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Determination of the aggregation threshold of non–UV-absorbing, neutral or charged surfactants by frontal- and vacancy-frontal analysis continuous capillary electrophoresis

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Abstract

Supplementing our recent work on UV-absorbing anionic surfactants, new protocols based on frontal analysis continuous capillary electrophoresis (FACCE) were developed for the investigation of the aggregation threshold of non-UV absorbing anionic, cationic and neutral surfactants, and exemplified with sodium dodecyl sulfate (SDS), tetradecyltrimethylammonium bromide (TTABr) and Brij 35. Contrary to UV-absorbing surfactants, the critical micelle concentration (CMC) determination of non-UV absorbing surfactants requires the use of a marker providing adequate detection capabilities. UV-absorbing markers were selected, according to the charge of the studied surfactant (neutral for SDS and TTABr, anionic for Brij 35). In all cases, the free marker concentration was quantified as a function of the total surfactant concentration. In addition, a modified implementation of FACCE, that we called vacancy FACCE (VFACCE), was employed for the case of the neutral surfactant. VFACCE entails first filling the capillary with the system components to be studied in the background electrolyte, next continuously introducing the plain BGE electrokinetically. The salient theoretical features of FACCE and VFACCE were compared. These new protocols were successfully applied to yield reliable CMC values within short operational time and with low sample consumption.

Keywords: Aggregation threshold; Critical micelle concentration; Vacancy frontal analysis continuous capillary electrophoresis; Surfactants

1. Introduction

Surfactants are nowadays chemical compounds of great interest because of their unique amphiphilic structures and self-aggregation properties above a threshold concentration that is called the critical micelle concentration (CMC). They have found widespread use in a great deal of industrial areas, including detergency, healthcare, pharmaceutical formulation, painting and so on. Numerous analytical techniques have been commonly implemented to obtain CMC and aggregation data, among which conductimetry [1], potentiometry [2], surface tension measurement [3], absorbance and fluorescence spectroscopy [4], light scattering [5], cyclic voltammetry [6] and NMR [7]. The choice of the technique depends primarily on the surfactant nature (ionic, non-ionic) and its spectroscopic properties but also on its local availability and easiness of use. More recently, capillary zone electrophoresis (CZE) has emerged as an alternative for the determination of the CMC values of UV-transparent, anionic and cationic surfactants with low sample requirement [8–14]. In a previous paper [15], we also introduced frontal analysis continuous capillary electrophoresis (FACCE) as a new electrophoretic implementation to determine such thresholds. Briefly, FACCE consists in electrokinetically injecting the surfactant sample into the capillary under the effect of an electrical field, which then results in the formation of distinct migration fronts for the free and aggregated surfactant forms. The main interest of the method is that it allows direct determination of the free surfactant concentration without disturbing the surfactant aggregation equilibrium. CMC was easily obtained by varying the total surfactant concentration. The effectiveness of this approach was exemplified with the CMC determination of octyl- and dodecylbenzenesulfonates. These UV-absorbing surfactants

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do not require the use of any marker, amenable to perturb the aggregation.

This paper shows how to extend the applicability of the frontal analysis continuous capillary electrophoresis method to non UV-absorbing surfactants of the different classes (anionic, cationic and neutral) using the current UV detection equipping commercial instruments. The FACCE method has been applied first in its classical form for the case of sodium dodecyl sulfate (SDS) and tetradecyltrimethylammonium bromide (TTABr) by simply circumventing the problem raised by analyte UV-transparency through the surfactant interaction with a chromogenic marker and that of the wall adsorption of cationic surfactants through the use of amine-coated capillaries. Then, a new version of FACCE, which we coin vacancy frontal analysis continuous capillary electrophoresis, was developed for the CMC determination of neutral, UV-transparent surfactants and applied to poly(oxyethylene 23 dodecanol) (Brij35).

2. Theoretical

2.1. Frontal analysis continuous capillary electrophoresis (FACCE)

Frontal analysis continuous capillary electrophoresis was introduced by Gao et al. [16] some years ago as a resurgence of moving boundary electrophoresis for the determination of interaction parameters. As depicted in Fig. 1A, the method consists in first equilibrating the capillary with a background electrolyte (BGE) and then continuously introducing the sample under the effect of an electrical field. This process results in the apparition of migration fronts of the free and complexed forms involved in the considered equilibrium in the order of decreasing apparent mobilities. A major advantage of this method is that it only provides partial separation of the interacting partners, as is the case for, e.g. dialysis, insuring the absence of equilibrium displacement. The method, however, has also some constraints. Especially, it requires a linear detector response with respect to the concentration of the form of interest and a long enough time-based plateau to allow for precise height measurement. According to this, FACCE is most properly implemented when the migration front to be exploited is detected first, as in this case, the operating parameters can be rather smoothly adapted to meet the preceding two conditions. Conversely, when the migration front of interest is not the first (for example the last one), the conditions above may not be fulfilled and problems arising from wall adsorption of one of the interacting species, high absorbance level or noisy signals may be encountered, resulting in inaccurate measurements. These drawbacks can be alleviated by employing a modified FACCE protocol, that we propose to call vacancy frontal analysis continuous capillary electrophoresis (VFACCE). This protocol consists in first hydrodynamically equilibrating the capillary with the sample species in equilibrium in the desired background electrolyte (BGE) and in next electrokinetically removing them from the capillary by continuously introducing the BGE only (Fig. 1B). This experimental protocol provides negative migration fronts for the free and complexed forms of the interacting species, in the order of the decreasing apparent mobilities.

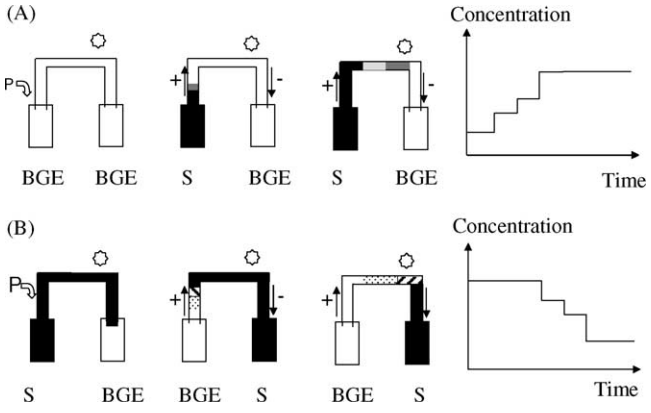

Fig. 1. Experimental protocol for (A) frontal analysis continuous capillary electrophoresis (FACCE) and (B) vacancy frontal analysis continuous capillary electrophoresis (VFACCE) methods. Case of three forms 1, 2, 3 in the order of decreasing apparent mobilities. Dark: 1 + 2 + 3; dark grey: 1; light grey: 1 + 2; hatched area: 2 + 3; dotted area: 3. *P*: pressure; BGE: background electrolyte; S: sample.

2.2. Application of FACCE to CMC determinations

As is the case with CZE methods whatever the surfactant is, the determination of the aggregation threshold of a non-UV-absorbing surfactant by FACCE methods requires the use of a UV-absorbing marker M. This being considered, the chemical equilibria involved remain the same in CZE and in FACCE modes, with marker M interacting with monomeric surfactant S and its micelle S_n (above the CMC). Briefly, the system can be described according to the following reactional scheme and corresponding equations, assuming 1:1 interactions between the marker and either the monomeric surfactant or the micelle:

$$\mathrm{M} + \mathrm{S} \rightleftarrows \mathrm{MS} \quad \text{(a)} \quad \text{with } K_\mathrm{S} = \frac{[\mathrm{MS}]}{[\mathrm{M}][\mathrm{S}]} \tag{1}$$

$$\mathrm{M} + \mathrm{S_n} \rightleftarrows \mathrm{MS_n} \quad \text{(b)} \quad \text{with } K_\mathrm{mic} = \frac{[\mathrm{MS_n}]}{[\mathrm{M}][\mathrm{S_n}]} \tag{2}$$

$$[\mathrm{M}]_\mathrm{t} = [\mathrm{M}] + [\mathrm{MS}] + [\mathrm{MS_n}] \tag{3}$$

$$[\mathrm{S}]_\mathrm{t} = [\mathrm{S}] + [\mathrm{MS}] + n[\mathrm{MS_n}] + n[\mathrm{S_n}] \tag{4}$$

where $[\mathrm{M}]_\mathrm{t}$, $[\mathrm{S}]_\mathrm{t}$, and n are the total marker concentration, total surfactant concentration and aggregation number, respectively.

When $[\mathrm{S}]_\mathrm{t} <$ CMC, only equilibrium (a) is involved and the solute interacts with the monomeric surfactant. In this

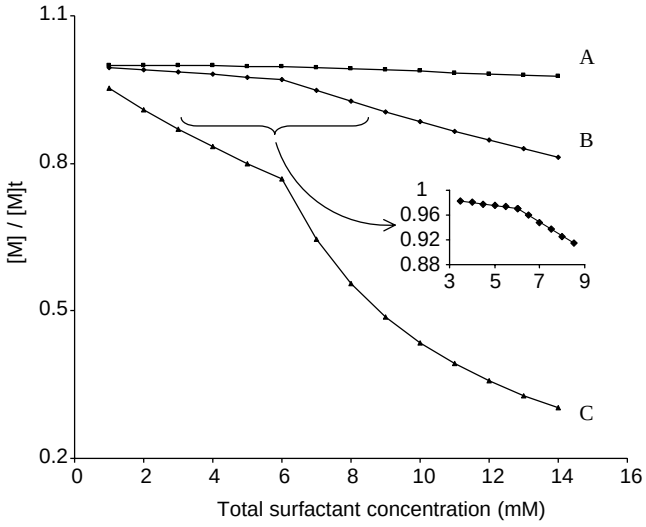

Fig. 2. Theoretical variation of the free to total marker concentration ratio $[M]/[M]_t$ as a function of the total surfactant concentration $[S]_t$, according to Eq. (7) with (A) $K_S = 0.5$ and $K_{mic} = 150$; (B) $K_S = 5$ and $K_{mic} = 1500$ and (C) $K_S = 50$ and $K_{mic} = 15000$. CMC = 6 mM, $n = 60$. See text for additional details.

case, mass balance for M leads to:

$$\frac{[M]}{[M]_t} = \frac{1}{1 + K_S[S]} \quad (5)$$

where [S] may be calculated as the positive solution of the following equation:

$$K_S[S]^2 + [S](K_S([M]_t - [S]_t) + 1) - [S]_t = 0 \quad (6)$$

As K_S is expected to be rather low (of the order of 5 mol^{-1} L according to the work by Jacquier and Desbène [8]) and as the concentrations $[S]_t$ and $[M]_t$ remain quite low compared to the inverse of the equilibrium constant, the assumption $[S]_t = [S]$ can be made.

When $[S]_t > CMC$, the mass balance for M is described by Eq. (3), which can be rearranged into:

$$[M]/[M]_t = \frac{1}{1 + K_S CMC + K_{mic}(([S]_t - CMC)/n)} \quad (7)$$

For Eq. (7) to be satisfied, note that [M] should be quite low compared to both K_S and K_{mic} reciprocals. Fig. 2 represents the double hyperbolic variation of $[M]/[M]_t$ versus $[S]_t$ in the whole concentration range according to Eqs. (5) and (7), for various K_S and K_{mic} values and with CMC = 6 mM and $n = 60$, which are representative of SDS in a low ionic strength medium [8]. The graph of [M] versus $[S]_t$, that was exploited experimentally in the FACCE method from the measurement of the height of the migration front of the marker, is homothetic of the preceding one. The CMC value can be determined from the discontinuity in the curve derivatives observed apart this concentration. It appears that the marker should therefore be selected according to its hydrophobicity, so that this discontinuity is made more apparent. This is in fact not so an easy task, as K_S and K_{mic} show the same tendency in terms of the marker hydrophobicity and as the aggregation number n is also involved. On the whole, it can be expected that K_{mic} will increase more rapidly with the marker hydrophobicity than K_S. In addition, considering the shape of the curves obtained for various K_S and K_{mic} values in Fig. 2, it appears that a marker of choice should be of intermediate hydrophobicity, which results in a situation such as that depicted in Fig. 2B, and enables to apply linear curve fitting. So, the markers need not be as hydrophobic as those best suited for a CZE method. This can be considered as a practical advantage with respect to the marker solubility in water media and hence to the detector signal to noise ratio of the marker. Moreover, the kinetics of the marker distribution between the aqueous medium and the surfactant aggregate needs not be fast, as is required in the dynamic CZE mode, since an incubation of the surfactant–marker system is allowed in the FACCE mode. This contributes to further enlarge the choice of an appropriate marker. Finally, it is to note that non-linear fittings of the [M] versus $[S]_t$ curve on both sides of the CMC value can give access to the system parameters K_S and K_{mic}/n, if a marker of strong enough hydrophobicity is used.

3. Material and methods

3.1. Surfactants and reagents

SDS (99% purity) was purchased from Fluka (Saint-Quentin-Fallavier, France), poly(oxyethylene 23 dodecanol) (Brij 35, unspecified purity) and TTABr (99% purity) were from Sigma (Saint-Quentin-Fallavier, France). Ethylparaben (ethyl *para*-hydroxybenzoate, EP, 99% purity) and sodium octylbenzenesulfonate (SOBS, 97% purity) used as chromogenic markers were obtained from Sigma–Aldrich (Saint-Quentin-Fallavier, France), respectively. Sodium mono- and dihydrogenophosphates used to prepare the background electrolytes were purchased from Sigma and sodium tetraborate decahydrate from Fluka. Water used throughout was produced by an Alpha Q system (Millipore, Molsheim, France).

3.2. Electrolytes and sample solutions

According to the surfactant studied, the BGE were 2.8 mM sodium phosphate buffer, pH 6.9 (5 mM ionic strength) for SDS, 10.9 mM sodium phosphate buffer, pH 6.4 (10 mM ionic strength) for TTABr or 20 mM sodium borate, pH 9.2 (10 mM ionic strength) for Brij 35. Stock surfactant solutions of 30 mM SDS in 2.8 mM sodium phosphate buffer, pH 6.9 (5 mM ionic strength), 7.5 mM TTABr in 16.35 mM phosphate buffer, pH 6.4 (15 mM ionic strength) and 1 mM Brij 35 in 20 mM borate buffer, pH 9.2 (10 mM ionic strength) were prepared. The stock marker solutions used were 1 mM ethylparaben in the BGE (for SDS) or in water (for TTABr) and 0.96 mM SOBS in the BGE (for Brij 35). These solutions were next mixed in appropriate

proportions with the corresponding plain BGE to generate appropriate series of solutions of variable known surfactant concentrations and constant marker concentration.

3.3. Capillary electrophoresis

Electrophoretic measurements were performed with a HP^{3D}CE (Agilent Technologies, Waldbronn, Germany) capillary electrophoresis system. This apparatus automatically realizes all the steps of the analytical protocols, including capillary conditioning, sample introduction, voltage application and diode array detection, and allows to run unattended method sequences. Data were handled by the Agilent Chemstation and Microsoft Excel softwares. According to the experiments, migrations were performed in bare fused silica capillaries (Phymep, Paris, France) 35 cm (26.5 cm to the detector) × 50 μm i.d. or eCAP amine-coated capillaries (Beckman, Gagny, France), 62.5 cm (54 cm to the detector) × 50 μm i.d. The running voltage was 10, 15 or 25 kV. The temperature of the capillary cartridge was set at 25 °C and the sample tray was also maintained at this temperature by an external water bath. The markers were detected by UV absorbance at 193 or 256 nm, according to cases.

For the FACCE experiments, BGE was first flushed into the capillary for 5 min under 935 mbar (about 20 capillary volumes). Next, a small plug (approximately 3 nL) of the surfactant/marker sample mixture was hydrodynamically injected for 2 s under 30 mbar. Then, a continuous electrokinetic introduction of the previous sample mixture in the same electrolyte was performed under the running voltage.

For the VFACCE experiments, capillary was initially equilibrated with the surfactant/marker mixture dissolved in the BGE, next a small plug (approx. 3 nL) of the BGE was hydrodynamically injected into the capillary (applying a 30 mbar pressure for 2 s), and finally BGE was continuously introduced under electrical field.

New bare fused silica capillaries were conditioned by successive flushes with 1 M NaOH, 0.1 M NaOH, water and finally equilibrated with the BGE, under 935 mbar for 10 min each (about 40 capillary volumes). Between runs, the capillaries were washed with 0.1 M NaOH for 3 min, water for 2 min and BGE for 5 min. Before first use, amine-coated capillaries were flushed with 0.1 M NaOH for 3 min, water for 10 min and the Amine Regenerator Solution (from Beckman) for 10 min. Between runs, amine coated capillaries were washed with 0.1 M NaOH for 3 min, water for 2 min and BGE for 5 min, as recommended by the supplier. Capillaries were rinsed with water and dried by air when not in use.

4. Results and discussion

4.1. Determination of the aggregation threshold for SDS

In a previous work [15], it was shown that the partial, continuous frontal electrophoretic separation of UV-absorbing anionic surfactants in the presence of an electroosmotic flow resulted in the direct, clear-cut visualization of a free surfactant zone, followed by these assigned to aggregated forms. Calibration of the height of the first detected front gave an easy access to the surfactant CMC. Unfortunately, this simple, direct approach cannot be straight applied to UV-transparent anionic surfactants and a chromogenic marker, interacting with the surfactant has to be selected in view of a spectrophotometric monitoring. It is worthy of note as to now that this marker needs not bind strongly the free surfactant, as the monitoring of the concentration of this latter form is not mandatory to obtain the CMC. More generally, the CMC threshold can be pinpointed from the variation of the free marker concentration as a function of the total anionic surfactant in the system, as was anticipated from the theoretical study (see especially Eqs. (5) and (7) and Fig. 2). Among the potential markers to be used, it is well known that most organic cationic compounds strongly bind anionic micelles. Nevertheless, neutral hydrophobic markers should be preferred for the present purpose to prevent wall interactions in bare silica capillaries. According to the guidelines stated in the theoretical part, ethylparaben (EP, ethyl *p*-hydroxybenzoate) was chosen to evaluate the merit of frontal electrophoresis and study the aggregation of emblematic, anionic surfactant SDS, the CMC of which in water is 8.1 mM at 25 °C [17]. Indeed, ethylparaben has an intermediate hydrophobicity (log $P = 2.11$ [18]), ensuring proper incorporation into the micelles, while remaining rather soluble in water (ca. 5 mM [18,19]). Fig. 3A shows a series of frontal electropherograms obtained with continuous electrokinetic introduction of 0.67 mM EP in the presence of varying concentrations of SDS. Under the experimental conditions employed (bare silica capillary, electrolyte and voltage polarity), neutral and anionic zones are carried to the detector in that order by a cathodic electroosmotic flow. The first detected migration front, F1, common to all electropherograms, is migrating at the electroosmotic velocity (corresponding to a mobility m_{eo} of 70×10^{-5} cm^2 V^{-1} s^{-1}) and therefore attributed to the neutral marker in its free form. Afterwards, different migration fronts can be distinguished according to the total SDS concentration. For $[S]_t$ values lower than 6 mM, a second migration front F2 appears with an inflection point corresponding to an electrophoretic mobility of -22×10^{-5} cm^2 V^{-1} s^{-1}. This front and the subsequent zone should be due to the neutral marker interacting with the monomeric surfactant. When $[S]_t$ values are greater than 7 mM, a third, spread front, F3, is developing for migration times above 4 min. In order to render this whole migration front more discernable, the introduction of the surfactant/marker mixture was also performed at the detection side of the capillary (so-called short-end injection) leaving an effective migration length of 8.5 cm. The resulting electropherograms are represented in Fig. 3B. The electrophoretic mobility corresponding to the inflection point of front F3 is about -50×10^{-5} cm^2 V^{-1} s^{-1}. So, this latter front should pertain to the marker incorporated inside the

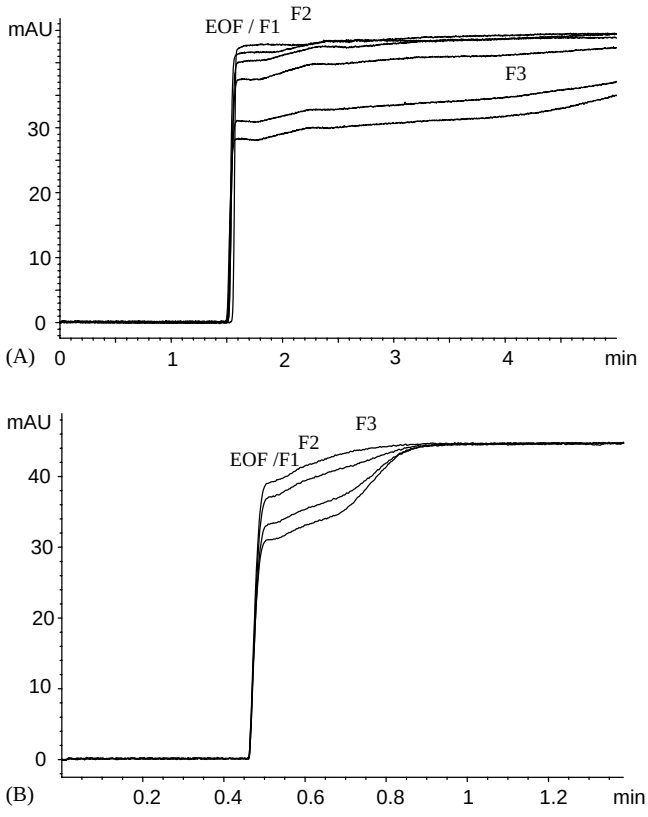

Fig. 3. Frontal electrophorerograms of SDS/ethyl paraben mixtures obtained with (normal) long end (A) and short end (B) sample introduction. Bare silica capillary, 35 cm (detection, 26.5 cm (A); 8.5 cm (B)) × 50 μm i.d. BGE: 2.8 mM sodium phosphate buffer, pH 6.9 (5 mM ionic strength). Applied voltage: +25 kV (A); −25 kV (B); initial current intensity, 4 μA. Temperature: 25 °C. UV absorbance detection at 256 nm. Sample: 0.67 mM ethylparaben and SDS at the following various concentrations in the BGE: top to bottom: (A): 1, 3, 5, 7, 9 and 10 mM; (B): 6, 7, 8 and 9 mM. Sample introduction: hydrodynamic (30 mbar, 2 s), followed by continuous electrokinetic (+25 kV (A), −25 kV (B)). EOF: electroosmotic flow. F1, F2, F3: migration fronts of free marker, marker-monomeric surfactant and marker-micelle complexes, respectively.

SDS micelle. Furthermore, a perfect overlay of the baseline and the final absorbance level can be noticed on these electrophoregrams, which indicates that the detector response coefficient remains independent of the form of the marker (free or associated with monomeric SDS or SDS micelle). This property was used during the experiments to better control the constancy of the total marker concentration. Finally, the variation of the height of the migration front of the free marker (F1) in terms of the total SDS concentration is plotted in Fig. 4. As the total marker concentration was kept constant and as this height was proportional to the free marker concentration (calibration graph not shown), the latter should display the trend predicted by Eqs. (5) and (7). Although hyperbolic variations on either side of the CMC are anticipated from the theoretical section, high regression coefficient values were obtained when treating the experimental points by linear least squares regression, which validates this most simple mathematical procedure. Accordingly, the CMC for SDS was evaluated as the abscissa of the intersection point of the two straight lines obtained, yielding a value of 6.1 mM for a 5 mM ionic strength, pH 6.9 sodium phosphate buffer. This value appears to be in very close agreement with those available in the literature, especially with the work by Jacquier and Desbène [9], who found CMC values of 6.9 mM at 3.3 mM Na^+, as in our conditions.

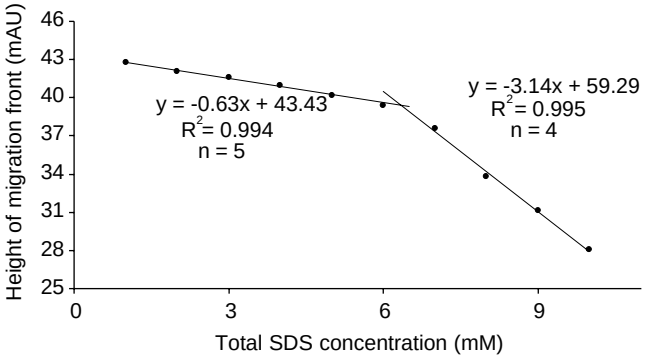

Fig. 4. Variation of the height of the migration front (F1) of the free marker (ethylparaben), measured at a constant electrophoretic mobility of -7×10^{-5} cm^2 V^{-1} s^{-1}, as a function of the total SDS concentration. Experimental conditions, see Fig. 3A.

4.2. Determination of the aggregation threshold for TTABr

The preceding method, developed for a UV-transparent, anionic surfactant cannot be applied for a cationic surfactant because of strong electrostatic and cooperative hydrophobic interactions with the negatively charged wall of the bare silica capillary. The continuous electrokinetic introduction of a cationic surfactant into a bare silica capillary according to the experimental protocol described above would result in the reversal of the electroosmotic flow (from cathodic to anodic) during the migration of the surfactant front, because of the progressive reversal of the polarity of the electrical double layer [20]. After some time, the free and micellar forms of the cationic surfactant would be dragged by the anodic flow, so that the corresponding front could not be detected, unless the voltage polarity was inverted. No quantitative measurement of the height of a migration front can therefore be performed in this case, due to surfactant adsorption. These problems were avoided by using an amine coated silica capillary, which ensures anodic electroosmotic flow due to its positive surface charge and also minimizes the surfactant adsorption through electrostatic repulsion. Likewise, cationic surfactants migrate counter-electroosmotically, but can be detected on the anodic side when injected under negative polarity, thus behaving quite symmetrically to anionic surfactants in bare silica capillaries. Accordingly, the migration fronts can be detected in order of the increasing effective mobilities, that is, neutral marker first, then monomeric surfactant, and micelle last. TTABr was selected to provide proof of principle. Also, the requirements for the marker can be considered as remaining unchanged, and ethylparaben

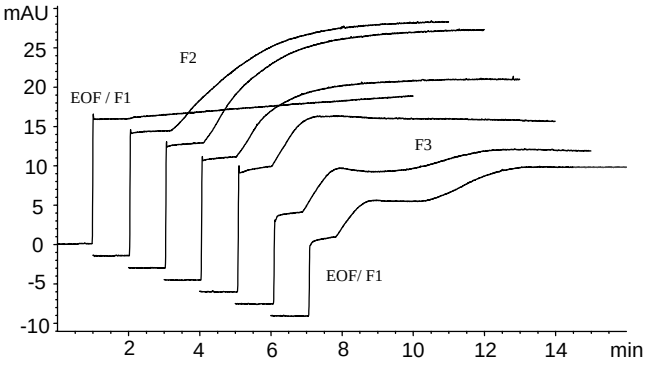

Fig. 5. Frontal electropherograms of tetradecyltrimethylammonium bromide (TTABr)/ethyl paraben mixtures. Amine coated silica capillary, 62.5 cm (detection, 8.5 cm) × 50 μm i.d. BGE: 10.9 mM sodium phosphate buffer, pH 6.4 (10 mM ionic strength). Applied voltage: +15 kV, initial current intensity, 6.4 μA. Temperature: 25 °C. UV absorbance detection at 256 nm. Sample: 0.33 mM ethylparaben and TTABr at the following various concentrations in water: left to right: 0, 0.75, 1.25, 1.50, 2, 3, 3.50 mM. Short-end introduction of the sample: hydrodynamic (30 mbar, 2 s), followed by continuous electrokinetic (+15 kV). EOF: electroosmotic flow. F1, F2, F3: migration fronts of free marker, marker-monomeric surfactant and marker-micelle complexes, respectively.

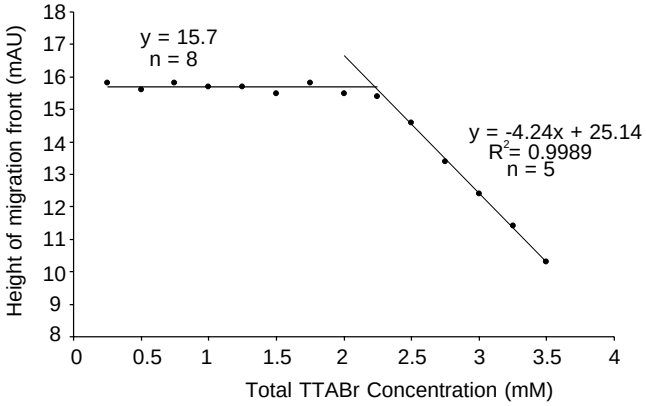

Fig. 6. Variation of the height of the migration front (F1) of the free marker (ethylparaben), measured at a constant electrophoretic mobility of $+9 \times 10^{-5}\,cm^2\,V^{-1}\,s^{-1}$, as a function of the total TTABr concentration. Experimental conditions, see Fig. 5.

was again chosen as the marker for TTABr, the CMC of which is 3.5 mM in water [17]. Fig. 5 shows a series of frontal electropherograms of TTABr-ethylparaben mixtures obtained by short-end introduction into an amine coated capillary for various concentrations of TTABr and a fixed concentration of ethylparaben. When no surfactant was present in the sample, only the marker front (F1) was detected, at a time corresponding to the electroosmotic velocity ($m_{eo} = -35 \times 10^{-5}\,cm^2\,V^{-1}\,s^{-1}$). The slight drift in absorbance observed after the front suggests that the marker was weakly adsorbed onto the capillary wall, presumably by hydrophobic interactions with the capillary coating. When TTABr was present in the sample, different migration fronts were detected, depending on the initial surfactant concentration. For concentrations up to 1.25 mM, the electropherograms exhibited a second, diffuse, front (F2) having an inflection point corresponding to electrophoretic mobilities of (-20.6 to -22.9) $\times\ 10^{-5}\,cm^2\,V^{-1}\,s^{-1}$. This front was attributed to the monomeric form of the surfactant associated to the marker. The dissymmetry and spreading of this front indicates that the adsorption of the marker was enhanced in the presence of the surfactant, which in turn suggests monomer surfactant adsorption. For surfactant concentrations ranging from 1.25 to 2 mM, the height of the front of the monomeric surfactant tended to decrease on increasing total surfactant concentration, suggesting the occurrence of oligomeric surfactant aggregates and a modification of the molar absorption coefficient of the marker included into these aggregates. For upper surfactant concentration, a third migration front, F3, was detected, the inflection point of which corresponded to an electrophoretic mobility of $-33 \times 10^{-5}\,cm^2\,V^{-1}\,s^{-1}$. This latter front was assigned to the surfactant micelles. The decreasing height of the total absorbance on top of this front with increasing total surfactant concentration seems to confirm that the absorption coefficient of the marker was lowered when incorporated into tetradecyltrimethylammonium aggregates. Fig. 6 shows the variation of the height of the migration front of the free marker (F1) as a function of the total surfactant concentration. The height of this front remains constant until the surfactant reaches a total concentration of ca 2 mM, then it declines linearly. As for SDS, the CMC value for tetradecyltrimethylammonium in this BGE (10 mM ionic strength sodium phosphate buffer, pH 6.4) can be determined from the abscissa of the intersection point of the two regression straight lines, which gives a value of 2.25 mM. This result is in good agreement with the CMC value in water (3.5 mM [17]) and in a 70 mM phosphate buffer, pH 6 (1.6 mM [12]). Eventually, it is to note that the quantitative monitoring of the height of the front of the free marker (F1) is perturbed neither by wall adsorption nor by the variation in the molar absorption coefficient, since it is detected first. The short-end, frontal introduction of the sample may also contribute here to minimize the incidence of these deleterious effects.

4.3. Determination of the aggregation threshold for Brij35

The last surfactant experienced to assess the performances of FACCE methods was the non-UV-absorbing, neutral surfactant Brij 35, which consists in an oligomeric distribution of polyoxyethylenedodecanol centered on the 23 mer. The reported CMC for this surfactant is 0.09 mM [17] and this value should be almost insensitive to ionic strength, since Brij 35 does not bear any electric charge. As the free form of the interactive marker should be separated from the surfactant-bound ones in order the CMC to be determined, the marker should be charged and hence rather amphiphilic in this case. We therefore elected to use the UV-absorbing, anionic surfactant sodium octylbenzenesulfonate (SOBS) at a concentration lower than its critical micellar concentration

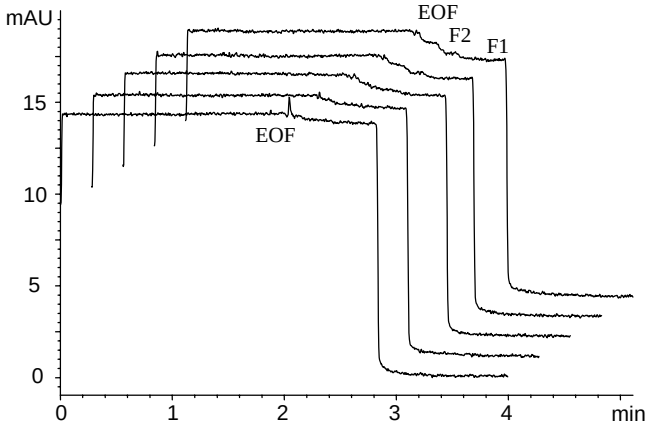

Fig. 7. Vacancy frontal electropherograms of Brij 35/sodium octylbenzenesulfonate (SOBS) mixtures. Bare silica capillary, 50 μm i.d. × 35 cm (detection, 26.5 cm). BGE: 20 mM sodium borate buffer, pH 9.2 (10 mM ionic strength). Applied voltage: 10 kV, initial current intensity, 5 μA. Temperature: 25 °C. UV absorbance detection at 193 nm. Initial hydrodynamic capillary conditioning with 0.064 mM SOBS and Brij 35 at the following various concentrations in the BGE: left to right: 0.05, 0.07, 0.09, 0.13, 0.15 mM. Hydrodynamic (30 mbar, 2 s), followed by continuous electrokinetic (10 kV) injection of BGE. EOF: electroosmotic flow. F1, F2: migration fronts of free SOBS and mixed Brij 35/SOBS micelles, respectively.

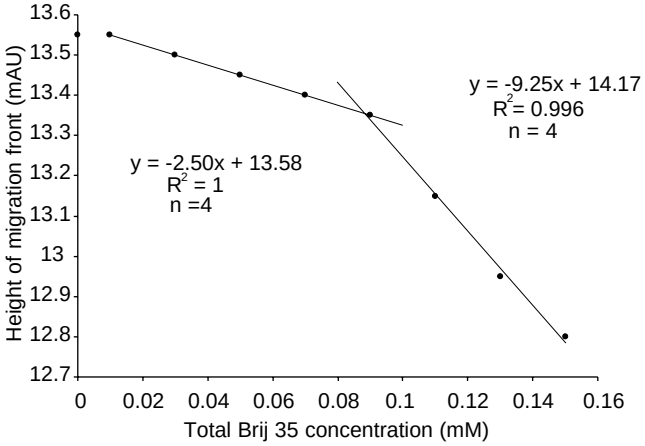

Fig. 8. Variation of the height of the migration front (F1) of the free marker (SOBS), measured at a constant electrophoretic mobility of $-17 \times 10^{-5}\,cm^2\,V^{-1}\,s^{-1}$, as a function of the total Brij 35 concentration. Experimental conditions, see Fig. 7. Each experimental point is the average obtained from duplicate frontal electrophoresis.

(10 mM in 10 mM sodium borate buffer, pH 9.2 [15]). For this marker, a bare silica capillary was more relevant, to minimize wall adsorption. Under low ionic strength borate buffer conditions, the electroosmotic flow dragged all the species of interest to the cathodic side of the capillary, so that introducing Brij 35/SOBS mixtures from the anodic side by a conventional FACCE protocol would result in a diffuse front of SOBS-labelled Brij micelles being detected first after the electrosmotic time, followed by a positive front of free SOBS. The quantitative treatment of such a frontal electropherogram would then require to measure the height of the final SOBS front, using the preceding Brij/SOBS plateau as baseline. This would be very unwieldy in practice, considering both Brij 35 polydispersity and occurrence of wall adsorption. For these reasons, we deemed it better to equilibrate first the capillary with the working Brij 35/SOBS mixtures in the BGE and then to continuously introduce the plain BGE into the capillary under electric field, a mode that we called vacancy frontal analysis continuous capillary electrophoresis (VFACCE). A representative set of electropherograms is given in Fig. 7. The small peak or baseline alteration appearing at 2.05 min was due to EOF. For Brij concentration lower than 0.09 mM, a single, negative front (F1), corresponding to an electrophoretic mobility of (−20 to −22) $\times 10^{-5}\,cm^2\,V^{-1}\,s^{-1}$, and therefore attributable to SOBS [15], was detected. For Brij 35 concentrations upper than 0.09 mM, the electropherograms exhibited close additional negative fronts (F2) of intermediate mobility between SOBS and EOF, which presumably corresponds to mixed Brij 35/SOBS micelles. Applying the VFACCE protocol allowed us to monitor the height of migration front of the free marker (F1) from the stable baseline of the BGE, leading to much more reproducible results for the small variation that needed to be measured. Increased signal to noise ratios were obtained using short wavelength (193 nm) detection. The height of this front was plotted against the total Brij 35 concentration in Fig. 8. The slow decrease in free marker concentration observed for Brij concentration under 0.09 mM might be due to Brij monomer/marker associations. A steeper decrease, however, was noticed for Brij concentrations in excess of 0.09 mM. The CMC value determined from the intersection point of the two regression straight lines was 0.089 mM, in excellent agreement with the literature value (0.09 mM [17]).

5. Concluding remarks

Extending preliminary results acquired for UV-absorbing anionic surfactants, this work provides proof of principle that FACCE is effective at determining aggregation threshold for any kind of surfactant, be it anionic, cationic or neutral, especially as regards accuracy and sample amount requirement. The time needed for the determination is directly linked to the aimed precision. Non UV-absorbing surfactants require the resort to a marker conferring proper detection conditions. Neutral markers can be selected for charged surfactants, whereas a charged, preferably anionic marker should be retained for neutral surfactants, so that partial FACCE separation of the free and surfactant-bound forms of the marker can take place. One advantage of the FACCE mode over the zone one is that surfactant-marker equilibrium is perturbed to a minor extent, just like in methodologies based on dialysis. Also, the choice of the hydrophobicity of the marker can be enlarged toward medium levels, making it easier as compared to zone electrophoresis,

due to the fact that the followed experimental parameter in FACCE is the free marker concentration. In addition, the kinetics of the micelle-BGE distribution needs not be as fast as for zone mode, which could be an asset for studying peculiar high molecular aggregates. Finally, it should be emphasized that this work introduces a vacancy set-up of frontal analysis capillary electrophoresis, VFACCE. VFACCE appears to be particularly well adapted when the migration front of interest presents the smallest apparent mobility, as is the case for the CMC determination of neutral surfactant, as it permits the height of this front to be quantified more precisely. Other applications of VFACCE can be contemplated in the realm of the determination of interaction constants.

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