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Capillary electrophoretic studies on the migration behavior of cationic solutes and the influence of interactions of cationic solutes with sodium dodecyl sulfate on the formation of micelles and critical micelle concentration

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Abstract

The migration behavior of cationic solutes and influences of the interactions of cationic solutes with sodium dodecyl sulfate (SDS) on the formation of micelles and its critical micelle concentration (CMC) were investigated by capillary electrophoresis at neutral pH. Catecholamines and structurally related compounds, including epinephrine, norepinephrine, dopamine, norephedrine, and tyramine, which involve different extents of hydrophobic, ionic and hydrogen-bonding interactions with SDS surfactant, are selected as cationic solutes. The dependence of the effective electrophoretic mobility of cationic solutes on the concentration of surfactant monomers in the premicellar region provides direct evidence of the formation of ion-pairs between cationic solutes and anionic dodecyl sulfate monomers. Three different approaches, based on the variations of either the effective electrophoretic mobility or the retention factor as a function of surfactant concentration in the premicellar and micellar regions, and the linear relationship between the retention factor and the product of a distribution coefficient and the phase ratio, were considered to determine the CMC value of SDS micelles. The suitability of the methods used for the determination of the CMC of SDS with these cationic solutes was discussed. Depending on the structures of cationic solutes and electrophoretic conditions, the CMC value of SDS determined varies in a wide concentration range. The results indicate that, in addition to hydrophobic interaction, both ionic and hydrogen-bonding interactions have pronounced effects on the formation of SDS micelles. Ionic interaction between cationic solutes and SDS surfactant stabilizes the SDS micelles, whereas hydrogen-bonding interactions weakens the solubilization of the attractive ionic interaction. The elevation of the CMC of SDS depends heavily on hydrogen-bonding interactions between cationic solutes and SDS surfactant. Thus, the CMC value of SDS is remarkably elevated with catecholamines, such as epinephrine and norepinephrine, as compared with norephedrine. In addition, the effect of methanol content in the sample solution of these cationic solutes on the CMC of SDS was also examined.

Keywords: Critical micelle concentration; Capillary electrophoresis; Micellization; Sodium dodecyl sulfate; Catecholamines; Cationic solutes

1. Introduction

Capillary electrophoresis (CE) is a powerful technique for the separation of a variety of analytes owing to the advantages of high efficiency, high resolution, rapid analysis, and very small volume of sample [1–3]. Capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) are the two most widely used separation modes of this technique. The separation principle of CZE is based on the difference of the electrophoretic mobility of analytes, whereas that of MEKC is based on the differential partitioning of analytes between the micellar and aqueous phases. Ionic analytes can be separated by both CZE and MEKC, while MEKC is commonly employed for the separation of non-ionic compounds. However, the separation of non-ionic compounds can occasionally be achieved by CZE when using a background electrolyte (BGE) containing an ionic surfactant at concentrations below the critical micelle concentration (CMC), provided that the selective interactions between neutral analytes and ionic surfactant monomers occur [4,5].

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Depending on the structures of analytes, various interactions, such as hydrophobic, ionic, and hydrogen-bonding interactions, may occur between the analytes and micelles. In the separation of non-ionic hydrophobic compounds by MEKC, hydrophobic interaction between the analytes and micelles is the predominant factor to govern the partitioning of analytes between the micellar and aqueous phases, whereas in the separation of ionic compounds, ionic interaction between the analytes and micelles becomes important, together with hydrophobic interactions, because ionic interaction can enhance the dissolution of cationic solutes into the anionic micelles drastically [6–8]. In the separation of ionic compounds with hydrophilic functional groups, such as cationic hydrogen-donating solutes, hydrogen-bonding interaction between the cationic hydrogen-donating solutes and anionic micelles may also affect the partitioning of cationic solutes into the micelles. It has been demonstrated that hydrogen-bonding interaction between the cationic solutes and mixed micelles composed of SDS and a non-ionic surfactant possessing polyether moieties, such as Tween 20 (polyoxyethylene sorbitan monolaurate) [6,9] or Brij 35 (polyoxyethylene (23) lauryl ether) [9], reduces the ionic interaction between the cationic analytes and SDS micelles considerably. Consequently, the selectivity and resolution of MEKC separations, can be optimized by varying the molar ratio of mixed micelles. As SDS micelles possess hydrogen bond donating characteristics, in addition to the predominant hydrophobic characteristics, it is thought that cationic solutes possessing hydrogen bond accepting characteristics are accessible to SDS micelles [8].

The effects of adding surfactants in a BGE in CZE are multiple. Surfactant monomers can interact with the wall surface of the capillary, and thus, alter the magnitude and the polarity of the electroosmotic flow (EOF) [10–15]; they can selectively interact with sample analytes and/or BGE components so that the selectivity and separation can be improved [16–23]; they may also change the viscosity of the BGE, thus affecting the magnitude of the EOF. On the other hand, the operation of MEKC separation requires a BGE solution containing one or more surfactants as micelle forming agents at concentrations above the CMC [24,25]. Consequently, the selectivity of analytes can be controlled and optimized with the use of various surfactants or mixed surfactants. As the micellization of a surfactant and the modification of the micellar phase can be reflected from the effective electrophoretic mobility of some appropriately selected solutes as a function of surfactant concentration, CE is a very useful tool to study the migration behavior of solutes and the interactions of solutes with both surfactant monomers and the micelles. In viewing of many advantageous applications of CE and the micellization process being a key parameter in the optimization of analytical conditions in CE, particularly in MEKC, a good understanding of the micellization of a surfactant is of fundamental importance.

CE has been proven to be a convenient technique to determine the CMC of a surfactant under the operating conditions of an electrophoretic system [25–36]. So far, three different approaches based on CE techniques have been proposed. The first method is based on the linear relationship of the retention factor of a solute with micelle concentration, using MEKC [25,26]. The second method is based on the variation of the effective electrophoretic mobility of a marker compound as a function of surfactant concentration in the premicellar and micellar regions. By plotting the effective electrophoretic mobility of a marker compound against surfactant concentration, a sharp change in slope can be observed at the CMC [27–32,34–36]. The third method is based on the measurements of the electric current of micellar electrolyte solutions as a function of surfactant concentration, using CE instrumentation at a given applied voltage [33]. This approach essentially consists of a CE version of the traditional method of measuring the CMC value by conductivity.

It has been demonstrated that, for some neutral and anionic hydrophobic solutes, the plots of k versus surfactant concentration can give straight lines almost passing through the same intercept with the slope increasing with the hydrophobicity of the solutes. The CMC value of a surfactant can be determined from the slope and the intercept of the line [25,26]. It has been known that a stronger hydrophobic interaction between the solute and micelles may yield a smaller error in the determination of the CMC value [25]. However, for solutes involving more complicated interactions, the CMC value determined by this method is not very reliable [8,25,37]. The drawback of the third method is that the slopes of the straight lines corresponding to the premicellar and micellar states of a surfactant in the two concentration ranges may not differ significantly so that the CMC value can not be unambiguously determined [33]. Moreover, as the current variation detected by a CE instrument is usually very small, the precision of this method is not very good [33]. Therefore, the method based on the mobility model is the method of choice for the determination of the CMC of a surfactant under the operating conditions of an electrophoretic system by CE technique.

The CMC value of a surfactant is not only affected by the operating conditions of an electrophoretic system and the nature of the micellar buffer electrolyte [34], including the nature of the surfactant, the type and the composition of the electrolyte solution, the presence of organic modifier and electrolyte modifiers, but also affected by the nature and the structures of solutes [31–34]. It is worthy to note that the CMC value of a surfactant may vary considerably from one solute to the other when the interactions between the solutes and micelles are very different [31].

It was previously reported that the CMC values of SDS determined by the linear retention model with ephedrine, norephedrine, epinephrine and norepinephrine as marker compounds, were 1.3, 3.1, 5.1, and 9.2 mM, respectively, in a phosphate buffer (50 mM) at pH 7.0 and 40 °C [8]. The CMC values determined with these cationic solutes varied greatly from one solute to the other. However, the reasons behind this phenomenon have never been discussed. Besides, the exper-

imental data near the CMC provided by the authors were insufficient. Therefore, further study is certainly needed in order to find out the accuracy of these values and the suitability of the method used.

The formation of ion-pairs between cationic solutes and anionic surfactant monomers is a controversial issue. The formation of ion-pairs was suggested [8]. However, the independence of the electrophoretic mobility of epinephrine and its precursors on the SDS concentration in the premicellar region was previously reported [6]. As these cationic solutes involve different extents of ionic and hydrogen-bonding interactions with the SDS monomers, it seems that the ion-pair formation between the cationic solutes and SDS monomers may not be ruled out.

Catecholamines and the structurally related compounds, including norepinephrine, epinephrine, dopamine, norephedrine, and tyramine are selected as cationic solutes. The structures of these cationic solutes are shown in Fig. 1. In this study, the dependence of the migration of these cationic solutes on the concentration of SDS in the premicellar and micellar regions is examined. Moreover, the CMC values of SDS micelles solubilized by the selected cationic solutes are determined by CE and the influence of the interactions between cationic solutes and SDS surfactant on the formation of micelles and the CMC are studied. Furthermore, the suitability of the method based on the linear retention model for the determination of the CMC value of SDS with these cationic solutes is discussed.

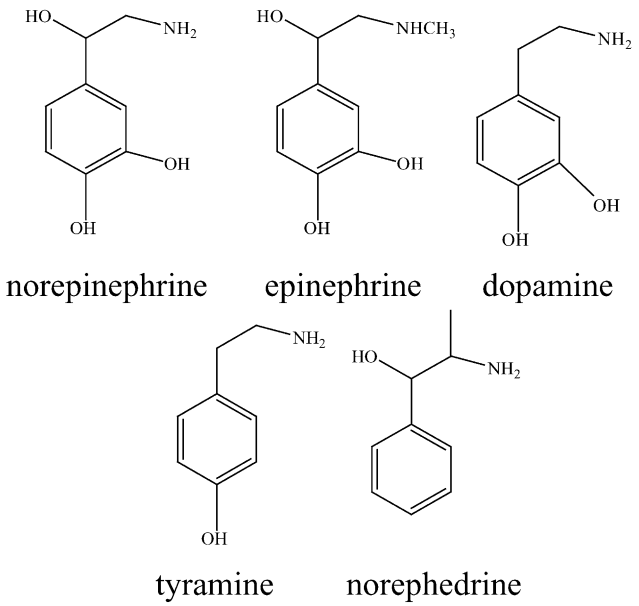

Fig. 1. The structures of the five cationic solutes studied.

2. Experimental

2.1. Apparatus

All CE separations were performed on a Beckman P/ACE System MDQ equipped with a photodiode array detector for absorbance measurements at 214 nm (Beckman Coulter, Fullerton, CA, USA). Uncoated fused-silica capillaries purchased from Polymicro Technologies (Phoenix, AZ, USA) were used. The dimensions of the capillary were 57 cm × 50 μm i.d. The effective length of the capillary was 50 cm from the injection end of the capillary. The CE system was interfaced with a microcomputer and a laser printer. System Gold software of Beckman was used for data acquisition. For pH measurements, a pH meter (Suntex Model SP-701, Taipei, Taiwan) was employed with a precision of ±0.01 pH unit.

2.2. Chemicals and reagents

Epinephrine, norepinephrine, dopamine, and tyramine were obtained from Sigma (St. Louis, MO, USA). Norephedrine hydrochloride was purchased from Aldrich-Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA. USA).

Standard solutions of epinephrine, norepinephrine and tyramine at a concentration of 20 μg/mL and that of norephedrine at concentrations of 80 μg/mL were prepared by dissolving analytes in an aqueous solution containing 2–10% (v/v) methanol. The pH of a phosphate buffer was adjusted to the desired pH value by monitoring the pH of the electrolyte solution with a pH meter while mixing various proportions of 50 mM disodiumhydrogenphosphate solution with the same concentration of phosphoric acid. All buffer solutions, freshly prepared weekly and stored in a refrigerator before use, were filtered through a membrane filter (0.22 μm).

2.3. Electrophoretic procedure

When a new capillary was used, the capillary was washed 30 min with 1.0 M NaOH solution, followed by 30 min with deionized water at 25 °C. Before each injection, the capillary was prewashed for 3 min with running buffer. After each injection, the capillary was postwashed for 3 min with deionized water, 3 min with 0.1 M NaOH, and 5 min with deionized water to maintain proper reproducibility of run-to-run injections. Sample injections were done in a hydrodynamic mode over 5 s under a pressure of 1.0 psi at 25 °C. Each sample analyte was injected individually. The measurements were run at least in triplicate to ensure reproducibility. A voltage of 20 kV was applied to keep the total current less than 70 μA. The detection wavelength was set at 214 nm. Peak identification was conducted by spiking with the analyte to be identified. Methanol was used as a neutral marker. The relative standard deviation of migration time is less than 0.6% (n = 5).

2.4. Mobility calculations

The effective electrophoretic mobility of analytes was calculated from the observed migration times with the equation:

$$\mu_{ep} = \mu - \mu_{eo} = \frac{L_d L_t}{V}\left(\frac{1}{t_m} - \frac{1}{t_{eo}}\right) \quad (1)$$

where μ_{ep} is the electrophoretic mobility of the analyte tested, μ is the apparent mobility, μ_{eo} is the electroosmotic mobility, t_m is the migration time measured directly from the electropherogram, t_{eo} is the migration time for an uncharged solute, L_t is the total length of capillary, L_d is the length of capillary between injection and detection, and V is the applied voltage.

3. Results and discussion

3.1. Methodological approaches of CMC determination

3.1.1. Method based on the mobility model

As in the case of a negative charged solute interacting with cationic surfactant monomers reported previously [30,31], the effective electrophoretic mobility of a positively charged solute (A^+) interacting with anionic surfactant monomers in the premicellar concentration region and partitioning in anionic micelles in the micellar region can be described, respectively, by the following equations:

$$\mu_{eff} = \frac{\mu_{A^+} + K_{A^+S}[S]\mu_{A^+S}}{1 + K_{A^+S}[S]} \quad \text{(below the CMC)} \quad (2)$$

and

$$\mu_{eff} = \frac{\mu_{A^+} + K_{A^+S}(\text{CMC})\mu_{A^+S} + K_{A^+M}[M]\mu_{mc}}{1 + K_{A^+S}(\text{CMC}) + K_{A^+M}[M]} \quad \text{(above the CMC)} \quad (3)$$

where μ_{A^+} is the electrophoretic mobility of a cationic solute, K_{A^+S} and μ_{A^+S} are the binding constant and electrophoretic mobility, respectively, of the ion-pair adducts, K_{A^+M} is the binding constant of a charged solute to the micelles, [S] the concentration of surfactant monomers, [M] the micelle concentration which is equal to (C_T − CMC)/n, where n is the aggregation number of the micelles and C_T is the total surfactant concentration.

In the case of complete complexation, K_{A^+S} is extremely large. Thus, $K_{A^+S}[S] \gg 1$ and μ_{eff} in Eq. (2) is essentially equal to zero when the concentration of surfactant monomer is close to or equal to the CMC. Consequently, Eq. (3) can be simplified to,

$$\mu_{eff} = \frac{K_{A^+M}[M]\mu_{mc}}{1 + K_{A^+S}(\text{CMC}) + K_{A^+M}[M]} \quad \text{(above the CMC)} \quad (4)$$

The CMC of the anionic surfactant can then be determined from the plot of μ_{eff} versus surfactant concentration at a particular surfactant concentration where a dramatic change in slope can be observed in the negative mobility region. This particular concentration is a good indication of the CMC of a surfactant. Alternately, the CMC of a surfactant can be precisely determined from the simulation of the mobility curve according to Eq. (4).

3.1.2. Method based on the retention model

In MEKC, the effective electrophoretic mobility of a neutral solute (μ_{eff}) can be described as:

$$\mu_{eff} = \mu_{mc}\frac{k}{(1+k)} \quad (5)$$

where k is the retention factor of the solute, μ_{mc} the mobility of the micellar phase, and $k/(1 + k)$ represents the mole fraction of the solute in the micellar phase. Eq. (5) can be rearranged and expressed as [26]:

$$k = \frac{\mu_{eff}}{(\mu_{mc} - \mu_{eff})} \quad (6)$$

In CE, the effective electrophoretic mobility of a solute is related to the migration times by Eq. (1). For a cationic solute with an anionic surfactant, an equilibrium is involved due to ion-pairing interaction between the cationic solute and surfactant monomers. In the case of complete complexation, k can be given by Eq. (6) [6]. Hence, the variation of k as a function of surfactant concentration is expected and the CMC of a surfactant can be determined from the plot of k versus surfactant concentration when micelle concentration is equal to zero.

3.1.3. Method based on the linear retention model

On the other hand, k can be linearly releated to the partition coefficient of a solute between the micellar and aqueous phase (P_{mw}) and the phase ratio which can be expressed as $\tilde{v}$ (C_T − CMC) with surfactant molecules at concentrations near the CMC [25]:

$$k = P_{mv}\tilde{v}(C_T - \text{CMC}) \quad (7)$$

where $\tilde{v}$ is the molar volume of SDS micelles, which is 0.2483 M^{-1} [38]. By plotting k against C_T, the CMC of a surfactant can be determined from the slope and intercept of the straight line.

3.2. Migration behavior of cationic solutes

Despite that the formation of ion-pairs between anionic surfactant monomer and cationic solutes, such as norepinephrine and epinephrine was suggested [8], the formation of ion-pairs was ruled out by Esaka et al. [6] because the dependence of the electrophoretic mobility on SDS concentration was not observed. We are curious to find out whether the migration of the cationic solutes studied depends on the SDS concentration or not.

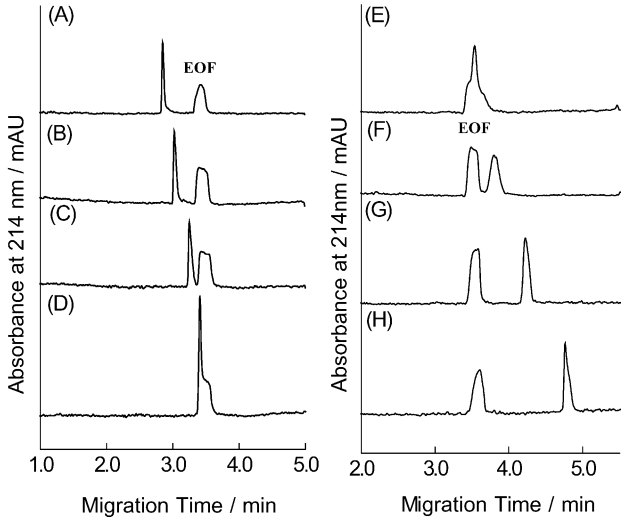

Fig. 2. Electropherograms of epinephrine obtained with a sample solution containing 2% (v/v) methanol and with addition of SDS surfactant at concentrations of: (A) 4.0; (B) 6.0; (C) 8.0; (D) 10.0; (E) 12.0; (F) 14.0; (G) 18.0; (H) 25.0 mM, in a phosphate buffer (50 mM) at pH 7.0. Capillary, 57 cm × 50 μm, i.d.; sample concentration, 20 μg/mL; detection wavelength, 214 nm. Other operating conditions, 20 kV, 25 °C.

3.2.1. Epinephrine

Fig. 2 shows some of the electropherograms of epinephrine obtained with a sample solution containing 2% (v/v) methanol, using a phosphate buffer (50 mM) containing SDS surfactant at varied concentrations at pH 7.0. Fig. 3A shows the variations of the electrophoretic mobility of epinephrine obtained. In the presence of SDS surfactant at concentrations less than 2 mM, epinephrine migrated as a cationic solute under the operating conditions and exhibited only little complexation with anionic dodecyl sulfate monomers. Thus, the analyte peak appeared before a neutral electroosmotic flow (EOF) marker in the electropherogram. In the presence of surfactant monomers at concentrations greater than 2 mM, the analyte peak appeared in the electropherogram at a position closer and closer to the EOF marker as the concentration of surfactant monomers

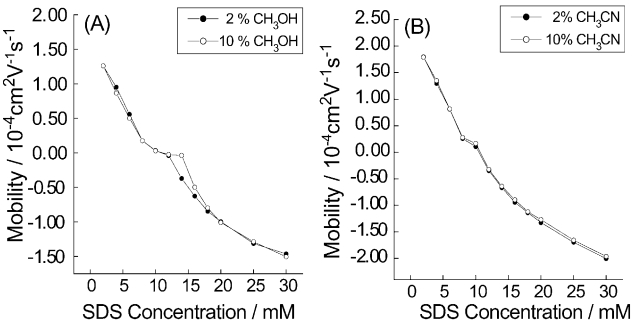

Fig. 3. Variations of the effective electrophoretic mobility of epinephrine as a function of SDS concentration in the range 2–30 mM, using a phosphate buffer (50 mM) at pH 7.0 with a sample solution containing: (A) 2% (●) and 10% (○) (v/v) methanol and (B) 2% (●) and 10% (○) (v/v) acetonitrile. Other operating conditions are the same as for Fig. 2.

increased (Fig. 2A–D). As shown in Fig. 3A, the effective electrophoretic mobility of epinephrine decreased markedly from $1.26 \times 10^{-4}\,cm^2\,V^{-1}\,s^{-1}$ to zero with increasing SDS concentration from 2 to 10 mM and the dependence of the effective electrophoretic mobility of epinephrine as a function of SDS concentration in this region was observed. This phenomenon provides direct evidence of the formation of ion-pairs between cationic epinephrine and anionic dodecyl sulfate monomers. Interestingly, with addition of SDS surfactant at about 10–11 mM, a sharper peak could be observed in the electropherogram when sample analyte was detected at 214 nm (Fig. 2D) as compared with the one shown in Fig. 2C. This sharp peak is attributed to the formation of ion-pairs between the cationic solute and anionic dodecyl sulfate monomers because the electrophoretic mobility of this species is nearly equal to zero. The appearance of this peak also indicates that the concentration of surfactant monomers is close to the CMC. Upon addition of SDS at 12 mM, the SDS micelles were formed and the analyte which was solubilized into the SDS micelles could be affirmatively detected (Fig. 2E) and the analyte solubilized in the SDS micelles migrated as an anion. Thus, the analyte peak appeared after the EOF marker (Fig. 2F–H) and the electrophoretic mobility of epinephrine (migrating toward the anode) increased with further increasing the concentration of SDS micelles from 12 to 30 mM (Fig. 3A).

The variation of the electrophoretic mobility of epinephrine obtained with the analyte dissolved in a sample solution containing 10% (v/v) methanol is also shown in Fig. 3A. As methanol interacts with epinephrine through hydrogen bonding, consequently, the methanol content in the sample solution may affect the formation of SDS micelles. When using a sample solution containing 10% (v/v) methanol, the sharp peak attributed to the formation of ion-pairs appeared in the electropherogram at about 10–13 mM, thus an elevation of the CMC of SDS is expected. The results of the present study suggest that hydrogen-bonding interaction between epinephrine and methanol in the sample solution may compete with the interaction between epinephrine and SDS monomers in the buffer solution, thus reducing the interactions of the analyte with SDS monomers.

In order to add further support, capillary electrophoretic experiments were performed with epinephrine dissolved in a sample solution containing acetonitrile under the same electrophoretic conditions. As the migration behavior of epinephrine dissolved in a sample solution containing acetonitrile is similar to that of epinephrine dissolved in a sample solution containing methanol. The sharp peaks attributed to the ion-pair adduct formed between epinephrine and SDS monomers appeared in the electropherograms in a narrow SDS concentration range (9–10 mM). For comparison, the variations of the electrophoretic mobility of epinephrine as a function of SDS concentration with a sample solution containing acetonitrile were shown in Fig. 3B. No significant difference in the electrophoretic mobility of epinephrine obtained with the analyte dissolved in 2 and 10% (v/v) acetoni-

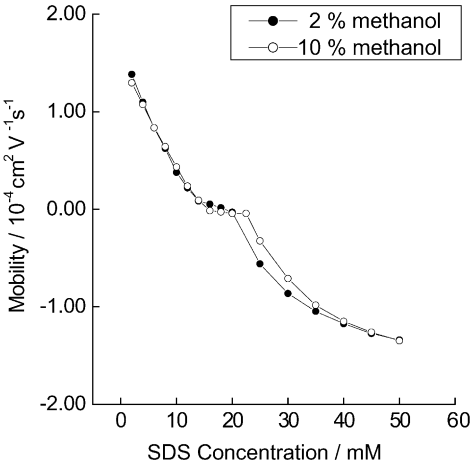

Fig. 4. Variations of the effective electrophoretic mobility of norepinephrine as a function of SDS concentration in the range 2–50 mM with the analyte dissolved in a sample solution containing 2% (●) and 10% (◯) (v/v) methanol, using a phosphate buffer (50 mM) at pH 7.0. Other operating conditions are the same as for Fig. 2.

trile solutions was observed. Presumably, this is due to the absence of hydrogen-bonding interaction between epinephrine and acetonitrile in the sample solution.

3.2.2. Norepinephrine

Fig. 4 illustrates the variations of the electrophoretic mobility of norepinephrine as a function of SDS concentration, using a phosphate background electrolyte (50 mM) containing SDS at pH 7.1 with sample solution containing 2 and 10% (v/v) methanol. Fig. 5 shows some electropherograms of norepinephrine obtained with a sample solution containing 2% (v/v) methanol. The migration behavior of norepinephrine, observed under the same electrophoretic conditions, is similar to that of epinephrine observed, except that the electrophoretic mobility of norepinephrine as a function of SDS concentration varies to a less extent than that of epinephrine. As the extent of the variation of the electrophoretic mobility reflects the magnitude of the binding constant of a cationic solute to SDS surfactant, the results indicate that norepinephrine interacts less strongly than epinephrine with both SDS monomers and SDS micelles. This is consistent with the results reported previously [8]. The distribution coefficients reported were 180 and 110 M^{-1} for epinephrine and norepinephrine, respectively [8]. Similarly, the sharp peak attributed to the ion-pair adduct appeared in the electropherogram with addition of SDS in the concentration ranges of 16–20 and 16–22 mM for norepinephrine dissolved in 2 and 10% (v/v) methanol solution at pH 7.1, respectively. The results reveal that, the formation of SDS micelles is relatively favorable with norepinephrine than with epinephrine under the same electrophoretic conditions. In view of the structural difference between norepinephrine and epinephrine, the additional hydrophobic contribution of the methyl group in the protonated secondary amino group of epinephrine plays a significant role in governing the migration behavior of epinephrine [8].

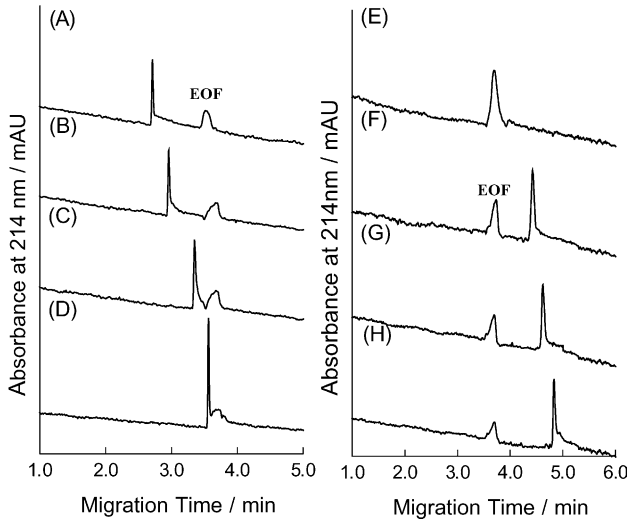

Fig. 5. Electropherograms of norepinephrine obtained with addition of SDS surfactant at concentrations of: (A) 2.0; (B) 6.0; (C) 12.0; (D) 16.0; (E) 20.0; (F) 30.0; (G) 35.0; (H) 40.0 mM, in a phosphate buffer at pH 7.0. Other operating conditions are the same as for Fig. 2.

3.2.3. Norephedrine

As the signal of norephedrine was very weak to be detected at sample concentration of 20 μg/mL, capillary electrophoretic measurements were performed at a higher concentration (80 μg/mL) of norephedrine. Fig. 6 shows the variation of the electrophoretic mobility of norephedrine obtained, using a phosphate buffer (50 mM) containing SDS at varied concentrations in the range 1–12 mM at pH 7.0 with sample solutions containing 2% (●) and 10% (◯) (v/v) methanol. The effective electrophoretic mobility of norephedrine decreased very drastically to a value near zero

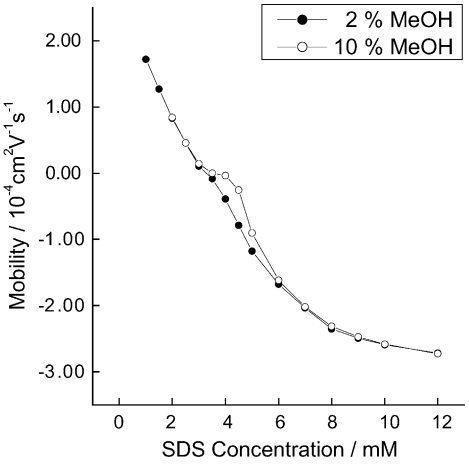

Fig. 6. Variations of the effective electrophoretic mobility of norephedrine as a function of SDS concentration in the range 1–12 mM with the analyte dissolved in a sample solution containing 2% (●) and 10% (◯) (v/v) methanol, using a phosphate buffer (50 mM) at pH 7.0. Other operating conditions are the same as for Fig. 2.

with increasing SDS concentration from 1 to 3 mM. This migration behavior indicates that norephedrine interacts very strongly with SDS monomers. Apparently, a much stronger interaction of norephedrine than those of epinephrine and norepinephrine with SDS monomers occurs. Fig. 7 shows some of the electropherograms of norephedrine obtained with a sample solution containing 2% (v/v) methanol. Again, the dependence of the electrophoretic mobility of norephedrine as a function of SDS concentration in the premicellar region was observed. Interestingly, a sharp peak attributed to the formation of ion-pairs between norephedrine and SDS monomers was observed (Fig. 7C–D) as in the case of norepinephrine and epinephrine, despite that a much broader peak was observed for norephedrine with addition of SDS monomers at 2.5 mM (Fig. 7B). The reasons behind this peculiar behavior are not clearly known. The observation of a sharp peak may probably be resulted from stacking due to the change in the local electrophoretic mobility of analyte molecules at the boundary between sample plug and separation buffer solution because of the formation of ion-pairs, and/or probably due in part to a decrease in the ionic interaction due to the zero charge of the ion-pairs, thus resulting in a reduction of the ionic interaction between norephedrine and the capillary wall, and consequently, the minimization of the adsorption of norephedrine onto the capillary wall. The sharp peak observed is in a rather narrow SDS concentration range 3.0–3.5 mM and the CMC value of SDS determined is about 4.0 mM.

In view of the structural difference between norephedrine and norepinephrine, the results reveal that, due to the lack of catechol moiety in norephedrine, hydrogen-bonding interaction between norephedrine and SDS surfactant is greatly reduced. As a result, the formation of SDS micelles is much easier and the CMC value of SDS becomes much smaller. This is consistent with the results reported previously that the distribution coefficient of norephedrine is much larger than that of norepinephrine [8].

3.2.4. Dopamine and tyramine

For a better understanding of the influence of hydrogen-bonding interaction between the phenolic hydroxyl moiety of hydrophobic cations and SDS surfactant on the micellization of SDS, dopamine and tyramine are also selected as cationic solutes. Fig. 8A and B show the variation of the effective electrophoretic mobility of dopamine and tyramine, respectively, as a function of SDS concentration in the ranges of 2–30 mM and 2–20 mM at pH 7.0 with sample solution containing 2 and 10% (v/v) methanol. The dependence of the electrophoretic mobility of dopamine and tyramine as a function of SDS concentration in the premicellar region was observed. The electrophoretic mobility of dopamine decreased more drastically (migrating toward the cathode) than those of epinephrine and norepinephrine with increasing SDS concentration in the premicellar region and increased more drastically (migrating toward the anode) than those of epinephrine and norepinephrine with increasing SDS concentration in the micellar region. Compared with the results obtained with norepinephrine, the present results clearly reveal that hydrogen-bonding interaction between the hydroxyl group of the alkyl chain of norepinephrine and the SDS surfactant plays an important role in the micellization of SDS. The lack of this hydroxyl group in dopamine resulted in a great reduction in the hydrogen-bonding interaction between dopamine and SDS monomers and micelles. Thus, SDS micelles are greatly stabilized.

As tyramine possesses only one phenolic hydroxyl group, it is expected that the strength of hydrogen-bonding interaction of SDS surfactant with tyramine should be stronger than that with norephedrine, but weaker than that with dopamine. Indeed, as shown in Fig. 8B, the electrophoretic mobility of tyramine (migrating toward the cathode) decreased relatively more drastically than that of dopamine with increasing SDS concentration in the premicellar region and increased

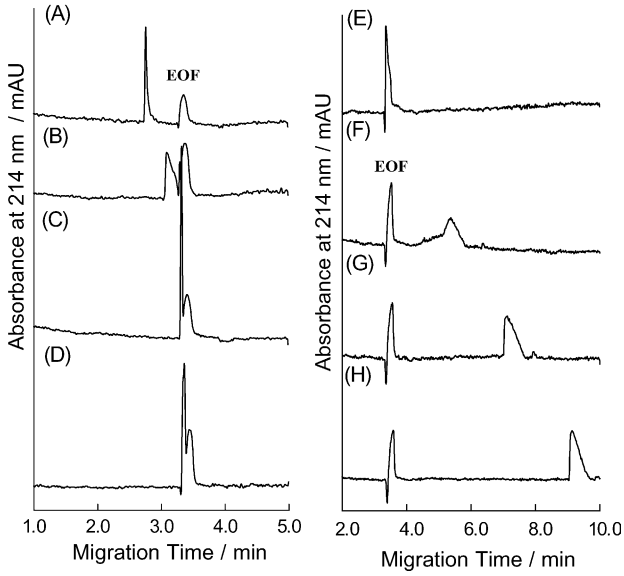

Fig. 7. Electropherograms of norephedrine obtained with addition of SDS surfactant at concentrations of: (A) 2.0; (B) 2.5; (C) 3.0; (D) 3.5; (E) 4.5; (F) 6.0; (G) 8.0; (H) 12.0 mM, in a phosphate buffer (50 mM) at pH 7.0 sample concentration, 80 μg/mL. Other operating conditions are the same as for Fig. 2.

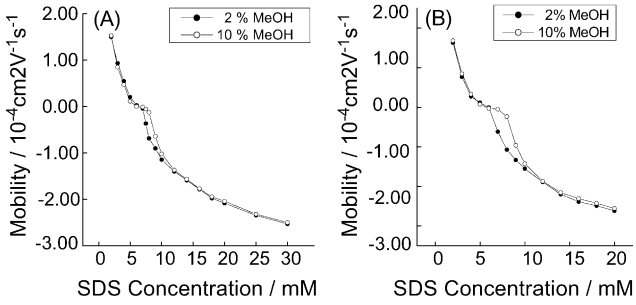

Fig. 8. Variations of the effective electrophoretic mobility of: (A) dopamine and (B) tyramine as a function of SDS concentration in the ranges of 2–30 mM and 2–20 mM, respectively, using a phosphate buffer (50 mM) at pH 7.0 with sample solutions containing 2% (●) and 10% (○) (v/v) methanol. Other operating conditions are the same as for Fig. 2.

relatively more drastically (migrating toward the cathode) than that of dopamine with increasing SDS concentration in the micellar region. Thus, the results provide further evidence that hydrogen-bonding interaction between the phenolic hydroxyl group of cationic solutes and SDS surfactant may weaken the ionic interaction between tyramine and SDS surfactant and retard the formation of SDS micelles, as compared with norephedrine as a cationic solute.

3.3. Determination of the CMC of SDS

3.3.1. Mobility model

As shown in Figs. 3, 4, 6, and 8, the variations of the effective electrophoretic mobility of cationic solutes (in the negative mobility region) as a function of SDS concentration exhibit a dramatic change in slope above a particular SDS concentration at which the cationic solute starts to solubilize in the micelles. This particular concentration is the CMC of SDS with a particular cationic solute as a marker compound. For example, the mobility curves shown in Fig. 3A illustrate that the CMC values of SDS with epinephrine dissolved in 2 and 10% (v/v) methanol solution using a phosphate buffer (50 mM) at pH 7.0 are 12.0 and 14.0 mM, respectively. Similarly, the CMC value of SDS with epinephrine dissolved in both 2 and 10% (v/v) acetonitrile solution at pH 7.0 is 10 mM. The CMC values of SDS can unambiqueously be determined from Figs. 3A, 4, 6, and 8 with these cationic solutes as marker compounds and are listed in Table 1.

3.3.2. Retention model

Eq. (6) was used to calculate the retention factor of analytes. Figs. 9 and 10 show the variations of the retention factor of epinephrine and norepinephrine, respectively, as a function of SDS concentration with sample solutions containing 2 and 10% (v/v) methanol. As illustrated, the retention curve of a selected cationic solute as a function of SDS concentration in the micellar region shown by the solid line deviates from a straight line indicated by the dashed lines and a dramatic change in slope of the retention curve can be observed before and after the occurrence of the solubilization of a selected cationic solute (or a selected ion-pair adduct) in the SDS micelles occurs. Again, the CMC value of SDS can unambiguously be determined from these plots when the micellar concentration is equal to zero. For example, the CMC values of SDS with epinephrine, norepinephrine and norephedrine (dissolved in 10% (v/v) methanol solution) as marker

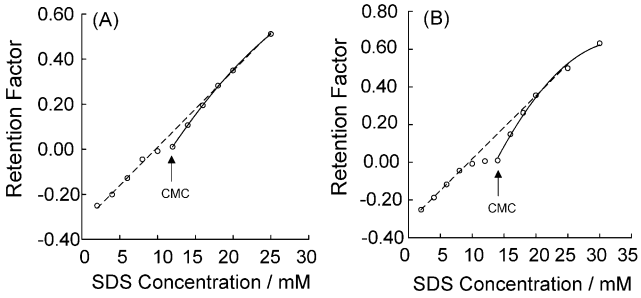

Fig. 9. Variation of the retention factor of epinephrine as a function of SDS concentration with a sample solution containing: (A) 2% and (B) 10% (v/v) methanol. Other operating conditions are the same as for Fig. 2.

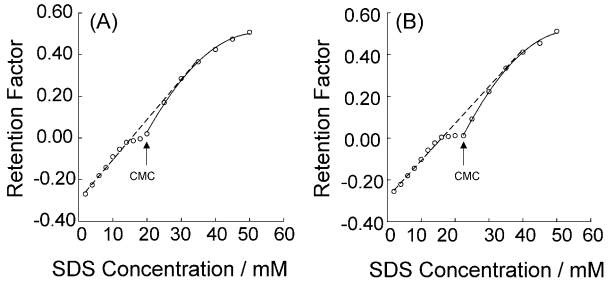

Fig. 10. Variation of the retention factor of norepinephrine as a function of SDS concentration with a sample solution containing: (A) 2% and (B) 10% (v/v) methanol. Other operating conditions are the same as for Fig. 2.

Table 1
The solute-solubilized CMC values of SDS determined[a]

Cationic solute	Methanol content (%)	CMC values of SDS (mM)			
		Mobility model	Retention model	Linear retention model	Literature value[b]
Norepinephrine	2	20.0	20.0	15.0	9.2
	10	22.0	22.0	16.5	
Epinephrine	2	12.0	12.0	9.6	5.1
	10	14.0	14.0	10.0	
Dopamine	2	6.3	6.3	5.2	3.7
	10	7.9	7.9	5.5	
Tyramine	2	6.0	6.0	4.1	–
	10	7.8	7.8	4.3	
Norephedrine	2	3.7	3.7	2.8	3.1
	10	4.3	4.3	3.0	

[a] Electrophoretic conditions: phosphate buffer (50 mM) at pH 7.0 and 25 °C with sample solutions containing 2% or 10% (v/v) methanol solution.
[b] From Ref. [8].

compounds determined are 14.0, 22.0, and 4.3 mM, respectively. The CMC values of SDS determined with this model, which are essentially consistent with the values obtained with mobility model are also given in Table 1.

3.3.3. Linear retention model

It has been known that the retention factor of hydrophobic neutral solutes can be linearly related to the concentration of SDS [25]. As the formation of ion-pairs between a cationic solute and anionic surfactant monomers occurs in a certain range of surfactant concentration, the linear relationship can no longer be observed, if sufficient experimental data in the concentration region near the CMC are obtained. As shown in Figs. 9 and 10, which illustrate the variations of the retention factor as a function of SDS concentration, all of the data points do not fall in a straight line. Thus, a straight line may falsely be obtained if insufficient experimental data are provided, especially in the concentration region near the CMC. For example, by deleting the data point at 12 mM of SDS concentration in Fig. 9A or the data points at 18 and 20 mM of SDS concentration in Fig. 10A, a straight line can be drawn in the plot of the retention factor versus SDS concentration, as observed previously by Strasters and Khaledi [8]. As indicated by the dashed lines in Figs. 9 and 10, the CMC of SDS estimated by the linear retention model occurred at a particular SDS concentration where the ion-pairs between the cationic solute and SDS monomers begin to form. For instance, the CMC values of SDS estimated with this model with norepinephrine dissolved in 2 and 10% (v/v) methanol solutions are 15.0 and 16.5 mM, respectively, and the CMC values determined with norephedrine dissolved in 2 and 10% (v/v) methanol solutions are 2.8 and 3.0 mM, respectively. The CMC values evaluated with this model, together with the CMC data reported previously, are also listed in Table 1. It should be pointed out that the CMC values determined by the retention method of this model deviate from the actual values. Hence, the suitability of the method based on the linear retention model is questioned for the determination of the CMC values of SDS with cationic solutes as marker compounds.

4. Conclusion

The dependence of the effective electrophoretic mobility of cationic solutes on the concentration of SDS monomers provides direct evidence of the formation of ion pairing between cationic solutes and SDS monomers. Hydrophobic interaction, ionic interaction and hydrogen-bonding interaction play important roles in the micellization of SDS. In addition to hydrophobic interaction, both ionic and hydrogen-bonding interactions between cationic solutes and SDS monomers and/or SDS micelles can greatly influence the micellization and consequently the solute-solubilized CMC values of SDS. The increase in the hydrophobic interaction stabilized SDS micelles, thus causing the lowering of the CMC value, whereas the increase in hydrogen-bonding interaction destabilized the micelles, thus resulting in the elevation of the CMC value. The CMC value of SDS can be precisely determined with the use of the mobility model and the retention model. However, the linear retention model is not suitable for the determination of the CMC of SDS with cationic solutes as marker compounds, particularly, with those possessing strong hydrogen-bonding interaction with both SDS monomers and micelles.

Acknowledgement

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