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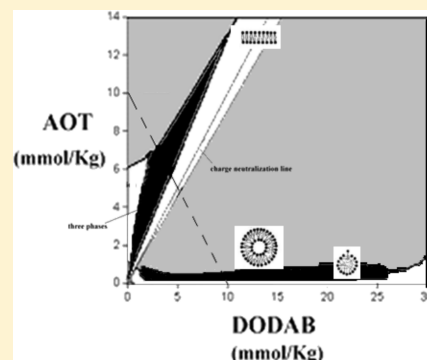
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1 Ion Distribution around Synthetic Vesicles of the Cat-Anionic Type

2 Carlotta Pucci,[§] Aurelio Barbetta,[§] Fabiola Sciscione,[§] Franco Tardani,[§] and Camillo La Mesa^{*,§}3 [§]Department of Chemistry, La Sapienza University, Cannizzaro Building, P. le A. Moro 5, I-00185 Rome, Italy4 **S** Supporting Information

ABSTRACT: Aqueous alkyltrimethylammonium bromides, or dialkyldimethylammonium ones, were mixed with sodium alkyl sulfates and dialkanesulfonates. Depending on the overall surfactant concentration, charge and/or mole ratios, cat-anionic vesicles were formed by mixing nonstoichiometric amounts of oppositely charged species. The resulting vesicles are thermodynamically and kinetically stable. ζ -potential and dynamic light scattering characterized the systems. As a rule, cat-anionic vesicles have sizes in the 10^2 – 10^3 nm range and bear significant amounts of surface charges. At fixed surfactant concentration, the vesicle surface charge density scales with mole ratios and tends to zero as the latter approach unity. Conversely, the hydrodynamic radius diverges when the cationic/anionic mole ratio is close to 1. The double-layer thickness and surface charge density are controlled by mole ratios and addition of NaBr, which plays a role in vesicle stability. The salt screens the surface charge density and modulates both vesicle size and double-layer thickness. Slightly higher concentrations of NaBr induce the transition from vesicles toward lamellar phases. The electrokinetic properties of cat-anionic dispersions were analyzed by dielectric relaxation experiments. The measured properties are sensitive to vesicle size distributions. In fact, the relaxation frequency shifts in proportion to vesicle polydispersity. Model calculations proposed on that purpose supported the experimental findings.



22 ■ INTRODUCTION

23 Ion distribution inside and around living cells controls many
24 biological processes and is responsible for their stability, shelf
25 life, and matter exchange with the medium.^{1–6} However,
26 information from in vivo experiments is hardly quantified,
27 because the experimental results strongly depend on pH, added
28 electrolytes, proteins, and other species present in biological
29 fluids. To rationalize ion distribution in the above systems,
30 therefore, simulations^{7,8} and experiments with natural or
31 reconstructed lipid-based vesicles^{9–11} are carried out. Unfortu-
32 nately, lipid-based vesicles are unstable and spontaneously re-
33 form the dispersions from which they are made. For the above
34 reasons, vesicular entities endowed with ad hoc properties are
35 considered. This is why thermodynamically and kinetically
36 stable vesicles mimicking some aspects of living cells are
37 currently dealt with.

38 Among those proposed so far, vesicles made by cat-anionic
39 (an acronym of CAT-ionic + AN-ionic, and indicated as CAT-
40 AN) mixtures of surfactants and/or lipids mimic some features
41 of cells. The rationale underlying the present working
42 hypothesis is justified by the fact that cell membranes are
43 made of cationic and anionic lipids and behave as CAT-AN
44 species in the conditions dictated by composition and medium
45 properties. The first studies on cat-anionic mixtures date to the
46 1980s. The possible formation of lamellar liquid crystalline
47 phases when oppositely charged surfactants were mixed in
48 stoichiometric amounts was demonstrated and produced poorly
49 soluble salts.^{12,13} Nonstoichiometric CAT-AN mixtures show a
50 rich polymorphic behavior; they form micelles of diverse
51 morphologies, microemulsions, lyotropic or thermotropic

liquid crystals, and vesicles, depending on the components
and their concentrations.^{14–18} It is well-known now that CAT-
AN vesicles electrostatically interact with polyelectrolytes,
proteins, and DNA^{19–22} and are versatile matrices for drug
delivery and transfection technologies.^{23,24} Those mentioned
above are excellent reasons for an exhaustive physicochemical
and/or biological characterization of their solution behavior.

The most important reason for using CAT-AN vesicles is due
to the fact that their stability is thermodynamic in origin.²⁵ The
formation of CAT-AN vesicles depends on a proper alkyl chain
length, polar head group size, and net charge. At constant
temperature and composition, such vesicles are characterized by
monomodal size distributions,²⁰ provided the CAT/AN mole
ratio, R , is not equal to 1. Their formation is controlled by the
overlapping energy and entropy terms, whose balance ensures
well-defined size distributions in the 10^2 – 10^3 nm range and
long-term stability.^{26,27} Therefore, sizes and charge are
potentially modulated. The above features suggest using
CAT-AN mixtures to investigate ion distribution around
vesicles. That quantity is related to the overall surfactant
content (i.e., CAT+AN), mole or charge CAT/AN ratios,
cosurfactants, salts, and polyelectrolytes and to the working
temperature.

This contribution focuses on ion distribution around CAT-
AN anionic vesicles, realistic models on the scale of biological
cells. Experimental support comes from dynamic light

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78 scattering, ζ -potential, and dielectric relaxation methods. They
79 jointly offer the opportunity to relate composition with vesicle
80 properties and give information on hydrodynamic radii, D_H ,
81 surface charge density, σ , and double-layer thickness, δ ,
82 respectively. Information on ion dynamics at or around charged
83 interfaces was considered too. Therefore, the experimental
84 determination of ion distribution around vesicles is potentially
85 at hand.

86 Quantitative studies on ion distribution around cells and
87 vesicles are scarce and sound theoretical approaches are
88 missing. This is because of the experimental drawbacks
89 encountered when dealing with metastable vesicles. To open
90 the way to an extensive use of the above methods and to
91 investigate vesicles and, in perspective, cultured cells, we rely on
92 models proposed for the electrokinetic properties of colloid
93 entities. In a first approximation, we also assume that they can
94 be considered fairly monodisperse.^{28–34} It is not yet well-
95 known whether size polydispersity is relevant. Neither is it
96 possible to transfer automatically models proposed for hard
97 colloids to soft and deformable ones. Therefore, experiments
98 were compared with current theories, taking into account the
99 properties of CAT-AN systems and recent advances in
100 electrokinetics.^{35–41} The manuscript is divided in different
101 sections, in which the experimental properties of CAT-AN
102 vesicles are reported. Experiments are discussed, taking into
103 account size polydispersity.

104 ■ EXPERIMENTAL SECTION

105 **Materials.** Cetyltrimethylammonium bromide, CTAB,
106 tetradecyltrimethylammonium bromide, TTAB, didodecyl-
107 methylammonium bromide, DDAB, and dioctyldimethylam-
108 monium bromide, DODAB, sodium dodecyl sulfate, SDS,
109 sodium octyl sulfate, SOS, and sodium bis-2-(ethylhexyl)-
110 sulfosuccinate, AOT, were from Sigma, TCI, or Fluka. The
111 cationic species and the alkyl sulfates were dissolved in hot
112 ethanol, precipitated by cold acetone, filtered, and vacuum-
113 dried. The purity for each surfactant was confirmed by
114 measuring the ionic conductivity and surface tension of the
115 respective aqueous solutions. The respective critical micelle
116 concentrations, CMC_p, agree with previous findings.^{42,43} AOT
117 was dissolved in hot methanol, filtered, cooled, dried,
118 redissolved in chloroform, filtered and cooled again, recovered,
119 and vacuum-dried. The whole procedure was repeated twice.
120 The final product is a birefringent waxy paste, with a melting
121 point of 152 ± 2 °C. Its solution properties and CMC conform
122 to previous work.⁴³

123 NaBr, Sigma, was vacuum-dried at 150 °C. Water was doubly
124 distilled over KMnO_4 and bubbled by N_2 , to reduce the
125 presence of CO_2 , which influences the dispersion conductivity.
126 At 25.0 °C the conductance of freshly distilled and degassed
127 water, χ , is $< 1 \times 10^{-7} \Omega^{-1} \text{ cm}^{-1}$.

128 Aqueous solutions of the single surfactants were prepared
129 separately, by weighing proper amounts of cationic, CAT, or
130 anionic, AN, species. Solutions of the above species having
131 exactly the same molality (mmol kg^{-1}) were mixed in due
132 proportions, to get different [CAT/AN] ratios, indicated as R .
133 The above quantity refers to both charge and mole ratios, and is
134 ≥ 1 . R values close to unity imply the precipitation of solids.

135 The individual samples were sealed in glass vials, sonicated,
136 and equilibrated at 25 °C. They were checked from time to
137 time until the appearance and turbidity remained constant.
138 Visual inspection and optical microscopy ascertained the

presence of solids or anisotropic phases. To enhance phase
separation, centrifugation at 6000 rpm was used.

Thermal cycling avoids the formation of multilayered vesicles
and gives bilayer ones.⁴⁴ When heated at 50–55 °C, the
dispersions may change from milky to an opalescent, or bluish,
appearance and remain as such when brought back to 25.0 °C.
Vesicle stability is substantial, as confirmed from the long-term
constancy in size.

Dynamic Light Scattering. Measurements were run by a
Malvern Zeta Nanosizer, working at 632.8 nm in backscattering
mode (173°) at 25.0 ± 0.1 °C. Size distributions were obtained
by CONTIN algorithms.⁴⁵ The light intensity fluctuations, I ,
give G_2 , according to

$$G_2(\vec{q}, t) = \left(\frac{\langle I(\vec{q}, t) \cdot I(\vec{q}, t + \tau) \rangle}{\langle I(\vec{q})^2 \rangle} \right) \quad (1)$$

where \vec{q} is the scattering vector and τ the delay time. $G_2(\vec{q}, t)$ is
related to the autocorrelation function for the electromagnetic
field, $g_1(\vec{q}, t)$, according to

$$G_2(\vec{q}, t) = A + B \cdot g_1(\vec{q}, t)^2 \quad (2)$$

where A is the baseline and B is the intercept. $g_1(\vec{q}, t)$ was
expanded in a second-order relation as

$$\ln[g_1(\vec{q}, t)] = -\Gamma_1 + \left(\frac{\Gamma_2}{2} \right) \cdot \tau^2 \quad (3)$$

Γ_1 in eq 3 gives the self-diffusion coefficient, D (in $\text{cm}^2 \cdot \text{s}^{-1}$),
related to D_H through the Einstein–Stokes relation. Γ_2 ,
conversely, is proportional to the polydispersity index, indicated
as PdI.

Electrophoretic Mobility. It was run by a Malvern Zeta
Nanosizer Laser-Doppler facility, at 25.0 ± 0.1 °C. Cells are
equipped with gold-coated electrodes. From electrophoretic
mobility, μ , the ζ -potential, ζ , was determined. In fact,⁴⁶

$$\zeta = \mu \cdot \left(\frac{4\pi\eta}{\epsilon^\circ} \right) \quad (4)$$

where η is the solvent viscosity and ϵ° is its static dielectric
permittivity. The double-layer thickness surrounding vesicles, δ ,
is $\ll D_H/2$ (see below), and Smoluchowski's approximation
holds.⁴⁷

Dielectric Relaxation. The permittivity, ϵ' , and loss, ϵ'' ,
were measured at 25.0 ± 0.1 °C by an HP 4194-A unit, in the
 10^5 – 10^8 Hz range. More details are given elsewhere.^{48,49} The
relaxation frequency, f^* , and amplitude, $\Delta\epsilon = (\epsilon' - \epsilon_\infty)$, were
obtained by

$$(\epsilon' - \epsilon_\infty) = \frac{\Delta\epsilon}{\left[1 + \left(\frac{jf}{f^*} \right)^2 \right]^{(1-\alpha)}} \quad (5)$$

where ϵ' is the dielectric permittivity at a given frequency, f
(Hz), ϵ_∞ its high-frequency limit, and $j = -1^{1/2}$. The exponent
in eq 5 ($0 \leq \alpha \leq 0.3$) is a fitting parameter that accounts for
the spreading of relaxation times;⁵⁰ it is related to the size
distribution functions. Errors on ϵ' are $\pm 2.0\%$, those on ϵ'' are
 $\pm 4.0\%$, when the uncertainty on $f^* \approx 5\%$. In the measuring
range an average relaxation process is present. No other
contributions were observed by fitting ϵ' versus ϵ'' values into
Cole–Cole plots.⁵¹

188 ■ RESULTS

189 **Phase Diagrams and Vesicle Location.** Vesicles were
 190 obtained by mixing two single, one double, and one single or
 191 two double-chain surfactants. AOT, DDAB, and DODAB are
 192 double-chained, when all other species have only one chain.
 193 SOS, SDS, TTAB, and CTAB form molecular, micellar, and
 194 hexagonal liquid crystals. Provided experiments are run at
 195 temperatures greater than T_K (the Krafft point),⁵² the phase
 196 location depends on the surfactant composition and temper-
 197 ature.^{53,54} The canonical sequence for double-chain species,
 198 conversely, is molecular, micellar, two-phase regions, lamellar
 199 phases.^{55–57} We do not discuss the state of micellar, solid,
 200 multicrystalline or liquid crystalline phases occurring in the
 201 respective diagrams and only focus on the vesicular area.

202 In the regions considered here water is in large excess, ≈ 98 –
 203 99 wt %, and ternary plots can be drawn as pseudobinary.
 204 Because of metathesis, addition of the surfactant in defect
 205 implies NaBr release and the formation of 1/1 CAT-AN
 206 species. Strictly speaking, thus, the systems are quaternary
 207 rather than ternary.

208 Mixing CAT and AN species in due proportions implies
 209 vesicle formation. The ratios among the species control the
 210 aggregate size and shape, the packing density, and the curvature
 211 elasticity.⁵⁸ The shape is controlled by the packing parameter,
 212 P ,⁵⁹ indicating if spherical, cylindrical, disklike micelles, vesicles,
 213 or diverse liquid crystalline phases occur. P is the ratio of alkyl
 214 chain(s) volume, V , divided by the effective area per headgroup,
 215 A , times the main chain length in extended conformation, L (P
 216 $= V/A \cdot L$). It is modulated by composition, R ratios,
 217 temperature, cosolutes, and added salt. Each aggregate shape
 218 is characterized by a P range;⁵⁸ when it is slightly less than
 219 unity, vesicles occur.

220 Apart from the CTAB/SDS, the TTAB/SDS, and the
 221 DODAB/SDS systems, the chain lengths of CAT and AN
 222 species are the same. In such cases, vesicle size depends only on
 223 V and A terms, both related to R and ionic strength. This is why
 224 it is possible to get a fine modulation of P . The location of the
 225 vesicular regions in the phase diagrams fulfills the packing
 226 constraint theory, Figures 1 and 2. Vesicles, in fact, are located
 227 between the micellar ($P \approx 1/3$), and the lamellar ($P = 1.0$), or
 228 solid phases ($P = 1.0$ for smectic solids). In DDAB-containing
 229 mixtures, micelles occur at very low concentrations, and vesicles
 230 coexist with lamellar dispersions in a wide composition range.
 231 Lamellar DDAB dispersions, in fact, are observed even at
 232 concentrations $< 10 \text{ mmol kg}^{-1}$.

233 NaBr screens the electrostatic repulsions between head
 234 groups and reduces the surface charge density and, therefore, A
 235 values. The same holds for R . The charge screening forces
 236 surfactant molecules to change their P values. This behavior is
 237 put in evidence by the significant changes in vesicle
 238 hydrodynamic radii, $D_H/2$, Figure 3. The transformation in
 239 other supra-molecular aggregates depends on composition, R ,
 240 and added electrolyte. With the overall surfactant content fixed,
 241 the transition from vesicles to lamellae occurs when added
 242 NaBr is ≈ 20 – $25.0 \text{ mmol kg}^{-1}$.

243 CAT-AN vesicles are thermodynamically stable and coexist
 244 with the surfactant ions from which they are made. In contact
 245 with the solvent they partition the components in such a way
 246 that the chemical potential of the i th species in the vesicle, μ_{iV} ,
 247 and in the bulk, μ_{iB} , is the same. The more soluble surfactant is
 248 preferentially partitioned in the bulk. When changes in
 249 temperature occur, the partition is sensitive to it,⁶⁰ and a

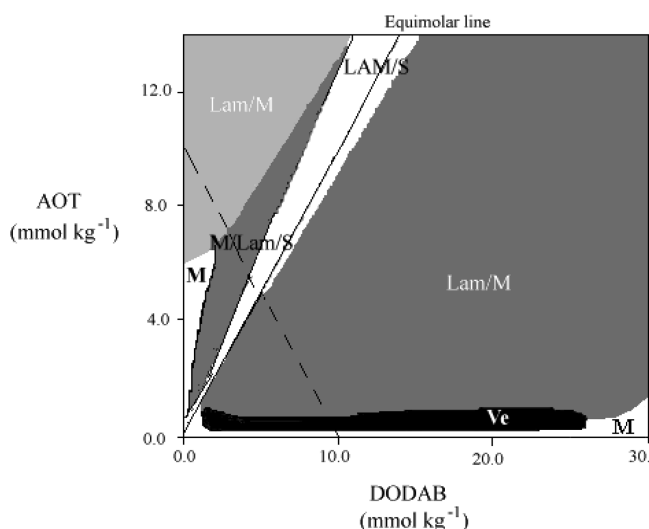


Figure 1. Pseudobinary phase diagram of the system water–AOT–DODAB, at 25.0 °C. Concentrations are in mmol kg^{-1} scale. The solution regions are indicated by the letter M, the vesicular ones by Ve, the two-phase lamellar/micellar region as Lam/M, and the solution/lamellar/solid region as M/Lam/S. The line dividing the figure is the charge neutralization (equimolar) line. The dashed line at $10.0 \text{ mmol kg}^{-1}$ indicates how R changes with mole ratios; its intersection with the charge neutralization line defines the $R = 1.0$ value.

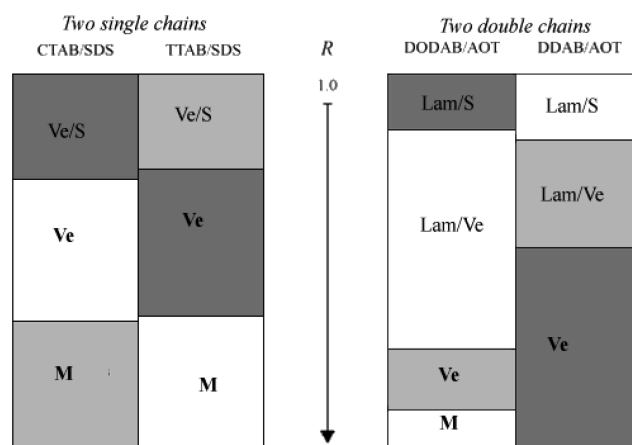


Figure 2. Scheme indicating the dependence on R for selected 1–1 and 2–2 surfactant mixtures. Data refer to the cationic-rich region at an overall surfactant content of $10.0 \text{ mmol kg}^{-1}$ and 25.0 °C. The meaning of symbols is as in Figure 1

thermally induced size modulation takes place. The effect on D_H is noticeable, with quasi-critical changes in size and formation of bilayer vesicles. The above behavior was described elsewhere⁴⁴ and shall not be dealt in this context.

Vesicle Size. Sizes span the 10^2 – 10^3 nm range, depending on the components, overall surfactant content, R , and added salt. The dispersions are milky or opalescent or may have a bluish color in the case of moderate vesicle sizes. At 6.0 mmol kg^{-1} and $R = 0.59$, for instance, vesicles in the CTAB/SDS system have a D_H of $340 \pm 50 \text{ nm}$. Close D_H values ($300 \pm 40 \text{ nm}$) are observed at the same R ratio in the TTAB/SDS system. The latter cationic species is a 14-chain analogue of CTAB (having 16 CH_2 units).

Similar considerations apply to double-chain surfactants. In the DDAB/SDS system D_H values are centered at $250 \pm 30 \text{ nm}$. Hydrodynamic radii relative to DODAB-based systems are

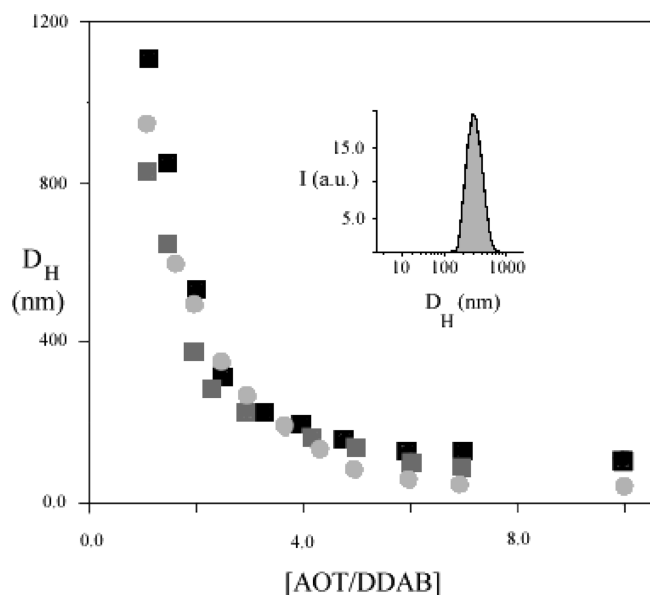


Figure 3. Hydrodynamic radii, D_H , nm, vs the [AOT/DDAB] R ratio, at 25.0 °C, for a surfactant content of 10.0 (gray circle), 20.0 (gray square), and 25.0 (black square), mmol kg^{-1} . In the inset is reported the size distribution of a 15.0 mmol kg^{-1} mixture, with the R ratio 3.02. Errors on D_H are $\pm 10.0\%$.

size also occur. We do not consider what happens when $|\zeta| \leq 279$ 25–30 mV, Figure 5. That threshold implies the onset of a 280 fs

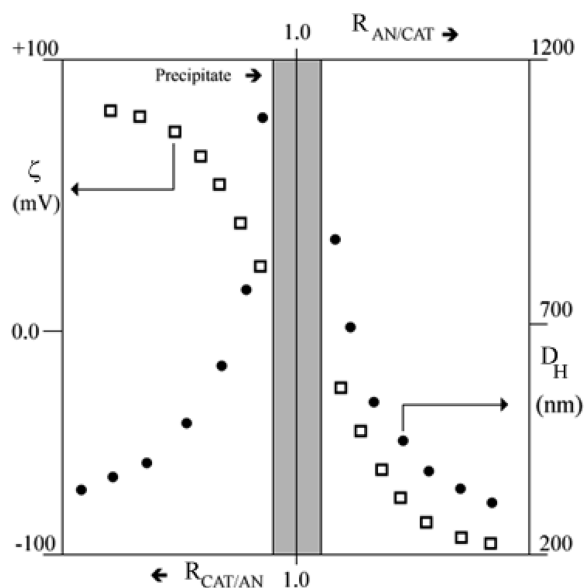


Figure 5. ζ -potential (\square) and D_H values (\blacksquare), for 6.0 mmol kg^{-1} DDAB/SDS mixtures at 25.0 °C, as a function of R . The gray area indicates the formation of precipitates.

266 ≈ 400 nm large, and more in the DODAB/SOS one. In all cases
267 a significant tendency to an increase in D_H values is observed
268 when R approaches unity, Figure 3. Large sizes are observed at
269 low concentrations too. Therefore, D_H mostly depends on R
270 and ionic strength.

271 **ζ -Potential and Surface Charge Density.** ζ -potential and
272 σ scale with R ; when the latter approaches 1.0, ζ and σ tend to
273 zero, with the occurrence of phase separation. In the AN-rich
274 region of the DODAB/AOT system ζ decreases in inverse
275 proportion to R , Figure 4, and approaches 0. The charge
276 neutralization process occurring upon addition of NaBr, or of
277 the surfactant in defect, finds analogy to classical colloid
278 titration.⁶¹ In association colloids, however, changes in particle

kinetic instability, related to the fact that the ratio of 281
electrostatic to thermal energy is close to unity.⁴⁶ At that 282
limit, the vesicular dispersions are destabilized and separation 283
occurs, with formation of solids or lamellar phases and 284
subsequent sedimentation or creaming. 285

Surfactant ions, counterions, vesicle released NaBr, and 286
added salt jointly contribute to the ionic strength and, 287
therefore, to the double-layer thickness. They exert a significant 288
role on ζ -potentials. For instance, addition of 25.0 mmol kg^{-1} 289
NaBr to the DODAB/AOT system, when $R = 8$ and CAT + 290
AN = 15.0 mmol kg^{-1} , results in changes of ≈ 50.0 mV, Figure 291
6. Above that threshold σ reduces and lamellar phases are 292
formed. In that region it is not possible to detect reliable ζ - 293
potentials. 294

Dielectric Relaxation. Figure 7a indicates the presence of 295
dielectric relaxation processes located in the megahertz range. 296

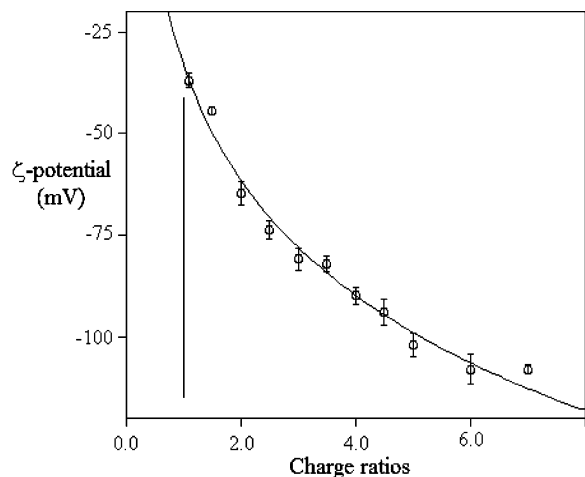


Figure 4. ζ -potential, in mV, vs the AOT/DDAB mole (and charge) ratio. Data refer to the AOT-rich region, for an overall surfactant content of 20.0 mmol kg^{-1} , at 25.0 °C. The full line is drawn for visual purposes; the vertical bar indicates the location of the precipitation line.

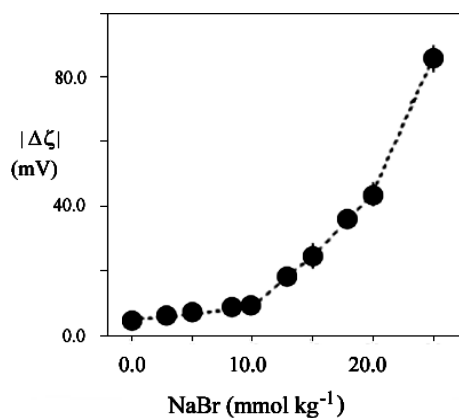


Figure 6. Changes in ζ -potential (mV) vs NaBr molality (mol kg^{-1}). Data refer to a 15.0 mmol kg^{-1} DODAB/AOT solution having R ratio 8.0, at 25.0 °C. The dashed line is for visual purposes.

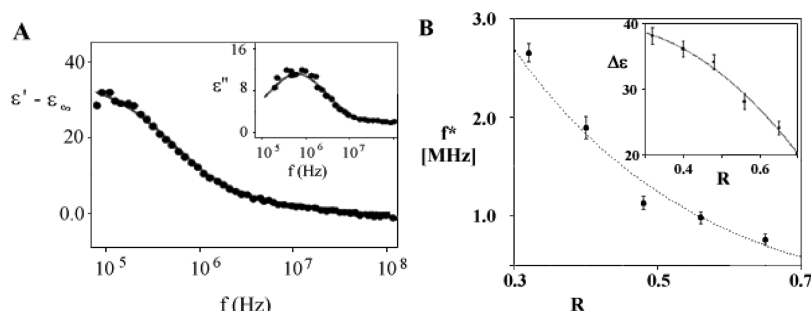


Figure 7. (A) Dielectric relaxation spectrum, $(\epsilon' - \epsilon_\infty)$, for a 12.0 mmol kg⁻¹ CTAB/SDS, $R = 0.65$, at 25.0 °C. The ϵ'' plot, in the inset, is characterized by a significant scattering. (B) Dependence of f^* (in MHz) and $\Delta\epsilon$, in the inset, on R for 12.0 mmol kg⁻¹ CTAB/SDS samples, at 25.0 °C. Uncertainties are indicated by bars.

At fixed surfactant content, $\Delta\epsilon$, ϵ'' , and f^* regularly depend on R , Figure 7b. Similar conclusions apply when NaBr is present in small amounts (data not shown). Both $\Delta\epsilon$ and f^* decrease on increasing R , when the spreading of dielectric relaxation times increases in proportion to it. That is, f^* depends on vesicle size and polydispersity, both related to R .

Dielectric relaxation spans in a frequency range^{36,41} in which Maxwell–Wagner contributions may occur.^{36,62} We do not have explicit evidence of the latter eventuality, or if they overlap with other relaxing modes. Anyhow, their presence cannot be ruled out “a priori”. The relaxation modes may be radial, or tangential. The former are due to the charge transfer in the double layer, when tangential ones to ion motions around vesicles. Both modes depend on the net vesicle charge. The tangential conductivity around vesicles is fast, depends on σ and is modulated by the mobility of free ions. Ion motion around vesicles is essentially due to Na⁺ and Br⁻, when the contribution due to surfactant ions is moderate, if any.

Attempts to rationalize the role of the above contributions to polarization are delicate items to face with. They involve many aspects related to the charge density and double-layer thickness. The role of the single contributions in controlling the above quantities is analyzed below.

DISCUSSION

Generalities. The determination of electrokinetic properties is relevant to quantify many features of colloid dispersions. Available information suffers from experimental uncertainties, which do not allow solving the inherent intricacies. Efforts are made in the following to get consistent results based on previous hypotheses and models. General consensus exists on the fact that many factors control electrokinetic phenomena. Among them are worth mentioning the electroneutrality constraints, the local equilibrium, matter exchange between volume elements, and boundary conditions. In addition, incompressibility, flows, and ion velocity must be accounted for.^{26–42} The following are also required:

1. The double-layer thickness, δ , $\ll D_H/2$, and Smoluchowski's approximation holds.^{47,63} The hypothesis is consistent with ionic strength values (≥ 1.0 mmol kg⁻¹).
2. The Poisson–Boltzmann equation can be solved in one direction, i.e., $\nabla^2\psi = (\partial^2\psi/\partial X^2)$.
3. Size polydispersity is moderate and its distribution function monomodal, Figure 3. In all cases $PdI \leq 0.3$.
4. The spreading of f^* and ζ values is moderate.
5. Vesicular dispersions are diluted (the volume fraction is $\phi \approx 10^{-3}$).

6. The viscosity and permittivity are close to that of the solvent.

Local equilibrium conditions apply to single vesicles in equilibrium with their ionic atmosphere. Considering each vesicle as a thermodynamic small system, therefore, a statistical distribution of values follows, and polydispersity can be accounted for. Vesicles may be deformed by electric fields. The above item is of minor relevance, since Lekkerkerker discussed the corrections to be introduced in the electrophoretic equations for anisometric entities.⁶⁴

Colloid systems are intrinsically polydisperse in size; CAT-AN vesicles conform to the above rule. It is not known how much polydispersity, controlled by thermodynamics,²⁶ influences the electrokinetic properties. For the above reasons, questions related to the single contributions should be clarified. According to experiments, the accuracy on ζ -potentials is a few millivolts wide. D_H , σ , and f^* data, Figures 3–7, indicate a spreading of the corresponding quantities. The confidence limits in σ and f^* depend on D_H and are related to vesicle size. For a population of 320 ± 30 nm and a ζ -potential of 40 ± 3 mV, for instance, the uncertainty on σ , calculated by the propagation of errors in D_H and ζ , is $\approx 15\%$. The uncertainty on f^* increases in proportion to R and D_H . That on δ , finally, depends on the medium ionic strength; its evaluation can be cumbersome (see below).

The interpretation of ζ -potential and dielectric relaxation is based on classical electrokinetic approaches. Vesicles are charged spheres located in a medium of viscosity η and static permittivity ϵ° . Ions moving around are characterized by their valence, z^\pm , self-diffusion coefficients, D^\pm , and equilibrium concentration, C^\pm_0 , taken far from the vesicle surface. If the above quantities are known, it is possible to develop suitable expressions for ζ values, $\Delta\epsilon$, and f^* . Toward this purpose, it is required to have realistic estimates of ionic strength, which depends on added salt, overall surfactant content, counterion binding, and R . Therefore, it is necessary to develop approaches for estimating it.

Ionic Strength. The ionic strength, I , is the sum of vesicle-released ions, free counterions, surfactant ones, and added electrolyte. The maximum amount of vesicle-released NaBr, C_{rel} , is

$$\left(\frac{C^0}{R + 1} \right) = C_{rel} \quad (6)$$

where C^0 is the overall surfactant content ($C^0 = C_{CAT} + C_{AN}$). The free surfactant contribution was evaluated from the experimental critical micelle concentrations and from those of

the single species, CMC_i . In multicomponent surfactant systems, in fact, the critical concentration of the mixture, CMC^* , is related to the individual CMC_i 's according to⁶⁵

$$\text{CMC}^* = \frac{\prod_i \gamma_i \cdot \text{CMC}_i}{\sum_i \gamma_i \cdot X_i \cdot \text{CMC}_i} \quad (7)$$

where X_i is the mole fraction of the i th species, related to R ($X_i = [(R/R + 1)]$) and γ_i is the activity coefficient. The formation of mixed micelles follows the regular solution theory;⁶⁶ therefore, γ_i can be expressed as

$$\gamma_i = \exp[\beta X_i^2] \quad (8)$$

where β is the interaction parameter among the species. In CTAB/SDS and TTAB/SDS mixtures $\beta \approx -18 \text{ RT}$ units, i.e., close to the decyltrimethylammonium bromide/sodium decyl sulfate system.⁶⁷ Similar β values were obtained in other mixtures. The concentration of surfactant ions calculated by eqs 7 and 8 is much lower than the individual CMC_i 's and decreases in presence of NaBr.

The ionic strength was evaluated by summing all the above contributions, and Debye's screening length was estimated accordingly, accounting for terms due to C_{rel} , CMC^* , and added salt. The calculated double-layer thickness, δ , lies in the 2–7 nm range and regularly depends on X_i , R , and NaBr content.

Electrophoretic Mobility. The charge distribution and electrokinetic effects are associated with ion motions toward, from, and around vesicles. σ is the value arising from such contributions. It is related to ζ -potentials according to the classical equation

$$\sigma = \left[\frac{\varepsilon^0 \zeta}{4\pi \delta} \right] \quad (9)$$

where δ is the double-layer thickness. Equation 9 is rewritten as

$$\sigma \delta = \left[\frac{\varepsilon^0 \zeta}{4\pi} \right] \quad (9')$$

to put in evidence that ζ is proportional to an electric moment per unit area. The distance between the vesicle surface of charges and the slippery plane is related to δ , and depends on I . The dependence of the double-layer thickness on composition was inferred by deriving eq 9' with respect to the amount of NaBr, or as a function of R . In both cases, when $\zeta \rightarrow 0$ the same holds for $(\sigma \delta)$. At that point, σ approaches zero and δ diverges. The amount of vesicle-released ions depends on R . We do not have realistic estimates on the vesicle net charge. However, it is possible to quantify how much binding depends on NaBr content or R . Surface binding models based on ζ -potentials were analyzed by a Langmuir-like approximation, adapted to ion adsorption on oppositely charged surfaces. Therefore, σ was expressed as⁶⁸

$$\sigma = \left[1 - \left(\frac{z\theta_s}{C_{\text{el}}} \right) \right] \cdot \left(\frac{e}{a^0} \right) \quad (10)$$

where $z \cdot e$ is the ion charge, C_{el} is the concentration of added salt, a^0 is the area per binding site ($\approx 0.6 \pm 0.1 \text{ nm}^2$), θ_s is the surface coverage, and $(e/a^0 = \sigma_0)$ is a charge per binding site. Rearrangement gives

$$\left(\frac{C_{\text{el}}}{z} \right) \cdot \left[1 - \left(\frac{\sigma}{\sigma_0} \right) \right] = \theta_s \quad (10') \quad 438$$

Equation 10' links changes in θ_s to ionic strength. In the systems considered here the increase is over 20% for an ionic strength $\approx 20\text{--}25 \text{ mmol kg}^{-1}$. θ_s depends on I and levels off above that NaBr content. Changes are not a linear function of added salt. This may be the reason for the effects observed in ζ -potentials, Figure 6. Ion condensation is large if $(\partial\sigma/\partial\text{NaBr})$ is large (low NaBr content), and negligible at high salt concentration; in words, charge neutralization occurs. The effect is evident close to the phase boundaries, where 20–25 mmol kg^{-1} NaBr induces the transition from vesicles to lamellar phases.

Dielectric Relaxation. Studies reported so far deal with uncharged and monodisperse hard colloids in supporting electrolyte. In such cases the dielectric properties give information on the double-layer thickness.⁶⁹ In fact, the double-layer contribution to conductivity is related to the polarizability amplitude, P^* , when the dielectric constant increment reflects the polarization dynamics.³⁶ Upon increasing the frequency, the dielectric increment scales with the double-layer thickness; the reverse behavior holds for conductivity. The two contributions dominate at low and high frequencies, respectively. The high-frequency behavior of $\Delta\varepsilon$ does not automatically match the double-layer dynamics. The situation can be different when charge-modulated entities are dealt with. For these reasons, we avoid adding extra constraints or adjustable parameters, and proceed according to classical routes.

Previous approaches allow getting information on the features of colloid particles. Some deal with intrinsic;^{36–41} others, with association colloids. Models linking the relaxation processes to composition were proposed for micellar colloids.⁷⁰ However, micelles are orders of magnitude smaller than vesicles and do not conform to Smoluchowski's approximation ($\delta \geq D_{\text{H}}/2$). Thus, routes proposed for intrinsic colloids were considered. Most current theoretical models reported so far are derived from an approach formerly developed by Shilov.⁷¹ For instance, Grosse developed a theory based on Shilov's one^{39–41} and derived the Poisson–Boltzmann equations for the number of ions (n), ρ , and φ , all related to σ and/or P^* .

The self-diffusion coefficients of ions, D^+ and D^- , must be considered in the calculations; they are responsible for radial and tangential motions around vesicles. Although the approximation is not realistic, most theories impose using $\lim_{c \rightarrow 0} D^\pm$. The limiting values for Na^+ , Br^- , DS^- , and CTA^+ ions are 1.33, 1.25, 0.8, and $0.4 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, respectively.^{72–74} (N.B. The last value is extrapolated from ref 74.) Only terms due to Na^+ and Br^- were considered, because those due to surfactants are of minor relevance. We performed some calculations and found that the inherent error is $\leq 5\%$.

$\Delta\varepsilon (= \varepsilon^0 - \varepsilon_\infty)$ depends on the volume fraction, ϕ , and was written as

$$\left(\frac{\varepsilon^0 - \varepsilon_\infty}{3\phi\varepsilon_\infty} \right) = \text{Re}(P^*) + \left(\frac{\chi}{\omega\varepsilon_\infty} \right) \text{Im}(P^*) \quad (11) \quad 491$$

where χ is the conductivity and P^* is the polarization term. Their values depend on ϕ , more correctly on particle sizes. Equation 11 contains three terms, when Maxwell–Wagner contributions are accounted for; accordingly, it is rewritten as⁴¹

$$\frac{\epsilon^\circ - \epsilon_\infty}{3\phi\epsilon_\infty} = \text{Re}(P^*) + \text{Im}(P^*)_\kappa + \text{Im}(P^*)_{\text{MW}} \quad (12)$$

where the first term refers to the dielectric increment, the second to the conductivity, and P^*_{MW} to Maxwell–Wagner contributions. P^* depends on the double-layer thickness; in the case of a single population of particles, it can be expressed as

$$\frac{P^*}{P^\circ} = \left[\kappa \left(\frac{D_H}{2} \right) \right]^3 \quad (13)$$

where κ is the inverse screening length and P° is the permittivity of bare particles. Accordingly, P^* is proportional to κ^3 and is related to the electric moments facing the outward vesicles. The differential form is expressed as

$$dP^* = \left(\frac{\partial P^*}{\partial \kappa} \right)_{D_H} d\kappa + \left(\frac{\partial P^*}{\partial D_H} \right)_\kappa dD_H \quad (13')$$

which gives information on the effect of both ionic strength (through κ) and D_H .

Calculations. Taking into account that stated before, we proceeded with model calculations based on the presence of a single relaxation process and assumed that the former hypotheses on D^\pm values and other terms were effective. In a first approximation, it was postulated that uncharged vesicular entities were present and that polarization was essentially due to adsorbing ions. This is a crude approximation, finding its rationale in the fact that added salt acts as a swamping electrolyte. Data were elaborated according to the model hypotheses.

It was found that the radial relaxation mode depends on I (on δ , therefore). Experimental f^* values indicate that the former hypothesis is realistic. The tangential contribution is much slower, being related to the motion of ions around large vesicles. No explicit Maxwell–Wagner terms were observed in the available frequency window, and we considered only the canonical contributions to permittivity, as in eq 11. Pertinent data were reported in terms of a single relaxation equation.

According to experiments, P^* depends on polydispersity and is related to $\langle D_H \rangle^3$, when κ is constant. For vesicles having sizes of 200 and 500 nm and present in variable amounts in a medium of well-defined ionic strength, changes in P^* are over 1 order of magnitude, depending on the relative weight of the two populations. The above behavior is also reflected by significant changes in f^* , Figure 8.

Comparison of Experiments and Theory. Dielectric relaxation spectra in Figure 8 indicate that polydispersity is relevant, at fixed temperature, I , and ϕ . f^* was calculated for uncharged vesicles of $D_H = 200$ nm. In monodisperse regimes, errors on f^* are moderate and the spreading of relaxation times is negligible. Thereafter, we accounted for progressive amounts of vesicles having $D_H = 500$ nm. Comparison was made by rescaling the polarizability terms in eqs 11–13. P^* values are related to the D_H/δ ratios. When monodispersity does not hold, a shift in f^* occurs; the effect is in proportion to $\langle D_H \rangle$. For instance, the computed f^* values for a mixture containing 70 and 30% of the two populations is twice than that observed for vesicles 200 nm large. The reasons for the results in Figure 8 are many-fold. In particular:

1. Uncorrect estimates of $\langle D_H \rangle$ or κ are responsible for large changes on P^* and on f^* .

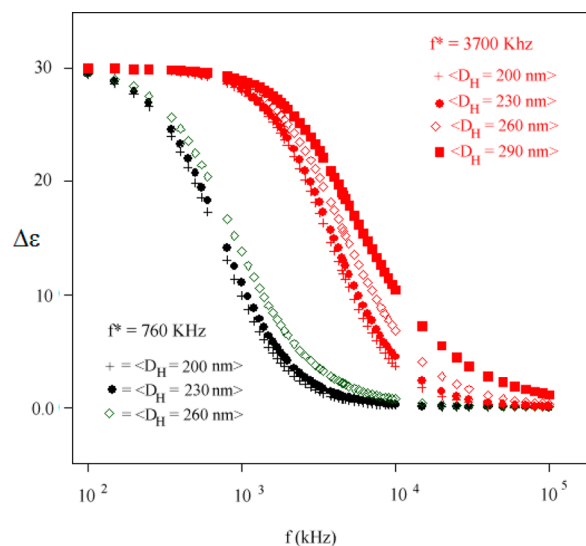


Figure 8. Calculated dielectric relaxation spectra for dispersions having 700 kHz, black, or 3700 kHz, red, as original relaxation frequency, and $\Delta\epsilon$ (i.e., $\epsilon^\circ - \epsilon_\infty$) = 30. The plots refer to two populations of vesicles having sizes of 200 and 500 nm, respectively. They refer to $1 \times 10^{-3} \phi$ dispersions in 5.0 mmol kg^{-1} NaBr, at 25.0°C .

2. The uncertainty in σ is large. That fact implies, in turn, errors in $d\rho$, $d\phi$, and P^* .
3. At finite concentrations, D^\pm terms are much lower than the limiting values;
4. The forces controlling ion motion around charged vesicles may be much more noticeable than in the case of uncharged colloids.

f^* , therefore, is proportional to the motion of ions in the double layer. The term due to ions moving tangentially around vesicles occurs at much lower frequencies and decreases in inverse proportion to vesicle size. It was observed, in particular, that δ is directly proportional to the measured dielectric relaxation time. These facts allow us to assume that the radial relaxation mode is active.

Some more points must be put in evidence. Ion binding onto vesicles is substantial at moderate NaBr content and levels off above a certain threshold. Vesicle ionization leads to changes in κ and P^* . Ion adsorption or release to/from the bulk is concomitant with changes in σ and, to a minor extent, in the solvent properties (i.e., viscosity and permittivity). Ion mobility and diffusion are substantially influenced by the charges facing outward vesicles. Therefore, free ions are sensitive to the electric field generated by vesicles, and to excluded volume, as well.

CONCLUSIONS

Detailed theories for electrokinetic effects refer to uncharged dilute colloids and do not account for the presumed strong interactions between surface charges and free counterions occurring in CAT-AN systems. In vesicular dispersions, f^* scales with ionic strength, as expected. The experimental results are of the correct order of magnitude for particle sizes and double-layer thicknesses close to those considered here. Notwithstanding the reported drawbacks, the agreement between experiments and expectations is satisfactory. The same holds for the ϕ dependence.

Theories proposed so far refer to a single population; thus, the spreading of particles size is not explicitly accounted for.

We found, conversely, that P^* depends on $(\kappa(D_H))^3$. Therefore, size dispersity contributions should be accounted for, whatever the theory. The proposed models hold for uncharged colloids in low-concentration regimes and strict monodispersity conditions. The present results, conversely, suggest that realistic estimates of polydispersity are required to get reliable results and to develop generalized theoretical models. We feel that a cross-combination of the uncertainties on σ and D_H will render theories more valuable. In fact, it is possible to express eqs 11–13, or more detailed formulations, in terms of average size and charge. Changes in composition influence the viscosity and permittivity of the solvent and should be considered in the theory; the same holds for the effective diffusion coefficients of ions present in the bulk.

ASSOCIATED CONTENT

Supporting Information

Effect of ionic strength on the relaxation frequency and dielectric relaxation amplitude for a vesicular dispersion. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

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