

Flexibility of Dilauroyl-Phosphatidyl-Nucleoside Wormlike Micelles in Aqueous Solutions

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We report an investigation of the structural properties of dilauroyl-phosphatidyl-uridine wormlike micelles by the combined use of static light scattering and small angle neutron scattering techniques. The q -range covered in the reciprocal space is large enough for a complete structural characterization of micellar aggregates, which can be deduced in terms of contour length, persistence length, and cross sectional radius. In particular, the structural parameters have been extracted through a nonlinear least-squares fitting of the experimental spectra by parametrized wormlike chain scattering functions, obtained with Monte Carlo methods.^{1,2} This procedure includes excluded volume interactions, micellar polydispersity, and intermicellar effects. The main result is an accurate estimate of micellar flexibility in terms of persistence length (230 Å). We also show that the persistence length for nucleoside derivative surfactants is not a simple function of the surfactant tail length. A modest change in the hindrance and in the chemical nature of the surfactant polar head, as the exchange of uridine with adenine, produces a significant change in the stiffness of the wormlike micelles. The results are compared to most wormlike systems currently investigated and the biologically relevant implications due to the nucleosidic nature of the phospholipid derivative polar head are discussed.

Introduction

We have recently investigated the structural properties of supramolecular self-assemblies formed from phospholipids functionalized with a nucleosidic headgroup.^{3–7} This work is motivated by the interest in the reproduction of complex functions typical of living systems, i.e., molecular recognition with a pattern mimicking nucleic acids using self-organized systems. The formation of supramolecular self-assemblies is cooperative and leads to variously shaped aggregates, like globular assemblies, rigid or flexible cylindrical micelles, or bilayered structures, as a result of the packing preferences of the constituting surfactant molecules. Information at a molecular level (i.e., the packing parameter) is amplified in the supramolecular arrangement and controls the phase behavior.

In nucleic acids the covalent sugar–phosphate backbone provides the cooperative effect that leads to the coupling of two polynucleotide strands, and the stacking interactions between neighboring bases display a prominent role in the stabilization of the duplex. In our case, base organization in a supramolecular array is favored by self-aggregation, and the extent of interaction between bases is modulated by the interfacial curvature of the surfactant film. We have previously reported the investigation of globular micelles⁴ and liposomes,³ where it was shown that the interaction between nucleic bases anchored onto the aggregate hydrophilic shell occurs through a combination of hydrogen bonding and base stacking, reproducing the molecular recognition pattern typical of nucleic acids.

More recently our attention has been devoted to the study of semiflexible wormlike micelles also called polymerlike micelles, formed by dilauroyl-phosphatidyl-nucleosides, and in particular to the uridine derivative (henceforth called DLPU).

The major morphologic characteristic of polymer-like micelles is the different length between the cross section diameter and the “long” axis. The former is correlated to the dimension of the amphiphilic molecule constituting the aggregate whereas the latter can, under proper experimental conditions, grow up to several micrometers. The theoretical approach to wormlike micellar solutions benefits from concepts adapted from polymer physics. However, important differences should be properly considered. From the static point of view, the molecular weight is ruled by thermodynamics and is not quenched, i.e., depends for most soft materials on the amphiphile volume fraction, solution composition, and/or ionic strength. Micelles are also transient structures that undergo breaking and recombination processes on a time scale that depends on the system and on the chemical conditions.⁸ Similarly to polymers, wormlike micellar solutions, above a crossover concentration c^* , enter in a semidilute regime, where cylindrical micelles entangle and many of their properties are similar to those of polymer solutions in the same regime.^{9,10}

In 0.1 M sodium phosphate solutions (pH 7.5), flexible cylindrical aggregates can already be detected at DLPU volume fractions as low as 0.06%^{11,12} (1.0×10^{-3} mol/L), as determined from Cryo-TEM inspection. We have recently reported a detailed structural investigation of these micelles as a function of lipid concentration, evidencing the concentration regimes characteristic of wormlike systems. Using a combination of scattering methods (mainly SANS, QELS, and SLS), it has been possible to highlight the unidirectional growth of the average molecular weight with increasing surfactant volume fraction ϕ , due to mass-action effects¹³ and, in agreement with the predictions of a mean-field approach, a broad size distribution of contour lengths.¹⁴

Both the cross section and the contour length of the aggregates are determined by information coded in the molecular subunit.

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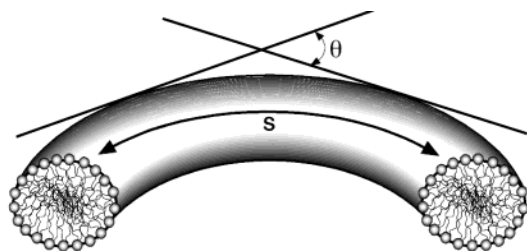


Figure 1. Schematic representation of a cylindrical wormlike micelle over a contour length s . The definition of persistence length follows from the decay of orientational correlation along the “long axis”, $\langle \cos \theta(s) \rangle = \exp\{-s/l_p\}$.

The first parameter is connected to the chain length of the amphiphile and is independent of the surfactant concentration. Contour length, instead, is determined by the concentration and by the extent of the free energy gain, ΔE_{exc} , associated with the surfactant packing along the cylindrical body of the aggregate, according to

$$\langle L \rangle \propto \sqrt{\left(\phi \exp\left(\frac{2\Delta E_{\text{exc}}}{k_B T}\right) \right)} \quad (1)$$

This quantity is sensitive to the variation of a control parameter (surfactant volume fraction, ionic strength, temperature and so on). However, the flexibility of aggregates, a fundamental structural parameter, is missing in this approach.

Polymer physics language and concepts can again be invoked to define a characteristic length scale correlated with macromolecular flexibility, i.e., the persistence length, defined as the characteristic length along the axial contour over which directional correlation decays. The persistence length l_p is related, for unidimensional objects, to the bending modulus k of the system through $l_p = k/k_B T$ (Figure 1).¹⁵

For synthetic and natural polymers this number can vary by orders of magnitude, ranging from 10 to 20 Å for vinyl polymers to 500 Å for highly charged electrolytes such as DNA.¹⁶ Micelles are instead generally characterized by relatively high intrinsic l_p 's because they have large cross sections, constituted by the hydrophobic core. Values ranging from 100 to 200 Å are common for nonionic amphiphiles, whereas for ionic surfactants a l_p around 300–600 Å can be reached. This wide spread of values suggests that micellar flexibility is strongly dependent on the surfactant monomer structure and that for ionic surfactants it is highly influenced by the ionic strength of the solution and by the counterion nature.

Despite the wealth of experimental data, mainly obtained through scattering techniques, a reliable evaluation of micellar flexibility, and therefore an assessment of its dependence on the chemical structure of molecular subunits of the aggregate and on control parameters, has been elusive until a significant breakthrough by Pedersen and Schurtenberger.^{1,2} These authors have evaluated through Monte Carlo simulations the scattering function for semiflexible wormlike chains needed to fit SANS data over an extended range of scattering vectors, including polydispersity and excluded volume correlations. This method has been first applied to successfully infer contour and persistence lengths of reverse lecithin micelles in hydrocarbons¹⁷ and subsequently used for the investigation of both nonionic and mixed nonionic/ionic direct micellar systems.^{18,19} More recently it has been applied by Magid and co-workers to scattering data of cationic (CTAX and CpyX)²⁰ and anionic (SDS) micellar systems.^{21,22}

The present paper extends the previous report by us on the structural characterization of wormlike micelles¹¹ and provides a deeper insight on micellar flexibility. The paper is organized in the following way: first scattering data are presented and discussed in a model-free fashion to emphasize general structural properties of micellar solutions; then attention is devoted to micellar flexibility and on its evaluation. A comparison with data available in the literature obtained with the same method is then reported, mainly to stress the importance of predictability of micellar flexibility from the chemical structure of the monomer. This is particularly important to devise structure–performance relationships of surfactant solutions. Finally, biologically relevant implications based on the nucleoside nature of the polar head are discussed.

Experimental Sections

Materials. 1,2-Dilauroyl-*sn*-glycero-3-phosphocholine (DLPC) was purchased from Avanti Polar Lipids (Alabaster, AL) and its purity checked by TLC. The lecithin was used as received because no oxidation or lyso products could be detected. Uridine, adenine, HCl, CHCl₃, MeOH, and NH₃ (33% aqueous solution) used in the synthesis and NaH₂PO₄ (>99%) and Na₂HPO₄ (>99%), were purchased from Fluka (Switzerland). Phospholipase D from *Streptomyces sp* AA586 was a generous gift from Asahi Chemical Industry Co., Ltd (Tokyo, Japan). Deuterium oxide (>99.5%) for SANS measurements was obtained by Euriso-Top, (Saclay, Gif sur Yvette, France).

Synthesis of Dilauroyl-Phosphatidyl-Uridine and -Adenosine. DLPU and DLPA were synthesized starting from the corresponding phosphatidylcholine in a two-phase system,²³ according to a modification of the method proposed by Shuto and co-workers,^{24,25} and obtained as an ammonium salt. Molecular structures of the two derivatives are reported in Figure 2.

Sample Preparation for SANS. The lyophilized powder was dissolved in 0.1 M deuterated phosphate buffer to minimize the incoherent background from hydrogen and obtain a high scattering contrast. In the present study only data pertaining to the dilute regime (until a surfactant volume fraction of 0.6%) have been taken into account.

Static Light Scattering. Light scattering measurements were performed on a Brookhaven Instrument apparatus equipped with a BI9000AT correlator and a BI200SM goniometer. The signal was detected by an EMI 9863B/350 photomultiplier. The light source was the second harmonic of a Nd:YAG diode laser ($\lambda = 532$ nm), Coherent Innova, linearly polarized in the vertical direction. Measurements were performed at 25 °C. Approximately 1 mL of sample solution was transferred into a cylindrical Hellma scattering cell that was sealed and centrifuged for about 4 h (the most viscous samples required overnight centrifugation) at about 5000g to remove dust particles from scattering volume. SLS were performed at 60 different angles in the range $15^\circ \leq \theta \leq 150^\circ$, corresponding to a scattering vector range $7.0 \times 10^{-4} \text{ Å}^{-1} < q = 4\pi n/\lambda \sin(\theta/2) < 3 \times 10^{-3} \text{ Å}^{-1}$, with the refractive index of the scattering medium $n = 1.33$ and 5320 Å wavelength. Data have been converted into absolute scattering intensity $\Delta R(q)$ (i.e., “excess Rayleigh ratios”) using toluene as reference standard, according to

$$\Delta R(q) = \frac{\langle \Delta I(q) \rangle}{\langle I_{\text{ref}}(q) \rangle} R_{\text{ref}}(q) \left(\frac{n}{n_{\text{ref}}} \right)^2$$

where $\Delta I(q)$ and $\langle I_{\text{ref}}(q) \rangle$ are the excess scattering intensity of the solution and the scattering intensity of the reference solvent

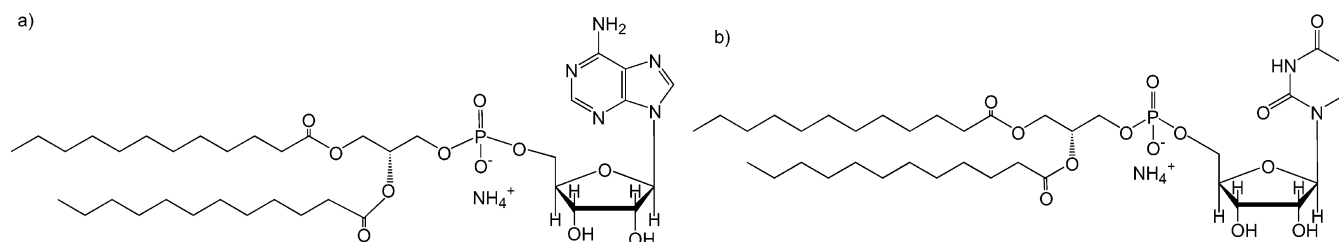


Figure 2. Schematic drawing of the chemical structures of dilauroyl-phosphatidyl-adenosine (a) and dilauroyl-phosphatidyl-uridine (b).

toluene, $R_{\text{ref}}(q) = 2.77 \times 10^{-5} \text{ cm}^{-1}$ is the Rayleigh ratio of toluene at 5320 \AA , and n and n_{ref} are the refractive indexes of the solution and the reference solvent, respectively.

Small Angle Neutron Scattering. Small angle neutron scattering experiments were performed at the spectrometer V4 (Bensch-Hahn Meitner Institut-Berlin). Three different configurations (i.e., sample–detector distances: 1, 4, and 16 m) allowed us to cover a range of wave vectors q ($4\pi/\lambda \sin(\theta/2)$) from 3.8×10^{-3} to 0.35 \AA^{-1} with 6.1 \AA neutron wavelength and $\Delta\lambda/\lambda < 10\%$. Samples were contained in a flat Hellma quartz cell of 1 mm path length. Scattered neutrons were detected by a two-dimensional position sensitive detector with 4096 active elements. The measured intensities were corrected, cell by cell, for background scattering, transmission, and detector efficiency and calibrated for absolute intensity referring to scattering of water. Data have been reduced according to standard BENS procedures²⁶ for small angle isotropic scattering. All experiments have been performed at 25°C . The solvent, 0.1M phosphate buffer at pH = 7.5 (0.1M PBS), has been measured and then subtracted from all the samples.

The spectra reported in this work are the combination of light and neutron scattering experiments, covering a $7 \times 10^{-4} \leq q (\text{\AA}^{-1}) \leq 0.35 \text{ \AA}^{-1}$ range. SLS data have been rescaled by the ratio between SLS and SANS optical constants, and then superimposed to SANS data. No further correction factor was applied to experimental data. The optical constant of static light scattering experiments ($K = 4\pi^2 n^2 (dn/dc)^2 / (N_A \lambda_0^4)$, where (dn/dc) is the refractive index increment) has been determined considering that $dn/dc = 0.161 \text{ mL/g}$; SANS optical constant has been evaluated by calculating the contrast from known chemical composition and molar densities.

Data Analysis. Data analysis for DLPW wormlike micelles has been performed by nonlinear least-squares fittings of the scattering curves. Here we give a short account of the fitting procedure, following the lines drawn by Pedersen and Schurtenberger, and according to the approach outlined by Magid.²¹

Neglecting intermicellar interactions, the scattered intensity of a micellar solution can be written as

$$I(q, c) = KcM_w \langle P(q, c) \rangle + B \quad (2)$$

In the eq 2 B is the background, K is a collection of constants characteristic of the probing radiation, given for neutrons by $[v^2(\rho_m - \rho_s)^2]/N_a$, where the specific micellar volume v is expressed in cm^3/g (obtained by density measurements), the scattering length densities of micelles ρ_m and solvent ρ_s are expressed in cm^{-2} , and for light by $4\pi^2 n^2 (dn/dc)^2 / (N_A \lambda_0^4)$, where n is the solvent refractive index, dn/dc is the refractive index increment, and λ_0 is the wavelength of the incident light. The background term in (2) includes scattering from the solvent and incoherent background. $\langle P(q, c) \rangle$ is the form factor averaged over the micellar molecular weight distribution. M_w , the weight averaged molecular weight of the micelles, is

$$M_w = N_L \langle L_c \rangle PM \frac{(z + 2)}{(z + 1)} \quad (3)$$

where N_L is the weight average aggregation number per unit length, $\langle L_c \rangle$ is the number average contour length of the micelle, PM the molecular weight of the monomer, and z is the Schultz–Zimm polydispersity index.

The scattered intensity from wormlike micelles typically displays three spectral regions containing information about three lengths in direct space: the contour length L_c , the persistence length l_p , and the cross sectional radius r_{cs} . For long wormlike aggregates, the cross section is generally well separated in the q space from the contour length and the persistence length. The total form factor of wormlike micelles can be factorized as²⁷

$$\langle P(q, c) \rangle = P_{cs}(q, r_{cs}) \cdot \langle P_{wc}(q, L_c(c), l_p) \rangle \quad (4)$$

where $P_{cs}(q, r_{cs})$ is the contribution from the cross section content and $\langle P_{wc}(q, L_c(c), l_p) \rangle$ is the contribution from the semiflexible chains whose contour lengths, L_c 's, are dependent on surfactant concentration c .

The cross section form factor for locally cylindrical micelles is given by

$$P_{cs}(q, r_{cs}) = [2J_1(qr_{cs})/(qr_{cs})]^2 \quad (5)$$

where $J_1(x)$ is the first-order Bessel function. The wormlike chain form factor, P_{wc} , has been introduced through a parametrized expression, derived on the basis of a series of Monte Carlo simulations, containing a contribution that accounts for intrachain excluded volume interactions, as illustrated in the original papers by Pedersen and Schurtenberger.^{1,2}

Polydispersity in contour lengths has been included by means of a Schultz–Zimm distribution, which leads to an averaged $\langle P_{wc}(q, L_c(c), l_p) \rangle$,^{28,29} with the polydispersity fixed at $M_w/M_n = 2$, as predicted by the ladder theory³⁰

$$\langle P_{wc}(q, L_c(c), l_p) \rangle = \int N(L_c) L_c^2 P_{wc}(q, L_c, l_p) dL_c / \int N(L_c) L_c^2 dL_c \quad (6)$$

The overall fitting procedure consists of two steps: in the first one the variational parameters are $\langle L_c \rangle$, l_p , and r_{cs} (q -dependent), B (background in eq 2), and KcM_w ; the obtained $\langle L_c \rangle$ has, however, only a qualitative value, because the fitting procedure is restricted to the SANS q range. Moreover, intermicellar interactions being neglected, the resultant (apparent) persistence length shows an unphysical dependence on the surfactant concentration, as already noticed by Pedersen, Schurtenberger, and co-workers.^{17,18}

In the second scan the only adjustable parameter is $\langle L_c \rangle$, whereas the persistence length, previously determined through extrapolation to infinite dilution, is included as a constant parameter. In this step the fitting procedure has been extended to the all q range to infer a more realistic $\langle L_c \rangle$ value.

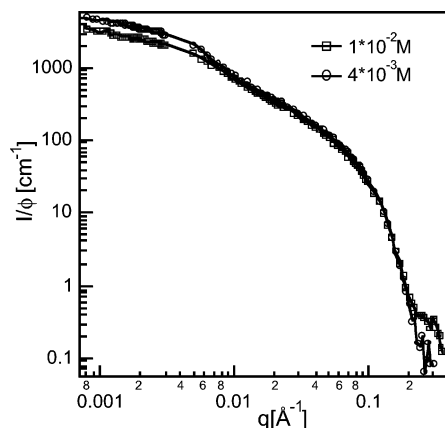


Figure 3. SANS spectra normalized for surfactant volume fraction from DLPU wormlike micellar solutions, merged in the low- q region with static light scattering data for $\phi = 0.0078$ and $\phi = 0.0031$.

Intermicellar interactions are now included in the scattering law that becomes

$$I(q,c) = KcM_w S_{\text{RPA}}(q,c) + B \quad (7)$$

where $S_{\text{RPA}}(q,c)$ is the random phase approximation (RPA) structure factor, frequently used in polymer physics. Monte Carlo simulations on wormlike micelles³¹ reveal that all structure factors obtained can be adequately reproduced by $S_{\text{RPA}}(q,c)$. The full structure factor in the random phase approximation can be expressed as

$$S_{\text{RPA}}(q,c) = \langle P(q,c) / \{1 + [S(0,c)^{-1} - 1]f_D(q^2 R_g^2)\} \rangle \quad (8)$$

where

$$f_D(x)^2 = 2(e^{-x} - 1 + x)/x^2 \quad (9)$$

$\langle P(q,c) \rangle$ is the single-chain scattering function for a wormlike micelle given by eqs 4 and 5 and f_D is the Debye function.

The osmotic compressibility, $S(0)^{-1}$, is related to the apparent molar mass M_{app} according to

$$M_{\text{app}} = M_w S(0) \quad (10)$$

Hence, we could include in our fits $S(0)$, by using the M_{app} value deduced from static light scattering data.

Results and Discussion

A combination of light scattering with small angle neutron scattering techniques results in an extended q -range and permits, in principle, an investigation over all the relevant length scales of wormlike aggregates.

Both static and dynamic light scattering data on DLPU micellar solutions, performed in an extended concentration range,¹¹ show a bell shaped trend for correlation lengths and apparent molar masses. This behavior indicates a crossover threshold to a semidilute regime, where micellar worms begin to entangle and the structural properties deduced by light scattering have a different meaning. This crossover is located around 0.6% lipid volume fraction for DLPU. The analysis reported in the present paper is, however, limited to a concentration range that is *below* the entanglement threshold, where individual wormlike chains are present.

Figure 3 reports SANS spectra merged with SLS data, normalized for the surfactant concentration, for two representative samples. An inspection of the different spectral regions

reveals some structural characteristics in a model-free fashion. The different normalized intensity in the low- q region indicates an obvious dependence of the overall aggregate dimensions on the surfactant concentration. In the intermediate q range, $I(q)$ follows a q^{-1} power law, suggesting a locally cylindrical structure, that is concentration invariant. Micellar growth is made possible by the relative high ionic strength of the medium, because an analogous concentration scan done in water without added electrolytes yields only a very modest micellar growth and a meaningful intermicellar correlation deriving from electrostatic repulsion.⁶

The ionic strength of the buffer solution corresponds to a 1:1 electrolyte concentration of 0.25 M with a Debye length of about 6 Å. An ongoing investigation on micellar flexibility at fixed surfactant concentration performed as a function of the ionic strength³² shows that, at the above ionic strength, the electrostatic contribution to the persistence length is almost negligible.

For a correct evaluation of ionic strength, surfactant dissociations should also be taken into account according to

$$\kappa^2 = 4\pi L_B [\text{Surf}] \left(\alpha + 2 \frac{[\text{Salt}]}{[\text{Surf}]} \right) \quad (11)$$

where κ^{-1} is the Debye screening length, L_B is the solvent Bjerrum length, and α is the degree of micellar ionization.

Considering the relative high ionic strength with respect to surfactant concentration and the small volume fraction range explored, we can safely assume an invariant ionic strength provided by the electrolyte along the concentration scan.

The coincidence of the scattering curves for $q > 0.01 \text{ Å}^{-1}$ indicates that the cross sectional micellar structure is basically constant with the lipid concentration.

In the intermediate-high q -region the cylinder cross-section can be determined through a Guinier-like plot, namely a plot of $I(q) \cdot q^2$ versus q^2 ; following an argument first described by Porod, it is possible to show that the following equation holds³³

$$I(q) = \left(\sum_N C_N \right) \frac{\pi}{q} N_L (b_m - \rho_s v_m)^2 e^{-R_c^2 q^2 / 2} \quad (12)$$

where C_N is the number density of molecules forming an aggregate of N monomers, N_L is the weight average aggregation number per unit length, and R_c is the weight average radius of gyration of the cross-section of the cylinder (for uniform rodlike particles $R_c = r_{cs}/\sqrt{2}$), $(b_m - \rho_s v_m)$ being the contrast. Therefore, realistic evaluation of the cross section radius of the micelles can be obtained from (12).

The reasonable separation of length scales of the aggregates allows a neglect of the overall micellar characteristics: at this stage we are interested only in the *local* cylindrical structure and disregard whether the micelle is rigid or flexible.

The Guinier-like plots are reported for each samples in Figure 4. The resulting cross section radius r_{cs} is around $21.0 \pm 0.5 \text{ Å}$, irrespective of the lipid concentration.

The aggregation number per unit length, N_L , can be deduced from the intercept, once known the contrast term $(b_m - \rho_s v_m)$. This quantity for DLPU wormlike micellar solutions is 1.5 ± 0.1 .

The hydrophobic chain length is about 15.4 Å according to a classical Tanford model, which is lower than the experimental cylinder cross section, indicating that SANS is actually monitoring both phospholiponucleoside hydrophobic chains and bulky and contrasted polar headgroups, in agreement with the two-shell model recently employed for SANS analysis of globular

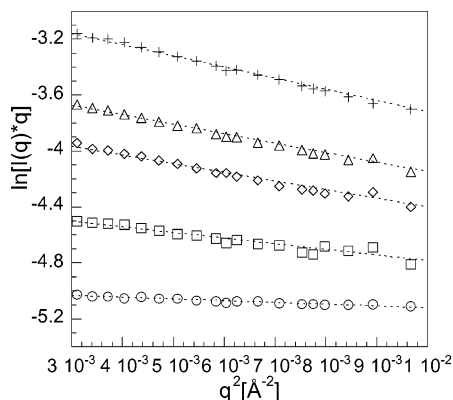


Figure 4. Guinier plot for the determination of the cross-sectional radius of gyration of DLPU wormlike micelles in the concentration range 1.0×10^{-3} to 1.0×10^{-2} M: (○) 1.0×10^{-3} M; (□) 2.0×10^{-3} M; (◇) 4.0×10^{-3} M; (△) 7.5×10^{-3} M; (+) 1×10^{-2} M.

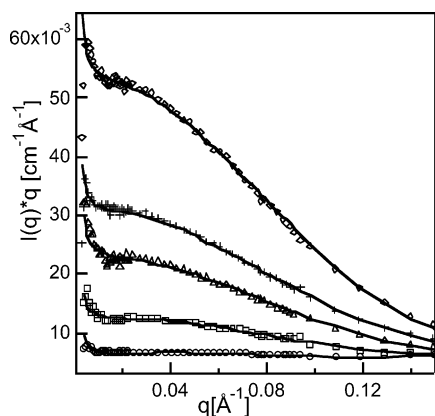


Figure 5. Holtzer plot of SANS spectra from DLPU wormlike in the concentration range 1.0×10^{-3} to 1.0×10^{-2} M: (○) 1.0×10^{-3} M; (□) 2.0×10^{-3} M; (△) 4.0×10^{-3} M; (+) 7.5×10^{-3} M; (◇) 1×10^{-2} M.

phospholiponucleoside micelles.⁷ However, in the data analysis presented here, the micellar cross section has been treated as homogeneous with respect to scattering lengths (21.0 ± 0.5 Å), to avoid additional adjustable parameters in the fitting.

The previous rough analysis leaves little doubt about micellar structure. However, a much deeper insight is necessary to achieve a quantitative evaluation of the persistence length of the aggregates.

A very convenient representation for spectra of semiflexible cylindrical aggregates is the so-called bending rod or Holtzer plot $I(q)*q$ vs q .^{34,35} This plot, shown in Figure 5, for a set of samples at different concentrations, highlights the region with scaling power -1 , typical of cylindrical structures. A plateau is clearly recognizable for low volume fraction samples, as well as a slope change at lower scattering vectors. The slope change supports a different scaling behavior due to the fact that neutrons are probing different lengths scales, i.e., from rigid rods to flexible chains.

This switch can be correlated to the persistence length of the aggregate in direct space, estimated by some authors to be $l_p \sim 1.9/q^*$.³⁵

The analysis of SANS data is complicated by three factors: the wide polydispersity of the micellar contour lengths, the presence of an intermicellar structure factor, whose contribution becomes larger as lipid concentration is increased, and an electrostatic contribution to the flexibility, due to the ionic nature of the surfactant. If this situation reflects the difficulties in

TABLE 1: Fitted Parameters for Micellar DLPU Solution in Phosphate Buffer 0.1 M

c (M)	$\langle L_c \rangle$ (Å)	l_p (Å)	r_{cs} (Å)	B (cm ⁻¹)
1.0×10^{-3}	5090 ± 10	240 ± 10	21.4 ± 0.5	$0.038 \pm 0.1\%$
2.0×10^{-3}	5430 ± 10	336 ± 10	21.4 ± 0.5	$0.036 \pm 0.2\%$
4.0×10^{-3}	5610 ± 10	350 ± 10	21.4 ± 0.8	$0.036 \pm 0.2\%$
7.5×10^{-3}	6416 ± 10	453 ± 10	21.3 ± 0.8	$0.039 \pm 0.3\%$
1.0×10^{-2}	6376 ± 10	500 ± 10	21.4 ± 0.5	$0.041 \pm 0.3\%$

treating polymeric scattering, in our case it is utterly complicated by the fact that each of the above quantities is volume-fraction dependent.

Persistence length is probably the most influenced parameter by the above-mentioned factors. Persistence lengths $l_{p,cs}$ obtained by using the crossover relation should be regarded as merely qualitative. In our case the crossover behavior is concentration-dependent, yielding an extrapolated value for $c \rightarrow 0$ of 118 Å. This result is obviously too low if compared to those typical for micelles, considering their finite cross section (about 40 Å in our case). L_p , like the end-cap energy of the ladder model, is an intrinsic property of the micelle, governed by the dependence of the molecular packing free energy on the interfacial curvature, and on the area per headgroup, namely on the local aggregation geometry.^{36–38}

The main contribution to the determination of the free energy cost to bend a portion of the micellar worm has been generally indicated in the literature as due to the hydrophobic chain length,^{39,40} i.e., the higher the cross section, the longer the directional correlation. This argument is indeed reasonable for small and chemically “non intelligent” polar headgroups, as for the alkyl sulfate series, but fails, for instance, if micellar growth is triggered by more sophisticated counterions (e.g., exchanging sodium salts with pyridinium salts²⁰), which influence not only contour length but also flexibility. In our case, considering the complexity of polar heads and their biological relevance, the polar head structure and the extent of interactions among neighboring molecules in the aggregate play a leading role in micellar flexibility.

We have therefore performed a model fitting as outlined in the Experimental Section. The results of this model fitting, obtained by neglecting any interaction effects, are also reported as solid lines in the Holtzer plots displayed in Figure 5. As can be observed, the agreement with experimental data is indeed satisfactory for the whole concentration range.

Table 1 reports the parameters extracted from the fitting. These data deserve some comments: the pronounced concentration-dependence of l_p is, as already stressed, unphysical and derives from the disregard of intermicellar interactions; therefore their inclusion in the model fitting of experimental data is necessary. A plot of the extracted persistence lengths reported in Table 1 as a function of lipid volume fraction reveals a linear trend, as shown in Figure 6, whose infinite dilution extrapolation yields a value of 230 Å. This value, if compared to those of other ionic micellar systems ($l_p \approx 300$ – 500 Å), usually characterized by a larger stiffness with respect to nonionic ones, looks more reasonable than the one obtained by the crossover relation.^{41,42} We can safely state that 230 Å is a reasonable evaluation of the real persistence length of wormlike DLPU micelles.

In this first step L_c values represent only an indication of the real contour length for two main reasons: the q range investigated is not extended enough, and intermicellar interactions are neglected. We can now fix the persistence length value of 230 Å throughout the whole concentration range and include intermicellar interactions through the quantity M_{app} , obtained

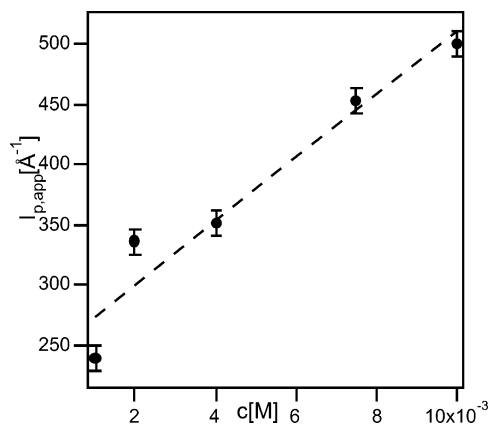


Figure 6. Apparent persistence lengths as a function of surfactant concentration. The extrapolated value for $c \rightarrow 0$ is 230 Å.

TABLE 2: L_c Values Obtained from Wormlike Fitting Extended to the Whole q Range Available (SLS + SANS)

c_{DLPU} (M)	M_{app} (g/mol)	$\langle L_c \rangle$ (Å)
0.0010	$1.19 \times 10^6 \pm 1.0\%$	2195 ± 10
0.0020	$1.43 \times 10^6 \pm 0.5\%$	3045 ± 10
0.0040	$1.53 \times 10^6 \pm 0.5\%$	4000 ± 10
0.0075	$1.38 \times 10^6 \pm 0.5\%$	5076 ± 10
0.0100	$1.02 \times 10^6 \pm 1.0\%$	5490 ± 10

from static light scattering data.¹¹ Best fit parameters are reported in Table 2 and the fitting curves are superimposed to experimental data in Figure 7.

This approach was first used for anionic wormlike micelles by Magid et al.²¹ to infer the persistence length of SDS micellar solutions as a function of the ionic strength. These authors observed a generalized increment of the contour lengths when intermicellar interactions are taken into account and a marked growth acceleration as surfactant concentration approaches the semidilute threshold. They regard this growth acceleration as due to the approximation used for intermicellar interactions and to the occurrence of multiple q -dependent factors at low q .

We observe a decrease in the fitting-extracted micellar contour lengths for lower DLPU concentrations and an increase of the micellar growth exponent to 0.4, which is closer to that predicted by the theory. Therefore, in our case, we regard L_c values (reported in Table 2) obtained by including interaggregate interactions and covering a more extensive q range ($q_{\text{min}} = 8 \times 10^{-4} \text{ Å}^{-1}$), as reasonable estimates of micellar contours. Cylindrical aggregates are customarily classified according to their average contour length $\langle L_c \rangle$ and persistence length ratio. When $l_p \ll \langle L_c \rangle$ the micelles are regarded as very flexible, i.e., wormlike; when $l_p \sim \langle L_c \rangle$, they are semiflexible, and when $l_p > \langle L_c \rangle$, the micelles are considered rigid rods.

Our results point out that DLPU micelles, in the concentration range explored, are characterized by $l_p \ll \langle L_c \rangle$ and can be thus regarded as wormlike micelles.

It would be interesting to see if the experimental persistence length is in agreement with the bending elasticity calculated through current molecular theories for surfactant wormlike systems. In the literature, the vast majority of experimental and theoretical studies concern the elastic properties of lipid monolayers and bilayers, and several theories at a molecular level have been formulated about the curvature and the stretching elasticity modulus of these systems.^{43–45} Recently, the bending rigidity (hence l_p) of wormlike cylindrical micelles has been estimated on the basis of a molecular level theory by Ben-Shaul and co-workers.⁴⁶ The theory employed is approximate because both the headgroup and the hydrocarbon chain contributions to

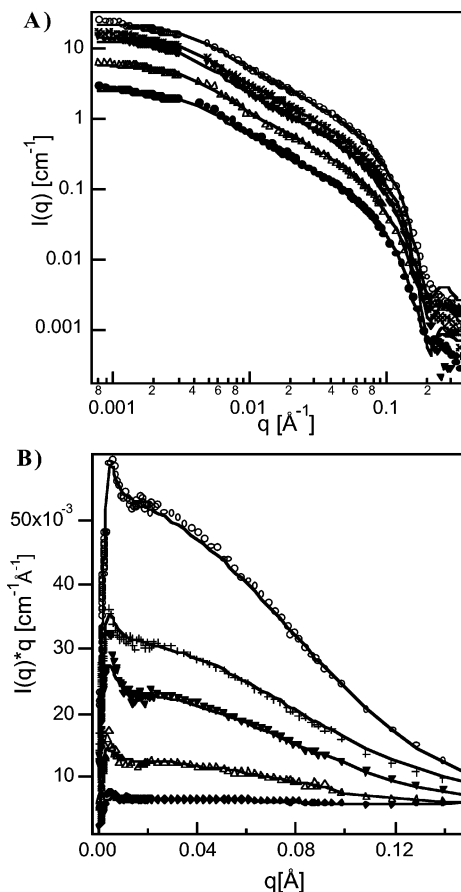


Figure 7. (A) Experimental SANS and LS scattered intensities, $I(q)$, for different DLPU surfactant concentrations in the dilute regime. Solid lines are the best fits obtained considering of intermicellar interactions in the fitting model. (B) Holtzer plot representation, $I(q) \cdot q$ vs q , for the same samples. Solid lines represent the best fits including intermicellar interactions. Key: (◆) 1×10^{-3} M; (Δ) 2×10^{-3} M; (▼) 4×10^{-3} M; (+) 7.5×10^{-3} M; (○) 1×10^{-2} M.

the packing free energies are treated at a mean field level. However, these authors find values of just a few nanometers (150–200 Å) for l_p of flexible wormlike micelles, values in qualitative agreement with that found for DLPU in the present study. The higher rigidity that we observe could be reasonably due both to the double chain presence and to headgroup interactions contributions.

In a recent work of Cohen Stuart^{2,47} the persistence length of nonionic wormlike micelles is calculated through a numerical self-consistent-field theory that accounts for molecular details. The authors propose a molecular interpretation of the bending rigidity (hence l_p) of wormlike micelles expressing the grand potential of toroidal micelles (i.e., the bending energy) in terms of the curvature in a Helfrich-like expansion up to the second order. They show that the bending rigidity of wormlike micelles is only a function of the surfactant tail length, as already reported in the literature,^{39,40} attributing the invariance of the flexibility with the headgroup size to the compensation of two opposite effects: a larger corona reduces the core size, meaning that both core and corona sizes affect micellar rigidity. However, this treatment is valid only for small curvatures, and already for curvatures of the order of one inverse persistence length, higher order terms of the series expansion of the bending modulus are no more negligible. Therefore, large headgroup sizes *do* influence significantly the stiffness of the wormlike micelles.

On this basis, we can say that higher DLPU l_p values with respect to the one expected with such a hydrophobic tail length,

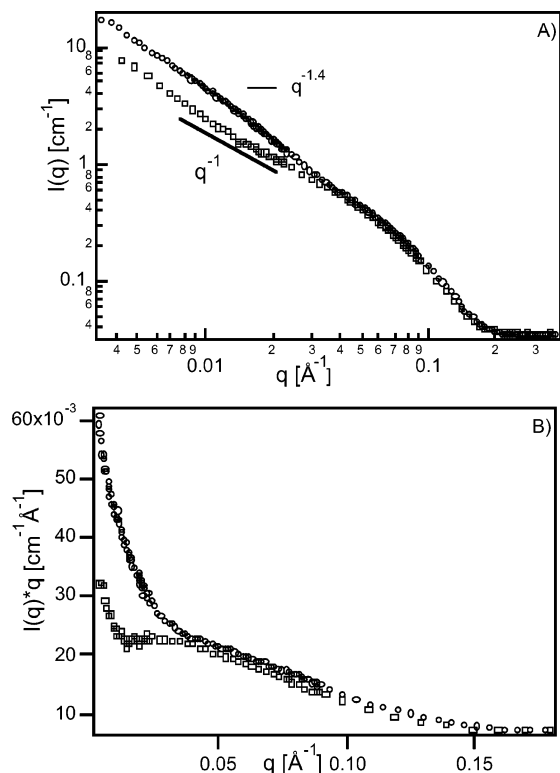


Figure 8. (A) Comparison between SANS spectra for DLPA and DLPU micellar solutions in 0.1 M deuterated phosphate buffer. (B) Holtzer plots for the same spectra. Total lipid concentration is 4 mM. Key: (○) DLPA; (□) DLPU.

could actually be ascribed to the fact that polar heads are not just merely hydrophilic steric units with mutual electrostatic repulsions, but can be possibly able to interact through stacking and H-bonding. The occurring of such interactions between bases at the interface could play a crucial role in the self-assembling and in bending energy.

This observation is further supported by a qualitative comparison with the behavior displayed, in the same concentration range and dispersing medium, by DLPA (1,2-dilauroyl-*sn*-glycero-3-phosphatidyl-adenine), the Watson–Crick counterpart of DLPU. DLPA and DLPU derivatives are characterized by the same alkyl portion and differ only in the nucleic base nature; in fact, uridine is a poor homostacker whereas adenosine, like all purine derivatives, has higher stacking constants.⁴⁸

In Figure 8a SANS spectra for 4 mM DLPA and DLPU in 0.1 M phosphate buffer (pH = 7.5) are presented. Although in the high-intermediate q region the two curves overlap, indicating the same local structure, in the low-intermediate q region we observe an evident discrepancy in the scaling behavior, which suggests a variation of the overall dimension and possibly a different shape on the mesoscopic scale.

The main difference between the two spectra is at first glance the lack of the q^{-1} region for DLPA that is better highlighted by the absence of a plateau region in the Holtzer-bending plot, reported in Figure 8B. The -1.4 scaling exponent cannot be correlated to any dimensionality of the aggregate but can surely be ascribed to a more complex phase behavior⁴⁹ of DLPA derivative, which will be explored in a forthcoming paper. What should be underlined here is how a slightly different nature of the polar head, in the case of smart surfactants, can drastically change self-assembling properties.

In this context it becomes particularly important the evaluation of the persistence length from experimental data, because the currently available theoretical models are not able to take into

account all the possible parameters involved in the determination of the phase behavior, even if they can give a qualitative trend of the flexibility of wormlike objects in function of the molecular details such as both the core and the corona size.

Conclusions

DLPU is an anionic phospholipidic surfactant with a nucleic acid polar head, namely uridine. Its aggregation, determined by the double lauroylic chain, is modulated by the properties of the polar head. In physiological conditions (0.1 M phosphate buffer at pH 7.5) the electrostatic screening can promote micellar growth along the axial direction, resulting in flexible and extremely long assemblies.

The transverse size of the self-assembled objects is dictated by molecular parameters and remains practically invariant as concentration is raised, i.e., the surfactant chain length, whereas micellar contours grows to about 0.5 μm for a 0.6% volume fraction.

DLPU wormlike micelles have been characterized in terms of their structural properties by a combined use of static light scattering and small angle neutron scattering techniques. The covered q range in the reciprocal space is such that a complete picture of micellar aggregates can be inferred according contour length, persistence length, and cross sectional radius.

In particular, the desired structural parameters have been extracted through a nonlinear least-squares fitting of the experimental spectra by parametrized wormlike chain scattering functions, obtained with Monte Carlo methods.^{1,2} Excluded volume interactions, micellar polydispersity and intermicellar effects are taken into account.

The main result presented here is an accurate estimate of micellar flexibility in term of persistence length, i.e., 230 \AA in 0.1 M PBS at pH 7.5 for DLPU micellar “worms”.

A classical single-chained anionic surfactant with similar chain length (SDS) does not show micellar growth in these concentration ranges and Debye screening length conditions: therefore the preferential packing along the cylindrical body is obviously ascribable to the presence of a double chain, together with (from a chemical point of view) the more sophisticated polar head of the phospholiponucleoside. To the best of our knowledge, an investigation on double-chained anionic surfactant micellar growth in direct conditions has not yet been reported; therefore a more precise comparison is at the moment precluded.

The reliable experimental access to the persistence length values for wormlike micellar systems will presumably have profound implications in development of theoretical models to predict flexibility properties of self-assembled aggregates as a function of the chemical nature of the monomer. These authors believe that, in the same way as was done in past decades for surfactant packing parameters for the preferred aggregation shape, this will allow the design at a molecular level of responsive complex fluids that could be of utmost importance to assess surfactant phase behavior for applications that require knowledge of flow behavior.

We are aware that the majority of wormlike systems investigated are constituted by chemically simple polar heads, whose contributions to micellar flexibility are the steric hindrance and the electrostatic repulsion.

The situation is considerably more complex in our case, where a phosphate-sugar-nucleic base (that is a nucleic acid building block) is the polar head and, notwithstanding the same negative charge, self-interactions are triggered and, at the same time, modulate the self-assembling pattern. As in real biological

systems, the sensitivity to slight chemical variations is extremely high and specific. To address this point, we have shown how a modest change in polar head hindrance and chemical nature, i.e., the exchange of uridine with adenosine, producing a really negligible alteration of the surfactant packing parameter can drastically alter phase behavior properties on the mesoscopic scale.

The strict parallelism between structural and dynamic properties of wormlike micellar solutions and polymer solutions, repeatedly stressed in the literature and in the present paper, acquires for DLP-nucleosides a different meaning, because the analogy with a polymeric counterpart can be further extended to the presence of chemical functionalities typical of nucleic acids, enlarging the comparison with natural polynucleotides rather than generic polymers.

It is worthwhile to stress that in Nature such variations have enormous and avalanche impact, as far as information is maintained, translated, and transmitted through chemical language; it is then of paramount importance that these self-assembled mimics reproduce partially this complexity.

In this context the experimental determination of micellar flexibility, meant as a mesoscopic gauge of molecular specificity, is of considerable importance because theoretical models will presumably not be able soon to take into account this wealth of diverse contributions to bending energy.

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