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Use of ionic liquids-like surfactants for the generation of unilamellar vesicles with potential applications in biomedicine

Cristian M. O. Lépori[a], N. Mariano Correa[b,c], Juana J. Silber[b,c], R. Darío Falcone[b,c], Manuel López-López[d] and M. Luisa Moyá[e]*

[a] Dr. C. M. O. Lépori. Instituto de Física Enrique Gaviola (IFEG), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) – Universidad Nacional de Córdoba (UNC), Medina Allende s/n, Ciudad Universitaria, X5016LAE, Córdoba, Argentina.

[b] Dr. N. M. Correa, Prof. J. J. Silber and Dr. R. D. Falcone. Departamento de Química, Universidad Nacional de Río Cuarto (UNRC), Agencia Postal # 3, C.P. X5804BYA, Río Cuarto, Argentina.

[c] Instituto para el Desarrollo Agroindustrial y de la Salud (IDAS), CONICET – UNRC. Agencia Postal # 3, C.P. X5804BYA, Río Cuarto, Argentina.

[d] Dr. M. López-López. Departamento de Ingeniería Química, Química Física y Ciencias de Materiales, Centro de Ciencia y Tecnología, Universidad de Huelva, Campus 'El Carmen', Facultad de Ciencias Experimentales, E-21071, Spain.

[e] Prof. M. L. Moyá. Departamento de Química Física, Universidad de Sevilla, c/ Prof. García González 1, 41012 Sevilla, Spain.

* Corresponding-Author: Dr. Cristian M. O. Lépori. E-mail: clepori@famaf.unc.edu.ar

ABSTRACT

The goal of this work is to understand the influence of the counterion nature on the organized systems formed by 1,4-bis-2-ethylhexylsulfosuccinate surfactants in aqueous solutions and, how these aggregates will have influence on the DNA-surfactants interactions. With this in mind, two ionic liquid-like surfactants were investigated: 1-butyl-3-methylimidazolium 1,4-bis-2-ethylhexylsulfosuccinate (bmim-AOT) and 1-hexyl-3-methylimidazolium 1,4-bis-2-ethylhexylsulfosuccinate (hmim-AOT). Measurements of dynamic light scattering (DLS), zeta potential, TEM images, fluorescence and UV-Visible spectroscopy were performed in order to study the characteristics of the vesicles formed by bmim-AOT and hmim-AOT. Regarding the determination of the interaction of the surfactants with DNA, circular dichroism was used.

The results obtained showed that bmim-AOT and hmim-AOT ionic liquid-like surfactants spontaneously form unilamellar vesicles in water at very low surfactant concentrations. The characteristics of these aggregates are dependent on the length of the tail of the counterions. The length of the hydrophobic chains of the counterions also influences on the DNA:surfactant interactions through hydrophobic effects.

KEYWORDS: ionic liquid-like surfactant, vesicles, bmim-AOT, hmim-AOT, DNA.

INTRODUCTION

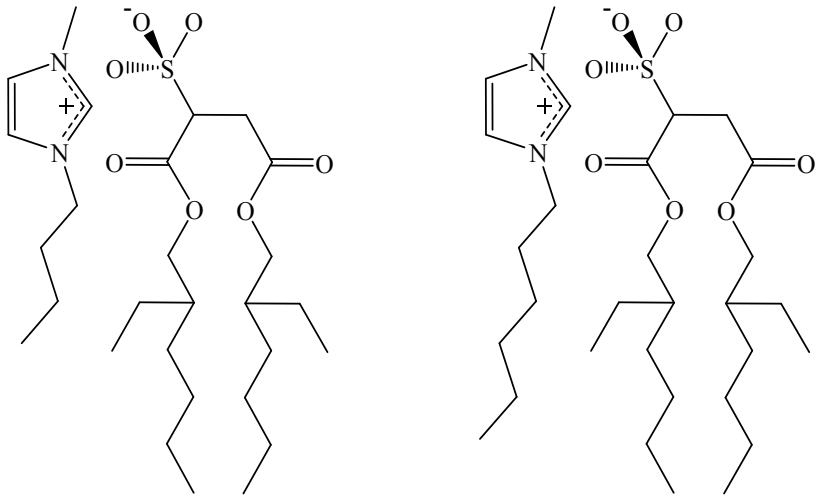
Ionic liquids (ILs) are a friendly class of compounds which have received significant attention as alternatives to conventional organic solvents.[1–3] The great impact that ILs have in chemistry is predominantly due to their properties that can be adapted in the synthesis procedure by a large variety of cation-anion combinations.[4] An attractive field of research deals with the synthesis of amphiphilic ILs.[5–7] These new materials, called *IL-like surfactants,* have been used to create a different kind of organized systems such as direct micelles, reverse micelles and vesicles.[3,5–11]

Vesicles are spherical organized systems formulated in water by some amphiphilic compounds (usually phospholipids). In these aggregates, the nonpolar bilayer surrounds an aqueous core.[12] Particularly, large unilamellar vesicles (LUVs) can be prepared using different techniques (such as ultra-sonication or extrusion) in order to convert multilamellar vesicles (formed spontaneously) into LUVs.[12,13] These vesicles can enhance drug stability, therapeutic effects, and uptake of the solubilized drug into the target site with reduced toxicity.[12] These organized systems can also be used for the development of new techniques for deoxyribonucleic acid (DNA) extraction, gene therapy and gene transfection.[14,15] However, it is mandatory to know which are the DNA-surfactant interactions present and, how they can modify, or not, the DNA structure. It is well-established knowledge that highly charged counterions have an impact on DNA compaction.[16,17] Therefore, the ILs can influence the DNA structure: the cations mostly interact with the DNA phosphate groups through electrostatic attractions, whereas the anions interact with the nucleobases.[18] For these reasons, the design of new IL-like surfactants that form organized systems in aqueous solution, with the ability to solubilize hydrophobic molecules, and that can also interact with DNA is a very interesting area to investigate. Recently[10] we reported the synthesis of the IL-like

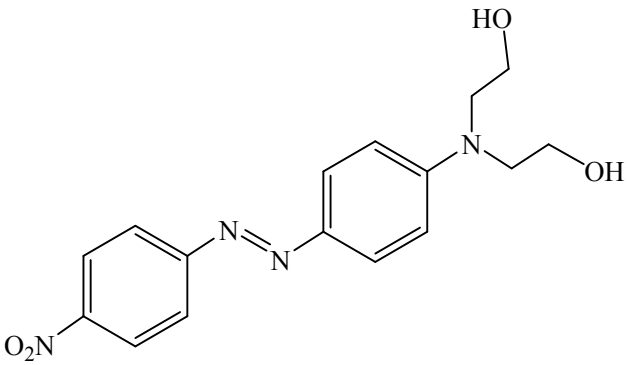
surfactant 1-butyl-3-methylimidazolium 1,4-bis-2-ethylhexylsulfosuccinate (bmim-AOT, Scheme 1), resulting from the mixture of sodium 1,4-bis-2-ethylhexylsulfosuccinate (Na-AOT) and 1-butyl-3-methylimidazolium chloride (bmim-Cl). This IL-like surfactant was isolated from the reactive mixture (removing the Na^+ and Cl^- counterions) and showed properties absolutely different from Na-AOT.[10] Particularly, we studied the physicochemical properties of reverse micelles in nonpolar solvents formed by bmim-AOT. It must be noted that, the properties of this surfactant in aqueous media were reported previously[19,20] but, it is not clear which kind of aggregates are form in water. Moreover, the very diluted concentration regime, where the aggregates-aggregates interactions are minimized, was not investigated. Thus, the kind of aggregates that bmim-AOT forms in water is not revealed until nowadays.

In the present work, we want to investigate the ability of this surfactant to form aggregates in aqueous solution, working in quite diluted solutions. Thus, we will show that, at bmim-AOT concentration lower than $1.5x10^{-3}$ M, unilamellar vesicles are formed. Futhermore, in order to investigate the effect that the counterions have on the properties of the formed aggregates, 1-hexyl-3-methylimidazolium 1,4-bis-2-ethylhexylsulfosuccinate (hmim-AOT, Scheme 1) was also synthesized. The study of this system will provide information about the influence of the counterion structure on these novel organized systems and, how may influence in their possible applications. In this way, physicochemical properties such as size, surface charge, micropolarity and microviscosity of the unilamellar vesicles were also investigated. Particularly, because we want to use these vesicles as a drug delivery agent[21] or for transfection of DNA molecules.[22] bmim-AOT and hmim-AOT vesicles were investigated using dynamic light scattering (DLS), zeta potential and transmission electron microscopy (TEM) techniques. Besides, the use of two different fluorescent molecular probes, 2,2'-[[4-[(4-

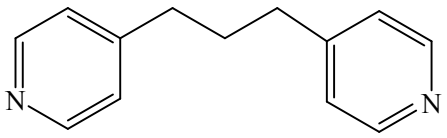
nitrophenyl)azo]phenyl]imino]bisethanol[23–25] (disperse red 19, DR9, Scheme 1) and 1,3-dipyrenylpropane,[26,27] (P3P, Scheme 1), which are incorporated into the vesicles, provided information about the polarity and the viscosity of their microenvironment.[28,29] Finally, the interactions between bmim-AOT and hmim-AOT vesicles and calf thymus DNA were studied using circular dichroism (CD).[30–34]



bmim-AOT **hmim-AOT**



DR19



P3P

Scheme 1. Molecular structures of the surfactants bmim-AOT and hmim-AOT, and the

molecular probes DR19 and P3P.

EXPERIMENTAL SECTION

Materials

1-hexyl-3-methylimidazolium chloride (hmim-Cl), bmim-Cl, Na-AOT, dichloromethane (DCM) and DR19 were from Sigma-Aldrich (> 99% purity). These compounds were kept under vacuum. P3P was purchased from Molecular Probes, Inc. and used as received.

The IL-like surfactant used, bmim-AOT, was obtained according the experimental procedure described in reference.[10] Hmim-AOT was prepared following the same methodology,[10] using as precursors hmim-Cl and Na-AOT (Scheme S1). The IL hmim-AOT obtained was a colorless and highly viscous liquid. The formation of hmim-AOT was confirmed by ^{1}H NMR technique. Figure S1 show that all protons corresponding to the anionic (AOT) and to the cationic ($hmim^{+}$) components are present. The chemical shifts values of the most relevant H of hmim-AOT obtained in $CDCl_3$ are included in Table S1. The NMR spectra for all compounds were performed in CITIUS (Research General Service for the University of Seville), using a Bruker Avance III 500 MHz spectrometer (500.2 MHz for ^{1}H). Also, FT-IR spectrum of hmim-AOT in CCl_4 was performed and compared with Na-AOT (Figure S2), observing the presence of the C-H stretching bands of the imidazolium ring. To obtain the FT-IR spectra a Nicolet IMPACT400 FT-IR spectrometer and IR cell of the type Irtran-2 (0.015 mm of path length) from Wilmad Glass (Buena, NJ) were used.

The vesicle solutions were prepared by mass and volumetric dilution, from a stock solution of surfactants prepared in deionized distilled water Super Q Millipore

(resistivity > 18 MΩ cm), and these samples were used directly in the different experiments without any other procedure, as it was made in other works.[6–8]

Calf thymus DNA (ctDNA, 99% purity) was purchased from Sigma-Aldrich. The ctDNA concentration (given by phosphate groups) was estimated spectrophotometrically at 260 nm (molar absorptivity of 6600 M^{-1} cm^{-1}).[35]

Hydrodynamic diameter and zeta potential measurements

A Zetasizer Nano ZS Malvern Instrument Ltd. was used for the measurement of hydrodynamic diameter and the zeta potential at 25 °C. The scattering angle used was 173°. CONTIN was used as the algorithm to obtain the hydrodynamic diameter values. All the experiments were carried out at several different surfactant concentrations however, systems with [surfactant] larger than $7x10^{-4}$ M were not prepared to the DLS measurements due to opalescence of the final solutions. At least six hydrodynamic diameters and zeta potentials were measured at each surfactant concentration and the average value (standard deviation) was considered.

Transmission Electron Microcospy (TEM)

For the TEM experiments, the micrographs were obtained with a JEOL 1200 EXII transmission electron microscope at a working voltage of 80 kV. The TEM samples were prepared by the negative-staining method. Phosphotungstic acid solution (2%) was used as the staining agent.[36]

Solubilization capacity and micropolarity of vesicle bilayer

Taking into account that DR19 is an azo dye insoluble in water, it was used to evaluate the solubilization capacity of the bmim-AOT and hmim-AOT vesicles. Thus,

an excess amount of DR19 in surfactant aqueous solutions was left stirring for two hours, at several surfactant concentrations. The resultant solution was filtered, using a standard gravity filtration method, and the dye concentration was determined spectrophotometrically at 495 nm and $\varepsilon = 23000\ M^{-1}\ cm^{-1}$.[23,24,28] The absorbance of the dye solutions was measured in a Hitachi UV-3900 spectrophotometer. The temperature was maintained at 25.0 ± 0.1 °C by using a water-jacketed cell compartment.

Determination of the microviscosity of vesicle bilayer

The vesicles containing P3P ($2x10^{-6}$ M) solutions were prepared following the method in reference.[26] The fluorescence emission spectra of P3P solutions were registered between 350 and 550 nm with an λ_{exc} = 346 nm. The intensities of the monomer emission (I_M) and the excimer emission (I_E) were recorded at the wavelength corresponding to the first vibronic peak of the monomer, located near 378 nm, and that of the excimer at around 490 nm, respectively.[26] Fluorescence measurements were done in a Hitachi F-2500 fluorescence spectrophotometer. The value of I_M/I_E ratio reported to each concentration of surfactant was the average of ten spectra. The temperature was kept at 25°C by a water flow cryostat connected to the cell compartment.

Circular dichroism

Circular dichroism (CD) experiments were performed in a Biologic Mos-450 spectropolarimeter. A circulation thermostatic bath kept the sample temperature at 25.0 ± 0.1 °C. A cuvette of 0.1 cm path length and a scan speed of 50 nm min^{-1} were used. Prior to use argon was passed through the solutions for more than two hours. The reported spectra were the average of ten runs with a 5 min equilibration before each scan.

RESULTS AND DISCUSSION

The solubility of bmim-AOT and hmim-AOT in water was investigated. Both ILs were soluble in water up to the evaluated concentration of 1 M, forming opalescent solutions stable over time. This type of opalescent solutions can be taken as indicative of the formation of aggregates of a considerable size since they show a great capacity to scatter the light.[37]

Different solutions of bmim-AOT and hmim-AOT in water were prepared and the samples were analyzed by DLS. Table 1 shows the apparent hydrodynamic diameter, d_{app}, values obtained for bmim-AOT and hmim-AOT in water at 25 ºC. Also in Figure S3, the DLS intensity plot for both surfactant in water at [surfactant] = $3x10^{-4}$ M is depicted. The DLS data show the formation of aggregates with diameters ranging from 146 - 164 nm for bmim-AOT and, from 93 - 120 nm for hmim-AOT, with low polydispersity indexes (within the range 0.2 - 0.4) in both cases. Moreover, in the range of $2x10^{-5}$ M - $7x10^{-4}$ M the sizes were independent of the surfactant concentration. The size ranges found for the two surfactants could suggest the formation of vesicles.[38]

The independency observed on the diameter values measured at different [surfactant] and, particularly the detection of aggregates even at low [surfactant] are good evidences that the aggregates formed (spontaneously) are vesicles and not direct micelles.[19,20,39–43] As it is well known the lack of critical aggregation concentration is a peculiarity that distinguishes vesicles[13] from direct micelles.[38] Moreover, the aggregates obtained at different [surfactant] concentrations do not present high polydispersity indexes, suggesting that they are unilamellar vesicles.[6,7]

Table 1. Apparent hydrodynamic diameters (d_{app}) and polydispersity indexes (PDI) of

bmim-AOT and hmim-AOT vesicles in water at different surfactant concentrations. T = 25 °C.

	bmim-AOT		hmim-AOT	
Surfactant concentration (10^{-4} M)	**d_{app} (nm)**	**PDI**	**d_{app} (nm)**	**PDI**
0.2	164 ± 10	0.4	120 ± 10	0.4
1.0	146 ± 10	0.3	94 ± 10	0.3
3.0	164 ± 10	0.2	90 ± 10	0.3
5.0	146 ± 10	0.2	93 ± 10	0.3
7.0	150 ± 10	0.3	118 ± 10	0.4

In order to further characterize the organized systems obtained, transmission electron microscopy (TEM) micrographs of the bmim-AOT and hmim-AOT aqueous solutions were registered. Figure 1 shows the TEM images obtained for both surfactants at [surfactant] = $3x10^{-4}$ M. Vesicles with diameters and homogeneous distributions comparable to those measured by DLS can be observed (see Figure S3). The average value of the vesicles observed in the TEM images is 186 ± 10 nm for the bmim-AOT vesicles and 103 ± 10 nm for the hmim-AOT vesicles.

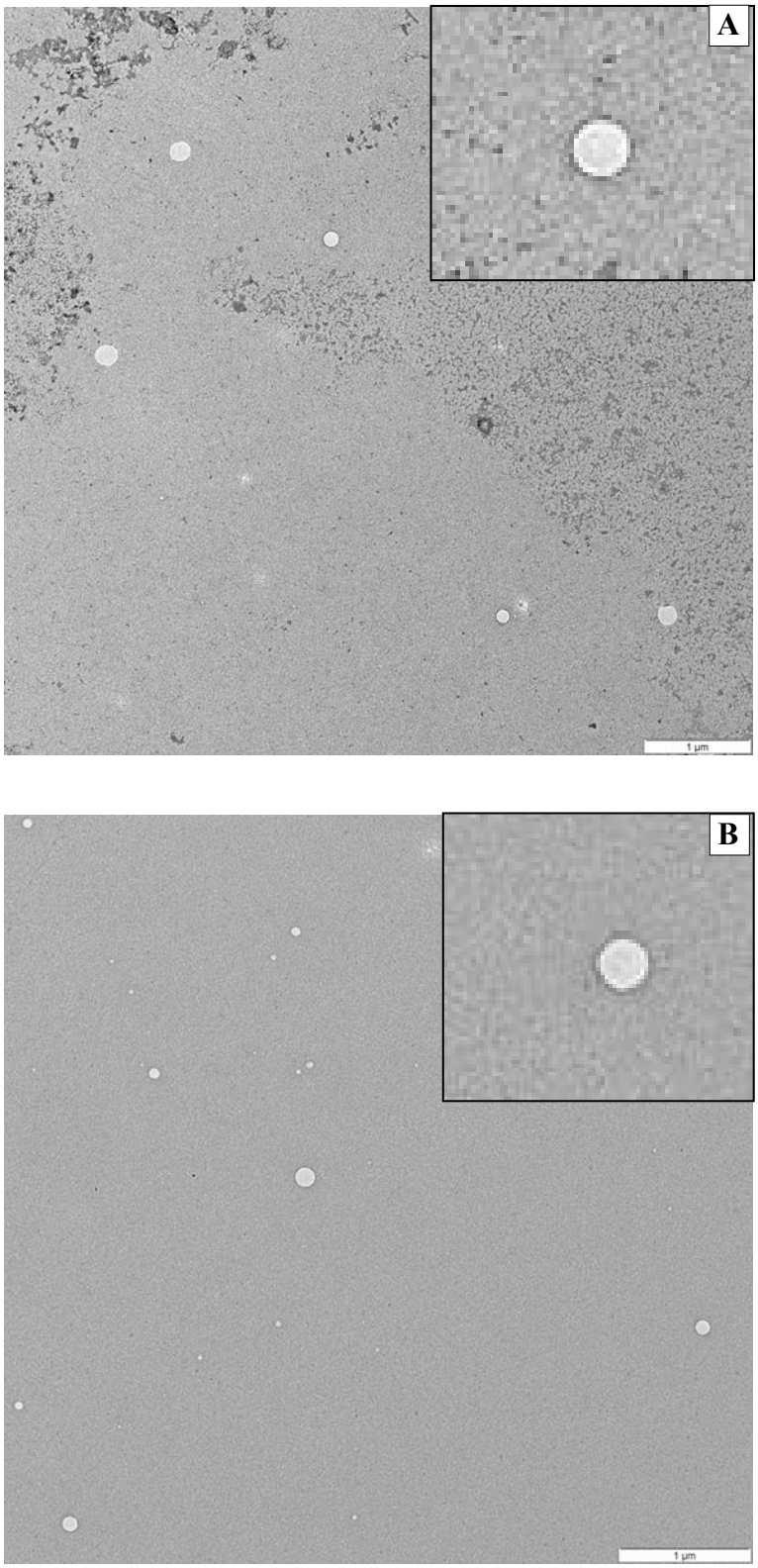


Figure 1. TEM images (negatively stained 2% phosphotungstic acid) of bmim-AOT (A) and hmim-AOT (B) vesicles. [Surfactant] = $3x10^{-4}$ M. Scale bar = 1 μm. The inset on the top-right corner corresponds to a zoom of a vesicle.

These results are very interesting because the Na-AOT surfactant does not have the capacity to form spontaneously unilamellar vesicles in water at the surfactant concentrations evaluated.[19,20,39–43] Several reports have shown data about the vesicle formation but use different methodologies to prepare them (such as extrusion).[44–49] Therefore, the chemical structure of the cationic components in the new surfactants strongly impact their aggregation process.[19,20,49]

As a complementary experiment, zeta potential measurements were carried out. This is an important parameter in order to determine the stability of vesicles and its interaction with biological system.[12] Zeta potential values corresponding to the different vesicle solutions investigated are listed in Table 2.

Table 2. Zeta potential values of bmim-AOT and hmim-AOT vesicles in water at different surfactant concentrations. T = 25 ° C.

	bmim-AOT	**hmim-AOT**
Surfactant concentration (10^{-4} M)	**Zeta potencial (mV)**	**Zeta potencial (mV)**
0.2	-21 ± 2	-14 ± 2
1.0	-22 ± 2	-18 ± 2
3.0	-20 ± 2	-15 ± 2
5.0	-22 ± 2	-19 ± 2
7.0	-21 ± 2	-15 ± 2

The results obtained show vesicles of bmim-AOT with an average zeta potential of -21 mV, while those of hmim-AOT have values around -16 mV. This suggests that the anionic part of the polar head of both surfactants is exposed to the outer region of

the vesicular bilayer. The fact that more negative charge is found in bmim-AOT vesicles compared to that in hmim-AOT vesicles could indicate the presence of a smaller amount of positive counterions in the vesicular interface of the water/bmim-AOT systems than in the hmim-AOT aggregates. It is important to mention that the solutions used for DLS and zeta potential measurements were stored for 30 days and they were used again obtaining sizes and zeta potential values very similar to the initials (values shown in Tables 1 and 2). Thus, the samples were stable and no flocculation or precipitation occurs.

Taking into account that the vesicles formed from traditional phospholipids have been widely used as nanocarriers of drugs, both water-soluble and lipid-soluble,[12] it seemed interesting to analyze the ability of the bmim-AOT and hmim-AOT unilamellar vesicles to incorporate a water-insoluble molecule such as the DR19 dye.[23–25] As DR19 is expected to be localized in the bilayer of the vesicles, this study is useful to estimate which of these two vesicles have the least polar bilayer.[28] An increase in the amount of dye localized in the bilayer would mean an decrease in the polarity of this region.[23–25, 28] Figure 2 shows the amount of DR19 dissolved in the vesicular solutions as a function of the surfactant concentrations.

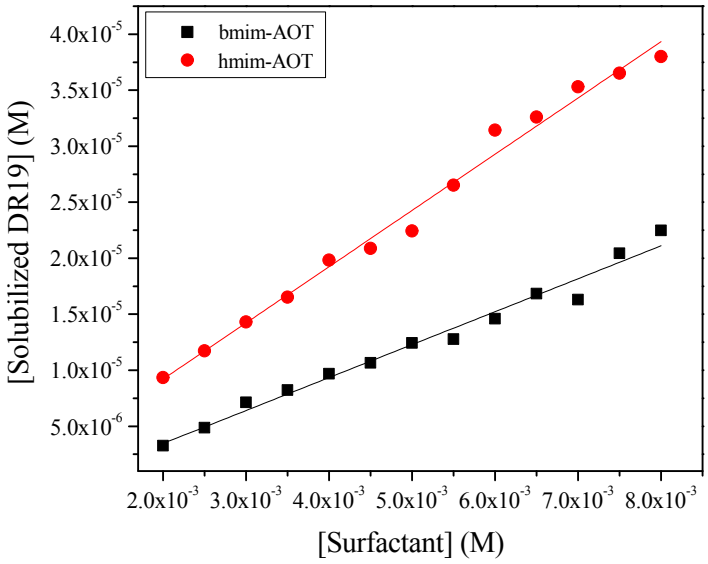


Figure 2. Variation of the DR19 concentration solubilized in the vesicular systems water/bmim-AOT and water/hmim-AOT as a function of the [surfactant]. T = 25 °C.

As can be seen in Figure 2, the bilayer of the hmim-AOT vesicles solubilizes more amount of DR19 than the vesicular system composed by bmim-AOT. This points out that the lipid bilayer of hmim-AOT could be less polar than that of bmim-AOT. This could be due to the fact that $hmim^+$, having a longer hydrocarbon chain than $bmim^+$, is more hydrophobic and consequently more included in the bilayer.

Then, it was interesting to obtain information about the microviscosity of both bilayers. With this in mind, the molecular probe P3P was used at a fixed concentration ([P3P] = $2x10^{-6}$ M), varying the concentration of surfactant. The dependence of the ratio of intensities of the emission bands of the monomer (I_M) and the excimer (I_E) in the fluorescence spectra of P3P on surfactant concentration is shown in Figure 3. The increase in the I_M/I_E ratio implies an increase in the microviscosity of the environment where the molecular probe is located.[26,29,50]

The I_M/I_E ratio follows a sigmoidal trend in both systems and, the higher ratio

found for hmim-AOT as compared to bmim-AOT for all the surfactant concentrations investigated, would indicate a larger rigidity of the interfacial layer of hmim-AOT vesicles than that of the bmim-AOT aggregates.

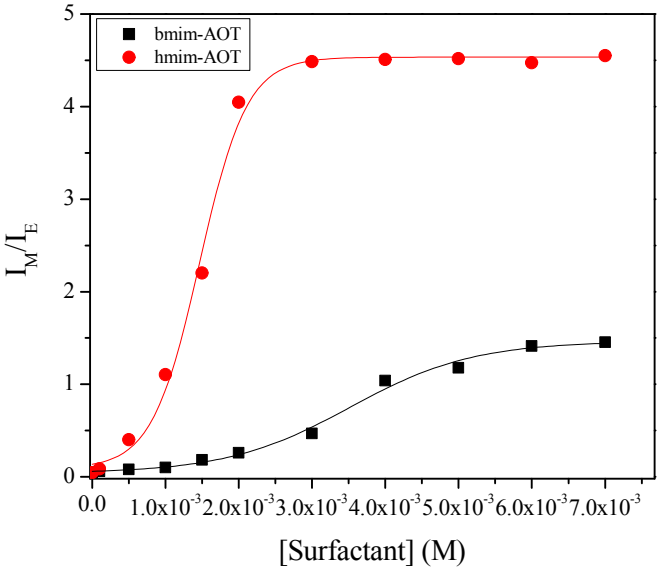


Figure 3. Variation of the I_M/I_E ratio with [surfactant] in the vesicular systems water/bmim-AOT and water/hmim-AOT. [P3P] = $2x10^{-6}$ M. T = 25 °C.

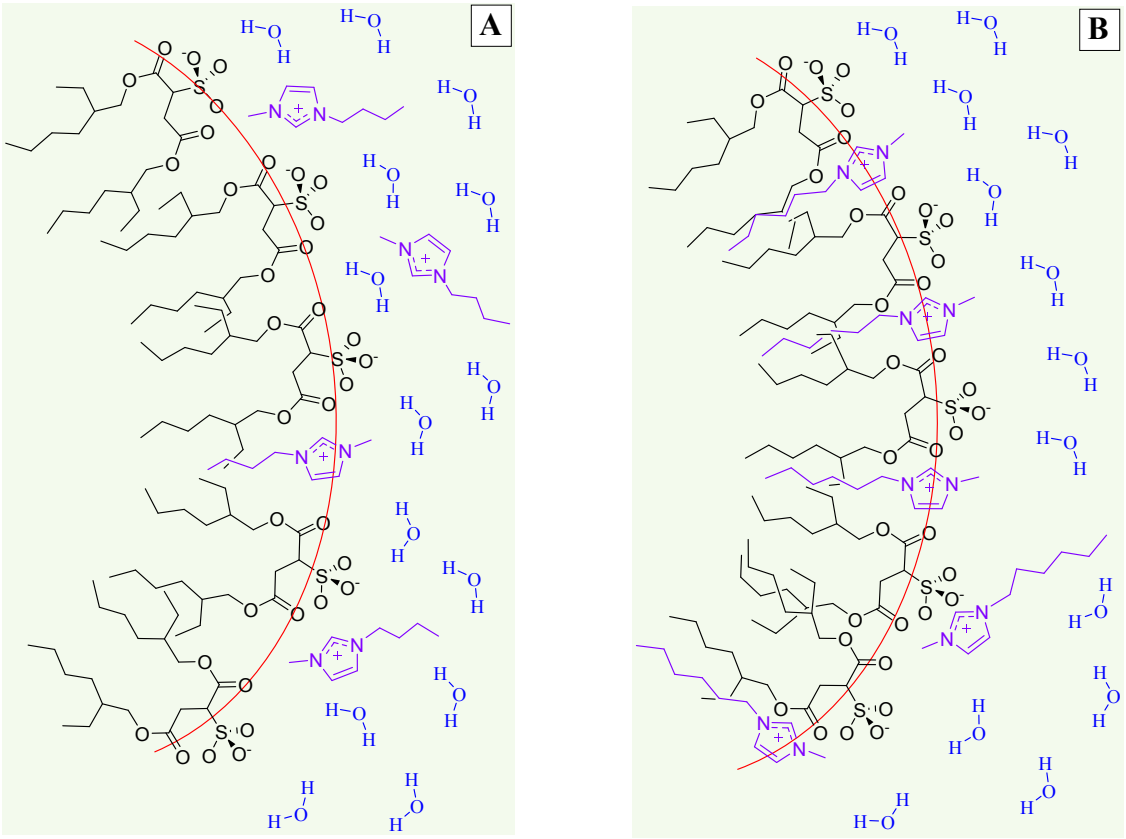
It can be observed in Figure 3 that the I_M/I_E ratio in hmim-AOT vesicles reaches a constant value of approximately 4.5, while in bmim-AOT vesicles this value is approximately 1.5. It is also interesting to note that the constant values are reached for [hmim-AOT] > $3x10^{-3}$ M while for [bmim-AOT] > $6x10^{-3}$ M. This could be explained by a stronger tendency of P3P to incorporate into the hmim-AOT vesicles due to its less polar bilayer (observed through the study that was performed with DR19), in comparison to that of bmim-AOT.[26] The higher microviscosity detected by P3P in hmim-AOT vesicles could imply that the bilayer of these vesicles is more compact than that of bmim-AOT vesicles. This result would agree with the size of vesicles obtained by DLS. Hmim-AOT vesicles with a more compact bilayer are smaller than bmim-AOT

vesicles. This smaller size could be the result of the $hmim^+$ hexyl tails being disposed in a more compact conformation in the vesicle bilayer than the $bmim^+$ butyl tails in the bmim-AOT aggregates. This behavior is similar to the observed for the catanionic IL-like surfactant benzyl-n-hexadecyldimethylammonium 1,4-bis-2-ethylhexylsulfosuccinate used to create vesicles which have a more compact bilayer with greater capacity to solubilize dyes than the DOPC vesicles.[8]

It is important to bear in mind that the size of the vesicles and the compactness of the bilayer depend, among many other variables, on the effective packaging parameter of the surfactant p, defined as $p = v/al_c$, where v and l_c are the volume and the length of the hydrocarbon chain, respectively, and a is the area of the surfactant head group.[51] The p value of the vesicles is within the range $1/2 < p < 1$, for direct micelles $p < 1/3$ and for bilayers $p \approx 1$. The size of the vesicles is larger when the p value is closer to 1. Therefore, all the factors that increase the v value, increase the packing parameter that affects the size of the vesicles.[52] At the surfactant concentrations used in this work, the Na-AOT precursor in water forms mainly direct micelles,[45,53,54] however, the results obtained in this work indicate that the two IL-like surfactants prepared form spontaneously vesicles in water. This can be explained as follows: the ionic nature of the polar head group of the precursors (Na-AOT, bmim-Cl and hmim-Cl) and, the need to act as counterions to each other in the new ILs-like surfactants produces changes in the p parameter compared to the Na-AOT precursor. Our hypothesis is that due to the replacement of the Na^+ counterion of AOT by $bmim^+$ and $hmim^+$, an increase in the effective area can be expected. However, the $bmim^+$ and $hmim^+$ counterions have a hydrocarbon tail which could be located close to the hydrocarbon tails of AOT, favoring hydrophobic interactions and significantly modifying the effective volume. In this way the v/a ratio would increase, causing an increment in the p value, which could go from p

< 1/3 (corresponding to Na-AOT) to *p* between 1/2 and 1 (corresponding to bmim-AOT and hmim-AOT vesicles).

In regard to the zeta potential values, they seem to indicate that there is a somewhat higher presence of $hmim^+$ than $bmim^+$ in the bilayer of the vesicles. This could result in a decrease in the electrostatic repulsions between the negative charges of the AOT, making the *v*/*a* ratio slightly lower in hmim-AOT vesicles than in those of bmim-AOT and, therefore, generating a more compact bilayer and smaller vesicles. A possible distribution of the ions at the bilayer in both vesicles is proposed in Scheme 2.

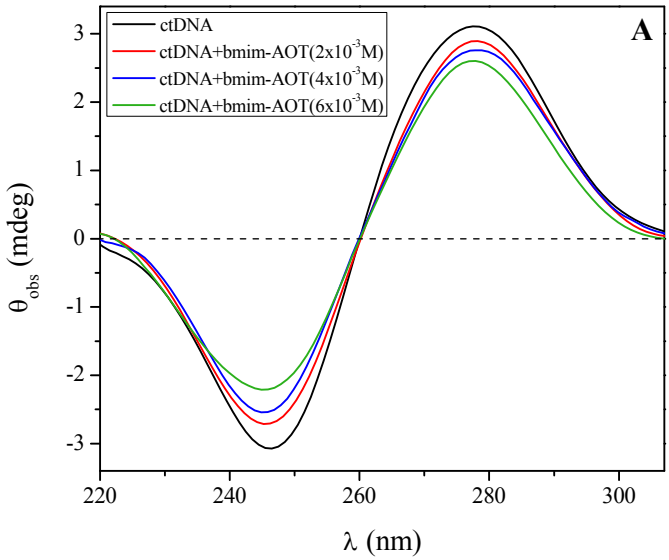


Scheme 2. Schematic representation of the vesicle interfaces of IL-like surfactants (A: water/bmim-AOT and B: water/hmim-AOT).

Once the vesicles systems were investigated, it would be very interesting to explore the possible DNA solubilization in the bilayer and, to investigate the DNA-surfactant interactions.

CD experiments were performed in order to investigate the interactions between DNA and the vesicles. Vesicular solutions of bmim-AOT and hmim-AOT were prepared at different surfactant concentrations and, the same amount of ctDNA ([ctDNA] = $5x10^{-5}$ M) was added to each of them. Using the CD technique, the characteristic bands of DNA between 220 - 310 nm were analyzed and plotted in Figure 4.

Double stranded DNA shows a positive band at λ = 278 nm and a negative band at λ = 246 nm, corresponding to the stacking of π-π bases and the helical structure, respectively.[55] CD spectra of solutions containing ctDNA with bmim-Cl and hmim-Cl were also registered, in order to know if the cationic precursors of both surfactants interact with the DNA (See Figure S4 in supporting information section).



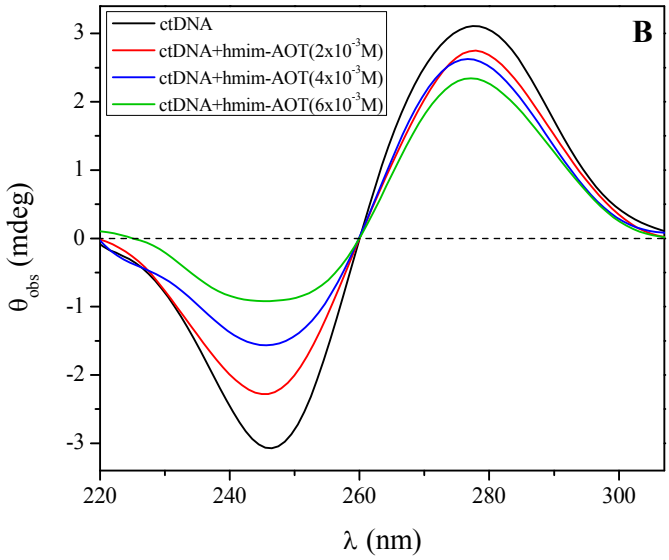


Figure 4. CD spectra of ctDNA interacting with the vesicular system water/bmim-AOT (A) and water/hmim-AOT (B). [ctDNA] = $5x10^{-5}$ M. T = 25 °C.

Figure 4 shows changes in the DNA CD spectra, which indicates that bmim-AOT and hmim-AOT vesicles interact with the polynucleotide. The intensity of both bands decreases upon increasing surfactant concentration, although the more substantial changes occur in the negative band. These results indicate that the vesicles interact with the DNA, partially modifying the polynucleotide conformation.[56] It is interesting to note that no changes in the DNA CD spectrum are observed in the solutions of bmim-Cl and hmim-Cl with DNA (Figure S4), indicating that the cationic precursors do not interact with the DNA. Additionally, the DNA CD spectrum in presence of the Na-AOT surfactant were taken (results not shown), and the DNA was not affected. This would indicate that it is necessary for the counterion to have a hydrophobic region so that it can interact with the polynucleotide. In order to compare between both vesicular systems, ellipticity (θ) of the negative band, at λ = 246 nm, was plotted in Figure 5 at different surfactant concentrations.

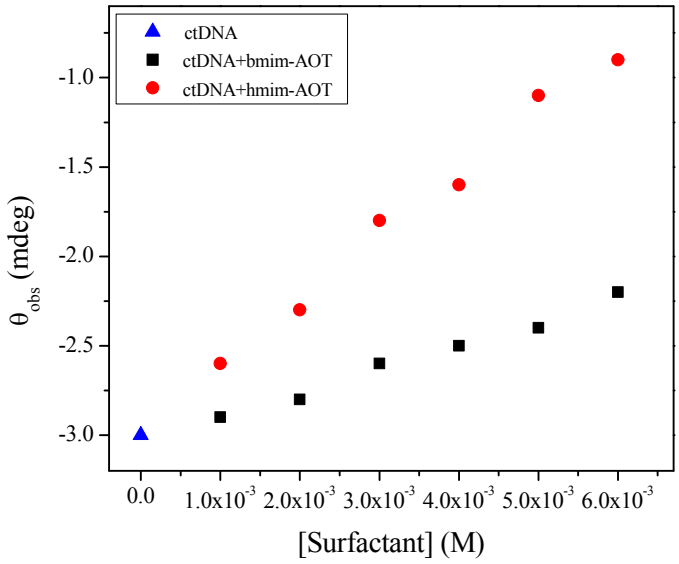


Figure 5. Variation of θ_{obs} with [surfactant] in the vesicular system water/bmim-AOT and water/hmim-AOT. [ctDNA] = $5x10^{-5}$ M. T = 25 °C. λ = 246 nm.

This Figure shows that hmim-AOT vesicles interact more strongly with DNA than bmim-AOT vesicles. One would expect stronger electrostatic interactions between bmim-AOT and DNA than for the hmim-AOT and DNA based on the zeta potential values listed in Table 2. However, hydrophobic interactions, which play a key role in the interactions between DNA and other species[32,57] will be stronger as the longer is the hydrophobic tail of the surfactant. Taking this into account, results in Figures 4 and 5 could be explained by the influence of the surfactant tail length on the vesicles:DNA interactions. In future work the interactions between the IL-like surfactants and DNA, and the cytotoxicity of these aggregates will be investigated in more depth since the design of biocompatible nanocarriers for genetic material is relevant in regard to gene transfection.

CONCLUSIONS

The results obtained in this investigation show that the replacement of Na^+ by $bmim^+$ and $hmim^+$ in the Na-AOT molecule drastically affects the physicochemical properties of the aggregates formed by the surfactant in water. The ionic liquids like-surfactants bmim-AOT and hmim-AOT have the ability to form spontaneously unilamellar vesicles in water. Hmim-AOT vesicles are smaller, with less negative surface charge, greater rigidity of the bilayer and greater capacity to solubilize molecules of low polarity, in comparison to bmim-AOT vesicles. It was also observed that hmim-AOT vesicles interact more with the DNA than bmim-AOT vesicles. This suggests the importance of hydrophobic interactions in the formation of surfactant-DNA complexes. The study of the interaction of surfactants with DNA deserves further investigation, since these aggregates could be efficient non-viral vectors in the transfection of genes.

ASSOCIATED CONTENT

Supplementary Information. NMR and FT-IR characterization of hmim-AOT, DLS data of aqueous solutions of bmim-AOT and hmim-AOT, circular dichroism spectra of ctADN whit bmim-Cl and hmim-Cl in water.

AUTHOR INFORMATION

Corresponding Author

*E-mail: clepori@famaf.unc.edu.ar

Notes

The authors declare no competing financial interests.

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REFERENCES

(1) Hallett, J. P.; Welton, T. Room-Temperature Ionic Liquids: Solvents for Synthesis and Catalysis. 2. *Chem. Rev.* **2011**, *111* (5), 3508–3576.

(2) Wasserscheid, P.; Welton, T. *Ionic Liquids in Synthesis: Second Edition*; Wasserscheid, P., Welton, T., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2008; Vol. 1.

(3) Falcone, R. D.; Correa, N. M.; Silber, J. J.; Levinger, N. E. Ionic Liquids in Soft Confinement. In *Ionic Liquid-Based Surfactant Science*; John Wiley & Sons, Inc: Hoboken, NJ, 2015; pp 283–301.

(4) Welton, T. Room-Temperature Ionic Liquids. Solvents for Synthesis and

Catalysis. *Chem. Rev.* **1999**, *99* (8), 2071–2083.

(5) *Ionic Liquid-Based Surfactant Science*; Paul, B. K., Moulik, S. P., Eds.; John Wiley & Sons, Inc: Hoboken, NJ, 2015.

(6) Villa, C. C.; Moyano, F.; Ceolin, M.; Silber, J. J.; Falcone, R. D.; Correa, N. M. A Unique Ionic Liquid with Amphiphilic Properties That Can Form Reverse Micelles and Spontaneous Unilamellar Vesicles. *Chem. - A Eur. J.* **2012**, *18* (49), 15598–15601.

(7) Lépori, C. M. O.; Silber, J. J.; Falcone, R. D.; Correa, N. M. Improvement of the Amphiphilic Properties of a Dialkyl Phosphate by Creation of a Protic Ionic Liquid-like Surfactant. *RSC Adv.* **2017**, *7* (71).

(8) Villa, C. C.; Correa, N. M.; Silber, J. J.; Moyano, F.; Falcone, R. D. Singularities in the Physicochemical Properties of Spontaneous AOT-BHD Unilamellar Vesicles in Comparison with DOPC Vesicles. *Phys. Chem. Chem. Phys.* **2015**, *17* (26), 17112–17121.

(9) Villa, C. C.; Silber, J. J.; Correa, N. M.; Falcone, R. D. Effect of the Cationic Surfactant Moiety on the Structure of Water Entrapped in Two Catanionic Reverse Micelles Created from Ionic Liquid-Like Surfactants. *ChemPhysChem* **2014**, *15* (14), 3097–3109.

(10) Lépori, C. M. O.; Correa, N. M.; Silber, J. J.; Falcone, R. D. How the Cation 1-Butyl-3-Methylimidazolium Impacts the Interaction between the Entrapped Water and the Reverse Micelle Interface Created with an Ionic Liquid-like Surfactant. *Soft Matter* **2016**, *12* (3), 830–844.

(11) Lépori, C. M. O.; Correa, N. M.; Silber, J. J.; Vaca Chávez, F.; Falcone, R. D. Interfacial Properties Modulated by the Water Confinement in Reverse Micelles Created by the Ionic Liquid-like Surfactant Bmim-AOT. *Soft Matter* **2019**, *15*

(5), 947–955.

(12) Pattni, B. S.; Chupin, V. V; Torchilin, V. P. New Developments in Liposomal Drug Delivery. *Chem. Rev.* **2015**, *115* (19), 10938–10966.

(13) New, R. R. C. *Liposomes : A Practical Approach*; Oxford University Press, 1990.

(14) Besteman, K.; Van Eijk, K.; Lemay, S. G. Charge Inversion Accompanies DNA Condensation by Multivalent Ions. *Nat. Phys.* **2007**, *3* (9), 641–644.

(15) Li, Y.; Wang, Y.; Huang, G.; Gao, J. Cooperativity Principles in Self-Assembled Nanomedicine. *Chem. Rev.* **2018**, *118* (11), 5359–5391.

(16) Estévez-Torres, A.; Baigl, D. DNA Compaction: Fundamentals and Applications. *Soft Matter* **2011**, *7* (15), 6746–6756.

(17) Manning, G. S. Counterion Binding in Polyelectrolyte Theory. *Acc. Chem. Res.* **1979**, *12* (12), 443–449.

(18) Egorova, K. S.; Gordeev, E. G.; Ananikov, V. P. Biological Activity of Ionic Liquids and Their Application in Pharmaceutics and Medicine. *Chem. Rev.* **2017**, *117* (10), 7132–7189.

(19) Brown, P.; Butts, C. P.; Eastoe, J.; Fermin, D.; Grillo, I.; Lee, H.; Parker, D.; Plana, D.; Richardson, R. M. Anionic Surfactant Ionic Liquids with 1-Butyl-3-Methyl-Imidazolium Cations: Characterization and Application. *Langmuir* **2012**, *28* (5), 2502–2509.

(20) Cheng, N.; Ma, X.; Sheng, X.; Wang, T.; Wang, R.; Jiao, J.; Yu, L. Aggregation Behavior of Anionic Surface Active Ionic Liquids with Double Hydrocarbon Chains in Aqueous Solution: Experimental and Theoretical Investigations. *Colloids Surfaces A Physicochem. Eng. Asp.* **2014**, *453* (1), 53–61.

(21) Onyesom, I.; Lamprou, D. A.; Sygellou, L.; Owusu-Ware, S. K.; Antonijevic, M.; Chowdhry, B. Z.; Douroumis, D. Sirolimus Encapsulated Liposomes for

Cancer Therapy: Physicochemical and Mechanical Characterization of Sirolimus Distribution within Liposome Bilayers. *Mol. Pharm.* **2013**, *10* (11), 4281–4293.

(22) Martínez-Negro, M.; Barrán-Berdón, A. L.; Aicart-Ramos, C.; Moyá, M. L.; de Ilarduya, C. T.; Aicart, E.; Junquera, E. Transfection of Plasmid DNA by Nanocarriers Containing a Gemini Cationic Lipid with an Aromatic Spacer or Its Monomeric Counterpart. *Colloids Surfaces B Biointerfaces* **2018**, *161*, 519–527.

(23) Menger, F. M.; Galloway, A. L. Contiguous versus Segmented Hydrophobicity in Micellar Systems. *J. Am. Chem. Soc.* **2004**, *126* (48), 15883–15889.

(24) Jaggi, N.; Gin, M.; Yadav, K. Absorption and Fluorescence Spectra of Disperse Red 19-An Azo Dye. *Indian J. Pure Appl. Phys.* **2013**, *51* (12), 833–836.

(25) Uliana, C. V.; Garbellini, G. S.; Yamanaka, H. Spectrophotometric Evaluation of the Behavior of Disperse Red 1 Dye in Aqueous Media and Its Interaction with Calf Thymus Ds-DNA. *J. Braz. Chem. Soc.* **2012**, *23* (8), 1469–1475.

(26) Zana, R. Microviscosity of Aqueous Surfactant Micelles: Effect of Various Parameters. *J. Phys. Chem. B* **1999**, *103* (43), 9117–9125.

(27) Kano, K.; Ishimura, T. Properties of Alkyl B-D-Glucoside and Alkyl b-D-Maltoside Micelles. *J. Chem. Soc. Perkin Trans. 2 Phys. Org. Chem.* **1995**, No. 8, 1655–1660.

(28) Martín, V. I.; de la Haba, R. R.; Ventosa, A.; Congiu, E.; Ortega-Calvo, J. J.; Moyá, M. L. Colloidal and Biological Properties of Cationic Single-Chain and Dimeric Surfactants. *Colloids Surfaces B Biointerfaces* **2014**, *114*, 247–254.

(29) Domínguez, R.; Rodríguez, A.; Maestre, A.; Robina, I.; Moyá, M. L. Synthesis and Physicochemical Characterization of Alkanedyil-α-ω-Bis(Dimethyldodecylammonium) Bromide, 12-s-12,2Br-, Surfactants with S=7, 9, 11 in Aqueous Medium. *J. Colloid Interface Sci.* **2012**, *386* (1), 228–239.

(30) Martín, V. I.; Sarrión, B.; López-López, M.; López-Cornejo, P.; Robina, I.; Moyá, M. L. Reversibility of the Interactions between a Novel Surfactant Derived from Lysine and Biomolecules. *Colloids Surfaces B Biointerfaces* **2015**, *135*, 346–356.

(31) Ostos, F. J.; Lebron, J. A.; Moyá, M. L.; Deasy, M.; López-Cornejo, P. Binding of DNA by a Dinitro-Diester Calix[4]Arene: Denaturation and Condensation of DNA. *Colloids Surfaces B Biointerfaces* **2015**, *127*, 65–72.

(32) López-López, M.; López-Cornejo, P.; Martín, V. I.; Ostos, F. J.; Checa-Rodríguez, C.; Prados-Carvajal, R.; Lebrón, J. A.; Huertas, P.; Moyá, M. L. Importance of Hydrophobic Interactions in the Single-Chained Cationic Surfactant-DNA Complexation. *J. Colloid Interface Sci.* **2018**, *521*, 197–205.

(33) López-López, M.; López-Cornejo, P.; González-Cortés, C.; Blanco-Arévalo, D.; Pérez-Alfonso, D.; Mozo-Mulero, C.; Oviedo, J.; Moyá, M. L. Influence of the Cyclodextrin Nature on the Decompaction of Dimeric Cationic Surfactant-DNA Complexes. *Colloids Surfaces A Physicochem. Eng. Asp.* **2018**, *555* (May), 133–141.

(34) García, J. P.; Marrón, E.; Martín, V. I.; Moyá, M. L.; Lopez-Cornejo, P. Conformational Changes of DNA in the Presence of 12-s-12 Gemini Surfactants (S=2 and 10). Role of the Spacer's Length in the Interaction Surfactant-Polynucleotide. *Colloids Surfaces B Biointerfaces* **2014**, *118*, 90–100.

(35) Liu, Y. J.; Chao, H.; Yuan, Y. X.; Yu, H. J.; Ji, L. N. Ruthenium(II) Mixed-Ligand Complexes Containing 2-(6-Methyl-3-Chromonyl)Imidazo[4,5-f][1,10]-Phenanthroline: Synthesis, DNA-Binding and Photocleavage Studies. *Inorganica Chim. Acta* **2006**, *359* (12), 3807–3814.

(36) Bello, V.; Mattei, G.; Mazzoldi, P.; Vivenza, N.; Gasco, P.; Idee, J. M.; Robic,

C.; Borsella, E. Transmission Electron Microscopy of Lipid Vesicles for Drug Delivery : Comparison between Positive and Negative Staining. *Microsc. Microanal.* **2010**, *16*, 456–461.

(37) Enoki, T. A.; Henriques, V. B.; Lamy, M. T. *Light Scattering on the Structural Characterization of DMPG Vesicles along the Bilayer Anomalous Phase Transition*; Elsevier Ireland Ltd, 2012; Vol. 165.

(38) Fendler, J. H. Atomic and Molecular Clusters in Membrane Mimetic Chemistry. *Chem. Rev.* **1987**, *87* (5), 877–899.

(39) Brown, P.; Butts, C.; Dyer, R.; Eastoe, J.; Grillo, I.; Guittard, F.; Rogers, S.; Heenan, R. Anionic Surfactants and Surfactant Ionic Liquids with Quaternary Ammonium Counterions. *Langmuir* **2011**, *27* (8), 4563–4571.

(40) Dey, J.; Bhattacharjee, J.; Hassan, P. A.; Aswal, V. K.; Das, S.; Ismail, K. Micellar Shape Driven Counterion Binding. Small-Angle Neutron Scattering Study of AOT Micelle. *Langmuir* **2010**, *26* (12), 15802–15806.

(41) Sheu, E. Y.; Chen, S.; John S. Huang. Structure and Growth of Bis(2-Ethylhexyl) Sulfosuccinate Micelles in Aqueous Solutions. *J. Phys. Chem.* **1987**, *91* (12), 3306–3310.

(42) Thapa, U.; Dey, J.; Kumar, S.; Hassan, P. A.; Aswalc, V. K.; Ismail, K. Tetraalkylammonium Ion Induced Micelle-to-Vesicle Transition in Aqueous Sodium Dioctylsulfosuccinate Solutions. *Soft Matter* **2013**, *9*, 11225–11232.

(43) Umlong, I. M.; Ismail, K. Micellization of AOT in Aqueous Sodium Chloride , Sodium Acetate , Sodium Propionate , and Sodium Butyrate Media : A Case of Two Different Concentration Regions of Counterion Binding. *J. Colloid Interface Sci.* **2005**, *291*, 529–536.

(44) Fan, Y.; Li, Y.; Yuan, G.; Wang, Y.; Wang, J.; Han, C. C.; Yan, H.; Li, Z.;

Thomas, R. K. Comparative Studies on the Micellization of Sodium Bis(4-Phenylbutyl) Sulfosuccinate and Sodium Bis(2-Ethylhexyl) Sulfosuccinate and Their Interaction with Hydrophobically Modified Poly(Acrylamide). *Langmuir* **2005**, *21* (9), 3814–3820.

(45) Lin, C.; Zhao, J.; Jiang, R. Nile Red Probing for the Micelle-to-Vesicle Transition of AOT in Aqueous Solution. *Chem. Phys. Lett.* **2008**, *464* (1–3), 77–81.

(46) Briz, J. I.; Velázquez, M. M. Effect of Water-Soluble Polymers on the Morphology of Aerosol OT Vesicles. *J. Colloid Interface Sci.* **2002**, *247* (2), 437–446.

(47) Zhang, Y.; Serrano-Luginbühl, S.; Kissner, R.; Milojević-Rakić, M.; Bajuk-Bogdanović, D.; Ćirić-Marjanović, G.; Wang, Q.; Walde, P. Enzymatic Synthesis of Highly Electroactive Oligoanilines from a P-Aminodiphenylamine/Aniline Mixture with Anionic Vesicles as Templates. *Langmuir* **2018**, *34* (31), 9153–9166.

(48) Fujisaki, T.; Ćirić-Marjanović, G.; Kissner, R.; Bajuk-Bogdanović, D.; Serrano-Luginbühl, S.; Schuler, L. D.; Khaydarov, A.; Kashima, K.; Ležaić, A. J.; Walde, P. How Experimental Details Matter. The Case of a Laccase-Catalysed Oligomerisation Reaction. *RSC Adv.* **2018**, *8* (58), 33229–33242.

(49) Srinivasa Rao, K.; Gehlot, P. S.; Trivedi, T. J.; Kumar, A. Self-Assembly of New Surface Active Ionic Liquids Based on Aerosol-OT in Aqueous Media. *J. Colloid Interface Sci.* **2014**, *428*, 267–275.

(50) Zana, R.; In, M.; Lévy, H.; Duportail, G. Alkanediyl-α,ω-Bis(Dimethylalkylammonium Bromide). 7. Fluorescence Probing Studies of Micelle Micropolarity and Microviscosity. *Langmuir* **1997**, *13* (21), 5552–5557.

(51) Cosgrove, T. *Colloid Science Principles, Methods and Applications*, 2nd ed.; John Wiley & Sons, 2010.

(52) Ramanathan, M.; Shrestha, L. K.; Mori, T.; Ji, Q.; Hill, J. P.; Ariga, K. Amphiphile Nanoarchitectonics: From Basic Physical Chemistry to Advanced Applications. *Phys. Chem. Chem. Phys.* **2013**, *15* (26), 10580–10611.

(53) Sanchez, F.; Moya, M. L.; Rodrigues, A.; Jimenez, R.; Gomez-Herrera, C.; Yanes, C.; Lopez-Cornejo, P. Micellar, Microemulsion, and Salt Kinetic Effects upon the Reaction Fe(CN) 2 (Bpy) 2 + S 2 O 82 -. *Langmuir* **1997**, *2* (14), 3084–3089.

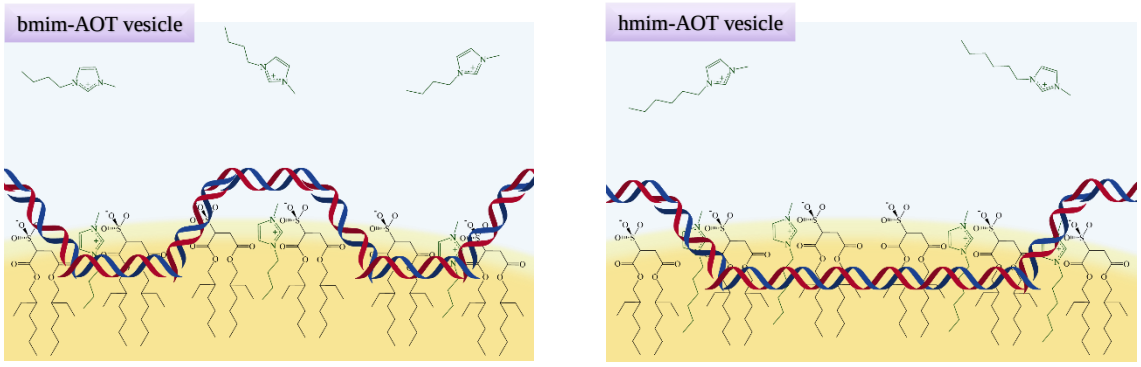
(54) Chatterjee, A.; Moulik, S. P.; Sanyal, S. K.; Mishra, B. K.; Puri, P. M. Thermodynamics of Micelle Formation of Ionic Surfactants: A Critical Assessment for Sodium Dodecyl Sulfate, Cetyl Pyridinium Chloride and Dioctyl Sulfosuccinate (Na Salt) by Microcalorimetric, Conductometric, and Tensiometric Measurements. *J. Phys. Chem. B* **2001**, *105* (51), 12823–12831.

(55) Neidle, S. *Nucleic Acid Structure and Recognition*; Oxford University Press: New York, 2002.

(56) Monnot, M.; Mauffret, O.; Lescot, E.; Fermandjian, S. Probing Intercalation and Conformational Effects of the Anticancer Drug 2-methyl-9-hydroxyellipticinium Acetate in DNA Fragments with Circular Dichroism. *Eur. J. Biochem.* **1992**, *204* (3), 1035–1039.

(57) Jumbri, K.; Ahmad, H.; Abdulmalek, E.; Abdul Rahman, M. B. Binding Energy and Biophysical Properties of Ionic Liquid-DNA Complex: Understanding the Role of Hydrophobic Interactions. *J. Mol. Liq.* **2016**, *223*, 1197–1203.

GRAPHICAL ABSTRACT



Due to the bilayer composition, hmim-AOT vesicles interact more strongly with DNA than bmim-AOT vesicles.