Structural Transitions in a Bicationic Amphiphile System Studied by Light-Scattering, Conductivity, and Surface Tension Measurements

Maria Isabel Viseu,*,† M. Mercedes Velázquez,‡ Cláudia S. Campos,† Inmaculada García-Mateos,‡ and Sílvia M. B. Costa†

Centro de Química Estrutural, Complexo I, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal, and Departamento de Química Física, Facultad de Química, Universidad de Salamanca, Plaza de la Merced s/n, 37008 Salamanca, Spain

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Vesicles were found to form easily in diluted aqueous mixtures of two cationic amphiphiles, the double-tailed, bilayer-forming didodecyldimethylammonium bromide (DDAB) and the single-tailed, micelle-forming dodecyltrimethylammonium chloride (DTAC), and therefore are believed to be spontaneous. A combination of equilibrium tensiometry, conductivity, and static light-scattering techniques enabled the determination of critical aggregation concentrations corresponding to the structural transitions *monomers* ↔ *vesicles* (CVC) and *vesicles* ↔ *micelles* (CMC), at several DDAB/DTAC molar ratios, thus delimiting the composition range of the vesicle stability, at 25 °C. It was found that the *monomers* ↔ *vesicles* transition, at lower total surfactant concentrations, is driven by the double-chained DDAB, while the *vesicles* ↔ *micelles* transition, at higher total surfactant concentrations, is mainly determined by the single-chained DTAC. A region of coexistence of vesicles and micelles was found, whose limiting lower and upper CMC values (CMC_L, CMC_U) were defined by static light-scattering measurements.

Introduction

Vesicles have recently attracted much interest due to their biochemical applications. In particular, synthetic quaternary ammonium compounds that are widely used to prepare vesicles, such as dioctadecyldimethylammonium chloride, DODAC, or bromide, DODAB, have outstanding properties as antigen-specific immunostimulators.[1] Several synthetic cationic vesicles have been successfully employed to interact with negatively charged surfaces or biomolecules such as antigenic proteins,[2] prokariotic[3] and eukariotic cells,[4] or viruses.[5]

However, the low stability of vesicles has limited their widespread application. Therefore, a great deal of research has been carried out to improve the stability of vesicles. Spontaneous vesicles, which are believed to be thermodynamically stable, were recently obtained in a few systems, mainly in aqueous mixtures of oppositely charged amphiphiles.[6−11]

In a previous work,[12] we found evidence of the formation of vesicles in a bicationic amphiphile system containing the double-tailed ("lipid" type, bilayer-forming) didodecyldimethylammonium bromide, DDAB, and the single-tailed ("detergent" type, micelle-forming) dodecyltrimethylammonium chloride, DTAC. The vesicles were obtained by dissolving DDAB in an aqueous DTAC solution, without sonication or other energy input except for a gentle manual mixing, and therefore are believed to be spontaneous. They appear in highly diluted solutions, at total surfactant concentrations intermediate from those where monomers and mixed micelles prevail. These vesicles were characterized at 25 °C by cryogenic transmission electron microscopy (cryo-TEM) and dynamic light-scattering (DLS) techniques, which provided complementary information on the aggregate form, mean size, and size polydispersity.[12]

In the last years, it became clear that vesicles and micelles are closely related structures which transform into each other by a slight change of composition or temperature.[7,13] Consequently, even though a phase diagram of the system DDAB-DTAC−water was presented,[12] the phase transition regions surrounding the vesicle region could not be clearly defined by the above techniques alone. In fact, only a few samples could be analyzed by cryo-TEM, and DLS is not sensitive to concentration but only to the aggregate size (related to its diffusion coefficient). Also, due to artifacts in the thin film preparation, such as surfactant adsorption and sorting effects,[14,15] cryo-TEM cannot give the exact composition

* Corresponding author. Telephone: 00-351-21-8419389. Fax: 00-351-21-8464455. E-mail: iviseu@alfa.ist.utl.pt.

† Instituto Superior Técnico.

‡ Universidad de Salamanca.

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limits for the various phases, because the concentration in the vitreous water film may differ from that of the bulk solution.

In the present paper, we obtain the critical concentrations corresponding to the pseudophase (or *structural*) transitions *monomers* ↔ *vesicles* (CVC) and *vesicles* ↔ *micelles* (CMC) of the system DDAB-DTAC−water at 25 °C. A combination of static light-scattering, conductivity, and equilibrium surface tension measurements was used for this purpose.

The *vesicles* ↔ *micelles* transition involves a considerable change in scattered light, and therefore it has been extensively studied in several systems by static light-scattering measurements. Typical examples are lecithin−surfactant systems,[16−21] which have some similarity with the present DDAB−DTAC system because both are composed of double- and single-chained amphiphiles. In fact, we found that the *vesicles* ↔ *micelles* transition was easily defined from static light-scattering data. However, it was not possible to assess from these measurements the critical aggregation concentration corresponding to the formation of vesicles from monomers, at lower surfactant concentrations.

On the other hand, equilibrium surface tension measurements have been widely used to obtain critical micelle concentrations (CMCs) of single-tailed surfactants, either pure[22] or in binary mixtures.[23,24] However, they have been used less often to obtain critical aggregation concentrations (CACs) of pure double-tailed amphiphiles, or mixtures that involve this type of amphiphiles.[25] This happens because double-tailed surfactants are sparingly soluble in water, and clear, homogeneous "solutions" (which are in fact dispersions) of these surfactants may only be obtained at the expense of a considerable energy input—such as vigorous mixing, heating, and/or sonication. Therefore, the aggregates obtained may not be true equilibrium structures.

However, double-tailed surfactants are more surface active than single-tailed ones, and therefore tensiometry might be a very adequate technique to study aggregation in systems involving these amphiphiles, even at very low concentrations. In this work, equilibrium surface tension measurements were extended into the vesicles region and enabled a quite clear definition of the *monomers* ↔ *vesicles* transition in the system DDAB-DTAC−water.

Finally, conductivity measurements have been extensively employed to determine the CMCs of pure and mixed ionic micelles. Therefore, we used this technique to obtain further information on the micelles formed in the present system.

Experimental Section

1. Materials and Preparation of Samples. Didodecyldimethylammonium bromide, DDAB, was obtained from Fluka with purity ≥98%, while dodecyltrimethylammonium chloride, DTAC, is an ion-pair chromatographic reagent purchased from TCI (Tokyo Kasei, Japan). The amphiphiles showed no minimum in the surface tension−concentration plots, confirming their purity, and therefore were used without further purification. The solvent water was purified with the Millipore Milli-Q system and presented a specific conductance of 0.5 ± 0.2 μS cm^{-1} and an equilibrium surface tension of 73.3 ± 0.8 mN m^{-1}, at 25 °C. This surface tension value slightly differs from the literature value, 72.0 mN m^{-1}.[26] Discrepancy is probably due to the measuring mode, the *standard mode*, designed for systems containing surface-active agents. We used the former value for comparative purposes, to study the effect of surfactant or surfactant mixtures on the water surface tension, obtained under the same experimental conditions.[27]

All concentrations are given on a molar basis. In binary systems, the composition is defined by the DDAB molar fraction relative to total surfactant, x_{DDAB}, and by the total surfactant concentration, C_0. Stock solutions of the amphiphile mixtures with prechosen x_{DDAB} values were prepared by dissolving DDAB in an aqueous DTAC solution and diluting to the final concentration with pure water. Care was taken not to use any external energy input except for gentle manual mixing or heating (if necessary), to obtain only the spontaneous aggregates. Stock solutions were prepared in the region of mixed micelles and then were diluted with pure water to the final solutions, some of which contained vesicles.

For the light-scattering experiments, x_{DDAB} ranged from 0.05 to 0.40 and C_0 from 1 to 30 or to 50 mM. For the surface tension measurements, samples were prepared with $x_{DDAB} = 0.10$ and 0.20 with C_0 ranging from $\approx 10^{-2}$ to $\approx 10^{2}$ mM. For the conductometric titrations, two samples were made with $x_{DDAB} = 0.10$ and 0.20, with C_0 equal to 30 and 50 mM, respectively. Pure DTAC and DDAB solutions were also prepared, to obtain their critical aggregation concentrations by both surface tension and conductivity titration measurements.

Except for the case of conductivity measurements, where a titration method was employed (see below), solutions were allowed to stand a few days at room temperature (20−25 °C), and at least 1 h at 25 °C just before measurements. These periods of time were considered sufficient to obtain the equilibrated structures, because kinetic light-scattering experiments showed that most DDAB−DTAC vesicles appear within a few minutes after dilution of an initial mixed micellar solution.[28]

2. Methods. a. Equilibrium Surface Tension Measurements. The equilibrium surface tension measurements were carried out in a drop volume tensiometer, model TVT1 from Lauda, Germany,[29] using a 2.5 mL syringe with an outer radius of the fitted steel capillary of 1.385 mm.

Measurements were performed in the so-called *standard mode* (STD), in which the drop formation consists of two different stages: at the beginning, it is formed with a rather high speed; then, the dosing rate decreases automatically with the increasing drop volume. By this procedure, hydrodynamic effects are avoided, because the final dosing rate just before the drop detachment is always very low.

In all measurements, the temperature in the tensiometer was controlled at 25.00 ± 0.01 °C by means of a thermostat/cryostat RM 6 B from LAUDA. The reproducibility in the surface tension measurements (average over 5 drops) was $\pm 0.01-0.05$ mN m^{-1} and the final accuracy in surface tension was ± 0.1 mN m^{-1}.

b. Conductivity Measurements. The electrical conductivity was measured at 25.0 ± 0.1 °C using a CDM 83 conductometer from Radiometer, Copenhagen, operated at 50 kHz and an Ingold conductivity cell of 0.974 ± 0.04 cm^{-1}. The cell constant was obtained by calibration with KCl solutions of known concentrations.[30]

Because the correct determination of the CMC by this method requires a large number of experimental data, a conductometric

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titration was employed. In pure surfactant solutions, each curve had 40 conductivity vs surfactant concentration data. For the binary mixtures, each curve contained 100−120 experimental points. During the titration, solutions obtained by successive dilutions were allowed to equilibrate a few minutes until a stable measurement (within the conductometer error) was obtained. The uncertainty in the conductance measurements was less than ±1% in the mS cm^{-1} scale and less than ±0.2% in the μS cm^{-1} scale.

c. Static Light-Scattering (SLS) Measurements. SLS measurements were performed in order to obtain the total intensity of scattered light. Measurements were done at 25.0 ± 0.1 °C in a standard luminescence spectrometer, model LS-50B from Perkin-Elmer, with the sample contained in a "fluorescence" quartz cell with a section of 1 × 1 cm^2. Incident light of 633 nm was used, and the scattered light was also collected at 633 nm at the scattering angle of 90°. The fluorimeter was operated in the *time−drive mode* in order to evaluate the measurement stability. Other operating conditions, such as "excitation" and "emission" slits, were kept constant whenever possible throughout the experiments; otherwise, a reference sample was used for comparison.

d. Phase-Contrast Microscopy. Two samples, a 2.24 mM pure DDAB solution and a mixed solution with $x_{DDAB} = 0.20$ and $C_0 = 18.0$ mM (or [DDAB] = 3.60 mM), were observed by phase-contrast microscopy at room temperature, using an Axiophot 2 microscope from Zeiss with a 440× lens.

Results and Discussion

General Behavior of DTAC and DDAB Amphiphiles and their Mixtures. Even though DTAC and DDAB have long alkyl chains of equal length (C_{12}) and similar headgroups (tri- and dimethylammonium, respectively), their pure aqueous solutions present quite different behavior resulting from the different number of chains in each surfactant.

The single-chained DTAC is easily soluble in water and typically forms highly curved spherical micelles above a critical concentration (CMC) of ≈20 mM, at 25 °C.[22]

The double-chained DDAB is only sparingly soluble in water, making difficult the evaluation of its critical aggregation concentration (CAC) in true equilibrium conditions. This may be the reason there is some discrepancy in the values obtained for the CAC of this amphiphile, at 25 °C: while some references indicate a CAC ≈ 0.05 mM,[31,32] another work presents an higher value, 0.16 mM.[33]

Also, the nature of the spontaneous aggregates formed by DDAB in water beyond the CAC does not seem to be fully established yet. Slightly above the CAC and up to ≈3 wt % DDAB, uni- and bilamellar vesicles have been observed using the cryo-TEM technique.[31,32,34] However, these are probably metastable because some energy is required to prepare the samples (generally described as a prolonged shaking).

Beyond a concentration of ≈3 wt %, DDAB "swells" in water and tends to form aggregates of nearly zero curvature, such as hydrated liquid crystals (lamellae).[35−39] Closed bilayer structures (vesicles) may also be obtained by an adequate energy input, using nonequilibrium methods such as the sonication of lamellar phases.[40] However, sonicated DDAB vesicles seem to be metastable and tend to revert to planar lamellae.

On the other hand, when DDAB and DTAC are dissolved together in water they produce a variety of aggregates, such as micelles, lamellar structures, and vesicles, depending on the relative DDAB:DTAC molar ratio and total surfactant concentration. In this system, vesicles may be readily obtained without any energy input except for a gentle mixing. In a previous paper,[12] these vesicles were characterized by cryo-TEM and DLS tecniques: they present a well-defined contour, are mostly spherical and unilamellar, but show a large size polydispersity with diameters ranging from ≈40−50 up to ≈500−600 nm. These vesicles were found to be *smaller* than pure DDAB vesicles, in agreement with the inclusion of the single-chained DTAC in the aggregates.

Apart from intact vesicles, other aggregates were also visualized by cryo-TEM in the DDAB−DTAC−water system:[12] vesicles with ruptured membranes, small bilayer disks (discoidal micelles), and globular micelles. Some of those aggregates were found to coexist in a few samples, such as intact and open vesicles, intact vesicles and disks, or vesicles and globular micelles.

Even though a few ruptured vesicles also appeared in the pure DDAB−water system, discoidal micelles could only be found in the mixed amphiphile system.[12] This is a good indication that disks must be composed of both surfactants, with the single-chained DTAC probably located mainly at the highly curved edges of the aggregates, protecting the DDAB hydrophobic chains from water. Ruptured vesicles and disks were assigned to intermediate structures between intact vesicles and globular micelles.

Globular micelles of the mixed system were found by cryo-TEM to be *bigger* than pure DTAC micelles,[12] according to the presence of the double-chained DDAB therein. Consequently, all the above aggregates (intact and open vesicles, globular and discoidal micelles) are probably composed of both surfactants, DDAB and DTAC.

When C_0 is increased at constant x_{DDAB}, two main *structural transitions* may be observed in the DDAB−DTAC−water system at 25 °C: *monomers* → *vesicles* and *vesicles* → *micelles*, with critical aggregation concentrations denoted by CVC and CMC, respectively. These transitions also appear in the reverse order, when C_0 is decreased at constant x_{DDAB}, and therefore will be denoted by a double arrow (↔). While the former transition seems quite sharp, the latter takes place over a large coexistence region of vesicles and micelles (probably including also ruptured vesicles and discoidal micelles), and so two limiting, lower and upper, CMC values (CMC_L and CMC_U) may be defined. In the present work, by using equilibrium surface tension, conductivity, and static light-scattering measurements, the critical concentrations of the different aggregation processes were determined as a function of x_{DDAB}.

Equilibrium Surface Tension Measurements. Equilibrium surface tension measurements were carried out in pure DDAB and DTAC solutions and also in binary mixtures with DDAB molar fractions of 0.10 and 0.20. For the mixtures, the range of total surfactant concentration was extended from ≈10^{-2} to ≈10^2 mM, to obtain both CVC and CMC. The relationship between equilibrium surface tension γ and total surfactant concentration C_0 is shown in Figure 1a. To assess which surfactant controls

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Table 1. Critical Aggregation Concentrations for the Pure DDAB and DTAC Surfactants and for Their Binary Mixtures with $x_{DDAB} = 0.10$ and 0.20, Obtained by Equilibrium Surface Tension and Conductivity Measurements[a]

surfactant or mixture	CVC ([DDAB]) surface tension	CVC ([DDAB]) conductivity	CAC lit. (ref 33)	CMC_L ([DTAC]) surface tension	CMC_U ([DTAC]) surface tension	CMC ([DTAC]) conductivity	CMC lit. (ref 22)
pure DDAB	0.18	0.14	0.16				
$x_{DDAB} = 0.20$	0.25			≈2.5	≈14	14.6	
$x_{DDAB} = 0.10$	0.22			≈4.5	≈11	14.3	
pure DTAC					22.4	22.5	20.3
pure DTAB							14.2−14.5

[a] For the mixtures, CVC (monomers ↔ vesicles) and CMC (vesicles ↔ micelles) are given in terms of [DDAB] and [DTAC], respectively. All values are in mM units. The CMC value for DTAB is given for comparison.

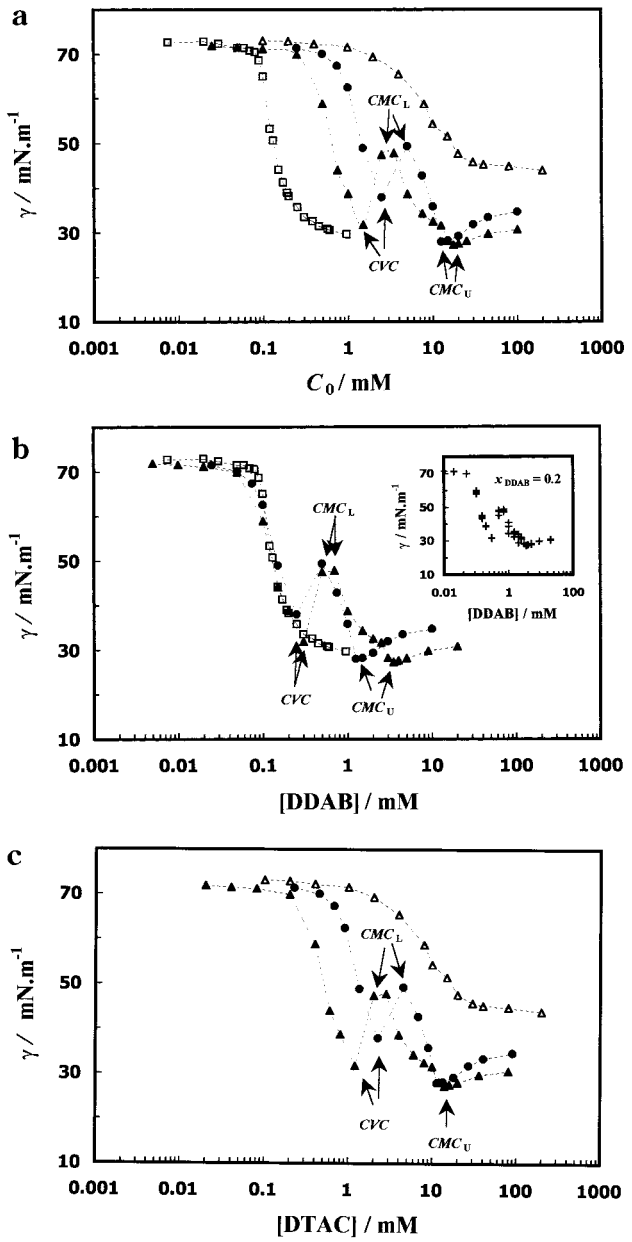

Figure 1. Equilibrium surface tension γ in the system DDAB-DTAC−water, as a function of (a) total surfactant concentration, C_0, (b) DDAB molar concentration, [DDAB], and (c) DTAC molar concentration, [DTAC]. Legend: open triangles, pure DTAC; open squares, pure DDAB; solid circles, binary mixture with $x_{DDAB} = 0.10$; solid triangles, binary mixture with $x_{DDAB} = 0.20$. The insert in (b) represents an example of the data reproducibility, for $x_{DDAB} = 0.20$.

the surface tension behavior in the mixtures, for the different concentration regimes, parts b and c of Figure 1 represent γ as a function of the individual DDAB and DTAC molar concentrations, respectively.

The surface tension of the *pure* surfactants decreases in the usual way with the increase in surfactant concentration (Figure 1a), and the curves inflect to give the CMC of DTAC (22.4 mM) and the CAC of DDAB (0.18 mM). These values are summarized in Table 1, together with a few literature values.[22,33] In the case of DDAB, the aggregates formed are probably *metastable vesicles*, and so this critical concentration is denoted by CVC in the table.

The curves show a different trend for the mixtures, presenting *three break points*: two of them where γ goes through a minimum (with $\gamma \approx 30$ mN m^{-1}) and another one, at intermediate C_0, where γ goes through a local maximum ($\gamma \approx 50$ mN m^{-1}).

The first part of these curves, at low C_0, is quite similar to the $\gamma - C_0$ curve of pure DDAB. This behavior is confirmed in the representation of Figure 1b, in terms of the DDAB molar concentration. As seen in the figure, all data below the first break point fall on a single curve. This fact indicates that DDAB is responsible for the surface properties of the mixtures in this concentration range, because it is the most surface-active component. In addition, this break point at [DDAB] ≈ 0.2−0.3 mM is close to the CVC of pure DDAB (0.18 mM). Therefore, in a first approach, this critical concentration (CVC) was ascribed to the formation of pure DDAB vesicles, from monomers.

However, after the first break point, the curves corresponding to the mixtures have positive slopes, attaining a local maximum (the second break point, denoted by CMC_L), while the pure DDAB curve stabilizes with a slightly negative slope. This effect is clearly visible in Figure 1b. The behavior observed in the mixtures in the concentration region between CVC and CMC_L suggests that a part of the DDAB molecules adsorbed at the interface is being withdrawn by the DTAC monomers, increasing the DDAB bulk concentration. Therefore, the aggregates formed at CVC must be *mixed* DDAB−DTAC *vesicles*. The same conclusion is achieved from phase-contrast microscopy (Figure 2), which shows that mixed vesicles are *smaller* than pure DDAB vesicles, in agreement with the inclusion of the single-chained DTAC in the aggregates.

The surface tension of the mixtures decreases again with C_0 after the second break point (Figure 1a), and this negative slope appears to be correlated with the decrease in γ for pure DTAC (see Figure 1c). So, this second break point seems to correspond to the onset of DTAC adsorption at the interface. This means that DTAC is now in excess in the bulk aggregates, and so it is probable that the formation of *mixed micelles* (from vesicles) starts at this concentration, CMC_L. A similar conclusion was obtained by SLS measurements, described below.

A third break point, named CMC_U and corresponding

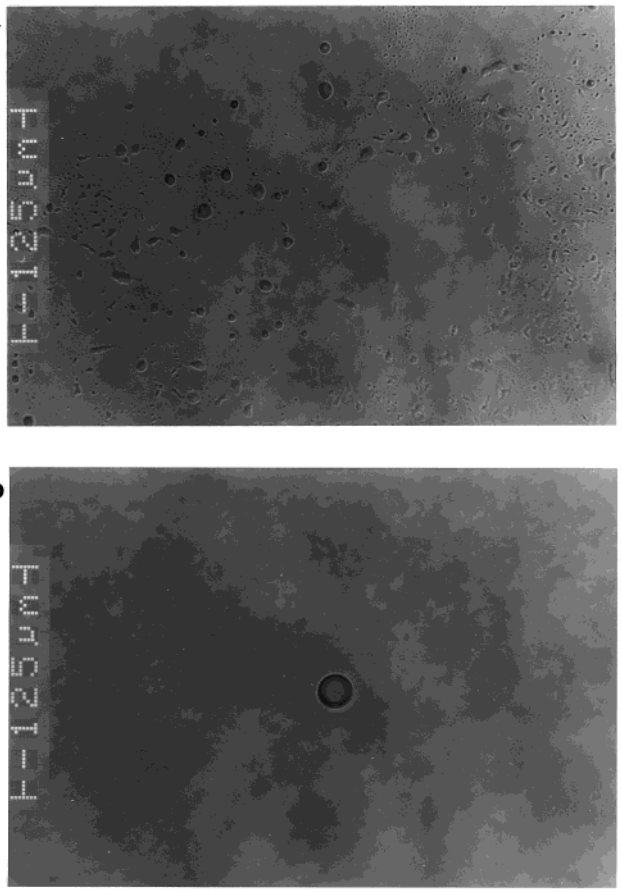

Figure 2. Phase-contrast micrographs showing **(**a**)** a polydisperse population of vesicles in a DDAB−DTAC aqueous sample with $x_{DDAB} = 0.20$ and [DDAB] = 3.60 mM and (b**)** a single vesicle in a pure aqueous DDAB sample with [DDAB] = 2.24 mM. Scale bars = 125 μm.

to the second minimum in surface tension (Figure 1), indicates a new transition process. It was attributed to the end of formation of mixed micelles from vesicles, i.e., to the point where vesicles completely disappear from solution. This result agrees with the SLS results presented below because solutions became transparent above CMC_U. Therefore, *micelles* and *vesicles* coexist in the concentration regime between CMC_L and CMC_U.

It is interesting to note that *three break points* were also found in the surface tension−molality curves of the catanionic amphiphile system DeTAB−SDeS (where DeTAB stands for decyltrimethylammonium bromide and SDeS for sodium decyl sulfate).[41] Because this type of surfactant mixture may form vesicles spontaneously, the three break points were assigned to the critical vesicle concentration and to the concentrations of the initial and final points of the vesicle−micelle coexistence region, respectively, in order of increasing molality at constant system composition. Even though important differences exist between the phase diagrams of catanionic surfactant systems and the present bicationic one,[12] it is worthwhile stressing the information obtained by surface tension measurements which, in both systems, were extended into the vesicles region.

However, we should note that the reproducibility in the surface tension is much smaller in the intermediate concentration region between CVC and CMC_U than in the other regions (see the insert in Figure 1b). This fact is likely due to adsorption and (consequent) kinetic effects during the surface tension measurement. Indeed, when the solution flows through the needle of the syringe to form a new drop, preferential adsorption of one surfactant (likely DDAB) onto the needle, at the other's expense, will change the aggregates composition and therefore will perturb equilibria between the bulk solution and the air−solution interface. Consequently, we found the need to confirm the values of the critical aggregation concentrations obtained by this technique (shown in Table 1) by complementary conductivity and light-scattering measurements.

Conductivity Titrations. Conductivity measurements have been widely used to obtain the CMC of pure or mixed ionic surfactants in water. The CMC of a pure surfactant is determined by the appearance of a discontinuity in the slope of conductivity as a function of surfactant concentration. In mixtures of two micelle-forming surfactants, a break point, which does not coincide with either of the individual CMC values, often appears and is interpreted as the critical concentration associated with the formation of mixed micelles.[42,43]

Conductivity titrations were used in this work to evaluate the critical aggregation concentrations of pure DTAC and DDAB surfactants and of two binary mixtures with $x_{DDAB} = 0.10$ and 0.20. To obtain the different break points observed by surface tension measurements, an extended concentration regime was explored for the mixtures. The results are shown in Figure 3.

As expected, only one break point was detected in the slope of the conductivity−concentration curves of the *pure* surfactants. CMC or CVC values, determined from the intersection of the tangents drawn before and after the break point, were 22.5 and 0.14 mM for the single-chained DTAC and the double-chained DDAB, respectively. Both values agree with those found by the surface tension results of the present paper and are summarized in Table 1. In addition, there is a general agreement between these values and the literature values using the same technique.[22,33] The CVC for DDAB (0.14 mM) is also expected to be slightly lower than that for the similar surfactant DDAC (due to the stronger binding of Br^- to the aggregates, as compared to Cl^-), and indeed a value of 0.176 mM was obtained for DDAC using conductivity measurements.[44]

For the mixtures, the initial (more concentrated) solutions were transparent, became turbid along the titration, and finally transparent again. Therefore, at least *two break points* could be expected in the slopes of the conductivity−concentration curves. However, only one discontinuity was detected in all the concentration ranges used (Figure 3), which does not agree with the CMC or CVC of the pure surfactants. Because it is similar, within the experimental error, to the CMC_U obtained by surface tension measurements (see Table 1), it was assigned to the upper limit of formation of *mixed micelles*, from *vesicles*.

Judging from these data, it seems that conductivity is more sensitive to the micelle association from vesicles (CMC) than to the vesicle formation from monomers (CVC). Furthermore, only one CMC was detected. These limitations probably result from the fact that, in mixtures, the discontinuity of the tangents slopes taken before and after the break point is not so abrupt as in the case of the

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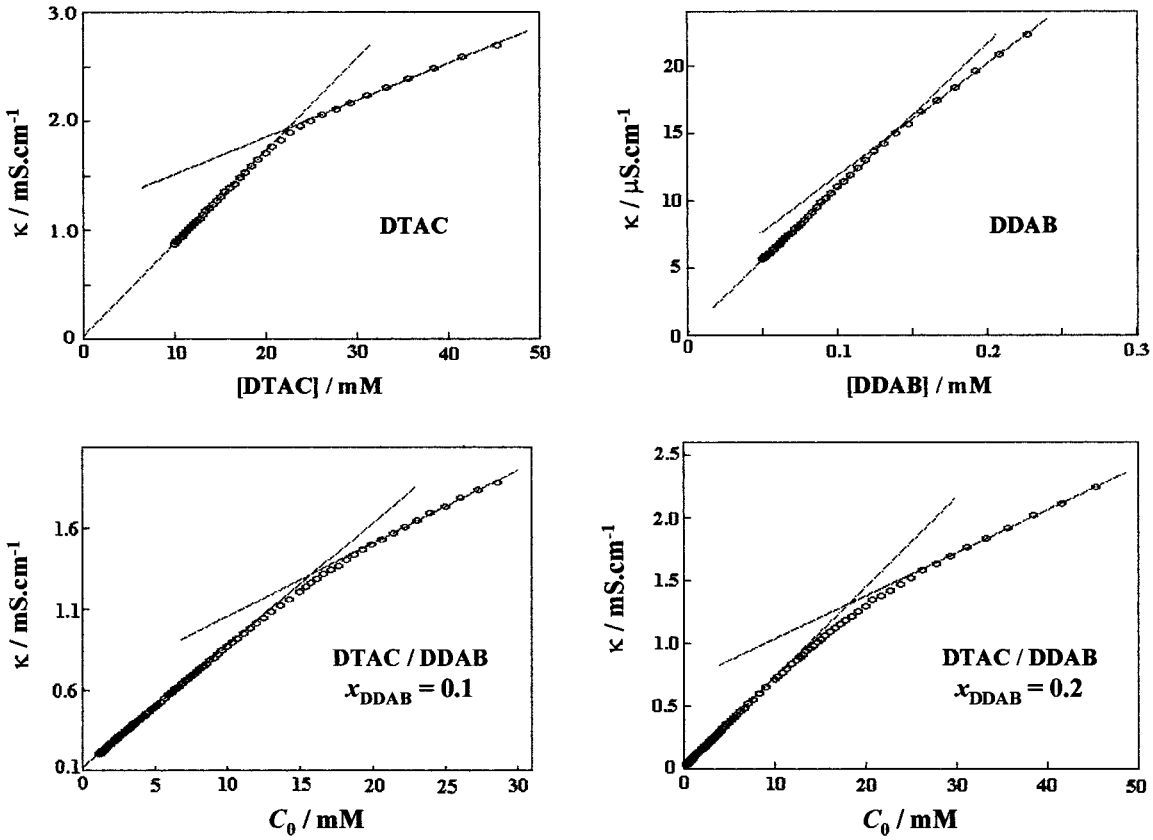

Figure 3. Conductivity κ in the system DDAB−DTAC−water, as a function of total surfactant concentration C_0, for pure DTAC, pure DDAB, and for two binary mixtures with $x_{DDAB} = 0.10$ and 0.20 (as indicated in each graph).

pure compounds (Figure 3). In fact, several experimental points in the mixtures do not fit any of the tangents. These data probably correspond to the *coexistence region* of vesicles and micelles.

A more reliable procedure to detect both critical micelle concentrations, CMC_L and CMC_U, proved to be the static light-scattering technique, as described below.

Static Light-Scattering Measurements. Static light-scattering (SLS) measurements were performed in order to obtain the total intensity of scattered light, which is related with both the dimensions and concentration number of the aggregates. Previous dynamic light-scattering (DLS) data gave independent information on the vesicles diameters as a function of the surfactants ratio and total concentration.[12] Therefore, when SLS results were combined with this information, simple relationships between the aggregates size and concentration were found.

Figure 4a presents the total intensity of scattered light I_S as a function of the total surfactant concentration C_0, for several DDAB−DTAC mixtures at constant x_{DDAB}. All curves show the same general trend and may be fragmented into three parts.

At low surfactant concentration, I_S increases almost linearly with C_0. Because this is the *vesicles* region, the constant slope suggests that these aggregates only increase in number (or concentration) when C_0 increases, keeping their size constant. This result agrees with DLS data on this system, which revealed that vesicles mean diameters are nearly constant when x_{DDAB} remains constant.[12]

At intermediate concentrations, I_S decreases abruptly from the maximum until nearly zero, as C_0 is increased. Because it is not expected that successively smaller vesicles are formed in this region,[12] this trend was interpreted as the progressive transfomation of vesicles into micelles, along a *coexistence region* of both types of aggregates. The same conclusion was obtained by the surface tension results presented above.

Finally, at high surfactant concentrations, solutions became transparent with almost zero I_S values, indicating that *micelles* are the only aggregates present. This is consistent with the surface tension and conductivity data presented above.

Therefore, only *two break points* were defined for each curve at constant x_{DTAC} (Figure 4a). These break points were named CMC_L and CMC_U, in order of increasing C_0, and both correspond to the *vesicles* ↔ *micelles* transition: lower and upper limits of the coexistence of vesicles and micelles, respectively.

As shown in the insert of Figure 4a, the reproducibility of the scattered light intensity is smaller in the concentration region between CMC_L and CMC_U than in the other regions. This was also observed for surface tension data between CVC and CMC_U and is most probably induced by perturbations in the equilibria between the different types of aggregates, vesicles and micelles (e.g., by small changes in temperature), which lead to instability in the measurements due to kinetic effects.

Figure 4b shows a different representation of the scattering data, in terms of the DDAB molar concentration. In this figure, all the data in the vesicles region nearly fall on a single curve, showing that the formation of *vesicles* is controlled by the double-chained DDAB. On the other hand, the two break points are clearly distinct for each curve, because the formation of *micelles* is mainly driven by the single-chained DTAC. These observations also agree with the surface tension results discussed above.

We should stress herein that the SLS technique was not sensitive to the *monomers* ↔ *vesicles* transition. Indeed, the slope of the curves in Figure 4b changes continuously

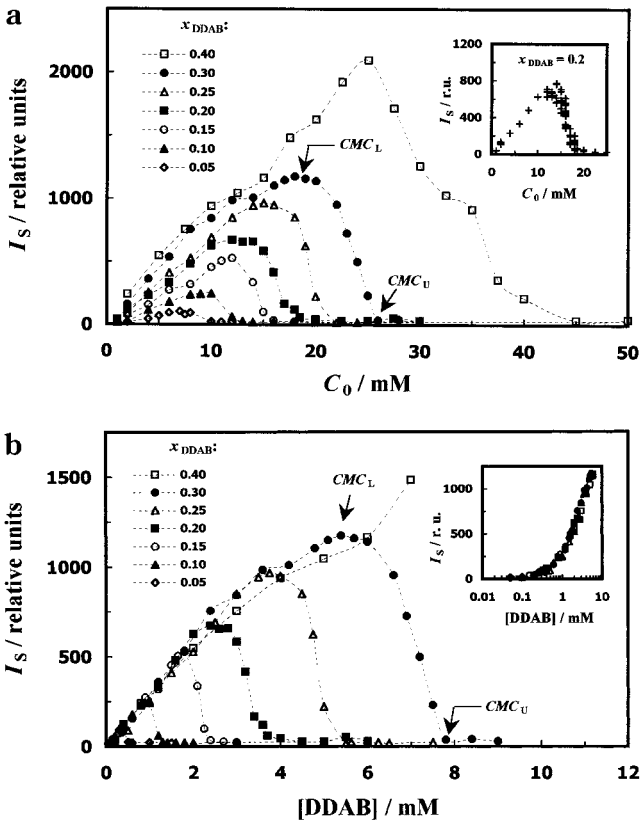

Figure 4. Total intensity of scattered light I_S in the system DDAB−DTAC−water, at several x_{DDAB} (see the inserted legend), as a function of (a) total surfactant concentration, C_0, and (b) DDAB molar concentration, [DDAB]. The inserts represent (a) an example of the data reproducibility, for $x_{DDAB} = 0.20$ and (b) an expansion of the lower concentration regime, in logarithmic scale.

Table 2. Lower (CMC_L) and Upper (CMC_U) Limits of the Critical Aggregation Concentration for the Vesicles ↔ Micelles Transition, Obtained by SLS as a Function of DDAB Molar Ratio, x_{DDAB}[a]

	CMC_L			CMC_U		
x_{DDAB}	C_0	[DDAB]	[DTAC]	C_0	[DDAB]	[DTAC]
0.40	25.0	10.0	15.0	40.0	16.0	24.0
0.30	19.0	5.7	13.3	26.0	7.8	18.2
0.25	17.0	4.3	12.8	21.0	5.3	15.8
0.20	14.0	2.8	11.2	18.5	3.7	14.8
0.15	12.5	1.9	10.6	15.5	2.3	13.2
0.10	9.6	1.0	8.6	12.5	1.3	11.3
0.05	7.7	0.4	7.3	10.0	0.5	9.5

[a] Values are given in mM units, in terms of the total surfactant concentration (C_0) and of each component concentration ([DDAB], [DTAC]).

at low [DDAB] values (see the insert in this figure, in logarithmic scale). This may be due to the fact that, at the CVC, a small number of vesicles are formed from a high number of monomers, and therefore the total scattered intensity changes slowly. As seen above, CVC could only be reliably detected by tensiometry.

The values of CMC_L and CMC_U are summarized in Table 2, for several x_{DDAB} ratios analyzed. They are indicated in terms of the total surfactant concentration, C_0, and of the corresponding DDAB and DTAC molar concentrations. These values are strongly dependent on the surfactants molar ratio, x_{DDAB}.

Figure 5 represents a partial phase diagram of the system at 25 °C, indicating the experimental values of CMC_L and CMC_U, at several x_{DDAB} values. The diagram

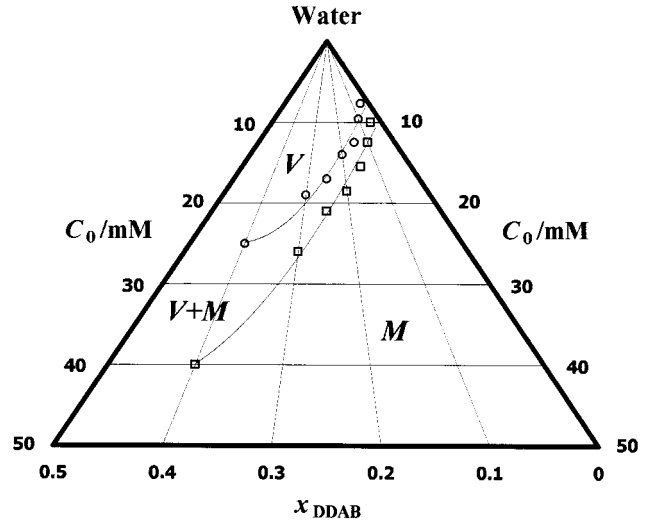

Figure 5. DDAB−DTAC−water phase diagram at 25 °C, showing the aggregates belonging to the isotropic solution phase L_1, in the diluted regime: V, vesicles; M, micelles. The circles and squares are the experimental CMC_L and CMC_U values, respectively, obtained by SLS measurements, and the lines are second-order polynomial trendlines.

is drawn in a molar basis, instead of the usual weight percent basis. The polynomial trendlines through the experimental data separate three main regions of the isotropic solution (L_1) phase: (i) vesicles (V); (ii) vesicles + micelles (V + M); and (iii) micelles (M). The present results agree with the phase diagram of the system presented previously,[12] but now the lower and upper limits of the structural transition *vesicles* ↔ *micelles* are defined.

We should finally note, as suggested by the cryo-TEM analysis,[12] that a part of this isotropic solution phase (L_1) probably coexists with a swollen liquid crystal lamellar phase (L_α), i.e., it belongs to a biphasic ($L_1 + L_\alpha$) region. The proportion of the L_α phase should increase with x_{DDAB}, but the limits of the $L_1 \leftrightarrow L_\alpha$ transition could not be defined therein.

Conclusions

By using static light-scattering, conductivity, and surface tension measurements, some *structural transitions* in aqueous mixtures of didodecyldimethylammonium bromide, DDAB, and dodecyltrimethylammonium chloride, DTAC, were observed as a function of the composition of the mixture. When the total surfactant concentration is increased at constant DDAB:DTAC molar ratio, three transitions were found: *monomers* ↔ *vesicles* (formed spontaneously in the surfactant mixtures); *vesicles* ↔ *micelles* (onset of micelle formation); and *vesicles* ↔ *micelles* (upper limit of micelle formation).

As usual, each one of the above techniques is specially designed to detect a particular structural transition. So, conductivity measurements detect mainly the *monomers* ↔ *micelles* or *vesicles* ↔ *micelles* transitions. On the other hand, static light-scattering measurements are specially sensitive to the *vesicles* ↔ *micelles* transition and enable a definition of the lower and upper limits of the coexistence region of both aggregates. Finally, surface tension measurements detect all the above-mentioned transitions, as well as the *monomers* ↔ *vesicles* transition, and therefore seem to be the most adequate method to characterize the different kinds of aggregates in the mixtures.

From all these results, it is possible to obtain a better description of the *vesicle*−*micelle* region corresponding to the diluted isotropic solution phase, L_1, of this system.

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