

Structural Parameters of Lamellar Phases Formed by the Self-Assembly of Dialkyldimethylammonium Bromides in Aqueous Solution

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Phase behavior of didodecyl and dioctadecyldimethylammonium bromide (DDAB and DODAB) in water was studied using X-ray techniques, differential scanning calorimetry and deuterium nuclear magnetic resonance measurements. Both surfactants self-assemble into lamellar liquid crystalline, either L_{α} (fluid) and L_{β} (gel), phases. The gel-to-fluid phase transition temperature (T_m) in DDAB lamellae was found to be higher than that observed for its vesicles, due to the formation of a stabilized gel phase. In addition, the lamellar phases formed by both amphiphiles differ in their swelling degree and bilayer thickness. DODAB in water formed rather thin bilayers, if compared with the length of the tails, suggesting effective chain interdigitation. These bilayers also presented higher swelling degree than those formed by the shorter-chain homologue DDAB. We propose that these structural properties are strongly influenced by the electrostatic repulsive forces acting on the system.

Keywords: double-chain cationic surfactants, lamellar phases, liquid crystals, swelling degree, interdigitation

Introduction

Surfactants are amphiphilic molecules with hydrophilic and hydrophobic parts composing its chemical structure. When dispersed in water, these molecules can spontaneously form molecular organized aggregates, such as micelles, vesicles and liquid crystalline phases.¹⁻⁴ The unique properties of these systems have generated extensive research interest. They possess biological relevance in membrane formation, for example, as well as the well-known industrial applications.⁵⁻⁷

The knowledge of structure of bilayers is important for our understanding of functioning of biological membranes and may be improved by the investigation of the structural properties of bilayers from natural or synthetic amphiphiles.⁸ Because double-tailed surfactants are especially useful to mimic membranes and vesicles such as biological membranes, the understanding of the phase properties of these surfactants was always of great interest.⁹

Dioctadecyldimethylammonium bromide (DODAB) is a synthetic double-tailed cationic surfactant and has found widely use in fundamental studies in colloid and membrane science because of its well-known vesicle-forming

properties.^{9,10} When dispersed in water, DODAB molecules can self-assemble into aggregates such as vesicles, at very low concentrations, and lamellar liquid crystalline phases, at higher concentrations.^{11,12} These lamellar phases can be described as a set of bilayers separated by aqueous layers, forming a one-dimensional network. For this surfactant in water, two main types of lamellar phases are described, hereafter named L_{α} (fluid) and L_{β} (gel) phases, which differ in the order state of their alkyl chains, the former being less ordered.¹²

Didodecyldimethylammonium bromide (DDAB) is another double-tailed surfactant, which also possesses great scientific interest. A number of studies have reported the phase diagrams of the binary DDAB/water system, where the presence of multilayer vesicles was verified at very low surfactant concentrations and three different lamellar phases were clearly identified at low and high concentrations, respectively, in this case differing on their degrees of swelling and the order of the chains in the bilayers.¹³⁻¹⁸

Because these amphiphiles dispersions can be used as biomembrane models⁸ and have wide applications in fields such as sensors¹⁹ and drug delivery systems,²⁰ many efforts have been made to understand their phase behavior and phase transitions mechanisms. As discussed below,

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there are many studies involving their phase behavior, but a more direct comparison on the effect of their alkyl chain length on the structures of the lamellae was not reported yet.

During the formation of lamellar phases, structural changes can occur in the bilayers under certain conditions, so that the surfactant molecules forming bilayers eventually reorganize themselves to form other types of molecular aggregates with particular structures. One of these peculiar structures is the interdigitated lamellar phase, that is characterized by thinner bilayers due to the interpenetration of the carbon chains of one monolayer with those of the opposing layer.²¹⁻²⁵ The phenomenon of interdigitation was also described for some of the dialkyldimethylammonium bromides,^{26,27} but a more clear explanation for this phenomenon is still lacking.

Another change that can occur in the structure of the bilayers composing a lamellar phase is in the variation of the repeat distance of bilayers upon hydration.^{22,26} The swelling degree of lamellae is an important parameter to be analyzed, because it can provide information about the (de)stabilization forces acting on the bilayers. Relatively few studies in the literature^{13,26} focused on the different swelling behavior for the lamellar phases formed by the dialkyldimethylammonium bromides surfactants, depending on the alkyl chain length, however, to our knowledge, the causes for this remain unclear.

In this paper, we report studies on the structural properties of the different lamellar phases obtained by the self-assembly of DDAB and DODAB surfactants in water by a combination of small-angle X-ray scattering (SAXS), X-ray diffraction (XRD), differential scanning calorimetry (DSC) and deuterium nuclear magnetic resonance (²H NMR) techniques. Combining these results, we produce a comprehensive set of structural information on their lamellar phases such as repeating distances (degree of swelling) and bilayer thicknesses, as well as the areas *per* surfactant molecules at the different ordering states of the bilayers.

Experimental

Chemicals

The surfactants DDAB and DODAB were purchased from Sigma-Aldrich (USA) with purity of 99.5% and used as received. NaCl, P.A., was bought from Synth (Brazil). D₂O, with purity of 99.9% in D atoms was received from Sigma-Aldrich (USA). Deionized water with a resistivity above 18.2 MΩ cm⁻¹, as obtained by a Milli-Q[®] system, was used in all experiments.

Sample preparation

Mixtures of surfactants in water, with concentrations from 0.1 to 85.0% in weight, were obtained by weighing the appropriate amounts of surfactants and mixing with water by stirring during 30 minutes at the desired temperature. Centrifugation at 5000 rpm was made, back and forth, to remove bubbles and to homogenize the samples, which were equilibrated for, at least, 30 days in the formulation temperature before characterization. The measurements, especially SAXS and DSC analyses, were repeated after different times of sample preparation (from 1 to 12 months) to ensure the equilibrium state of the investigated systems.

Techniques

Small angle X-ray scattering (SAXS)

The measurements were performed at the SAXS1 beamline of the Brazilian Synchrotron National Laboratory, LNLS, in Campinas, Brazil. The samples were positioned in a cell with two flat mica windows and a thermal bath was used for temperature control. The wavelength of X-ray was 1.608 Å and the used sample-to-detector distance was around 0.6 m. The obtained charge-coupled device (CCD) images were integrated and treated with the software Fit2D.

X-ray diffraction (XRD)

Cu ($K_{\alpha} = 1.5406 \text{ \AA}$) radiation was used to obtain XRD data of samples at different temperatures in a Shimadzu XRD-7000 diffractometer with a scan rate of 2 degree min⁻¹.

Differential scanning calorimetry (DSC)

The DSC measurements were performed in the Microcal VP high sensitivity calorimeter (Microcal Inc. Northampton, USA) equipped with 0.542 mL twin total-fill cells. Scanning was performed at a heating rate of 10 °C h⁻¹ from 10 to 80 °C, with null pre-scan, producing fully reproducible results when comparing consecutive runs. Transition enthalpies were obtained by integrating the area under the thermograms along the temperature range. The calorimetric analyses were made in triplicate.

Deuterium nuclear magnetic resonance (²H NMR)

The NMR spectra were obtained for samples prepared in water with the addition of 10% (v/v) of D₂O in 5 mm glass tubes in a Bruker DMX spectrometer operating at a frequency of 500 MHz.

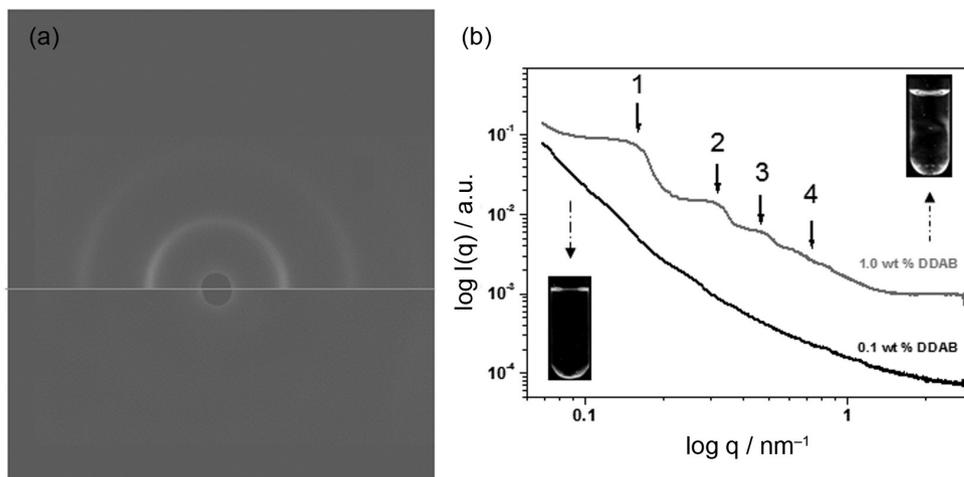


Figure 1. (a) 2D SAXS patterns for 1.0 wt.% DDAB in water sample (top) and 0.1 wt.% DDAB in water sample (bottom); (b) SAXS profiles for the same samples. Insets in (b) show the birefringence of the samples under crossed polarizers. All data were obtained at 25 °C.

Results

Phase behavior of DDAB and DODAB in water

The structures of the liquid crystals were first analyzed by small angle X-ray scattering (SAXS) experiments. Very dilute isotropic samples (0.1 wt.% DDAB in water) showed a scattering intensity, I , monotonously decreasing with increasing scattering vector, q . At higher concentrations (above 1.0 wt.%), SAXS patterns display correlation peaks due to the short-range positional order of the formed surfactant bilayers. Typical X-ray scattering patterns obtained from isotropic and anisotropic samples prepared with DDAB are presented in Figures 1a and 1b. The anisotropic character of the birefringent phase is visible under crossed polarizers and, together with the presence of Bragg reflections of 1, 2, 3, etc., proves the lamellar nature of these liquid crystals. Other images of birefringent samples observed under crossed polarizers are presented in Figure S1 (Supplementary Information section).

By SAXS analyses, it was possible to characterize these lamellar phases formed by the self-assembly of the double tail surfactants in solution. As mentioned above, the scattering pattern of a typical lamellar phase is characterized by the reflections 1, 2, 3, etc.²⁸ Two coexisting phases are seen as two sets of reflections. In the DDAB/water system, by the SAXS pattern, the birefringent phase observed at concentrations below 40.0% of surfactant, coexisting with isotropic solution, was identified as a lamellar fluid phase named as L_{α} phase. In concentrations above 40.0% of DDAB in water, the SAXS curves showed a sequence of scattering peaks that could be associated with two different lamellar phases. As examples, Figures 2a and 2b show representative SAXS curves for the lamellar phases

formed by DDAB in water. The indexing of the peaks will be described below.

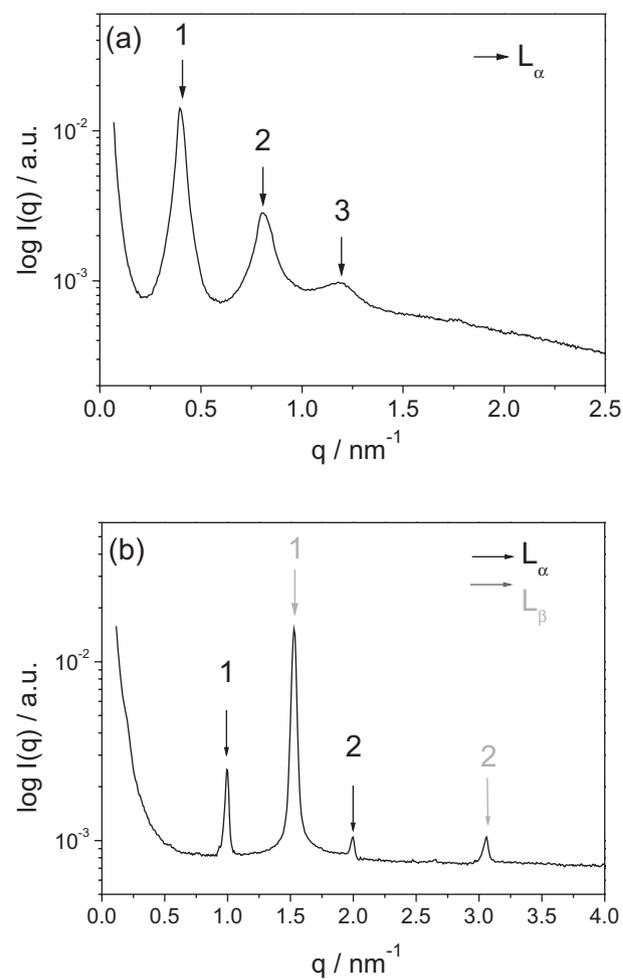


Figure 2. SAXS curves at 25 °C for (a) a diluted 5.0% DDAB in water sample and (b) a sample prepared with 50.0% of surfactant with scattering peaks indexed.

These two coexisting lamellar phases were identified as the L_α (fluid) and L_β (gel) phases. The main difference between these two lamellar phases is the organization of carbon chains in the bilayers. In the L_α phase, the chains are in fluid state, while in the L_β phase, they are in a solid-like state, in this case, making the membranes more rigid. As described elsewhere,²⁹ the fluid phase possesses larger repeat distance of the bilayers (d), calculated from the position of the first scattering peak in SAXS curves using equation 1:

$$d = 2\pi / q \quad (1)$$

where q is the scattering vector. By knowing that the fluid phase possesses a higher repeat distance of bilayers and that the scattering pattern for a lamellar phase corresponds to peak ratios of 1, 2, 3, etc., we assumed the first and third scattering peaks to be related to the fluid phase and the other two peaks referring to the gel phase, as indexed in SAXS pattern presented in Figure 2b.

Figures 3a and 3b show SAXS curves for a diluted and concentrated sample prepared with DODAB in water, with the Bragg peaks indexed. It was possible to identify the presence of the lamellar L_α fluid phase at concentrations below 40.0% (also coexisting with isotropic solution) and only the L_β phase in the samples prepared with higher concentrations of DODAB in water.

From the observation of SAXS curves, it can be noted that for both DDAB and DODAB bilayers, the L_α phase displayed broader scattering peaks, if compared with those of the L_β phase. This can be assigned to the greater flexibility of the bilayers that form the fluid phase, reflecting smaller degree of organization of carbon chains in that phase, according to the Caillé theory for the scattering of lamellar phases.²⁸

The gel phase was also characterized by a diffraction peak with distance (calculated by Bragg equation) of 0.42 nm, described in literature as the correlation distance among the carbon chains in the L_β phase.³⁰ XRD diffractograms for samples prepared with DDAB and DODAB amphiphiles presenting the lamellar L_β phase are shown in Figure 4.

Deuterium NMR spectra were also used qualitatively to characterize the lamellar liquid crystals. This spectroscopic technique has been largely used because in anisotropic systems a doublet quadrupolar splitting can be observed in the NMR spectra, which provides information about the small orientational ordering of water within anisotropic mesophases on account of interactions between water and the polar headgroups of the surfactants or lipids composing the bilayers.^{31,32}

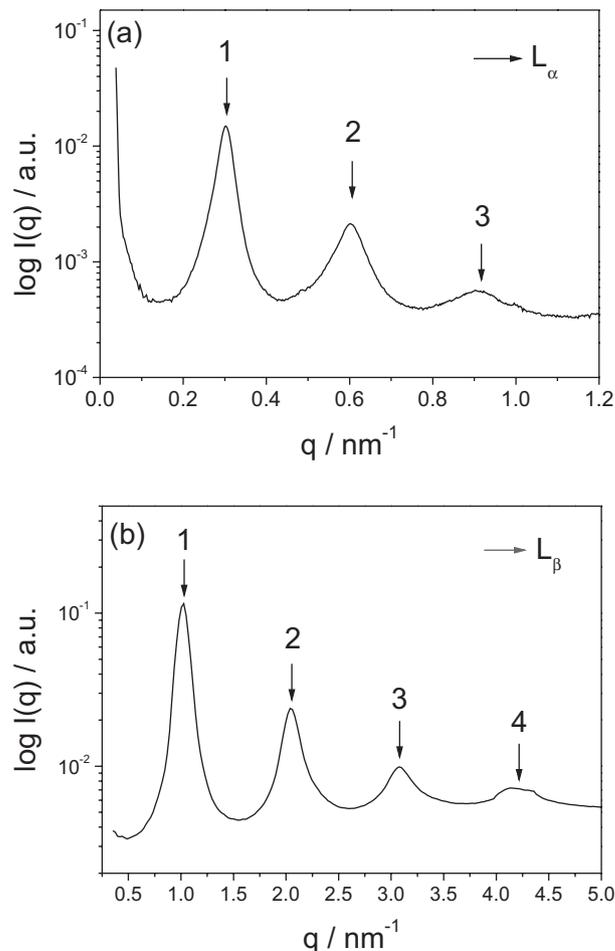


Figure 3. SAXS profiles at 25 °C for (a) 10.0%; (b) 50.0% DODAB in water samples with indexed peaks of lamellar fluid and gel phases, respectively.

In this way, the $^2\text{H}_2\text{O}$ NMR spectra obtained, at 25 °C, for samples prepared with different amounts of DDAB in water are presented in Figure 5. It is possible to note that, at higher concentration of surfactant, sufficient to form the lamellar liquid crystals, two doublets from quadrupolar splitting are seen in the spectra, each one corresponding to the lamellar phases coexisting in this range of concentration at room temperature, in accordance with the SAXS data. In the diluted sample case (1.0% of DDAB), only an unresolved doublet peak can be observed in the spectrum, indicating the small anisotropic orientation of the system in this surfactant concentration. Similar behavior was obtained in the DODAB/water system.

Upon increasing temperature, transformations between these liquid crystalline phases are followed by changes in molecular conformation and packing states.³³ Generally, below the gel-to-liquid crystalline phase transition (L_β - L_α) temperature, T_m , the surfactant molecules in bilayers present more solid-like alkyl chains with restricted molecular motion. Above T_m , the surfactant molecules

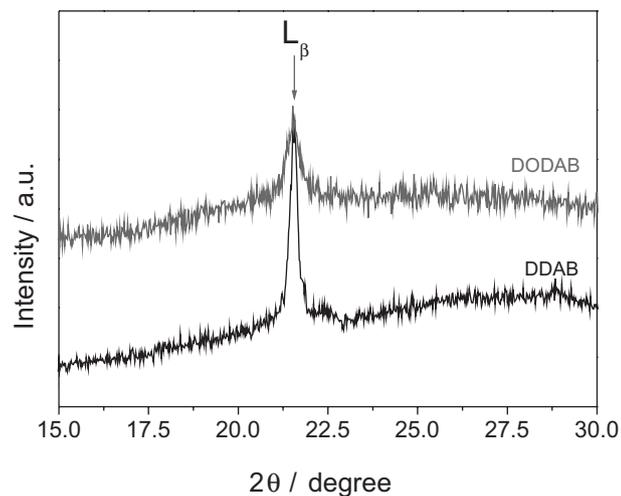


Figure 4. XRD diffractograms for 50.0% DDAB and DODAB samples with the L_{β} phase peak indexed. The data were obtained at 25 °C.

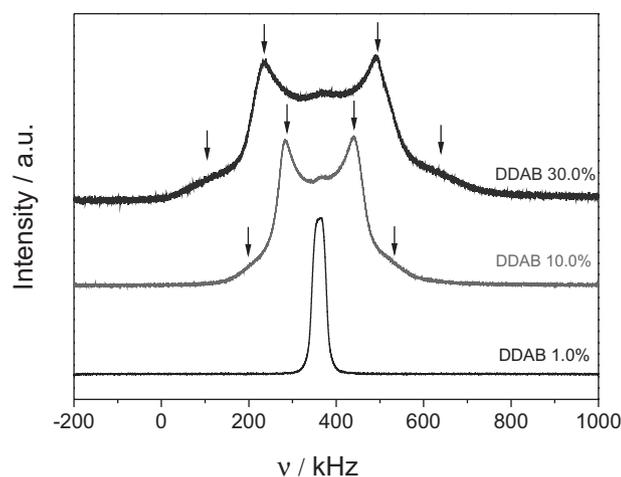


Figure 5. $^2\text{H}_2\text{O}$ NMR spectra for samples prepared with different amounts of DDAB in water, followed by the addition of 10% (v/v) of D_2O and homogenization. The data were obtained at 25 °C.

are in the fluid liquid crystalline state, in which alkyl chains conformational disorder predominates.³⁴ DSC was employed to determine the thermal phase behavior and estimate changes in the alkyl chain order in lamellar phases formed by DDAB and DODAB in water. Figure 6 shows the DSC thermograms obtained for 50.0% surfactant samples, showing an endothermic transition around 31 °C for DDAB and 42 °C for DODAB bilayers in the lamellar phase.

By integrating the peaks in Figure 6, we can determine the enthalpy change (ΔH) associated with the phase transition. Table 1 shows the measured ΔH values of the gel-to-liquid crystalline transition for DDAB and DODAB bilayers, as well as other parameters obtained from the DSC thermograms.

This transition was confirmed by XRD experiments that showed the absence of characteristic Bragg peak of the gel phase above the T_m (see Figure S2, Supplementary

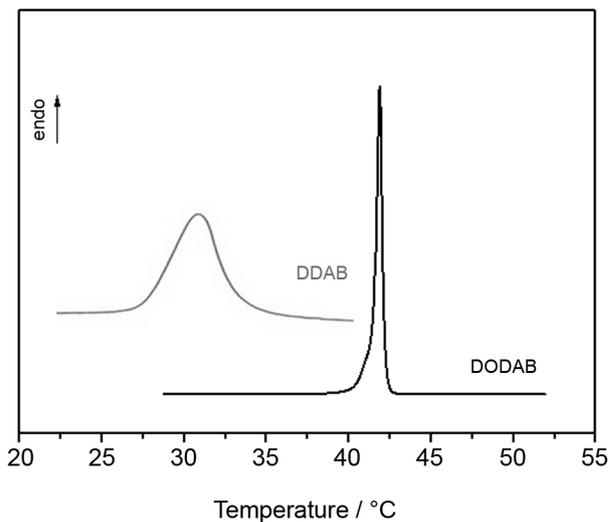


Figure 6. DSC thermograms for 50.0 wt.% DDAB and DODAB samples.

Table 1. Transition temperatures (T_m), peak width ($\Delta T_{1/2}$) and transition enthalpies (ΔH), with standard deviation in parenthesis, for DDAB and DODAB lamellae

Amphiphile	$T_m / ^\circ\text{C}$	$\Delta T_{1/2} / ^\circ\text{C}$	$\Delta H / (\text{kJ mol}^{-1})$
DDAB ^a	31	5	5 (1)
DODAB ^b	42	0.6	45 (1)

^aDidodecyldimethylammonium bromide (DDAB);

^bdioctadecyldimethylammonium bromide (DODAB).

Information section) for DDAB and DODAB bilayers. For DODAB bilayers, the gel to fluid phase transition was represented by a narrow peak, which is characteristic of a very cooperative process, where the amphiphilic molecules present strong interactions and do not melt independently.^{10,35} This is in full agreement with previous results reported elsewhere for DDAB and DODAB vesicles, as well as for other amphiphiles.^{34,35} As a comparison, the DSC thermograms of the gel-to-fluid phase transition in DDAB and DODAB vesicles, showing the narrow peak transition are presented in Supplementary Information section (Figure S3), accompanied by the thermal parameters obtained from the DSC data (Table S1). The vesicles used in this study were prepared according to Feitosa *et al.*³⁶

In the case of DDAB bilayers, the characteristic phase transition was not so cooperative, as can be noted by the broader peak transition. This can be explained by the fact that if a mixture of gel and fluid phases is composing the bilayers, the transformation from this mixture to only the fluid phase must involve a region with two coexisting phases. Under these circumstances, heat capacity no longer shows a distinct narrow peak at a given temperature; instead, a melting process takes place over a temperature range. Besides presenting coexistence of both lamellar phases in a broader temperature range, the DDAB bilayers

in lamellar form also presented higher T_m values than that observed in the vesicles of the same surfactant. This could indicate the formation of a stabilized gel phase, as will be discussed below.

Structural properties of lamellar phases

The swelling of the lamellar phases formed by the self-assembly of DDAB and DODAB and the bilayers thickness were investigated. The swelling extent is represented as the increasing in repeat distance of the bilayers (d) calculated from the first scattering peak in SAXS curves using equation 1, as described above. Figure 7 shows the repeat distance of the bilayers formed by both of the surfactants against the inverse of volumetric fraction of amphiphile in the samples at 25 °C. Details on how the volumetric fraction of surfactants in the samples were calculated, based on the work of Montalvo *et al.*,³⁷ are described in the Supplementary Information section (Table S2).

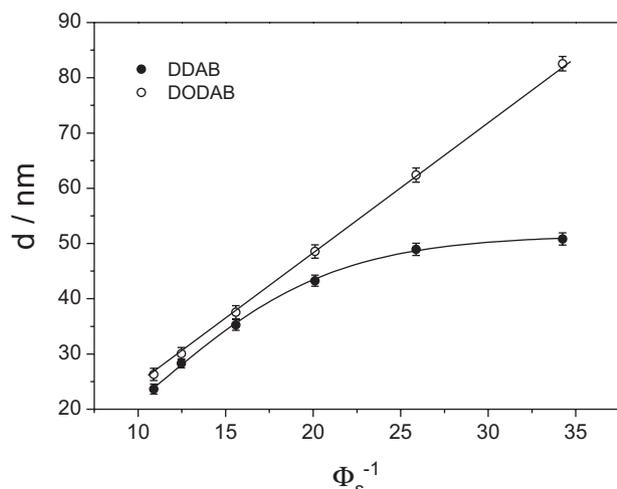


Figure 7. Swelling curves at 25 °C for DDAB and DODAB lamellae: the repeat distance of bilayers (d) as a function of the inverse volumetric fraction (Φ_s^{-1}) of surfactants in water.

This swelling behavior of DDAB lamellae upon dilution is usually expected during the swelling of a lyotropic lamellar phase. Once the maximum swelling is reached, water can no longer be inserted into the interlamellar space, the repeat distance remains constant and the excess of water is expelled, leading to phase separation.³⁸ This observation can be noted in the diluted DDAB samples, where it is possible to note the presence of a liquid crystalline birefringent phase coexisting with isotropic solution (Figure S1b).

Oppositely, in the same concentration range, the DODAB lamellae swell continuously upon addition of water. This behavior was already described for the lamellar phases formed by some dialkyldimethylammonium

bromides but the molecular origin of this phenomenon has not been well studied.²⁶ The non-ideal swelling can be observed in a number of situations and is strongly correlated with the (de)stabilizing forces acting on the bilayers.

The novelty of the present investigation comes from the application of SAXS analyses to derive structural information of these lamellae. The scattering vector, q , is correlated to the bilayers thickness, d_{HC} , and to the volumetric fraction of surfactant in the samples, Φ_s , according to the equation 2:

$$q = \frac{2\pi}{d_{HC}} \Phi_s \quad (2)$$

By replacing equation 2 into equation 1, it is possible to correlate the repeat distance and the bilayer thickness (equation 3):

$$d = \frac{d_{HC}}{\Phi_s} \quad (3)$$

This approximation, which has been employed in different lamellar systems,^{26,32,38} is valid taking into account a system composed by two phases, water and oil, where the aqueous phase represents the water molecules among the bilayers and the polar headgroups of the surfactants and oil represents only the carbon chains in the bilayers. As implied above, this relationship only holds for a pure lamellar system and formation of a pure aqueous phase due to saturation of the lamellar phase with water leads to formation of a macroscopic aqueous phase and to the deviation observed for DDAB at higher dilution (as seen in Figure 7).

Assuming that, by the extrapolation of swelling curves to 100.0% surfactant concentration it is possible to obtain the bilayer thickness. DDAB bilayers were found to be 2.30 ± 0.04 nm in thickness, in accordance with previous reports in literature.^{13,39} For DODAB bilayers, the thickness found was 2.41 ± 0.06 nm, thinner than estimated by molecular dynamics.⁸

One could argue that this comparison is not valid, because at 25 °C, DDAB bilayers exist as a mixture of fluid and gel phases, while DODAB bilayers are only in the gel phase in this temperature. Some measurements were made in two different temperatures, 18 °C and 45 °C (safely below and above the T_m for both of the systems). It was surely expected that these parameters vary with temperature (for example, an increase in the bilayer thickness) but the same trend was observed, that is, a higher swelling and thinner bilayers for DODAB amphiphile (Figures S4a and S4b in the Supplementary Information section).

Based on references 40 and 41 we estimated the cross-sectional area *per* surfactant (a_s) in the gel and fluid bilayers formed by DDAB and DODAB amphiphiles in aqueous solution. Details on how this parameter was calculated are presented in the Supplementary Information section. These data are presented in Table 2 and are in accordance with previous reports in literature for bilayers formed by these amphiphiles and other homologue-chain lipids.^{13,39,42}

Table 2. Area *per* surfactant (a_s) estimated for DDAB and DODAB bilayers in different states

Surfactant	DDAB ^a		DODAB ^b	
	Gel	Fluid	Gel	Fluid
$a_s^c / \text{\AA}^2$	65	68	89	94

^aDidodecyldimethylammonium bromide (DDAB); ^bdioctadecyldimethylammonium bromide (DODAB); ^c a_s : area *per* surfactant.

As expected, the area occupied by the surfactants in the bilayers are greater in the fluid phase, once in this state, the molecular mobility predominates, unlike the gel phase, in which the surfactant molecules are more densely packed forming rigid bilayers.³⁴ Although both surfactants possess the same polar headgroup (a quaternary ammonium cation), the cross-sectional area (which takes into account the hydrophilic group) occupied by the amphiphiles is greater in the DODAB bilayers. This intriguing fact will be discussed below.

Discussion

The phase behavior of DDAB in water has been studied earlier as a function of concentration of the amphiphile in solution.^{13-15,31} In these previous studies, a contrasting difference with the present results was the attribution of the coexistence, at room temperature, of a swollen and another collapsed lamellar fluid phase (also denoted as L_{α}' and L_{α}'' phases). In this study, as already described above, we have found, at 25 °C, the swollen L_{α} phase coexisting with the L_{β} phase (this last confirmed by XRD data, in addition to SAXS results), instead of a collapsed lamellar fluid phase.

In most of the cases involving lamellar phases based on surfactants in water, the coexistence of two fluid phases, L_{α}' and L_{α}'' , are common to be found due to specific interactions between headgroups, which affect the short-range repulsive force that controls the swelling of the concentrated lamellar phase.¹⁸ The coexistence of gel and fluid lamellar phases have been found in mixtures of different lipids or surfactants.^{33,42} This is attributed to the formation of domains of each amphiphile along the bilayers, each one fusing on their characteristic T_m .

The reason for this discrepancy is not clear at this stage. Apparently, purity of amphiphiles used in both studies is similar and, in the present study, significant care was taken to ensure sample equilibration, by repeatedly analyzing samples at intervals between 1 and 12 months, with the same SAXS and DSC results. Equilibration could be an issue for highly viscous samples and systems out-of-equilibrium may lead to erroneous results.

Although these previous studies have characterized the lamellar phases formed by DDAB in water by X-ray techniques, very few calorimetric studies were made to accurately determine the transition temperature of the gel to fluid phase.

The main difference is in the temperature ascribed to T_m which was used as ca. 16 °C elsewhere¹⁰ and was located around 31 °C from the present DSC results. A T_m of 16 °C was found in vesicles³⁶ but not for the lamellar phases formed by DDAB, as described above. In addition to the gel and fluid phases, the bilayers may present a subgel or a coagel phase depending on the sample history, concentration and presence of additives.⁴¹⁻⁴⁵

This coagel phase has been observed for concentrated amphiphilic samples, corresponding to poorly hydrated gel bilayers, and results from a loss of interlamellar water, accompanied by a partial dehydration of the polar headgroups and a further ordering of the alkyl chains.¹¹ This coagel phase presents a characteristic endothermic peak associated with the gel-to-fluid phase transition at temperatures higher than the typical T_m .⁴⁶ The increase in the phase transition temperature suggests that the higher concentration of bromide counterions, arising from less water available, may stabilize the gel phase by shielding the polar ammonium headgroups.

Indeed, the adsorption of negative species onto positively charged bilayers induces an increase in the transition temperature,^{44,46} as observed for DDAB lamellae. From this explanation, we propose that DDAB bilayers are less charged than DODAB bilayers, due to the adsorption of bromide counterions in the positive surface of membranes. This is an important point that will be discussed further later.

Despite the thermal phase behavior of DODAB vesicles being extensively investigated,^{34,35,43-46} the liquid-crystalline phases formed at higher concentrations of the surfactant have been less well studied. Schulz and co-workers¹² presented a phase diagram for the DODAB/water system, also indicating the presence of two different lamellar phases, as described here. However, an accurate investigation of the structural properties of the lamellar phases, and their correct identification were not achieved in their study.

Laughlin *et al.*⁴⁷ presented a phase diagram for DODAC (dioctadecyldimethylammonium chloride) in water that

indicates the presence of two lamellar phases, a fluid one coexisting with isotropic water and another ordered gel phase, similar to what has been attributed in the present report. It is important to reemphasize that the phase transition temperature for DODAB bilayers in lamellar phases was the same as that described for its vesicles, agreeing with our previous hypothesis that the DODAB bilayers are less shielded by the counterions.

For DODAB, the bilayer thickness was 2.41 ± 0.06 nm, much smaller than the length predicted for one extended octadecyl chain, around 2.42 nm, according to Tanford's equation.⁴⁸ Three possibilities to explain this difference may be taken into account: (i) the alkyl chains are not fully extended in the gel phase; (ii) the alkyl chains are tilted or (iii) the chains are interdigitated.

The first possibility, that the alkyl chains are not fully extended, was supported by the fact that DODAB alkyl chains can be stretched only in the subgel phase.³⁴ This phase is obtained by cooling the bilayers below 15 °C, resulting in a further stretching, increase in packing of the alkyl chains and presents a characteristic pre-transition peak at 36 °C in the thermograms.^{34,44}

Although taking into account that the alkyl chains of DODAB bilayers are not fully extended in the gel phase, this still does not explain the thin bilayer obtained in this study, because in the literature thicker bilayers have been already described for subgel phase of DODAB.^{34,46} It was also hypothesized that the alkyl chains could be tilted in the bilayers but the tilting angle of the alkyl chains would have to be too shallow (around 7 degrees) to account to such a small thickness, so we discarded this possibility.

Therefore, these results suggest that the alkyl chains of DODAB molecules should be interdigitated in the bilayers. When interdigitation occurs, the hydrocarbon chains of the amphiphilic molecules in a monolayer constituting a bilayer extend beyond the region of the bilayer midplane and, as a result, interpenetrate into the other opposing monolayer. The resulting structure is characterized by a small membrane thickness, if compared with a common membrane, where the hydrophobic thickness is typically smaller than, but not too far from, twice the length of the hydrophobic tails of the surfactant.^{49,50}

As discussed above, we hypothesized that DODAB bilayers are more positively charged than those formed by DDAB molecules. To our knowledge, information about the degree of dissociation for the dialkyldimethylammonium surfactants and its aggregates in water are still lacking. Nevertheless, earlier reports revealed that there is a higher surface charge density for DODAB bilayers if compared with those formed by DDAB.^{27,51,52} This means that there should be a lower amount of bromide counterions bound to

DODAB ammonium headgroups in the aggregates.

The larger area *per* headgroup displayed by DODAB in the bilayers (Table 2) also indicated that, in this case, the ammonium polar headgroups are less counterbalanced by the bromide counterions, possessing a higher effective area due to the net positive charge. This could cause greater electrostatic repulsion, increasing average distance among the headgroups. Although the ion-membrane interaction is still under investigation and discussion in different systems due to the complexity of overall balance forces involved, the present data are encouraging for future work on this topic.

Therefore, at low temperatures, because close contacts between the positively charged ammonium headgroups are unfavorable, the alkyl chains of DODAB are forced to interdigitate in order to condense the tail groups while keeping the neighboring headgroups apart.^{49,50} The phenomenon of interdigitation, caused by electrostatic repulsion, could explain the thinner bilayers formed by the eighteen-each-chain carbon atoms surfactant, closer to the expected length for only one stretched octadecyl chain. Because DDAB bilayers possess headgroups more shielded by the bromide counterions, repulsion is lower and interdigitation of tails is less necessary, oppositely to DODAB.

Characterization of samples formulated above the transition temperature to the fluid phase confirmed this hypothesis (Figure 8) as can be noted by the increase of bilayers thickness. This observation can be explained by the fact that, at higher temperatures (above the T_m), thermal energy imparts greater mobility to the surfactant molecules, making the carbon chains of the surfactant occupy a greater lateral space.

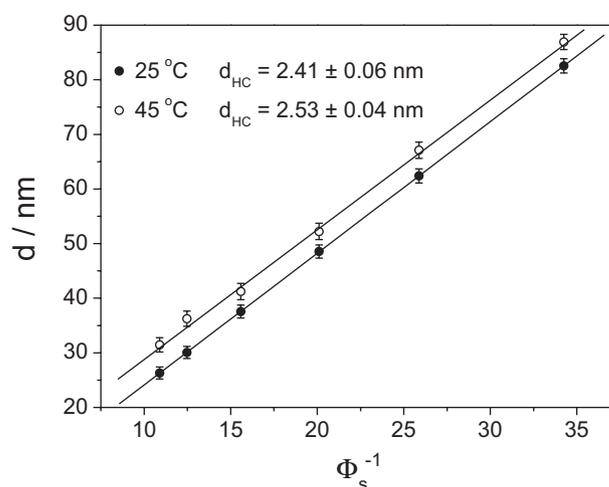


Figure 8. Repeat distance of bilayers (d) against the inverse of volumetric fraction of surfactant (Φ_s^{-1}) DODAB at 25 and 45 °C; d_{HC} represents the bilayer thickness.

This increment in the molecular mobility can be noted by the increase in the area *per* surfactant in the bilayers

in the fluid state (data presented in Table 2). This greater space may be sufficient to maintain the charged polar headgroups apart, reducing the repulsion among them.⁴⁹ If the repulsive forces between the charged ammonium headgroups decrease, interdigitation is less pronounced and there should be an increase in the bilayer thickness.

Stability of lamellar phases can be described as a balance between repulsive and attractive forces involving the bilayers. The total interaction energy *per* unit area can be approximated to the sum of the electrostatic (repulsive), Van der Waals (attractive) and hydration (attractive and repulsive) forces.^{22,24} A lamellar phase cannot be stable if there is no repulsive force acting between two bilayers because the coalescence interaction is attractive. The electrostatic and hydration (short-ranged) forces between charged bilayers are in most cases responsible for the repulsion.⁵³

When equilibrium between the attractive and repulsive forces is reached, the repeat distance among the bilayers reaches its maximum value upon dilution. This is what happens with DDAB bilayers. A greater swelling is, however, possible if ionized groups are incorporated into the bilayers. Due to repulsion forces between these electric double-layers, the swelling capacity of the lamellar system is greatly increased. It corresponds to what has been experimentally observed for the DODAB bilayers in the lamellar phase.

The characterization of samples formulated with added NaCl (1.0 wt.%) confirmed the hypothesis of the influence of the electrostatic repulsion in the swelling behavior of DODAB lamellae, as well as in the bilayer thickness, as shown in Figure 9. As described elsewhere,^{53,54} the chloride counterions shield some of the cationic surfactant headgroups, decreasing the repulsion between them. Therefore, it is less necessary to the tails assume the interdigitated form.

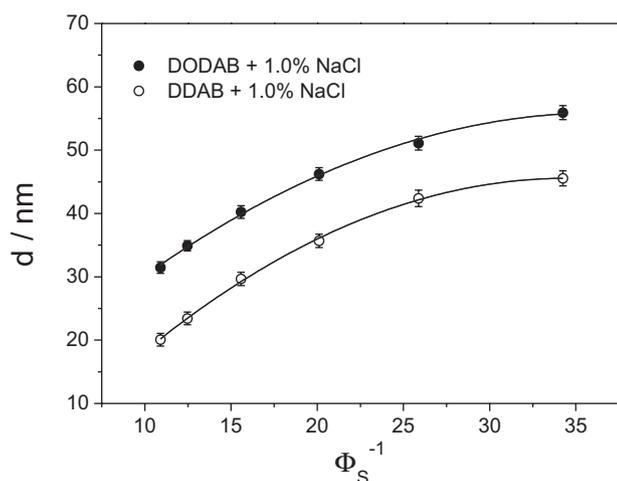


Figure 9. Repeat distance of bilayers (d) against the inverse of volumetric fraction of surfactants (Φ_S^{-1}) DDAB and DODAB in the presence of salt (1.0% of NaCl). All data were obtained at room temperature.

This fact can be seen by the increase in the bilayer thickness formed by DODAB in the presence of salt (2.55 ± 0.05 nm against 2.41 ± 0.06 nm in pure surfactant/water system). In addition, in the presence of salt, the repulsion among the bilayers also decreases, hence the lower swelling observed from the smaller repeat distances.

Conclusions

Both of the investigated surfactants self-assemble, in water, into lamellar liquid crystalline phases, whose structural properties, such as swelling and bilayers thickness, can vary with the alkyl chain length and can be strongly influenced by electrostatic forces acting at the bilayers. For DODAB lamellae, as a result of higher degree of counterion dissociation, there is a considerable repulsion between headgroups, forcing the tails to interdigitate and, thus, keeping polar groups apart, resulting in thinner bilayers. The electrostatic repulsion among the bilayers, contributes to larger interlayer spaces and, as a result, a considerable swelling. The increase in the phase transition temperature from gel to the fluid phase in the DDAB bilayers suggests that the bromide counterions, from the surfactant dissociation, may stabilize the gel phase by shielding the polar ammonium headgroups.

The knowledge of structural bilayer behavior is fundamental to design lamellar materials with improved properties and functions, due the enormous research in the core of various applications of DDAB and DODAB lamellae. Thus, the information obtained in the present study provides grounds for a better understanding of the ion-membrane interactions, as well as the overall balance of electrostatic forces acting on the bilayers, producing data that are encouraging for future work, for example, on the elucidation of the dynamic processes involved in the biomembranes formation and their functioning, topic in which the double-tailed surfactants possess large applications.

Supplementary Information

Supplementary data are available free of charge at <http://jbcs.sbq.org.br> as PDF file.

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References

- Laughlin, R. G.; *J. Am. Oil Chem. Soc.* **1990**, *67*, 705.
- Zana, R.; *Langmuir* **1996**, *12*, 1208.
- Nagarajan, R.; Ruckenstein, E.; *Langmuir* **1991**, *7*, 2934.
- Wennerström, H.; Lindman, B.; *Phys. Rep.* **1976**, *52*, 1.
- von Rybinski, W.; Hill, K.; *Angew. Chem. Int. Ed.* **1998**, *37*, 1328.
- Nagarajan, R.; *Langmuir* **2002**, *18*, 31.
- König, B.; Dietrich, U.; Klose, G.; *Langmuir* **1997**, *13*, 525.
- Jamróz, D.; Kepczynski, M.; Nowakowska, M.; *Langmuir* **2010**, *26*, 15076.
- Kunitake, T.; Okahata, Y.; *J. Am. Chem. Soc.* **1977**, *99*, 3860.
- Brito, O. R.; Marques, E. F.; *Chem. Phys. Lipids* **2005**, *137*, 18.
- Wu, F. G.; Wang, N. N.; Yu, Z. W.; *Langmuir* **2009**, *25*, 1339.
- Schulz, P. C.; Rodríguez, J. L.; Soltero-Martínez, F. A.; Puig, J. E.; Proverbio, Z. E.; *J. Therm. Anal. Calorim.* **1998**, *51*, 49.
- Dubois, M.; Zemb, T.; *Langmuir* **1991**, *7*, 1352.
- Caboi, F.; Monduzzi, M.; *Langmuir* **1996**, *12*, 3548.
- Zemb, T.; Belloni, M.; Dubois, M.; Marcelja, S.; *Prog. Colloid Polym. Sci.* **1992**, *89*, 33.
- Marques, E. F.; Regev, O.; Khan, A.; Lindman, B.; *Adv. Colloid Interface Sci.* **2003**, *100*, 83.
- Dubois, M.; Zemb, T.; Fuller, N.; Rand, R. P.; Parsegian, V. A.; *J. Chem. Phys.* **1998**, *108*, 7855.
- Silva, B. F. B.; Marques, E. F.; Olsson, U.; Pons, R.; *Langmuir* **2010**, *26*, 3058.
- Savariar, E. N.; Ghosh, S.; Gonzalez, D. C.; *J. Am. Chem. Soc.* **2008**, *130*, 5416.
- Samad, A.; Sultana, Y.; Aqil, M.; *Curr. Drug Delivery* **2007**, *4*, 297.
- Kranenburg, M.; Vlaar, M.; Smit, B.; *Biophys. J.* **2004**, *87*, 1596.
- Nagle, J. F.; Tristram-Nagle, S.; *Biochim. Biophys. Acta* **2000**, *1469*, 159.
- Takeda, K.; Okuno, H.; Hata, T.; Nishimoto, M.; Matsuki, H.; Kaneshina, S.; *Colloids Surf., B* **2009**, *72*, 135.
- Kranenburg, M.; Smit, B.; *J. Phys. Chem. B* **2005**, *109*, 6553.
- Winter, I.; Pabst, G.; Rappolt, M.; *Chem. Phys. Lipids* **2001**, *112*, 137.
- Haas, S.; Hoffmann, H.; Thuning, C.; Hoinkis, E.; *Colloid Polym. Sci.* **1999**, *9*, 856.
- Ryhanen, S. P.; Alakoskela, J. M. I.; Kinnunen, P. K. L.; *Langmuir* **2005**, *21*, 5707.
- Fruhwrith, T.; Fritz, G.; Freiverger, N.; Glatter, O.; *J. Appl. Crystallogr.* **2004**, *37*, 703.
- Fernandez, R. B.; Riske, K. A.; Amaral, L. Q.; Itri, R.; Lamy, M. T.; *Biochim. Biophys. Acta* **2008**, *1778*, 907.
- Percebom, A. M.; Piculell, L.; Loh, W.; *J. Phys. Chem. B* **2012**, *116*, 2376.
- Fontell, K.; Ceglie, A.; Lindman, B.; Ninham, B. W.; *Acta Chem. Scand.* **1986**, *40*, 247.
- Agzenai, Y.; Lindman, B.; Alfredsson, V.; Topgaard, D.; Renamayar, C. S.; Pacios, E. S.; *J. Phys. Chem. B* **2014**, *118*, 1159.
- Seddon, J. M.; Cevc, G.; Marsh, D.; *Biochemistry* **1983**, *22*, 1280.
- Saveyn, P.; Van der Meeren, P.; Zackrisson, M.; Narayanan, T.; Olsson, U.; *Soft Matter* **2009**, *5*, 1735.
- Feitosa, E.; Adati, R. D.; Hansson, P.; Malmsten, M.; *PLoS One* **2012**, *7*, 1.
- Feitosa, E.; Adati, R. D.; Alves, F. R.; *Colloids Surf., A* **2015**, *480*, 253.
- Montalvo, G.; Khan, A.; *Langmuir* **2002**, *18*, 8330.
- Gabriel, P. J.-C.; Franck, C.; Lemaire, B. J.; Desvaux, H.; Davidson, P.; Batail, P.; *Nature* **2001**, *413*, 504.
- Grillo, I.; Penfold, J.; Tucker, I.; Cousin, F.; *Langmuir* **2009**, *25*, 3932.
- White, S. H.; King, I. G.; *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 6532.
- Bernardes, J. S.; Norrman, J.; Piculell, L.; Loh, W.; *J. Phys. Chem. B* **2006**, *110*, 23433.
- Kucerka, N.; Tristram-Nagle, S.; Nagle, J. F.; *J. Membr. Biol.* **2005**, *208*, 193.
- Cocquyt, J.; Olsson, U.; Olofsson, G.; Van der Meeren, P.; *Langmuir* **2004**, *20*, 3906.
- Cocquyt, J.; Olsson, U.; Olofsson, G.; Van der Meeren, P.; *Colloid Polym. Sci.* **2005**, *283*, 1376.
- Benatti, C. R.; Epan, R. M.; Lamy, M. T.; *Chem. Phys. Lipids* **2007**, *147*, 27.
- Rozenfeld, J. H. K.; Duarte, L. E.; Barbosa, L. R. S.; Lamy, M. T.; *Phys. Chem. Chem. Phys.* **2015**, *17*, 7498.
- Laughlin, R. G.; Munyon, R. L.; Burns, J. L.; Coffindaffer, T. W.; Talmon, Y.; *J. Phys. Chem.* **1992**, *96*, 374.
- Tanford, C.; *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd ed.; John Wiley: New York, 1980.
- Rozenfeld, J. H. K.; Duarte, E. L.; Oliveira, T. R.; Lonez, C.; Ruyschaert, J. M.; Lamy, M. T.; *Langmuir* **2013**, *29*, 11102.
- Oliveira, T. R.; Duarte, E. L.; Lamy, M. T.; Vandrenbanden, M.; Ruyschaert, J. M.; Lonez, C.; *Langmuir* **2012**, *28*, 4640.
- Lukac, S.; *J. Phys. Chem.* **1983**, *87*, 5045.
- Cavalli, A.; Dynarowicz-Łątka, P.; Oliveira, O. N.; Feitosa, E.; *Chem. Phys. Lett.* **2001**, *20*, 89.
- Tristram-Nagle, S.; Harries, D.; Kucerka, N.; Nagle, J. F.; Parsegian, V. A.; *J. Lipid Res.* **2006**, *47*, 302.
- Petrache, H. I.; Zemb, T.; Belloni, L.; Parsegian, V. A.; *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 7982.

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