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Determination of critical micelle concentration by capillary electrophoresis
Application to organo–saline electrolytes

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Abstract

Having previously reported [J. Chromatogr. A, 718 (1995) 167] the theoretical approach of the determination of the critical micelle concentration (CMC) by capillary electrophoresis and validated this new technique with the determination of the CMC of sodium dodecyl sulphate in a simple electrolyte, we demonstrate in this paper the universality of this technique in studying the evolution of the CMC of the same surfactant in various complex electrolytic solutions. The electrolytic salts (sodium borate and phosphate) and the solvents (methanol, ethanol, acetone and acetonitrile) evaluated in this study correspond to the most used salts and solvents in micellar electrokinetic chromatography. This unique possibility offered by this new technique to study the micelle formation under identical electrophoretic conditions as used during the separation appears to be most interesting for the understanding of the separation forces and their optimisation. Moreover, a comparative study of the effect of added organic solvent to the electrolytic medium has been undertaken.

Keywords: Critical micelle concentration; Micelles; Buffer composition; Surfactants; Sodium dodecyl sulphate; Naphthalene

1. Introduction

Surfactants, amphiphilic molecules constituted of two well-defined parts of different polarity (hydrophobic tail and polar if not ionic head) present, when their concentration is over their critical micelle concentration (CMC), the very interesting characteristics of considerably increasing the solubility of apolar compounds in aqueous solutions.

This sharp increase of the solubility of poorly soluble compounds in aqueous solutions results in either the adsorption of these compounds on the micellar surface or on the deeper penetration of these compounds into the micellar core. Both the micellisation process and the micellar solubilization are essentially due to hydrophobic interaction.

This important property of micellar aqueous solutions thus presents the origin of a vast application field, in the pharmaceutical industry for example. From a chromatographic point of view, this property has given rise to numerous developments:

1. In thin-layer chromatography, since 1979 [1], micellar phases have proven to be of great interest

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for the separation of solutes of very different polarity in a single analysis.

2. In high-performance liquid chromatography, since 1980 [2], micellar aqueous solutions have been used as mobile phases either in reversed-phase or in normal-phase partition chromatography [3]. Indeed, these phases allowed a polarity tuning as a function of the surfactant concentration without presenting a problem for electrochemical detection when an elution gradient was performed [4], or even favouring the use of fluorimetric detection [5].
3. More recently, in 1984, these micellar phases have been the object of study for the separation of neutral molecules in capillary electrophoresis [6]. This new separation technique, called micellar electrokinetic chromatography (MEKC), was an important step in the development of capillary electrophoresis, this technique being hitherto confined to the separation of ionic species.

The optimisation of the analytical conditions in MEKC has been the subject of an important field of research since 1984 and has allowed the resolution of a number of diverse and various organic matrixes [7].

However, most of these studies dealt with the optimisation of the operating conditions (nature and concentration of the electrolytic salt, nature and concentration of organic solvent) concerning the relative interactions of the solutes between the micellar and the aqueous phases. For all that, few authors understood the influence of these operating parameters on the evolution, or the existence, of the micellar phase. As the study of the micellisation process is a key parameter in the optimisation of analytical conditions in chromatographic techniques using micellar phases, and particularly in MEKC, the determination of the CMC under given operating conditions proves to be essential.

Taking into account the complexity of the chromatographic media used in such analyses, the different physico-chemical properties used for the determination of the CMC (surface tension, conductivity, etc.) do not appear to be suitable any longer.

In view of the above problem, we have recently developed a new approach for the determination of CMC based on capillary electrophoresis [8]. In view of the first results, it appears that capillary electrophoresis is a preferred technique for the determination of CMC of anionic surfactants as it appears to be not only fast but also easily automated.

In order to define the potential of this new technique for the determination of critical micelle concentrations, as well as to enlarge its application range to the various media used in micellar chromatography, we studied the evolution of the CMC of sodium dodecyl sulphate (SDS), the most used surfactant in MEKC, as a function of the essential variables of MEKC, i.e.: (1) the nature and concentration of bulk salts, (2) the nature and concentration of organic solvents.

This paper summarises the results of this study confronted with a few literature data.

So far, concerning the influence of bulk salt, the published studies have dealt only with the influence of concentration of sodium chloride [9]; conversely, in the case of organic solvents, their influence has been studied in pure water, i.e. without bulk salt [10–12].

2. Experimental

2.1. Reagents

Buffer and sample solutions were prepared with water purified by reversed osmosis and filtration using a Milli-Ro+Milli-Q system (Millipore, Molsheim, France). The reagents used as electrolytes, i.e. borax and trisodium phosphate, were of analytical grade (99%) from Aldrich (La Verpillère, France). The SDS was of 99% purity and purchased from Sigma (Saint Quentin Fallavier, France).

The different organic solvents, i.e. methanol, ethanol, acetone and acetonitrile were of RS HPLC grade (Carlo Erba, Rueil Malmaison, France)

As stated in our previous study, the compound used as test sample for the determination of CMC was naphthalene of 99% purity from Aldrich.

2.2. Apparatus

All experiments were carried out on a P/ACE 2100 system (Beckman, Fullerton, CA, USA) controlled by a PS/2 computer (IBM, Greenock, UK),

using a P/ACE software (Beckman). Data collection was performed with the same software.

Samples were loaded by a 1-s pressure injection at the anodic end of a fused-silica capillary of 57 cm×50 μm I.D. The UV detection was performed through the capillary at 50 cm of the inlet at 214 nm.

The pH values of the electrolytes were measured using a Beckman Model Φ pH meter at the temperature of the analyses.

The separations were performed three to five times for each operating condition (nature and concentration of inorganic salt, nature and content of organic solvent), in order to obtain reliable statistics on the measurements.

3. Results and discussion

As capillary electrophoresis should enable the study of the micellisation process in any aqueous solution, and in particular when the solution contains a certain amount of salt and organic solvent (organo–saline solutions), we proceeded with the evaluation of the CMC of SDS in the various electrolytic solutions used in MEKC. Thus, we studied the evolution of the CMC of SDS in purely saline solutions (borax and trisodium phosphate at different concentrations), then in organo–saline solutions, the organic solvents chosen being methanol, ethanol, acetone and acetonitrile. In fact, these solvents, covering a broad lipophilic range, are the most currently employed in MEKC, either to adjust the aqueous phase lipophily, or to increase the selectivity of the chromatographic system, or, at last, to modify the retention time window.

3.1. Evolution of the CMC as a function of the nature and concentration of bulk salt

In aqueous solutions, added electrolytic salts reduce the CMC of most surfactants, especially for surfactants. Indeed, the addition of an electrolyte of the same nature as that of the counter-ion of the tensioactive molecule provokes a decrease of the CMC which has been quantified empirically by Corrin and Harkins [10] by the equation:

$$\log \mathrm{CMC} = -a + b \log c_i \quad (1)$$

where a and b are constants for a given tensioactive molecule, counter-ion and temperature, and c_i is the total counter-ion concentration.

The observed decrease is principally due to a reduction of the repulsive electrostatic forces between the ionic heads via a screening effect and, therefore, to a decrease of the contribution of these groups to the free energy term opposing the micellisation process.

Concerning the non-ionic surfactants, the effect of added salts is much less dramatic and is observed only at higher salt concentrations. The discussion of this phenomenon introduces the more general notion of 'salting in' and 'salting out' of non-electrolytes by the electrolyte. This influence can be expressed by [13]:

$$\log C_0/C = k\, m_E \quad (2)$$

where C_0 and C are the solubilities of the compound without and with electrolyte at a concentration m_E, k being the Setchenov constant.

In the framework of our study, which dealt with the determination of the effect of added salts on the CMC of SDS, we therefore attempted to estimate the empirical constants a and b of Eq. (1) in the case of borax and trisodium phosphate. Fig. 1 represents the evolution of the CMC of SDS at 25°C as a function of these two salts.

In order to calculate the empirical constants a and b for each studied salt, we then plotted the evolution of the decimal logarithm of the CMC as a function of the decimal logarithm of the total concentration of sodium salt in solution. The two linear regressions obtained by the least squares method are reported in Table 1.

The obtained values, even though mutually coherent, appear to be slightly different from those reported by Stigter [9] in the case of sodium chloride and one can wonder if these differences come from an incertitude in using capillary electrophoresis or if they are a consequence of the nature of the inorganic salt studied and are of a thermodynamic nature.

In fact, if the graphs reported in Fig. 1 are compared with the evolution of the CMC of SDS in the case of sodium chloride, one can observe that the

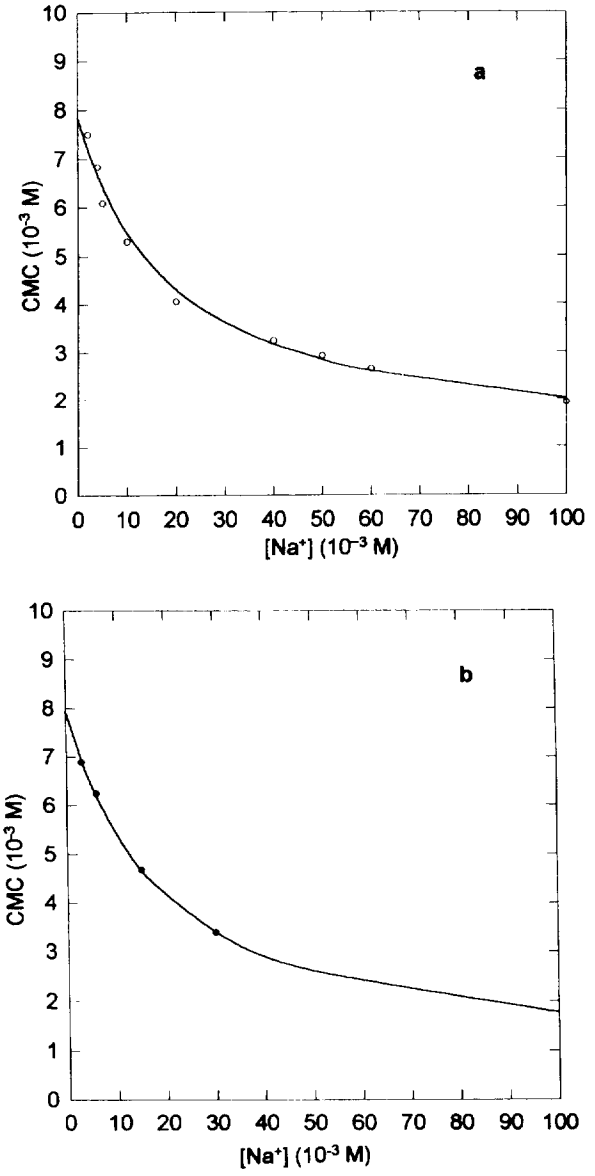

Fig. 1. Evolution of the CMC of SDS as a function of added salt concentration (a) in the case of borax and (b) in the case of trisodium phosphate.

obtained values start to diverge at a sodium concentration higher than 30 m*M* (Fig. 2).

These differences can therefore be well explained by a different salting-out effect of the monomeric surfactant depending on the nature of the added inorganic anion (borate, phosphate, chloride). Nevertheless, without any literature data on this subject, the doubt concerning these differences can not be removed.

In order to demonstrate the versatility of this new

Table 1
Parameters of Eq. (1) in the case of borax, trisodium phosphate and sodium chloride

	Salt		
	$Na_2B_4O_7 \cdot 10\ H_2O$	Na_3PO_4	NaCl [9]
a	3.23	3.33	3.511
b	−0.53	−0.59	−0.680
ρ	>0.994	>0.9993	>0.9998
CMC_0 (mM)[a]	7.82	7.93	8.12

[a] Critical micelle concentration without bulk salt (extrapolated values in the case of borax and trisodium phosphate, measured in the case of sodium chloride [9]).

technique for the determination of critical micelle concentrations, we studied the potentialities of this technique in the case of hydro/organic solutions.

3.2. Evolution of the CMC as a function of the nature and concentration of organic solvents

We attempted to evaluate the effect of the concentration of organic solvents on the CMC of SDS. As the aim of this study was a better understanding of the biphasic system and therefore of the phenomena governing the electrophoretic separation, we limited the choice of the organic solvents studied to those most employed in MEKC, i.e. (1) methanol and ethanol concerning the protic solvents, (2) acetone and acetonitrile concerning the aprotic solvents.

From a practical point of view, this study was performed under similar operating conditions as before, i.e. (1) Naphthalene has been kept as sample compound, (2) borax has been chosen as bulk electrolyte, at a 0.005 M concentration (pH 9.2 at 25°C).

In Fig. 3, the obtained CMCs are reported together as a function of their volume percentage in the different organic solvents studied.

From this figure it can be seen that acetone and acetonitrile modify the CMC of SDS in a similar way. Indeed, these two solvents, having pronounced lipophilic characteristics, stabilise SDS micelles in relation to the electrolyte when their added volume percentage is low (<10%, v/v), and 'denature' micelles at higher concentrations. These two phe-

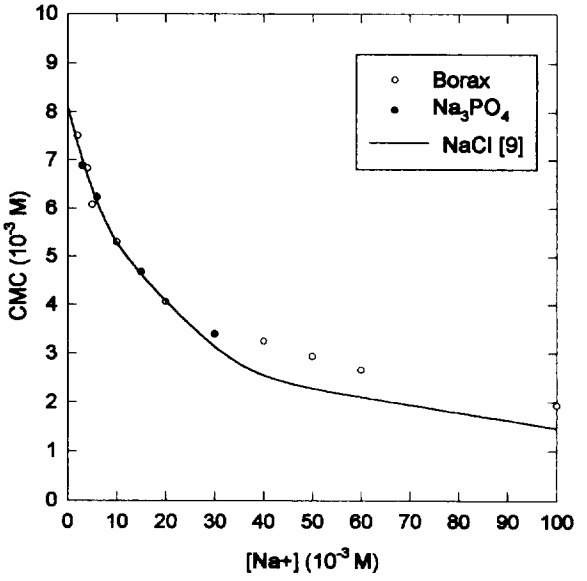

Fig. 2. Evolution of the CMC of SDS as a function of the added sodium salt concentration.

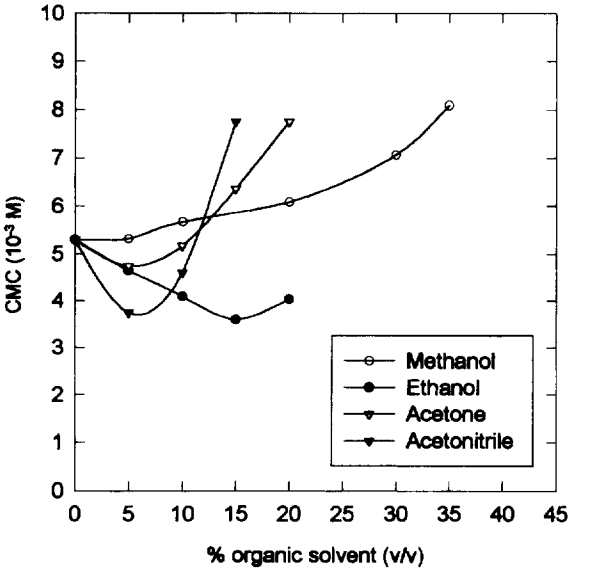

Fig. 3. Evolution of the CMC of SDS as a function of the added organic solvent content (electrolyte: borax 0.005 M).

nomena are more pronounced for acetonitrile than for acetone.

In the case of the protic solvents, their behaviour is largely different from each other. Indeed, the behaviour of ethanol, even if close to that of the aprotic solvents, is peculiar. In fact, this solvent has a stabilising effect over a very broad range of volume percentages. Moreover, this effect appears to be weaker than that of the aprotic solvents since, even if of a comparable magnitude as that of acetonitrile, the minimum in the CMC evolution occurs at a much higher content: 15% (v/v) in the case of ethanol compared to 5% (v/v) in the case of acetonitrile and acetone.

Despite the less pronounced stabilising effect of this solvent, no micellisation could be observed after 25% (v/v), i.e. a limit volume percentage close to the values observed for of acetone and acetonitrile.

In contrast, methanol, a typical protic solvent with little lipophilic character, does not appear to disturb the micellar system much, since the increase of the CMC is small even for volume percentages as high as 35%.

Such observations are confirmed by the few literature data about the effect of added organic solvent on the micellar aggregation of SDS [10–12]. Conductimetric studies by Emerson and Holtzer [12] allowed these authors to rationalise such behaviour taking the dielectric and lipophilic characteristics of the different solvents as critical parameters.

Indeed, according to these authors, the addition of solvents with a low dielectric constant to aqueous solutions should lead to an increase of the repulsive forces between the ionic heads of the tensioactive molecules resulting in a CMC increase. Also, the addition of strong lipophilic solvents should lead to an increase of the solubility of the monomeric surfactant and be responsible for an increase of the CMC value.

Based on such considerations, methanol, a typical solvent with a high dielectric constant and poor lipophilic character, induces a small denaturing effect. In contrast, acetone, which has a low dielectric constant and strong lipophilic character, should lead to an important increase of the CMC.

Nevertheless, these authors explain the decrease of the CMC at low organic solvent content by the tensioactive character of these small molecules and the possibility of their inclusion in the micellar core as co-surfactants, leading to the formation of more stable, mixed micelles than those of SDS alone.

Acetonitrile, a solvent not studied by these authors but with characteristics close to those of acetone, should give similar effects.

Lastly, ethanol should lead to a behaviour close to that of acetone, as described by Emerson and Holtzer [12]. However, our study did not underline the denaturing characteristics of this solvent. Nevertheless, this observation does not imply an inadequacy of the new proposed technique for the determination of CMC, as Flokhart [11] observed very different behaviours of ethanol as a function of temperature. In particular, his conductimetric study shows a stabilising effect of ethanol at low temperature (10°C), and a denaturing effect at 55°C.

As the studies conducted by Flokhart [11] and Emerson and Holtzer [12] were performed without any electrolyte salt in the medium, the behaviour of ethanol we observed can probably be explained by a reinforcement of the aqueous solution cohesion due to the addition of salt and be close to the one observed by Flokhart [11] at a low temperature of 10°C.

Analysis of these results proves that the proposed electrophoretic technique is well adapted to the study of the micellisation process in complex electrolytes (presence of electrolytic salts and organic solvents), media not compatible with the reference techniques used today, i.e. conductivity or surface tension measurements.

When higher contents of organic solvents are added in the electrolyte, no micellar aggregation is observed. For example, the evolution of the electrophoretic mobility of naphthalene, μ_{ep}, as a function of the total concentration of SDS, C_T, is shown in Fig. 4 for a volume concentration of acetonitrile of 40%.

If no micellar aggregation occurs in this case, this curve should correspond to an evolution of the electrophoretic mobility due only to the solvatophobic partition of naphthalene between the aqueous phase and the monomeric surfactants and described as previously established [8] by the equation:

$$\mu_{ep} = \frac{K_{solv} C_T}{1 + K_{solv} C_T} \cdot \mu_{solv} \qquad (3)$$

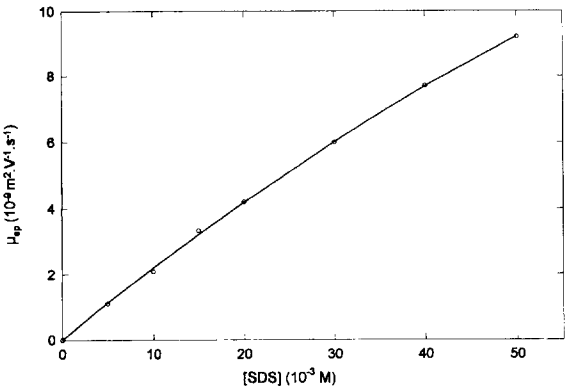

Fig. 4. Evolution of the electrophoretic mobility of naphthalene as a function of the total SDS concentration (electrolyte: borax 0.005 *M*, acetonitrile 40% v/v).

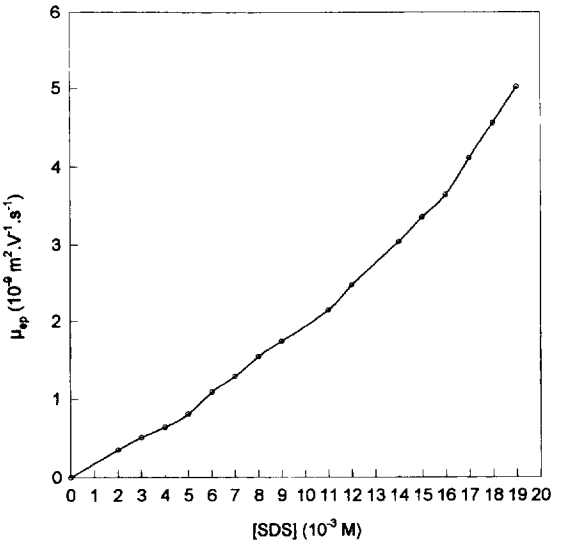

Fig. 5. Evolution of the electrophoretic mobility of naphthalene as a function of the total SDS concentration (electrolyte: borax 0.005 *M*, acetone 30% v/v).

where μ_{solv} is the electrophoretic mobility of the 'solvatophobic complex', K_{solv} is the partition constant of the solute between the aqueous phase and the monomeric surfactants, and C_T is the total concentration of surfactant in solution.

Eq. (3) can be rewritten as

$$\frac{1}{\mu_{ep}} = \frac{1}{\mu_{solv}} + \frac{1}{\mu_{solv} K_{solv} C_T} \quad (4)$$

When $1/\mu_{ep}$ is plotted as a function of $1/C_T$, in this case of high acetonitrile content (40%, v/v), the non-micellisation of SDS is effectively proved by the linear regression obtained ($1/\mu_{ep} = 16.71 \cdot 10^6 + 44.76 \cdot [1/C_T]$, linear regression coefficient $\rho \geq 0.9994$).

Moreover, this excellent linearity allows the values of μ_{solv} and K_{solv} to be determined, at $55.9 \cdot 10^{-9}$ $m^2\ V^{-1}$ and 3.73 mol^{-1} l, respectively.

Nevertheless, for less important organic solvents contents, acetone 30% (v/v) for example, we can not conclude to a total non-micellisation (Fig. 5). In fact, one can notice in this case a succession of small breaks in the slope of the curve of the evolution of the electrophoretic mobility of naphthalene as a function of the total SDS concentration, breaks which can be attributed to the formation of small surfactant–solvent mixed aggregates with a higher relative mobility than that of the monomeric surfactants [10]. It is nevertheless impossible to speak in this case of micellar aggregation in the sense of a concerted association of an important number of tensioactive molecules in view of the very small slope changes and their multiplicity.

4. Conclusion

Capillary electrophoresis appears to be a valuable technique for the determination of the CMC of tensioactive molecules. In fact, this technique appears to be not only quick but also easy to be carried out because of its automatization. Moreover, as demonstrated above, this new technique is very interesting as it allows the determination of CMC values in any electrophoretic medium, and especially in organo–saline solution, a medium which can not be studied with the currently used techniques of CMC determination.

First applied on SDS as the most used anionic surfactant in MEKC, we are considering studying, in the near future, the potential of this new technique for the determination of the CMC of cationic and non-ionic surfactants.

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