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Discussion

Determination of critical micelle concentration and interactions between cephalosporins and charged surfactants

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In a recent contribution to this journal, Mrestani et al. [1] reported on the characterization of interaction between cephalosporins and charged surfactants using capillary zone electrophoresis (CZE). They proposed a complex-formation model to calculate the aggregation constants and the stoichiometric coefficients between cephalosporins and dodecyltrimethylammonium bromide (DTAB). We disagree on the interpretation of the results obtained and the applicability of the model proposed because these authors erroneously determined the critical micelle concentration (CMC) of DTAB to be 18.103 m*M*, thus leading to a misinterpretation of the interaction between cephalosporins and DTAB. We question the correctness of the CMC value determined by them using conductivity measurements because it is even greater than the value reported in the literature, which is 15 m*M*, measured in pure water at 25°C [2]. It has been proven that capillary electrophoresis (CE) is a convenient and useful technique for the determination of the CMC values of surfactants [3–5]. Thus, in the evolution of the effective electrophoretic mobility as a function of surfactant concentration, a sharp change in slope may be observed at the CMC. Judging from the plots of the variation of electrophoretic mobility of cefazolin, cefotaxime, cephapirin and cefuroxime as a function of DTAB concentration shown in Figs. 2–5 of Ref. [1], we believe that the CMC value of DTAB determined in 20 m*M* phosphate buffer solution in the presence of cephalosporins (with a sample concentration at 500 μg/ml) should be about 10 m*M*. Unfortunately, Mrestani et al. [1] did not realize that micelles formed above this concentration.

In order to check whether the CMC value of DTAB measured by conductivity method by Mrestani et al. [1] is incorrect, we carried out basically the same experiments by measuring the conductivity of the same concentration of phosphate buffer solution containing DTAB at various concentrations ranging from 4.0 to 30.0 m*M* at pH 7.5, using a conductivity meter (Suntex SC-170, Taipei, Taiwan) calibrated with a 0.01 *M* KCl solution to a value of 1413 μS/cm (at 25°C). It was observed that the conductivity of the buffer solution increased linearly from 3.03 to 3.56 mS/cm with increasing DTAB concentration from 4.0 to 12.0 m*M*, then increased also linearly, but to a less extent, from 3.67 to 4.24 mS/cm when DTAB concentration increased from 14.0 to 30.0 m*M*. Thus the CMC of DTAB de-

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termined by conductivity in this buffer electrolyte at pH 7.5 is 13.0 m*M*.

The CE experiments were also performed in order to compare the CMC value of DTAB determined by CE with the addition of cephalosporins as test solutes. Fig. 1 shows the variation of the electrophoretic mobility of cephalosporins (with a sample concentration of 100 μg/ml) as a function of the DTAB concentration in the range 6–30 m*M* at pH 7.5. A sharp change in slope was observed with DTAB concentration at about 11.6±0.1 m*M*. As expected, this sharp change in slope occurs at a relatively greater concentration of DTAB than that obtained by Mrestani et al. [1] because the sample concentration we used is five times smaller than that used by Mrestani et al. [1]. As cephalosporins are present as sodium salts, the ionic strength of the buffer electrolyte in CE experiments due to the presence of anionic test analytes is comparatively higher than that of the buffer solution in conductivity measurements. The fact that a sharp change in slope for the variation of electrophoretic mobility of cephalosporins as a function of DTAB concentration was observed at about 12.0±0.1 m*M* when using a sample concentration of 50 μg/ml clearly indicates that the CMC value is affected by the concentration of ionic solutes. Hence, by extrapolation, the CMC value of DTAB determined by CE in the absence of anionic solutes is expected to be about 12.4 m*M*. This result is quite consistent with the value determined from our conductivity measurements.

Recently, it has been proven that an approach essentially consisting of a CE version of the traditional method of measuring CMC values by conductivity can be used with high confidence [6]. In this approach, the values of the electric current measured at different surfactant concentrations at a given voltage using a CE instrument are plotted

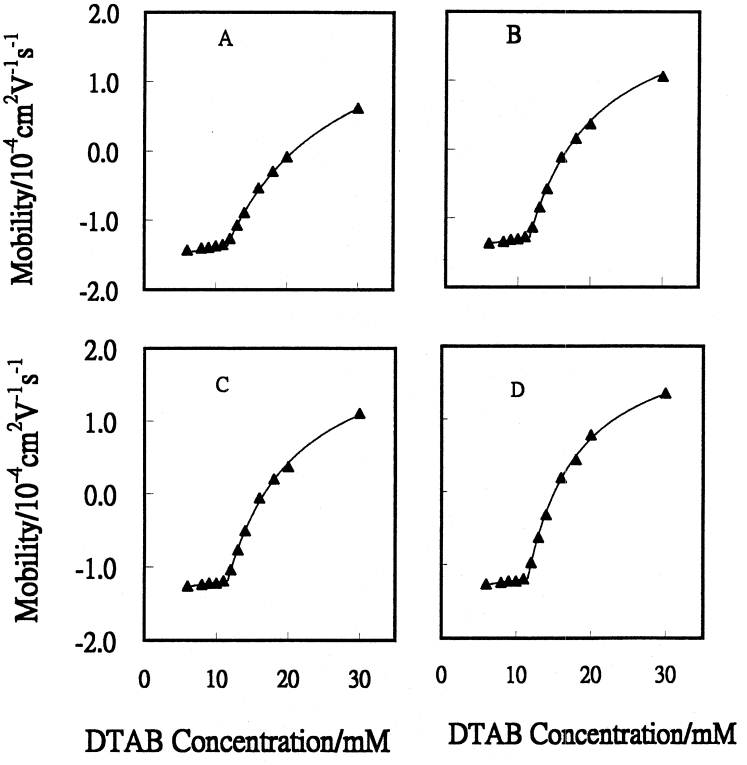

Fig. 1. The variation of electrophoretic mobility of cephalosporins as a function of DTAB concentration in the range 6–30 m*M*: (A) cefuroxime, (B) cefotaxime, (C) cefazolin and (D) cephapirin. Buffer electrolyte, 20 m*M* phosphate buffer at pH 7.5; capillary, 67 cm×50 μm I.D.; applied voltage, −20 kV; detection wavelength, 255 nm; temperature, 25°C; sample concentration, 100 μg/ml; CE system, SpectraPhoresis model 1000 (Thermo separation products).

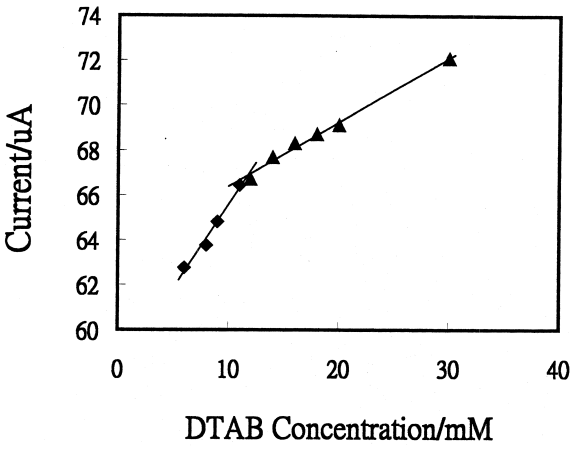

Fig. 2. Plots of electric current versus DTAB concentration in the range 6–30 m*M*. Test analytes and electrophoretic conditions are the same as for Fig. 1.

against the surfactant concentrations in a certain range above and below the CMC. To add further support, the CMC value of DTAB was also determined by this method. As shown in Fig. 2, two straight lines with different slopes were obtained and the CMC value of DTAB determined from the intersection point of these two lines occurred at 11.7 m*M*. Similarly, with a sample concentration of 50 μg/ml, the CMC value of DTAB was determined to be 12.1 m*M*. Table 1 summarizes the CMC values of DTAB obtained by various approaches. All these results indicate that, depending on the actual ionic strength of the buffer electrolyte containing ionic solutes (0~100 μg/ml), the CMC values of DTAB determined in 20 m*M* phosphate buffer at pH 7.5 lie in the range 11.6–13.0 m*M*.

Evidently, the dramatic change in the electrophoretic mobility of cephalosporins or the electric current of the electrophoretic system as a function of the DTAB concentration is a good indication of the CMC of DTAB. The dramatic increase in electrophoretic mobility is mainly due to the interactions of DTAB micelles with cephalosporins in micellar electrokinetic chromatography (MEKC), instead of the interaction of cephalosporins with DTAB surfactant monomers in various forms of complexation in CZE. In addition, as a small but significant change in the electrophoretic mobility of cephalosporins was observed when the DTAB concentration increased from 4 to 11 m*M*, the interaction between cephalosporins and DTAB monomers may not be neglected.

Table 1
The CMC value of DTAB determined[a]

Method	Sample concentration (μg/ml)		
	100	50	0
CE (mobility)	11.6	12.0	(12.4)[b]
CE (current)	11.7	12.1	(12.5)[b]
Conductivity	–	–	13.0

[a] CMC in unit of m*M*.
[b] Extrapolated value.

In a phenomenological approach, the effective electrophoretic mobility of an anionic solute in MEKC using a cationic surfactant can be expressed as

$$\mu_{eff} = \alpha_{A^-}\mu_{A^-} + \alpha_M\mu_{mc} \quad (1)$$

where α_{A^-} and α_M are the mole fractions of an unassociated anion in the aqueous phase and the anion associated with cationic micelles in the micellar phase, respectively, and μ_{A^-} and μ_{mc} represent the electrophoretic mobilities of an unassociated anion and the micelles, respectively.

Based on a theoretical treatment of mobility similar to the one described previously [5,7], also combined with an ion-interaction model [8,9], the effective mobility of cephalosporins in MEKC using a cationic surfactant can be specifically defined by the following equation:

$$\mu_{eff} = \frac{1}{1 + K_{A^-\cdot S^+}[S] + K_M[M]} \cdot \mu_{A^-} + \frac{K_M[M]}{1 + K_{A^-\cdot S^+}[S] + K_M[M]} \cdot \mu_{mc} \quad (2)$$

where [S] is the concentration of surfactant monomers under the conditions of reversed electroosmotic flow (reversed EOF), [M] is the micelle concentration and $K_{A^-\cdot S^+}$ and K_M represent the binding constants of an anionic solute (A^-) to cationic surfactant monomers and to cationic micelles, respectively. When the concentration of surfactant molecules is below the CMC where [M]=0, Eq. (2) is reduced to

$$\mu_{eff} = \frac{1}{1 + K_{A^-\cdot S^+}[S]} \cdot \mu_{A^-} \quad \text{(below the CMC)} \quad (3)$$

On the other hand, when the surfactant concentration is above the CMC, the effective electrophoretic mobility is given by

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

As described previously [5], the CMC value can be determined experimentally or, more precisely, by a curve-fitting approach. The simulation of the mobility curves of cephalosporins as a function of the surfactant concentration in MEKC and in CZE were performed using Eqs. (3) and (4), respectively, through the utilization of Excel software. The most suitable values of the binding constants and limiting mobilities of these cephalosporins could be obtained by varying these parameters until the predicted mobility curves were best fitted to the observed mobility curves. Thus the best-fitted mobility curves allow us to evaluate the binding constants of cephalosporins to surfactant monomers ($K_{A^-\cdot S^+}$) and to micelles (K_M), respectively, and the intersection of the two simulated mobility curves permits us to determine the most suitable CMC value of the surfactant. Table 2 gives the best-fitted values of binding constants of cephapirin, cefotaxime, cefazoline and cefuroxime to DTAB surfactant molecules evaluated at pH 7.5, together with the mobility data of cephalosporins. We believe that the magnitudes of these binding constants are much more reasonable than those reported by Mrestani et al. [1].

In conclusion, the CMC value of DTAB determined by Mrestani et al. [1] with conductivity measurements is erroneous and the complex-formation model proposed to calculate the aggregation constants of cephalosporins to monomeric charged surfactants in CZE is not applicable and perhaps conceptually misleading. Instead, an interpretation mainly based on the partition of analytes between the aqueous phase and micellar phase which involves the interactions between anionic solutes and cationic micelles in MEKC should be adopted.

Table 2
The mobility data of cephalosporins and binding constants of cephalosporins to DTAB in 20 m*M* phosphate buffer at pH 7.5 in the presence of cephalosporins[a]

Sample solutes	Mobility (10^{-4} cm^2 V^{-1} s^{-1})		Binding constants (M^{-1})	
	μ_{A^-}	μ_{mc}[b]	$K_{A^-\cdot S^+}$	K_M
Cefuroxime	−1.57	2.30	14	77
Cefotaxime	−1.48	2.30	13	128
Cefazolin	−1.38	2.30	12	125
Cephapirin	−1.38	2.30	12	179

[a] Solute concentration = 100 mg/ml.
[b] Micelle marker = oil yellow AB, DTAB concentration = 20 m*M*.

References

[1] Y. Mrestani, R.H.H. Neubert, H.H. Ruttinger, J. Chromatogr. A 802 (1998) 89.
[2] S. Terabe, in: Micellar Electrokinetic Chromatography, Beckman Instruments, Fullerton, CA, USA, 1993, p. 9.
[3] J.C. Jacquier, P.L. Desbene, J. Chromatogr. A 718 (1995) 167.
[4] H. Nakamura, A. Sano, K. Matsuura, Anal. Sci. 14 (1998) 379.
[5] C.E. Lin, T.Z. Wang, T.C. Chiu, C.C. Hsueh, J. High Resolut. Chromatogr. 22 (1999) 256.
[6] A. Cifuentes, J.L. Bernal, J.C. Diez-Masa, Anal. Chem. 69 (1997) 4271.
[7] C.E. Lin, C.C. Hsueh, T.Z. Wang, T.C. Chiu, Y.C. Chen, J. Chromatogr. A 835 (1999) 197.
[8] J.K. Strasters, M.G. Khaledi, Anal. Chem. 63 (1991) 2503.
[9] T. Kaneta, S. Tanaka, M. Taga, H. Yoshida, Anal. Chem. 64 (1992) 798.