsorbing CTAC with the formation of hemimicelles causes the reversal of the electroosmotic flow. On the other hand, the reversed electroosmotic mobility in sections III and IV is no longer affected by pH. This suggests that the adsorption of the surfactant ions in this region should result from Van der Waals attraction rather than coulombic force.

Fuerstenau and co-workers [14] calculated the Van der Waals energy per methylene group between adjacent chains of adsorbed surfactant, ϕ, from the measurement of the zeta potential of quartz. This value is in agreement with the values for the formation of the micelle in a bulk solution. The evidence is substantially in favour of the hemimicelle theory. We have previously

reported the effect of cationic surfactants having different alkyl chain lengths ($n = 10$, 12, 14) on the electroosmotic mobility [18]. The value of ϕ obtained from our results [from the slope of the plot of carbon number of the alkyl chain *versus* the logarithm of the concentration of the surfactants at zero electroosmotic mobility (C_0)] is $1.05kT$, and this value is in excellent agreement with Fuerstenau's results. This provides proof of the formation of hemimicelles on the capillary wall, although the conditions in the previous work were different from those in this study. A schematic illustration of hemimicelle formation on the capillary wall is shown in Fig. 2. Initially, cationic surfactant ions adsorb on the capillary wall as individual ions and neutralize the charge of the capillary wall. Then the adsorbing cationic surfactants begin to associate into two-dimensional patches of ions and form hemimicelles. Accompanied by the hemimicelle formation, the electroosmotic mobility changes considerably. When the net charge on the capillary wall changes from negative to positive owing to the adsorption of excess cationic surfactants due to the formation of hemimicelles, reversal of the electroosmotic flow occurs.

The effect of the cationic surfactant on the electrophoretic mobility of solutes was investigated. We first studied the migration behaviour of some organic acids as test solutes. The effective electrophoretic mobilities of the acidic solutes depend on the pH of the background electrolyte based on the acid dissociation equilibria. The effective electrophoretic mobility was obtained by subtracting the electroosmotic mobility from the observed electrophoretic mobility. The acid dissociation constants of these organic acids were evaluated according to the linear model of Khaledi *et al.* [19] as shown in Table I. These values are near 4.5 and some values agree with the literature values [20]. The order of the migration velocity at pH 4.6 (hydrocinnamic acid > 2-propanoic acid = cinnamic acid > tropic acid > benzoic acid) was the same as that of the dissociation constants. At pH 7.0, where all solutes should exist in the dissociated form, the order of the migration velocity was tropic acid > 2-propanoic acid = hydrocinnamic acid > cinnamic acid > benzoic acid, which is in agreement with the order of their molecular masses. We considered that these organic acids were appropriate model solutes to study the interaction with CTAC.

Fig. 3 shows the dependence of the effective electrophoretic mobility of benzoic acid on CTAC concentration. The pH of 7.0 of the background electrolyte guarantees that benzoic acid is in the dissociated form. An interesting behaviour was observed in the range of CTAC concentrations from 0 to 25 mM. The curve showing the relationship between CTAC concentration and the effective electrophoretic mobility of benzoic acid also possesses four distinct sec-

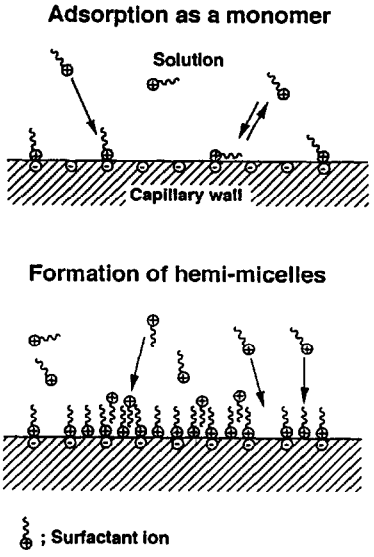

Fig. 2. Schematic illustration of hemimicelle formation on the capillary wall.

TABLE I

ACID DISSOCIATION CONSTANTS (pK_a) OF ORGANIC ACIDS

Compound	pK_a	Lit. [19]
Tropic acid	4.2	4.12
Benzoic acid	4.2	4.204
2-Phenylpropanoic acid	4.5	4.38
Hydrocinnamic acid	4.7	4.664 (35°C)[a]
Cinnamic acid	4.5	4.438

[a] Temperature specified for one value only.

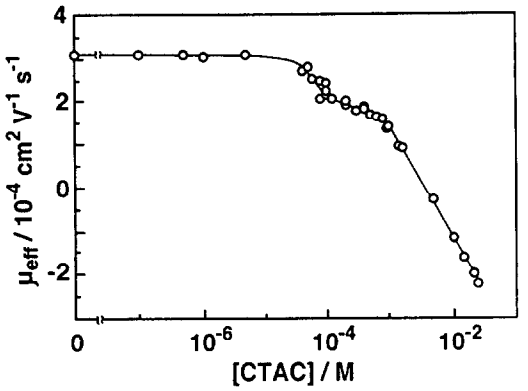

Fig. 3. Effect of CTAC concentrations on the electrophoretic mobility of benzoate ion. Buffer: 10 m*M* phosphoric acid–20 m*M* Tris–citric acid (pH 7.0). Other conditions as in Fig. 1.

tions: (I) a portion section where there is no change in the effective electrophoretic mobility, (II) a section where the electrophoretic mobility decreases considerably, (III) a section where the slope is gentle and (IV) a section where the effective electrophoretic mobility decreases rapidly again. We can relate this behaviour to the dependence of the electroosmotic mobility on CTAC concentration. In section I, CTAC behaves as a monomer individually at the surface of the capillary wall and in the bulk solution. There is only the ion association equilibria between the solutes and the cationic surfactants in this section. The interaction is not so strong that the change in the electrophoretic mobility of benzoic acid is very small in this section. In section II, where the surfactants form hemimicelles on the capillary wall, benzoate ions interact strongly with the hemimicelles and the electrophoretic mobility decreases considerably. As the second layer does not form in section III as mentioned above, the interaction with hemimicelles is held constant in this portion. However, the effective electrophoretic mobility of benzoate ion decreases slightly owing to the ion association equilibria between benzoate ions and surfactant monomers in the bulk solution. In section IV, the effective electrophoretic mobility decreases considerably again because benzoate ions begin to interact with the micelles in the bulk solution.

The migration behaviour of anions under ion association equilibrium below the cmc could be presented by the following equation [11]:

$$\frac{1}{\mu_{eff}} = \frac{K_{IA}}{\mu_{ep}} \cdot C_{sf} + \frac{1}{\mu_{ep}} \quad (1)$$

where μ_{eff} is the effective electrophoretic mobility, K_{IA} is the ion association constant between the anion and CTAC and C_{sf} is the CTAC concentration. The curves of C_{sf} *vs.* μ_{eff}^{-1} for benzoic acid and tropic acid are shown in Fig. 4. Two clear inflection points can be seen. In the solution without CTAC, the electrophoretic mobility of benzoate ion is larger than that of tropate ion because the molecular mass of benzoic acid is smaller than that of tropic acid. At CTAC concentrations, >0.1 m*M* (the first inflection point), the effective electrophoretic mobility of benzoate ion becomes smaller than that of tropate ion because benzoate ion interacts more strongly than tropate ion with hemimicelles of CTAC. μ_{eff}^{-1} increases linearly on the basis of the ion association equilibria until the CTAC concentration reaches the cmc in the bulk solution. The second inflection point indicates the cmc of CTAC in the bulk solution under this experimental conditions. Beyond this point, μ_{eff}^{-1} increases considerably owing to the interaction with the micelles formed in the bulk solution. The slope of the μ_{eff}^{-1} *vs.* C_{sf} line provides the ion association constant between organic anions and CTAC on the basis of eqn. 1. Fortunately, there is little change in the electroosmotic mobility

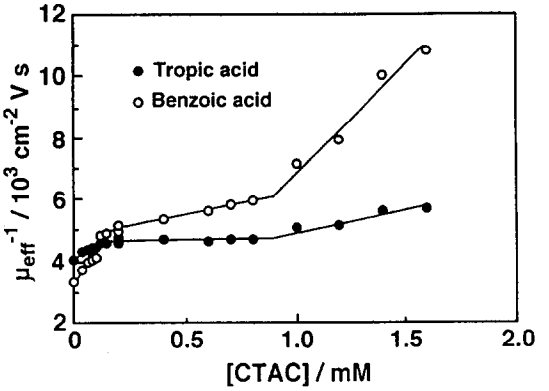

Fig. 4. C_{sf} *vs.* μ_{eff}^{-1} plots for (○) benzoate and (●) tropate ions. Conditions as in Fig. 3.

within the range of concentrations 0.2–1.0 m*M* (section III in Fig. 1). Hence we can determine the conditional ion association constants directly from the slope of the line plotted within this limited concentration range. The values obtained were 280 l mol^{-1} for benzoate ion, 280 l mol^{-1} for 2-phenylpropanoate ion and 350 l mol^{-1} for hydrocinnamate ion. The slope for tropate ion was so small that we could not estimate it. The intercept of the C_{sf} *vs.* $\mu_{eff^{-1}}$ line gives the effective electrophoretic mobility of the anion interacting with hemimicelles.

The effective electrophoretic mobility of the anion changes notably even at very low concentrations. The change in the electrophoretic mobility of benzoic acid and tropic acid resulting from the interaction with the hemimicelles (below the first inflection point in Fig. 4) has the almost same slope as that with the micelles in the bulk solution (beyond the second inflection point in Fig. 4). It is speculated that the interaction with the hemimicelles will be similar to that with the micelles in the bulk solutions and the interaction may consist of both hydrophobic interaction and electrostatic interaction.

Chromatograms of organic anions (A) without and (B) with 0.08 m*M* CTAC are shown in Fig. 5. The analyte ions migrate to the cathode in Fig. 5A, whereas they migrate to the anode in Fig. 5B. It is notable that in spite of the much lower concentration of CTAC than the cmc, the migration order in Fig. 5B is different from that in Fig. 5A. As cinnamic acid has the strongest interaction with hemimicelles, the peak shows serious tailing.

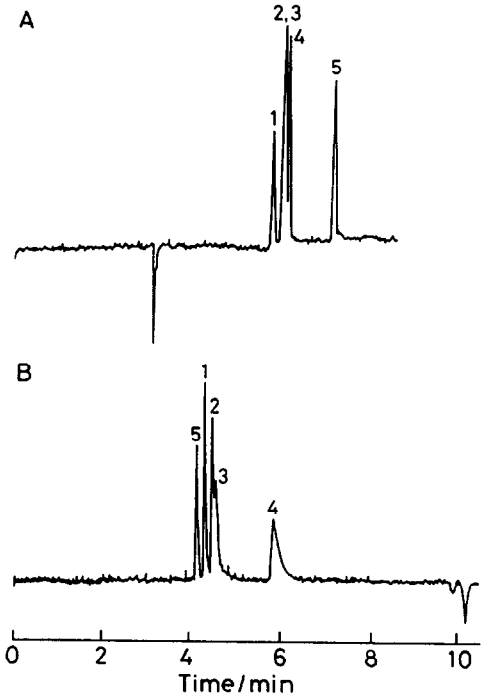

Fig. 5. Chromatograms of organic anions (A) without CTAC and (B) with 0.08 m*M* CTAC. Peaks: 1 = tropic acid; 2 = 2-phenylpropanoic acid; 3 = hydrocinnamic acid; 4 = cinnamic acid; 5 = benzoic acid. Buffer: 10 m*M* phosphoric acid–20 m*M* Tris–citric acid (pH 7.0). Migration voltage: 15 kV (9 μA). Other conditions as in Fig. 1.

CONCLUSIONS

The electroosmotic mobility was greatly influenced by a cationic surfactant and showed four stepwise changes, including reversal, with increasing CTAC concentration. The alternation of the electroosmotic mobility can be interpreted by the adsorption of CTAC on the capillary wall due to both the electrostatic interaction and Van der Waals attraction. At very low concentrations, CTAC adsorbed as individual ions by the electrostatic interaction between CTAC and silanol groups on the capillary wall, then it began to associate into hemimicelles by Van der Waals attraction between the hydrophobic carbon chains of CTAC. The reversed electroosmotic mobility can be explained by the excess positive charges of adsorbing CTAC on the capillary wall. The formation of hemimicelles facilitates the adsorption of CTAC beyond the amount required to neutralize negative charges of silanol on the capillary wall. The electrophoretic mobilities of organic anions also showed a similar behaviour to that of the electroosmotic mobility, reflecting the state of the capillary wall. On increasing the CTAC concentration, the electrophoretic mobilities of organic anions decrease as a result of the interaction of ion association with CTAC monomers, the interaction with hemimicelles and the interaction with the micelles in the bulk solution. The interaction with monomer and hemimicelles of a cationic surfac-

tant such as CTAC could provide a unique separation selectivity in the CZE and MECC separation of anions and also the interaction with the micelles in a bulk solution.

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