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Structural characterization of Di-C₁₂ P-uridine worm-like micelles: ionic strength dependence

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Abstract

Through a combination of small angle neutron scattering and static light scattering we have investigated the aqueous self-assembly of 1,2-dilauroyl-sn-glycero-3-phosphatidyl-uridine (DLPU). This phospholipid reproduces on its polar head the chemistry and the charge of an RNA monomer. The results indicate that DLPU forms flexible cylindrical micelles. The structural properties of the aggregates have been determined for three fixed surfactant volume fractions as a function of the medium salinity. The desired structural parameters have been inferred from non-linear least-square fitting of the whole scattering curve (SLS + SANS) through a parametrized worm-like chain scattering function. Surfactant concentration and ionic strength affect the contour length and the effective flexibility, leaving the cross section virtually unchanged. DLPU micelles become more flexible upon salt addition since the salt-dependent contribution to the micellar rigidity decreases. The intrinsic contribution is directly coded in the chemistry of molecular subunits, and depends for DLPU, besides on the steric hindrance of the amphiphile, on intermolecular interactions acting between polar heads and triggered by self-assembly. This contribution is invariant with medium conditions and can be considered as a mesoscopic gage of molecular specificity, while the response to ionic strength of ionic surfactant solutions represents a tuneable, responsive property.

(Some figures in this article are in colour only in the electronic version)

Introduction

The phase behavior of amphiphilic self-assembling systems has been the subject of several investigations in recent decades (Israelachvili 1992, Safran 1994, Gelbart and Ben Shaul 1996). Spherical micelles are the simplest self-assembled aggregates and their formation is the result of a delicate interplay between packing constraints of the surfactant molecules and of both solvent-surfactant and surfactant-surfactant interactions. A general feature of amphiphilic aggregates is the structural evolution to non-spherical shapes that can in some cases become elongated flexible cylindrical micelles, also termed thread-like or polymer-like micelles. Several reports have

in fact shown that aqueous solutions of non-ionic or ionic surfactants can exhibit a considerable micellar axial growth, as a response to a variation of control parameters (typically temperature or ionic strength), to form very large anisotropic aggregates (van de Sande and Persoons 1985, Schurtenberger and Cavaco 1994, Schurtenberger *et al* 1996, Magid *et al* 2000a, 2000b). For charged systems a qualitative explanation of this phenomenon can be found in the salt dependence of the surfactant packing parameter. In fact, the spontaneous curvature of the surfactant film, due to its chemical structure and charge, controls self-assembling packing preferences, determining the structural properties of the aggregate (Safran 1992). For $c > c_{mc}$, a further increase in concentration causes micelles to grow due to mass-action effects. For typical

ionic surfactants, that prefer spherical packing, this growth is generally very limited; however, at high ionic strength, intramicellar repulsive interactions are screened, favoring a locally cylindrical packing that provides a possibility for a uniaxial growth (Israelachvili *et al* 1976).

The major morphologic feature of polymer-like micelles is the inhomogeneous length scale of the cross section and the ‘long’ axis, that can, under proper experimental conditions, grow to several micrometers. The cross-sectional radius is directly connected to the length of the amphiphile and it is relatively insensitive to concentration and to experimental conditions. The contour length is determined by concentration and by the extent of free energy minimization that a surfactant molecule gains for packing along the cylindrical body of the aggregate instead of the hemispherical end-caps, and is given by

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

where ϕ is the surfactant volume fraction, $\langle L_c \rangle$ is the mean contour length and ΔE_{exc} is the difference in chemical potential for a surfactant molecule packed in the hemispherical end-caps and in the cylindrical body of the micelle. This quantity depends on the variation of the above-mentioned control parameters (surfactant volume fraction, ionic strength, temperature and so on).

This classical approach explains micellar growth on a molecular basis but it is restricted to stiff rod micelles, while flexibility is disregarded. Early investigators soon realized from scattering data (Porte *et al* 1980, Appel *et al* 1982a) that cylindrical micelles should present a more or less pronounced flexibility; this feature was confirmed by cryo-TEM visualization in direct space (Lee *et al* 1990, Egelhaaf *et al* 1998). These findings led to a straightforward experimental and theoretical analogy with polymeric solutions and much of the experimental data analysis has benefited from concepts and language from polymer physics (de Gennes 1979).

Micellar rigidity primarily depends on molecular details of the surfactant monomer that contributes to the so-called intrinsic flexibility. Moreover, experimental conditions such as the ionic strength, for charged systems, further influence this feature, as qualitatively illustrated by Candau and co-workers (Candau and Hirsch 1987, Lequeux and Candau 1994).

A characteristic length scale related to the macromolecular flexibility is the persistence length, that could be 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 $l_p = k/k_B T$ (Lifshitz and Pitaevskii 1980).

We have studied for some years self-assembling and recognition properties of phospholipids with a nucleosidic moiety on the polar head. Base–base interactions are triggered in these nucleosidic derivatives by the formation of supramolecular structures, similarly to polymeric arrangement of nucleic acids that makes it possible for each base on one strand to form a H-bond adduct with its Watson–Crick partner on the complementary strand.

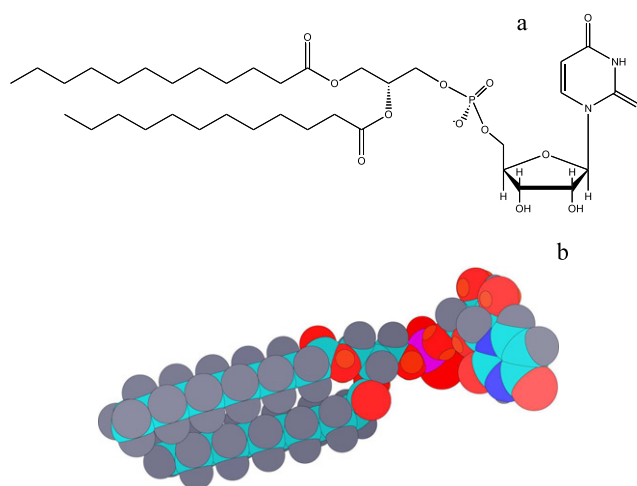


Figure 1. Schematic drawing of DLPU chemical structure (a) and its molecular structure (b) obtained by a geometrical optimization performed with MM⁺ force field in a box of water molecules. Water molecules are omitted.

Base organization in a supramolecular array is then made possible by self-aggregation, and the extent of interactions between bases is modulated by interfacial curvature, that depends on the dimensionality of the aggregate. ‘Short chain’ derivatives, such as 1,2-dioctanoyl-sn-glycero-3-phosphatidyl-nucleosides, form above their cmc globular aggregates in water and in 0.1 M pH 7.5 phosphate buffer (PBS) in a wide range of lipid concentration, while ‘long-chain’ derivatives, such as 1,2-dioleoyl-sn-glycero-3-phosphatidyl-nucleosides and

1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidyl-nucleosides organize into bilayered structures (lamellar phases and vesicles). Both micelles and vesicles exhibit strong evidence of base–base interactions generated by the surfactant aggregation, with interplay of both stacking and hydrogen bonding, notwithstanding base exposure to aqueous environment (Berti *et al* 1997, 1999, 2000). These amphiphiles, beside a fundamental interest concerning self-assembling issues, can be lipophilic prodrugs if their polar head is conveniently chosen among pharmacologically active modified bases (MacCoss *et al* 1978, Hostetler *et al* 1991). Moreover, the RNA-like polar head chemical structure of these surfactants makes them attractive candidates as delivery agents for non-viral gene transfection.

We have previously reported an investigation on worm-like phases formed by 1,2-dilauroyl-sn-glycero-3-phosphatidyl-uridine (DLPU, figure 1) as a function of lipid concentration (Baldelli Bombelli *et al* 2002a, 2002b, 2004a). We have defined these aggregates as ‘living polynucleotides’ because self-assembly performs a role similar to polymerization in polynucleotides and base stacking constants have an active role in determining the macromolecular conformation. It has recently been shown (Baldelli Bombelli *et al* 2006) that simple substitution of the lipid polar head with adenosine, uridine’s complementary base in RNA, induces a more complex self-assembling behavior. Both nucleolipids form worm-like micelles in freshly prepared buffered solutions, but DLPA assemblies show a concentration-dependent evolution to form

helically twisted superstructures. This different behavior is due to stronger stacking interactions and H-bonds between adenine bases, inducing an intramolecular and intermolecular coordination. These results demonstrate that the nature of the base is of paramount importance for the mesoscopic properties of the aggregates; on the same basis, micellar flexibility can be considered as a mesoscopic gage of intermolecular interactions established along the micellar contour. Therefore, its determination can provide valuable insights in terms of predictability of structural and dynamical properties.

However, micellar flexibility, determined in terms of persistence length (Baldelli Bombelli *et al* 2004a), also contains a non-negligible contribution due to electrostatic repulsion of like charges at the micellar surface that cannot be quantified at fixed salinity. This electrostatic contribution is explicitly taken into account in the present work and estimated through a combination of scattering techniques. The final result is the evaluation of the electrostatic persistence length and hence of the intrinsic persistence length of the system. A comparison with data available in the literature on flexibility of ionic worm-like micelles and polyelectrolytes is also reported, in order to highlight possible similarities.

1. Experimental section

1.1. Materials

1,2-dilauroyl-sn-glycero-3-phosphatidyl-choline (DLPC) was purchased from Avanti Polar Lipids (Alabaster, AL, USA) and its purity checked by TLC. The lecithin was used as received since no oxidation or lyso products could be detected. Uridine, HCl, CHCl_3 , MeOH, and NH_3 (33% aqueous solution) used in the synthesis and NaH_2PO_4 (>>99%) and Na_2HPO_4 (>>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 provided by Euriso-Top (Saclay, Gif sur Yvette, France).

1.1.1. Synthesis of 1,2-dilauroyl-sn-glycero-3-phosphatidyl-uridine. DLPU was synthesized starting from the corresponding phosphatidylcholine in a two-phase system according to a modification of the method proposed by Shuto and co-workers (Shuto *et al* 1987, 1988), and obtained as an ammonium salt. The molecular structure is reported in figure 1.

1.1.2. Sample preparation. The lyophilized powder was dissolved in 0.10–0.35 M NH_4Cl solutions prepared in D_2O , chosen to minimize the incoherent background from hydrogen and enhance the scattering contrast of aliphatic chains in SANS experiments. For each sample, pH has been adjusted to 7.0. In the present study we have investigated samples in the dilute regime, except for $I = 0.25$ and 0.35 M, when the highest lipid volume fraction investigated is approaching the semi-dilute threshold. The composition of the samples investigated in this study is summarized in table 1.

Table 1. Composition of the samples investigated by SANS and SLS experiments.

Sample	C_{DLPU} (mM)	ϕ_{DLPU}	$C_{\text{NH}_4\text{Cl}}$ (M)
1	4	0.0023	0.10
2	7	0.0041	0.10
3	10	0.0059	0.10
4	4	0.0023	0.18
5	7	0.0041	0.18
6	10	0.0059	0.18
7	4	0.0023	0.25
8	7	0.0041	0.25
9	10	0.0059	0.25
10	4	0.0023	0.35
11	7	0.0041	0.35
12	10	0.0059	0.35

We should mention that for a correct evaluation of the ionic strength, surfactant dissociation should also be taken into account according to

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

where α is the degree of ionization, $\kappa^{-1} = \sqrt{\frac{\epsilon_0 \epsilon_r k_B T}{2e^2 N_A I}}$ the Debye screening length and $L_B = \frac{e^2}{4\pi \epsilon_0 \epsilon_r k_B T}$ is the Bjerrum length (7.14 Å for water). In the present case surfactant dissociation can be regarded as negligible considering the relatively low cmc and high ionic strength.

1.2. Methods

1.2.1. 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 is the second harmonic of a diode pumped Coherent Innova Nd-YAG laser ($\lambda = 532$ nm), linearly polarized in the vertical direction impinging on the sample with 50 mW power. Measurements were performed at 25 °C. Approximately 1 ml of sample solution was transferred into the cylindrical Hellma scattering cell, that was then sealed and centrifuged for about 1 h at 5000 g in order to remove dust particles from the scattering volume.

1.2.2. Data analysis for static light scattering. Static light scattering experiments (SLS) were performed in the $25^\circ \leq \theta \leq 150^\circ$ range, corresponding to an $8 \times 10^{-4} \text{ \AA}^{-1} < q = \frac{4\pi n}{\lambda} \sin(\vartheta/2) < 3 \times 10^{-3} \text{ \AA}^{-1}$ scattering vector range, with the refractive index of the scattering medium $n = 1.33$ and the wavelength of the incident light $\lambda = 5320 \text{ \AA}$. Data have been converted into absolute scattering intensity $\Delta R(q)$ (i.e. ‘excess Rayleigh ratios’) using toluene as reference standard, according to (Jerke *et al* 1998)

$$\Delta R(q) = \frac{\langle \Delta I(q) \rangle}{\langle I_{\text{ref}}(q) \rangle} R_{\text{ref}}(q) \left(\frac{n}{n_{\text{ref}}} \right)^2, \quad (3)$$

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

Assuming a scattering law of the form (Schurtenberger *et al* 1996)

$$\frac{Kc}{\Delta R(q)} = \frac{1}{M_{\text{app}}} [1 + q^2 \xi_S^2] \quad (4)$$

a plot of $Kc/\Delta R(q)$ versus q^2 yields the apparent molar mass M_{app} and the static correlation length, ξ_S . In equation (4) c is the surfactant concentration and $K = 4\pi^2 n^2 (dn/dc)^2 / (N_A \lambda_0^4)$ is constant for a given wavelength, where (dn/dc) is the refractive index increment.

The refractive index increment for DLPU in 0.1–0.35 M NH_4Cl solutions is $1.40 \times 10^{-1} \text{ ml g}^{-1}$, as determined by differential refractometry using an RF-600 from C N Wood, Newton, PA, USA.

1.2.3. Small angle neutron scattering. Small angle neutron scattering experiments were performed at the spectrometer KWS-1 (Research Reactor in Juelich) at $T = 25 \text{ }^\circ\text{C}$. Four different configurations (i.e. sample–detector distances: 1.25, 2, 8 and 20 m) allowed us to cover a range of wavevectors $q = 2.5 \times 10^{-3} - 0.25 \text{ \AA}^{-1}$ with 7 \AA neutron wavelength and $\Delta\lambda/\lambda < 20\%$.

Corrections for background and sample holder contributions were made according to the standard procedures. Scattering intensities were converted to absolute units (cm^{-1}) by using calibration with low-density polyethylene (LUPOLEN). Samples were contained in a flat Hellma quartz cell of 1 mm path length. The medium, 0.1–0.35 M NH_4Cl solutions in D_2O , was treated as a sample and then subtracted from the corresponding surfactant solutions.

1.2.4. Data analysis for small angle neutron scattering. SANS data analysis for DLPU worm-like micelles has been performed with a non-linear least-squares fitting of the scattering curves.

A detailed description of the procedure is given in a previous paper (Baldelli Bombelli *et al* 2004b), where references to the original literature are also provided. Here we give a very short account as a guide for the reader through data analysis.

In the random phase approximation the scattered intensity of a worm-like micellar solution can be written as

$$I(q, c) = KcM_w S_{\text{RPA}}(q, c) + B \quad (5)$$

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 $\text{cm}^3 \text{ g}^{-1}$ (obtained by density measurements), the scattering length densities of micelles, ρ_m , and solvent, ρ_s , are in cm^{-2} , M_w is the weight averaged molecular weight and B the background term. $S_{\text{RPA}}(q, c)$ is the random phase approximation (RPA) structure factor. Monte Carlo simulations on worm-like micelles (Pedersen and Schurtenberger 1999) reveal that structure

factors obtained can be adequately reproduced by $S_{\text{RPA}}(q, c)$. The full structure can be expressed as

$$S_{\text{RPA}}(q, c) = \langle P(q, c) \rangle / \left\{ 1 + [S(0, c)^{-1} - 1] f_D \left(q^2 \overline{R_g^2} \right) \right\}, \quad (6)$$

where

$$f_D(x)^2 = 2(e^{-x} - 1 + x)/x^2, \quad (7)$$

$\langle P(q, c) \rangle = P_{\text{cs}}(q, r_{\text{cs}}) \cdot \langle P_{\text{wc}}(q, L_c(c), l_p) \rangle$ is the form factor averaged over the micellar molecular weight distribution and f_D is the Debye function, i.e. the scattering law of a Gaussian chain, while $S(0)^{-1}$ is the osmotic compressibility.

$P_{\text{cs}}(q, r_{\text{cs}})$ is the cross section form factor and $\langle P_{\text{wc}}(q, L_c(c), l_p) \rangle$ is the worm-like chain form factor, introduced through a parametrized expression that is derived on the basis of a series of Monte Carlo simulations (Pedersen and Schurtenberger 1996a, 1996b) containing a contribution that accounts for intra-chain excluded volume interactions.

The fitting procedure, whose details have already been reported (Baldelli Bombelli *et al* 2004b), consisted of two steps: in the first one, where intermicellar interactions are neglected, the persistence length, determined for a fixed ionic strength as a function of surfactant concentration, is evaluated through extrapolation to infinite dilution (Jerke *et al* 1997, 1998). In the second scan, intermicellar interactions are explicitly included and the previously determined persistence length is built in as a constant parameter.

The osmotic compressibility, $S(0)^{-1}$, can be inferred from static light scattering through the apparent molar mass M_{app} according to

$$M_{\text{app}} = M_w S(0). \quad (8)$$

The spectra reported in this work are the combination of light and neutron scattering experiments, covering a $7 \times 10^{-4} \leq q[\text{\AA}^{-1}] \leq 0.35q$ range. SLS data have been rescaled by the ratio between SLS and SANS optical constants, and then superimposed on SANS data.

2. Results and discussion

Figures 2(A)–(C) show SLS spectra, plotted as $Kc/\Delta R(q)$ versus q^2 , for the three studied surfactant concentrations at different medium salinities.

The effect of raising salt concentration, similar for each surfactant volume fraction, leads to a scattering intensity increase (i.e. to a $K/\Delta R$ decrease) over the whole q -range. This feature reveals a growth of the overall dimension of the aggregates upon ionic strength increase, as commonly encountered for other ionic surfactants. Micellar growth is promoted by a more effective screening of intramicellar electrostatic repulsions, i.e. by the decrease of Debye screening length of the medium.

Assuming a Lorentzian scattering law of the form of equation (4), a plot of $Kc/\Delta R(q)$ versus q^2 gives quantitative information on the correlation lengths ξ_S and apparent molar masses M_{app} . For $c < c^*$ (with c^* the crossover concentration between dilute and semi-dilute regimes), the correlation length can be related to an effective size of single aggregates, that is the radius of gyration of the micelles, through $\xi_S =$

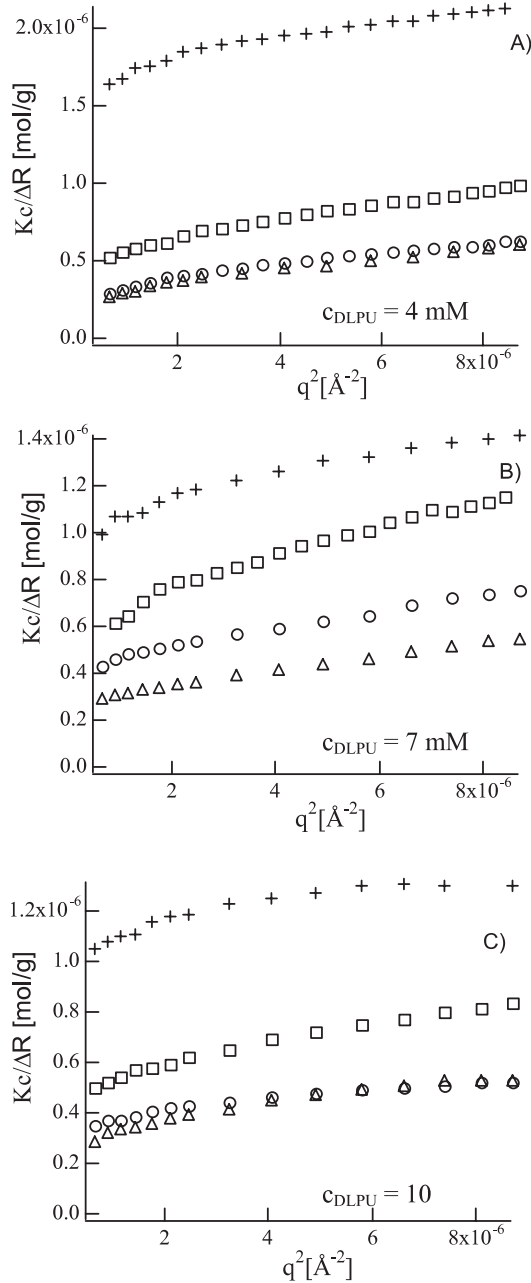


Figure 2. Plots of $Kc/\Delta R(q)$ versus q^2 of DLPU SLS spectra. The fitting of these plots, according to equation (4), yields the apparent molar mass M_{app} and the static correlation length ξ_s , reported in table 2. NH_4Cl concentration: +, 0.1 M; \square , 0.18 M; \circ , 0.25 M; \triangle , 0.35 M. DLPU concentration: (A) 4 mM, (B) 7 mM and (C) 10 mM.

$R_g/\sqrt{3}$, with $R_g = \langle R_g^2 \rangle_z^{1/2}$. The apparent molar mass M_{app} contains the actual micellar size ($\langle M \rangle_w$) and a term accounting for intermicellar interactions. In the semi-dilute regime the correlation length can be considered as a ‘screening length’ for intermicellar interactions in the network, and can be visualized as its mesh size.

The results reported in table 2 highlight meaningful differences for the effect of surfactant concentration on micellar structure between the lowest salinity medium and the higher ones.

Table 2. Structural parameters obtained from static light scattering data.

Sample	M_{app} (g mol ⁻¹) ^{a,d}	ξ_s (Å) ^{b,d}	ΔR_0 (cm ⁻¹) ^c
1	6.5×10^5	288	6.1×10^{-4}
2	1.0×10^6	315	1.9×10^{-3}
3	1.1×10^6	325	2.9×10^{-3}
4	1.8×10^6	410	2.0×10^{-3}
5	2.1×10^6	504	3.0×10^{-3}
6	2.0×10^6	335	5.3×10^{-3}
7	3.7×10^6	485	3.6×10^{-3}
8	2.8×10^6	372	4.3×10^{-3}
9	2.0×10^6	330	7.0×10^{-3}
10	4.7×10^6	580	4.7×10^{-3}
11	3.7×10^6	335	7.0×10^{-3}
12	2.8×10^6	300	8.5×10^{-3}

^a Apparent molar mass.

^b Static correlation length.

^c Forward scattered intensity reported as excess Raleigh ratio.

^d The uncertainty on M_{app} is $\pm 3\%$ and on ξ_s is $\pm 6\%$.

M_{app} and ξ_s are proportional to lipid concentration for $I = 0.10$ M, while for $I = 0.18$ M they show a small decrease for the maximum surfactant concentration and for $I \geq 0.25$ M decrease with surfactant volume fraction. Instead, the excess forward Rayleigh ratios, ΔR_0 , are still increasing whereas the former two quantities already show a decreasing trend; this effect is correlated to the osmotic compressibility $S(0)^{-1}$ that affects M_{app} values as explained in the data analysis section. The inversion indicates a decrease of osmotic compressibility and corresponds to a situation where micelles continue growing and become more and more entangled to form a transient network that breaks and recombines.

The crossover concentration between dilute and semi-dilute regimes, c^* , can be expressed according to

$$c^* = (k_B T L_B r_{cs} a^2 / \Delta E_{exc}). \quad (9)$$

Therefore the salinity of aqueous solutions, modulating the screening of charges along micellar contour, affects this threshold both through the term a (the effective charge per unit micellar length) and the scission energy ΔE_{exc} (free energy term defined in equation (1)).

In the present case the threshold occurs in the concentration range between 7.0 and 10 mM for ionic strengths greater than 0.1 M, while for lower salinities it lies presumably beyond our experimental range.

We have previously shown (Baldelli Bombelli *et al* 2002c, 2002d) that DLPU in 0.1 M PBS (pH = 7.5) forms worm-like phases already at a surfactant concentration of 1.0 mM (volume fraction 0.06%) and we have reported the structural evolution of the system as a function of the lipid volume fraction. These aggregates are characterized by a marked structural anisotropy ($L_c/r_{cs} > 100$ for the lowest volume fraction studied) and by a relatively high degree of flexibility. In the same work we have shown the crossover concentration between dilute and semi-dilute regimes to be around 10 mM (that is the highest surfactant concentration investigated in this paper), while a parallel concentration surfactant scan performed in

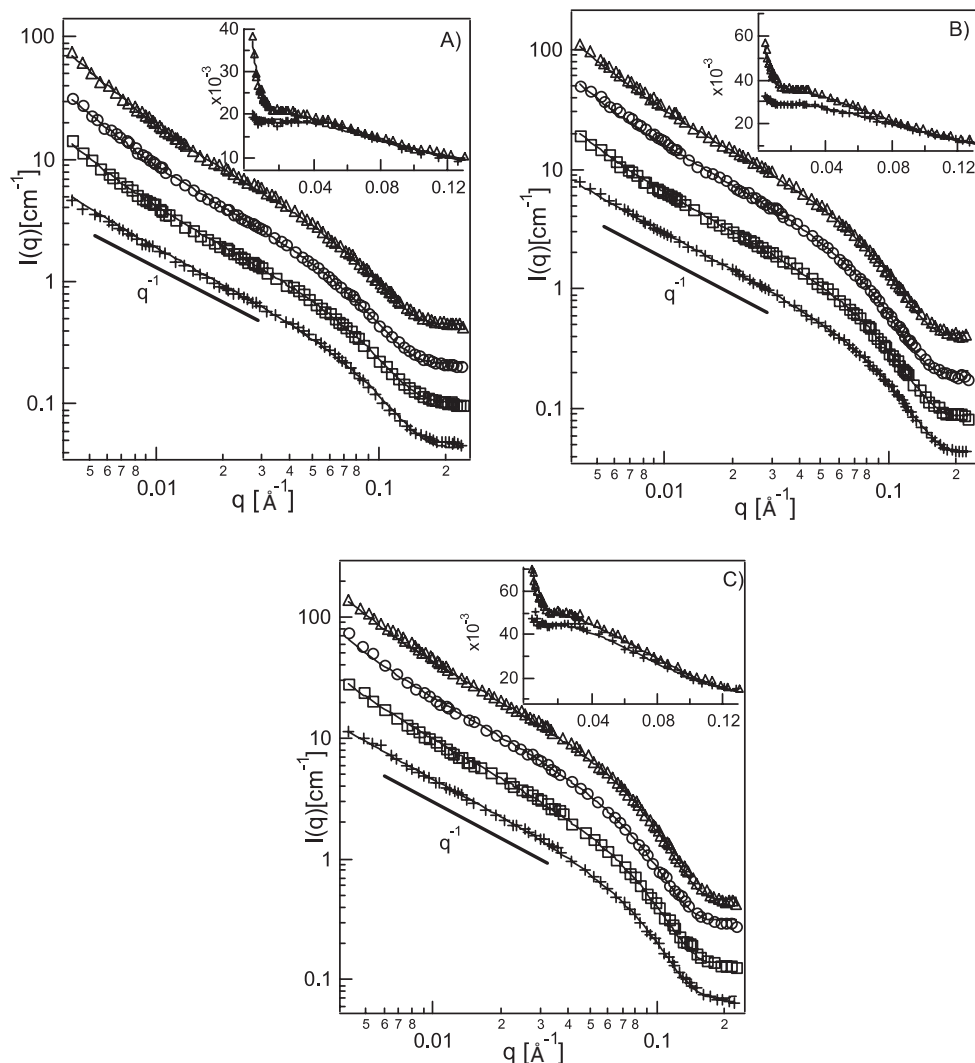


Figure 3. SANS spectra for DLPU micelles in NH_4Cl solutions; the solid lines represent the best fit according to equation (5). Each curve (both experimental spectra and fitting curves) has been offset on the y-axis by a factor of 2 with respect to the next below. The insets show the bending-rod representations of both experimental and fitting curves for the lowest (0.1 M) and highest (0.35 M) salt concentrations at a given surfactant volume fraction. (A) DLPU 4 mM. (B) DLPU 7 mM. (C) DLPU 10 mM. $[\text{NH}_4\text{Cl}]$: +, 0.1 M; \square , 0.18 M; \circ , 0.25 M; \triangle , 0.35 M.

water without added electrolytes revealed only a very modest micellar growth (Berti *et al* 2007). Therefore, the screening of electrostatic charges induces a meaningful difference in the chemical potential between a surfactant molecule located in the cylindrical body and in the hemispherical end-cap favoring the growth along the micellar contour.

A comparison with the previously obtained results for DLPU (Baldelli Bombelli *et al* 2002c) in 0.1 M PBS reveals that a close similarity between M_{app} and ξ_S values is obtained in the present case for a lower ionic strength, namely $I = 0.18$ M.

These two solutions (0.1 M PBS and 0.18 M NH_4Cl) have the same cation concentration, but a purely electrostatic argument would rather predict a closer similarity for the same ionic strength. However, a series of experimental observations, mainly performed on 1,2-dioctanoyl-*sn*-glycero-3-phosphatidyl-nucleoside monolayers at the air/water interface and globular micelles, have unexpectedly shown that anions have a Hofmeister-like impact on self-assembling properties of these anionic nucleolipids. This investigation (Fortini *et al*

2004) has shown that some surfactant properties, such as the surface excess or the cmc, correlate with anion features according to a pattern that resembles benzene salting out constants. It should be mentioned that DNA, notwithstanding its high charge density (one charge per 1.7 Å) follows a Hofmeister series for helix-coil transition temperatures (Hamaguchi and Geiduschek 1962, Franks and Lieb 1979). Without going into details of the possible interpretation of the underlying mechanism in terms of solvent quality or adsorption at the interface, we point out that the different growth behavior should be ascribed to a modulation of the chemical potential term contained in equation (1) and it is connected to the presence of nucleic bases on the surfactant head group.

Light scattering results agree with the picture of locally cylindrical structures growing along the micellar contour, but decisive evidence of the occurrence of such structures is not accessible in this q -range. Small angle neutron scattering provides a higher spatial resolution and allows inference of the local structure of worm-like micelles. Figures 3(A)–(C)

report SANS spectra in a double logarithmic representation of respectively 4 mM, 7 mM and 10 mM DLPU micellar solutions in 0.1–0.35 M NH_4Cl .

In the intermediate q region the scattered intensity follows a q^{-1} power law, a fingerprint of a locally cylindrical structure, invariant with surfactant and salt concentration.

SANS spectra of semi-flexible cylindrical aggregates can be opportunely represented as ‘bending-rod’ or Holtzer plots, $I(q) * q$ versus q (Denkiger and Burchard 1991). The insets in figures 3(A)–(C) show the Holtzer plots for the lowest (0.1 M) and highest (0.35 M) salt concentrations and highlight the q^{-1} region as a plateau. The slope change at lower scattering vectors reveals the fact that neutrons are probing different length scales, from rigid rods to flexible chains. This scaling switch has been exploited for a coarse evaluation of flexibility of aggregates, considering $l_p \sim 1.9/q$ (Marignan *et al* 1989). In analogy with polyelectrolytes, the persistence length of charged micellar worm-like chains can be, in the simplest approximation, described as arising from two decoupled contributions (Dautzenberg *et al* 1994):

$$l_{p,t} = l_{p,0} + l_{p,e}. \quad (10)$$

The intrinsic persistence length, $l_{p,0}$, is characteristic for a given surfactant and accounts for the effective flexibility of uncharged chains, while the electrostatic persistence length, $l_{p,e}$, depends on the electrostatic screening of the solution. Accordingly, any observed dependence on ionic strength should be ascribed to the electrostatic part.

An *a priori* evaluation of the intrinsic persistence length can be achieved by using the approach of Appell and Porte (Appel *et al* 1982b) readapted in a recent paper for SDS worm-like micelles in NaCl solutions (Magid *et al* 2000b).

In this approach cylindrical micelles are modeled as pearl necklaces (Stigter 1966) with the requirement that three consecutive beads of diameter d cannot overlap so that the ensemble of allowed configurations assumed by the string can lead to an estimation of $l_{p,0}$. In fact, from this assumption the center of bead n is joined to that of bead $n + 1$ or bead $n - 1$ by direction vectors of magnitude d , the angle θ between them can have with equal probability any value from 0 to 120° and the relationship $\langle \cos \theta \rangle = \exp(-\frac{2d}{l_{p,0}}) = 0.25$ holds.

Applying this model to DLPU micelles, we can substitute d in the previous relation with values ranging from 44 Å, twice the cross-sectional radius as determined by SANS, to 65 Å, that is the hydrodynamic diameter of DLPU cross section. This leads to $l_{p,0}$ values in the range 60–90 Å. The resulting intrinsic persistence length takes into account *only* the steric hindrance contribution due to the finite cross-sectional size of the micelle and can be regarded as a qualitative evaluation.

l_p 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 head group, namely on the local aggregation geometry (Israelachvili *et al* 1976, Israelachvili 1992, Gelbart *et al* 1994). The main factor contributing to the determination of the free energy cost to bend a portion of the micellar worm has been generally indicated in the literature as the hydrophobic chain length (Safran 1999, 2002), i.e. the higher the cross section the longer the directional

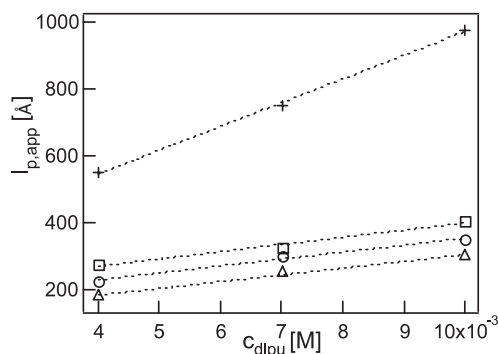


Figure 4. Dependence of total persistence lengths on DLPU concentration at different ionic strengths. Salt concentration: +, 0.1 M; □, 0.18 M; ○, 0.25 M; △, 0.35 M.

correlation. This argument is indeed reasonable for traditional surfactants, such as for the alkyl-sulfate series, but fails, for instance, when micellar growth is triggered by hydrotopic salts (for instance exchanging sodium salts with pyridinium salts (Magid *et al* 2000c)), that influence both contour length and flexibility. In this case a local electrostatic and hydrophobic modulation propagates on a higher length scale: therefore, a simple steric argument based on surfactant hydrophobic hindrance might not be sufficient to explain flexibility.

For DLPU, the chemistry of biologically relevant polar heads implies mutual interactions in the aggregate, not limited to electrostatic repulsions, that may well play an important role in micellar flexibility. The contribution of specific interactions between nucleic bases on the polar head, notwithstanding electrostatic repulsion, represents one of the main factors in determining micellar flexibility: an exchange of uridine with adenosine (or with a 1:1 adenosine uridine mixture), in the same experimental conditions and with the same hydrophobic portion and monomer size, has a dramatic effect on micellar structure, leading to the disappearance of the q^{-1} scaling for low surfactant volume fractions (Baldelli Bombelli *et al* 2006). Any modification of surface charge density will affect the headgroup environment and possibly base–base interactions; the ionic strength is therefore expected to influence the flexibility of the system not only through a simple electrostatic screening but also through the modulation of the interaction pattern between bases at the interface. A comparison with conventional micellar systems appears therefore interesting and worth investigating.

The persistence length has been obtained from SANS data through the procedure described in section 1, as follows. Full SANS curves have been first interpreted without taking into account intermicellar interactions, and the relative best fitting curves are reported in figures 3(A)–(C) as solid lines. The obtained structural parameters are summarized in table 3.

In this step the obtained $\langle L_c \rangle$ values, $\langle L_c \rangle_{\text{SANS}}$, are only qualitative, since the scattering vector range investigated is not extended enough and intermicellar interactions are neglected.

The resulting persistence lengths are biased by the neglect of intermicellar interactions and show a not genuine proportionality to surfactant concentration, as reported in figure 4. The extrapolation to infinite lipid dilution permits the

Table 3. Structural parameters obtained through worm-like non-linear least-squares fitting for DLPU micellar solutions as a function of medium salinity.

Sample	$\langle L_c \rangle_{\text{SANS}}$ (Å) ^a	$\langle L_c \rangle_{\text{SANS/SLS}}$ (Å) ^b	r_{cs} (Å) ^c	B (cm ⁻¹) ^d	$2 * l_{\text{p,app}}$ (Å) ^e	$l_{\text{p,c} \rightarrow 0}$ (Å) ^f
1	5021 ± 150	5011 ± 148	21.0 ± 0.6	0.044 ± 0.002	1099 ± 33	260 ± 25
2	5000 ± 155	5601 ± 165	21.2 ± 0.8	0.044 ± 0.002	1500 ± 41	
3	5270 ± 165	6160 ± 185	21.5 ± 0.6	0.071 ± 0.003	1950 ± 49	
4	4500 ± 130	5000 ± 135	22.0 ± 1.2	0.052 ± 0.002	550 ± 17	183 ± 25
5	5000 ± 143	5792 ± 173	22.0 ± 0.9	0.044 ± 0.002	650 ± 21	
6	5303 ± 156	6594 ± 183	22.4 ± 0.8	0.069 ± 0.003	811 ± 32	
7	5505 ± 165	5523 ± 143	23.6 ± 1.0	0.055 ± 0.002	450 ± 11	145 ± 21
8	5555 ± 180	6108 ± 165	24.0 ± 1.1	0.051 ± 0.002	600 ± 23	
9	6103 ± 195	6115 ± 155	23.8 ± 1.2	0.078 ± 0.003	700 ± 32	
10	5017 ± 151	5553 ± 174	23.5 ± 0.9	0.057 ± 0.003	356 ± 13	102 ± 15
11	6000 ± 173	6110 ± 155	23.4 ± 0.6	0.053 ± 0.003	500 ± 18	
12	6020 ± 177	5990 ± 143	23.6 ± 0.7	0.065 ± 0.003	603 ± 21	

^a Average contour length obtained by fitting SANS data.^b Optimized average contour length obtained by fitting combined SANS and SLS data.^c Cross section radius.^d Background.^e Apparent persistent length.^f Extrapolated persistence length.

determination of the real total persistence length for a given ionic strength, shown in the last column of table 3.

In the second step of the fitting procedure the persistence length is fixed to the corresponding extrapolated value and intermicellar interactions are included through the quantity M_{app} , that is an experimental parameter obtainable from static light scattering data, according to equation (8). For the other variational parameters, the best results obtained in the previous step are used as input values. Moreover, to obtain $\langle L_c \rangle$ evaluations, SANS data have been merged to SLS, to have an appropriate momentum transfer range and get reliable information on this quantity in real space. The best fitting curves are reported in figures 5(A)–(C) and the other resulting structural parameters are in close agreement with those previously determined shown in table 3. Chen *et al* (2006) have recently reported a SANS study on cationic worm-like micelles where they compare a modified fitting procedure to the well accepted model developed by Pedersen and Schurtenberger (1996a). These authors show that the inclusion of l_p as fitting parameter in the second step of the fitting significantly improves the quality of the curves. Although lower than in the first fitting step, they find a dependence of the micellar persistence length on surfactant concentration and do not rule out that it can be genuine. In our case, the inclusion of l_p as fitting parameter does not improve evidently the quality of the fitting and the final l_p values (if considered variational parameters in the second fitting step) differ from the extrapolated persistence length at infinite dilution within the experimental uncertainty. However, our experimental range is narrower both in terms of lipid concentration and of ionic strength and therefore we cannot fully appreciate a possible variance of l_p .

A plot of the total persistence lengths as a function of salt concentration, reported in figure 6, shows a trend ascribable to a decrease of the electrostatic contribution, $l_{\text{p,e}}$, as the screening on the micellar contour becomes more effective.

The functional prediction of the electrostatic contribution to the persistence length is rather debated in the literature: several theoretical models have been developed to estimate the influence of electrostatic interactions on flexibility of polyelectrolytes (Stevens and Kremer 1995). Odijk (1979), Skolnick and Fixman (1977) and Fixman (1982) modeled the electrostatic contribution to chain persistence length of polyelectrolytes near the rigid-rod limit. In this assumption, the contributions to total rigidity are not coupled, and the electrostatic contribution depends on the ratio of the Debye length, κ^{-1} , and the mean distance between charged groups, a , as follows:

$$l_{\text{p,e}} = \frac{L_B}{4} \cdot \left(\frac{\kappa^{-1}}{a} \right)^2. \quad (11)$$

In these limits $l_{\text{p,e}}$ exhibits a simple scaling with I^{-1} and the intrinsic and electrostatic contributions to rigidity are simply additive, as shown in equation (10); however, if the chain is intrinsically flexible, a more complex behavior and a scaling with $I^{-1/2}$ has been predicted by Ha and Thirumalai (1995) and Barrat and Joanny (1993) through different models. In this case the total persistence length value, beside the above mentioned scaling behavior, depends on the relative value of $l_{\text{p,0}}$ to L_B/a^2 .

Recently Buhler and Boue (2004) have reported an investigation about the flexibility of hyaluronan in aqueous solutions as a function of ionic strength, where they show that resulting experimental values of the electrostatic persistence length scale with κ^{-2} as predicted by OSF theory.

For mixed non-ionic/ionic worm-like micelles an agreement with OSF has been found: the authors highlight a better agreement between the theory and the data for the lowest ionic strengths (Sommer *et al* 2002).

The experimental scaling of electrostatic persistence lengths as a function of the salinity according to OSF (Odijk 1977, Skolnick and Fixman 1977) theory is not always

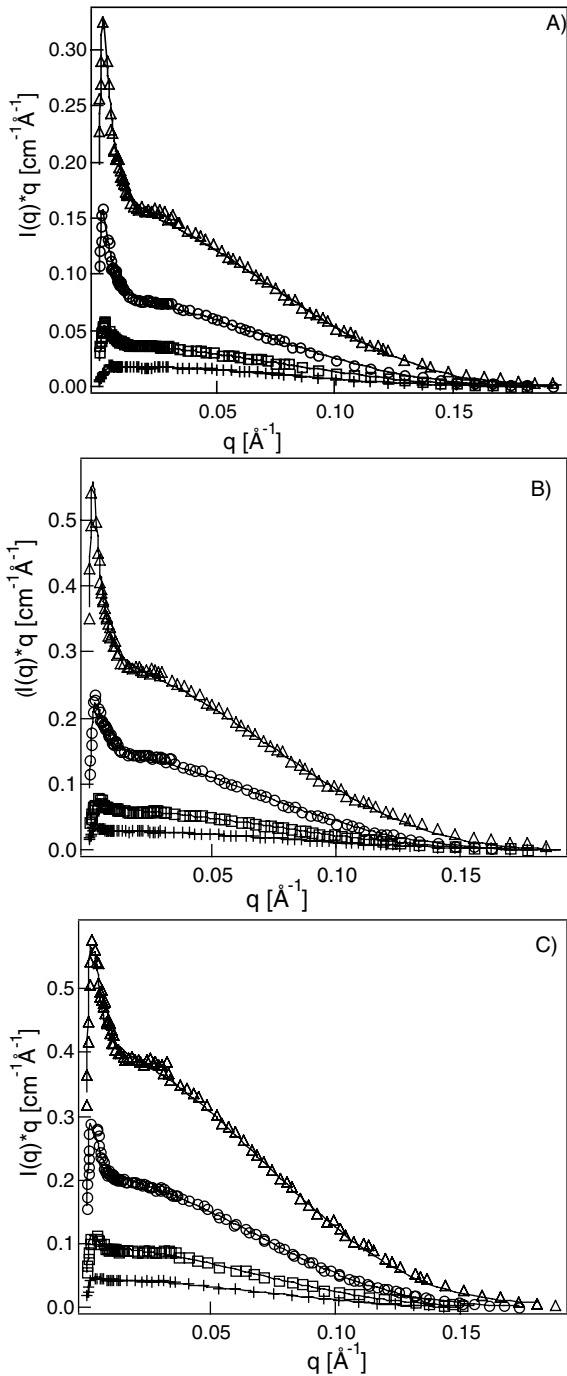


Figure 5. Holtzer plot representations, $I(q) * q$ versus q , of SLS and SANS spectra for DLPU micelles in NH_4Cl solutions; solid lines represent the best fit of worm-like micelles including intermicellar interactions. Each curve (both experimental spectra and fitting curves) has been offset on the y-axis by a factor of two with respect to the next below. (A) DLPU 4 mM. (B) DLPU 7 mM. (C) DLPU 10 mM. $[\text{NH}_4\text{Cl}]$: +, 0.1 M; \square , 0.18 M; \circ , 0.25 M; \triangle , 0.35 M.

followed: it scales with $c_s^{-0.5}$ for polysaccharides in aqueous solution (Ghosh *et al* 1990), while for SDS micelles (1–1.5 M added electrolyte) Magid *et al* (2000b) found an exponent between 0.5 and 1 for either $l_{p,e}$ or $l_{p,t}$.

It is then clear that the issue is under lively debate in the literature of polyelectrolytes, while the discussion is at

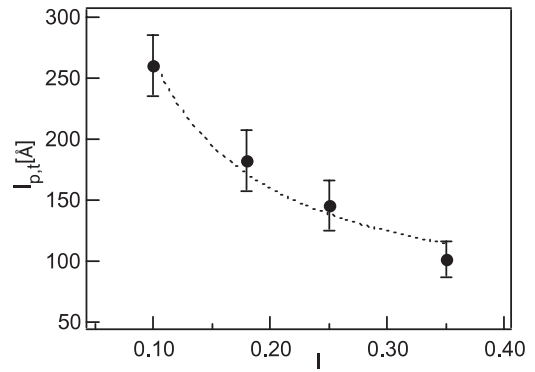


Figure 6. Total persistence length $l_{p,0}$ determined from SANS data as a function of salt concentration. The continuous line represents a guide for the eye according to a scaling law I^{-1} .

its early stages for polymer-like micelles, where a theoretical background that considers the finite cross section is still missing. Only very recently, Lauw *et al* (2007) have predicted by means of the self-consistent-field theory the persistence length of ionic worm-like micelles as a function of the pH and ionic strength and found a dependence of the electrostatic persistence length, $l_{p,e}$, on the Debye screening, κ^{-1} length as κ^{-p} , where p is in the range 2–1.5 (equal to scaling as $I^{-1} - I^{-0.75}$). Without entering into theoretical details, largely beyond the aim of the present paper, if we assume that the intrinsic contribution is anyhow salinity invariant, the asymptotic value for high ionic strength can be regarded as the intrinsic contribution to the rigidity.

An inspection of the plot reported in figure 6 reveals that at 0.35 M ionic strength we are not yet in the invariant regime. This is somewhat surprising, considering the low value of the screening distance (5.1 Å): therefore, electrostatic rigidity that acts on a length scale lower than the Debye length affects properties on the mesoscale, such as flexibility. Similar results have been found however for SDS worm-like micelles, whose Kuhn length determined from scattering data has been still found to vary in the range 1.0–2.0 M (Magid *et al* 2000b).

To extract an asymptotic value, we have to assume a functional dependence: the *a priori* evaluation according to the approach proposed by Appel and co-workers (Appel *et al* 1982b) yields an intrinsic value greatly above the Debye length: we can therefore tentatively apply OSF predictions.

Assuming a generic scaling law with I^{-1} the extracted asymptotic value for infinite ionic strength, that should give an evaluation of the intrinsic contribution, results for DLPU in around 60 Å; this experimental value shows a satisfying agreement with the simple *a priori* ‘steric hindrance’ approach 60–90 Å, confirming that the rigidity of DLPU micelles is highly dominated from low (0.1 M) to moderately high (0.18 M) salinity by electrostatic interactions.

In summary, even regarding the intrinsic persistence length found as indicative, it is clear that the electrostatic contribution is predominant at low ionic strength and that it decreases with increasing salt concentration according to a -1 power law. However, this contribution never levels off even for the highest salt concentration.

3. Conclusions

We have studied DLPU self-aggregation properties in aqueous solutions as a function of the ionic strength for three different surfactant concentrations. DLPU has a nucleic base as polar head and forms in PBS flexible cylindrical micelles already at volume fractions as low as 0.06%. Micellar contour length depends on surfactant concentration and/or added salt concentration. The experimental access to a wide scattering vector range, obtainable by combination of SLS and SANS, provides a complete structural characterization of the aggregates. The desired structural parameters have been extracted through a non-linear least-square fitting of the experimental spectra by a parametrized worm-like chain scattering function, obtained with Monte Carlo methods (Pedersen and Schurtenberger 1996a, 1996b).

Each monomer has a negative charge on the polar head that is partially screened in self-assembled ‘polyanions’: therefore, the flexibility of these objects is highly dependent on the ionic strength of the medium. We have focused our attention on the accurate evaluation of the aggregates’ flexibility in order to highlight its dependence on the charge density on the micellar contours. We could distinguish the electrostatic contribution to the persistence length from the intrinsic one and we have found that the electrostatic persistence length decreases with salt concentration according to a scaling law I^{-1} . DLPU micelles become more flexible with salinity increase. However, the electrostatic contribution to rigidity does not vanish even for high ionic strength. This behavior, already noticed for SDS worm-like micelles, is expected.

The parallelism between structural properties of worm-like micellar solutions and polymer solutions acquires for DLP-nucleosides a different meaning: due to the presence of chemical functionalities typical of nucleic acids, the analogy with a polymeric counterpart can be further extended to the comparison with natural polynucleotides rather than generic polymers. In this context the experimental determination of micellar flexibility, meant as a mesoscopic gage of molecular specificity, turns out to be of considerable importance.

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