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Structural features of a cationic gemini surfactant at full hydration investigated by energy dispersive X-ray diffraction

Giulio Caracciolo [a], Giovanna Mancini [b], Cecilia Bombelli [a], Ruggero Caminiti [a,*]

[a] *Dipartimento di Chimica and INFM, Università 'La Sapienza', P.le Aldo Moro 5, 00185 Rome, Italy*
[b] *IMC-Sezione Meccanismi di Reazione, c/o Dipartimento di Chimica, Università 'La Sapienza', P.le Aldo Moro 5, 00185 Rome, Italy*

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Abstract

Energy dispersive X-ray diffraction (EDXD) measurements were conducted on fully hydrated samples of the cationic gemini surfactant (2S,3S)-2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N*,*N*-dimethylammonium)butane dibromide, **SS**, as a function of temperature. The surfactant molecules self-assemble into multilamellar vesicles with a well-defined *d*-spacing which decreases as temperature increases. The derived structural parameters, such as bilayer thickness, size of the water region interbilayer, number of water molecules per surfactant molecule reveal reduced bilayer fluctuations, as a function of increasing temperature, consistent with the observed reduction of the water layer.

1. Introduction

Gemini surfactants are a wide class of amphiphilic molecules consisting of two identical surfactant units linked by a hydrocarbon spacer [1–3]. They present atypical physical and chemical properties compared to those of simple surfactants and lipids such as low critical micellar concentrations and 1000-fold higher surface activities [4,5]. In particular the variable length and flexibility of the linker can modulate the mobility and the packing of the monomer within the aggregate [6,7]. Cationic gemini surfactants have attracted a lot of attention in colloid and surface chemistry due to their peculiar characteristics [8]. Understanding the correlation between the chemical structure of a surfactant and the physical properties of the self-assembled aggregates in aqueous solution has been a central focus over the last two decades and could have a wide biological relevance. Recently, Gemini surfactants have also emerged as efficient transfection vehicles for carrying genetic material into eukaryotic cells especially if they can further be developed to compete with viral vectors in efficiency [9–12]. To this end, interest in the structure, morphology and physical properties of cationic Gemini surfactants is justified in view of the possibility that surfactant-based rational drug delivery systems can rely on physical predictions.

In this Letter we report an energy dispersive X-ray diffraction (EDXD) study, as a function of temperature, on fully hydrated liposomal preparations of the cationic gemini surfactant (2S,3S)-2,3-dimethoxy-1,4-bis(*N*-hexadecyl-*N*,*N*-dimethylammonium)butane dibromide, **SS** (chemical formula: $C_{42}H_{90}Br_2N_2O_2$; chemical structure reported in Fig. 1), as a function of temperature between 290 and 320 K [13].

In the present study we have used dimyristoyl phosphatidylcoline (DMPC) as a model system to test the applicability of our experimental technique to the study of lipid systems in excess water condition. The diffraction data were analyzed using a recently proposed method [13].

* Corresponding author. Fax: +39-06-490631.
E-mail address: r.caminiti@caspur.it (R. Caminiti).

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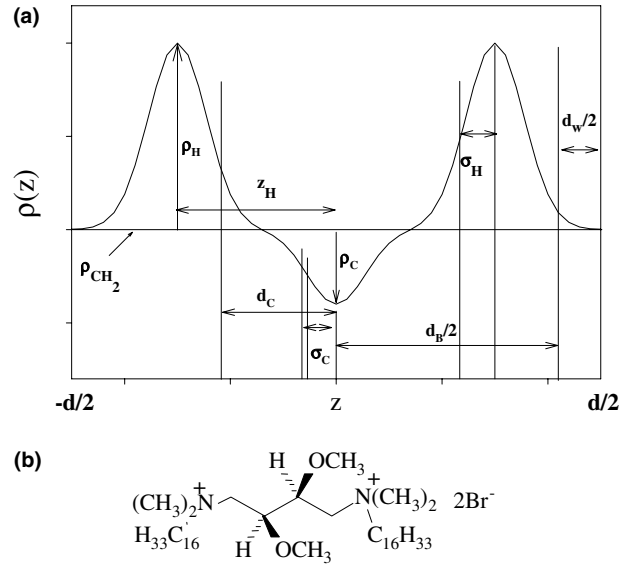

Fig. 1. (a) Electron density profile model. The distance z, calculated from the center of the bilayer, is along the normal to the bilayer; (b) the chemical structure of **SS** is schematically reported.

2. Experimental

2.1. Sample preparation

The aqueous dispersion of DMPC liposomes was prepared according to the procedure described by Hope et al. [14] A film of lipid (total 20.0 μmol) was prepared on the inside wall of a round bottom flask by evaporation of its $CHCl_3$ solution and was stored in a dessiccator overnight under reduced pressure. After addition of 1 ml of PBS buffer solution (10^{-2} M pH 7.4), the mixture was vortex-mixed and then freeze-thawed six times from liquid nitrogen to 313 K. Extruded vesicles were prepared passing the dispersion 10 times through a 100 nm polycarbonate membrane (Whatman Nucleopore®). The extrusions were carried out at 307 K, well above the transition temperature of DMPC (298 K), using a 2.5 ml extruder (Lipex Biomembranes, Vancouver, Canada). **SS** samples were prepared by quaternization of the proper diastereomer of 2,3-dimethoxy-1,4-bis(*N*,*N*-dimethylamino)butane with a 20% excess of 1-bromohexadecane in benzene at ambient temperature. The white precipitates were purified and characterized as previously described [9]. A 0.012 M aqueous solution of **SS** was prepared by adding 1 ml of bidistilled water to 10 mg of surfactant and gently heating till complete solubilization. The lipid films were subsequently fully hydrated from a water-saturated vapor for hydration. This procedure allows to prepare fully hydrated double layers [15].

2.2. X-ray diffraction experiments

The diffraction experiments were carried out using an energy dispersive X-ray diffractometer elsewhere described [16]. This instrument is composed of two optical benches pivoting around a single central axis perpendicular to the vertical scattering plane. This common rotation axis is at the sample position. The diffracted intensity is measured as a function of the exchanged momentum $q = cE\sin\theta$ ($c = 1.014$ Å^{-1} keV^{-1}) so that we can perform our measurements at a fixed diffraction angle θ, having a white incident polychromatic beam and an energy dispersive detector. The polychromatic radiation is produced by a standard X-ray tube mounted on one arm while the EG&G solid-state acquisition system is on the other one. The benches are moved by two linear actuators whose minimum step movement leads to a minimum angle incrementum and reproduction of 0.005°.

X-ray measurements of the DMPC dispersions were carried out at a diffraction angle $\theta = 0.4°$ covering a q-range between 0.08 and 0.32 Å^{-1}. X-ray diffraction patterns from **SS** dispersion were recorded as a function of temperature at the suitable diffraction angles $\theta = 0.7°$ and $\theta = 0.95°$ covering a larger q-range between 0.14 and 0.8 Å^{-1}. The samples were placed in a suitable cell. The temperature of the sample holder was controlled by a circulating liquid bath to within ±0.5 K. Previous to exposing the sample, it was equilibrated for a period of 2000 s for each given temperature. Typical acquisition times, depending on the sample concentration and the desired accuracy of experimental data, varied between 1000 and 5000 s. No structural modification or damage occurred during data acquisition because of the low X-ray dose to which the samples were exposed as EDXD experiments proceeded. The plain evidence for no damage to biological samples was carely discussed in a previous paper [17]. This fact strictly depends on the low X-ray dose to which the samples are exposed as EDXD experiments proceed. Indeed, the overall dose ($\sim 10^6$ photons $\text{s}^{-1}\,\text{mm}^{-2}$) is less than 100-fold that of a typical flux absorbed during data collection using moderate-intensity synchrotron radiation facilities. All the diffraction data were corrected for the transport function of the detector and for the parasitic effects arising from the water and the mylar windows of the sample cell. Since Bremmstrahlung radiation is a continuous function of energy, EDXD patterns have to be normalized to the incident radiation. The measurement of the incident spectrum is collected by placing the diffractometer arms in straight position (0° inclination angle) and acquiring the direct beam spectrum. Diffraction data were in turn divided to this white spectrum.

3. Results and discussion

EDXD experiments were first conducted on fully hydrated samples of DMPC at 300 K. At this temperature the phospholipid is in the biologically relevant liquid crystalline phase (smectic A) in which the acyl chains are melted and experience *trans-gauche* isomerizations [18]. This lipid/water system has been largely studied ([19] and references therein) as a model system of biological membranes especially due to its anomalous behaviours close to the main transition (liquid crystalline $\rightarrow$ ripple phase) temperature ($T \sim 298$ K). The powder data were analyzed using a recently proposed full q refinement method [13]. Even if liposomal suspensions usually give rise to a few number of weak Bragg reflections, nevertheless the developed theoretical apparatus allows to obtain structural information such as membrane thickness, hydrocarbon chain length and the number of interbilayer water molecules per lipid without additional volumetric measurements. According to the model the diffracted intensity is analyzed in terms of

$$I(q) = \frac{S(q)|F(q)|^2}{q^2}. \tag{1}$$

The proposed method utilizes a $S(q)$ modified Caillé theory (MCT) structure factor given by the following expression:

$$S(q) = N + 2\sum_{k=1}^{N-1}(N-k) \times \cos(kqd)\mathrm{e}^{-(d/2\pi)^2 q^2 \eta_1 \gamma}(\pi k)^{-(d/2\pi)^2 q^2 \eta_1}, \tag{2}$$

where N is the mean number of coherently scattering bilayers, γ is the Euler's constant, d is the lamellar periodicity and η_1 is the Caillé parameter

$$\eta_1 = \frac{\pi k_B T}{2\sqrt{BK_C}d^2}, \tag{3}$$

which depends on the bulk modulus of compression B and the bending rigidity of the bilayer K_C [13]. In the case of un-oriented lipid vesicles the Caillé theory yields η_1 whereas no information can be obtained about K_C and B independently.

The form factor $F(q)$ is the Fourier transform of the electron density profile $\rho(z)$ which is modeled as a summation of two Gaussians representing the polar region and the methyl group (Fig. 1)

$$F(q) = \sqrt{2\pi}[2\sigma_H \exp(-\sigma_H^2 q^2/2)\cos(qz_H) + \sigma_C \rho \exp(-\sigma_C^2 q^2/2)]. \tag{4}$$

The widths of the Gaussians are given by σ_H and σ_C whereas $\rho = \rho_H/\rho_C$ is the ratio between the electron densities of the headgroup, ρ_H and the hydrocarbon tails, ρ_C, both normalized to the methylene electron density ρ_{CH_2}. Interestingly, the model relies on the assumption that, along the normal to the bilayer, a region of constant electron density is occupied by the methylene groups on the fatty acid chains, between the headgroups and the methyls. This region can be modeled as a strip of constant electron density. Fig. 1 also displays that the bilayer thickness can be retrieved by the following geometric relation:

$$d_B = 2(z_H + 2\sigma_H), \tag{5}$$

whereas the hydrocarbon chain length is

$$d_C = z_H - \sigma_H, \tag{6}$$

where σ_H, according to [19], is the half-width at half-maximum of the Gaussian representing the polar region and can be assumed as a realistic estimate of the headgroup size. Simple geometrical considerations allow to calculate the water layer thickness d_w,

$$d_w = d - d_B, \tag{7}$$

and the total number of water molecules per lipid molecule, including the molecules inserted into the lipid bilayer

$$n_w = \frac{A(d/2 - z_H)}{V_w}, \tag{8}$$

where A is the area per lipid and V_w is the volume of one water molecule (~ 30 Å^3). For determining A, different methods have been proposed ([19] and references therein). Among these, the approach suggested by Lemmich [20] and reported by Pabst et al. [13] does not require volumetric data

$$A = \frac{1}{\rho_{CH_2}(\rho - 1)}\left(\frac{\rho n_C^e}{d_C} - \frac{n_H^e}{d_H}\right), \tag{9}$$

where n_C^e is the number of hydrocarbon electrons, n_H^e the number of headgroup electrons and d_H is the headgroup size which can be estimated from the full width at half maximum of the Gaussian which represent the headgroup itself.

The EDXD pattern from the liquid crystalline phase of unoriented multilamellar DMPC liposomes is shown in Fig. 2. A lamellar periodicity $d = 63.8$ Å was obtained for the DMPC sample as determined from the sharp first and second-order Bragg peaks. Considering the well-defined multilamellar structures observed in electron microscopy (EM) images (data not reported), the pattern of Fig. 2 confirm the presence of multilamellar vesicles and can be interpreted in terms of the (0 0 1) reflections of multilamellar structures with a repeat distance $d = 2\pi/q_{001}$.

Each multilamellar vesicle consists of concentrical spheres with the normal to the bilayers isotropically distributed in space. Such unoriented samples display a weak diffraction power which is taken into account by

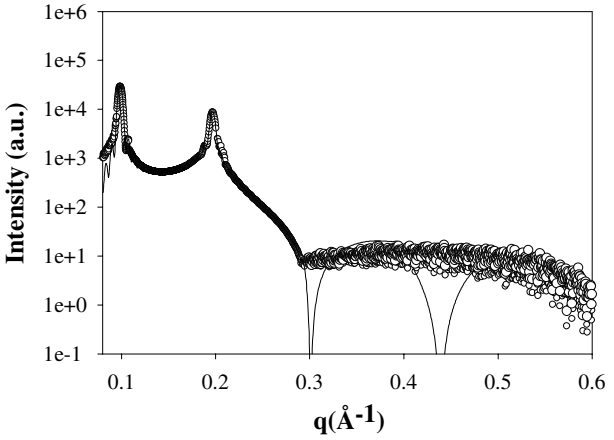

Fig. 2. EDXD pattern of DMPC multilamellar liposomes at 300 K. The solid line represents the best fit to diffraction data obtained applying the full q refinement model (for details see the text).

the Lorentz factor $1/q^2$ in Eq. (1). Fig. 2 also shows the best fit (solid line) to the experimental data obtained applying Eq. (1) and the structural fit results are reported in Table 1.

Nagle and Tristram-Nagle ([21] and references therein) reported only two orders of diffraction for unoriented DMPC bilayers in excess water conditions. Unfortunately, it is well established that detailed structural information, such as the widths of the headgroup and the methyl, cannot be anyway retrieved trough Gaussians if less of four orders of diffraction are recorded and only the Bragg peaks are considered. Partly dehydration of the sample via osmotic experiments is a common way to circumvent this problem. Detailed structural information on the fully hydrated system is obtained through subsequent extrapolations to zero osmotic pressure which, unfortunately, can be inherent to uncertainties. Pabst et al. [19] collected intensity data up to $q = 0.55$ Å^{-1} but, even in this study, the Bragg reflection information content consisted of only two orders of diffraction plus diffuse scattering. On the other hand, the above discussed MCG theory [13] also describes the diffuse scattering and finds out structural information hidden in the diffuse background scattering between Bragg peaks. Applying it, Pabst et al. were able to derive fundamental structural parameters of fully hydrated DMPC bilayers suggesting an attractive explanation to the pretransitional anomalous swelling of DMPC. The used procedure appeared to be a very promising method to derive structural parameters even if the number of recorded Bragg peaks is less than four. This is the main reason for applying MCG theory in the present study. Combining Eqs. (6) and (9) the area per lipid $A = 58.9$ Å^2 was calculated and this value seems to be in very close agreement to the value reported by Pabst [19] at the same temperature. For this calculation, the methylene electron density $\rho_{CH_2} = 0.292$ e Å^{-3} was calculated using the methylene volume reported by Nagle and Tristram-Nagle [21] for the liquid crystalline phase of lecithins.

Table 1
Structural parameters of the full q refinement model applied to the EDXD data of DMPC at 300 K

Parameter	Value
N	17 ± 1
d (Å)	63.8 ± 0.1
d_B (Å)	46.8
d_C (Å)	13.3
d_w (Å)	16.5 ± 0.1
n_w	29
z_H (Å)	16.8
σ_H (Å)	3.29
σ_C (Å)	4.45
ρ	-1.15 ± 0.001
η_1	0.081
k^* (10^{-2} a.u.)	1.3

* k is the scaling factor of the model to the diffracted intensity.

Inserting then this value into Eq. (8) the number of water molecules per lipid molecule $n_w = 29$ was also obtained. In addition, information about the number of waters per lipid, required to fully hydrate multilamellar arrays was also obtained from thermogravimetric measurements which closely confirmed the calculated value. In conclusion, all the structural parameters obtained applying the full q refinement model to our powder diffraction data are in good agreement with those elsewhere reported [13,21]. Thus, these preliminary measurements on the well-characterized DMPC/water system in the liquid crystalline phase revealed that EDXD can be successfully used to derive structural information from unoriented multilamellar liposomes at full hydration which actually represent a frequent measurement condition.

As above discussed, the relationship between the chemical structure of a surfactant and the physical properties of the self-assembled aggregates in aqueous solution is not a trivial argument and is currently attracting a great deal of attention [7]. The structural properties of the cationic gemini surfactant **SS** were investigated under experimental conditions analogous to those employed in the case of DMPC. The fully hydrated samples were explored as a function of temperature between 290 and 320 K. We were particularly interested in this 'physiologically relevant' temperature range because of the promising use of **SS** as DNA condensing agent in binary surfactant mixture employed in gene delivery [7]. A detailed picture of the thermal behaviour of **SS** is plotted in Fig. 3. At 290 K the diffraction pattern shows a pronounced diffraction maximum and its higher order reflections at $q = 0.219$ Å^{-1}, $q = 0.438$ Å^{-1} and $q = 0.655$ Å^{-1}, respectively. According to EM images (data not reported) we take these reflections to be the crystallographic reflections of

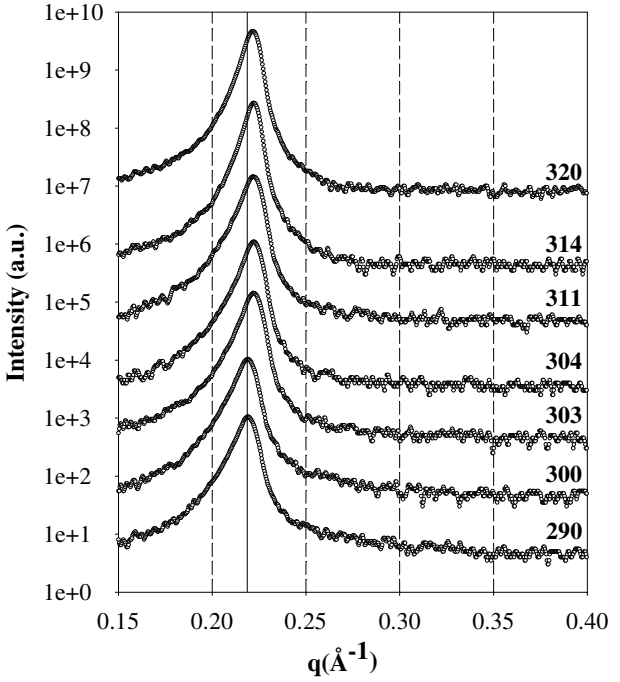

Fig. 3. First-order Bragg peak of **SS** multilamellar aggregates as a function of temperature (expressed in Kelvin). The solid line is a guide to the eye.

multilamellar structures with a characteristic d-spacing of 28.7 Å. This finding confirm, that **SS** molecules, in aqueous solution, assemble into multilamellar vesicles with a well-defined stacking repeat distance. Firstly, the small value of the repeat unit implies that the C_{16} acyl chains are melted and conformationally disordered in contrast to the nearby *all-trans* configuration in lipid crystals. Otherwise, the very small d-spacing could also indicate that **SS** has interdigitated chains.

Moreover, this finding could suggest that the thickness of the water layer between opposing bilayers is extremely thin.

In order to elucidate the physical properties of the aggregates, all the powder data were analyzed using the full q refinement model. Fig. 4 shows a typical best fit to our experimental data. The structural results obtained using Eqs. (5)–(8) are listed in Table 2.

It is noteworthy to evidence that the method gives an appreciable fit for the full q-range investigated, i.e., including the diffuse scattering between Bragg peaks which is modulated by the bilayer form factor.

It is not an irrelevant point. Indeed, the usually applied MCT data analysis only works in proximity of Bragg peaks neglecting diffuse scattering between them [22].

Starting at 290 K and on increasing temperature, the bilayer shows a continuous decrease in thickness (Fig. 5a) and the thin water layer experiences about the same reduction (Fig. 5b). Thus we can assume that the observed decrease in d can be essentially attributed to a

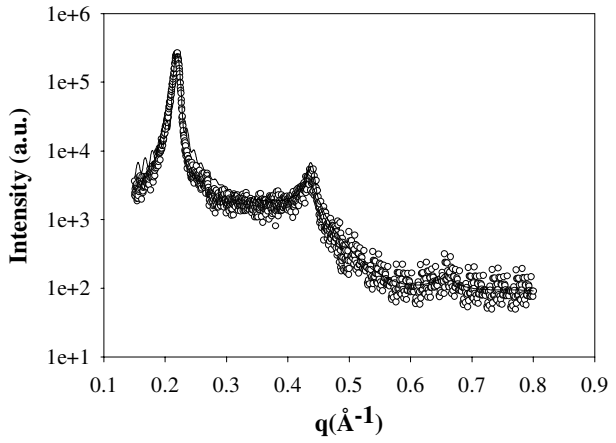

Fig. 4. Diffraction pattern of **SS** at 290 K. The solid line is the best fit to the data.

Table 2
Structural parameters of the full q refinement model applied to the EDXD data of cationic Gemini surfactant **SS** at 290 K

Parameter	Value
N	19 ± 1
d (Å)	28.7 ± 0.1
d_B (Å)	24.6 ± 0.1
d_C (Å)	10.5 ± 0.1
d_w (Å)	4.1 ± 0.1
n_w	3.5
z_H (Å)	11.02 ± 0.06
σ_H (Å)	0.51 ± 0.06
σ_C (Å)	2.51 ± 0.06
k^* (10^{-2} a.u.)	1.3

* k is the scaling factor of the model to the diffracted intensity.

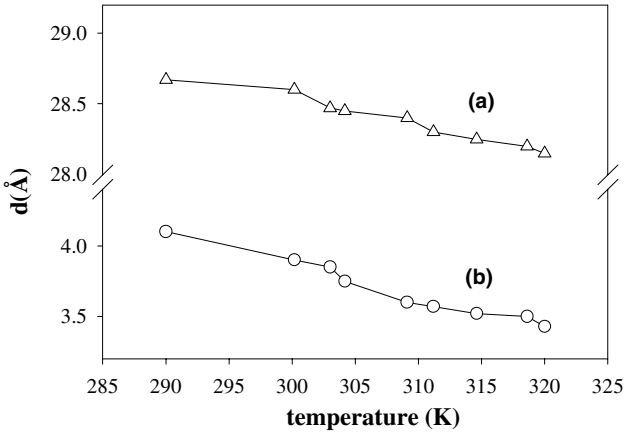

Fig. 5. Thermal behaviour of the d-spacing (a), the water layer thickness d_w (b), as a function of temperature in the range 290–320 K in fully hydrated **SS** samples.

reduction of the thickness of the water layer. This effect was also observed in cooling scans (data not reported) with only slight differences in the relative amplitudes of diffraction maxima.

The reduction in d-spacing observed in the investigated temperature range is similar to that found in 1-palmitoyl-2oleoyl-sn-glycero-3-phosphocoline (POPC) multilamellar vesicles by Pabst et al. [23] and in many other lipid/water systems [24–26]. The physical mechanism underlying the observed diminution in d could also be related to an increasing bending rigidity modulus. The thermally induced reduction of membrane flexibility leads to reducted density thermal fluctuations which would reduce repulsive forces between bilayers and therefore reduce d_w. Furthermore, it has recently been proposed that the degree of water ordering increases with decreasing interlamellar separation [27]. These effects can drastically restrict the motional freedom in direction along the membrane normal. As a result, the amplitude of the diffraction maxima increase and the first-order full-width at half-maximum (FWHM) decrease as temperature increases. This interpretation is enforced by the evolution of the normalized diffracted intensity which monotonously increases with growing temperature as evident in Fig. 3 and confirmed by other studies [23,24]. Additionally we observe that the Bragg peaks become shorter and the curve between them becomes higher, the farther from the origin.

These effects are imputable to the 'second-order disorder' caused by the random variations in the distance from one bilayer to the next [28,29]. This is also confirmed by the observation that the FWHM's increase with increasing transfer momentum. The total number of water molecules can be estimated if the lateral area A per lipid molecule is effectively known.

From Eq. (9) a value $A = 34$ Å^2 is directly calculated.

Combining Eqs. (8) and (9) we found $n_w = 3.3$ waters per **SS** molecule at 290 K whereas thermogravimetric measurements revealed the very close value $n_w = 3.5$. This relatively small number of tightly bound water molecules could probably indicate a very rigid packing of the headgroups. Furthermore the same nature of the interfacial region and, in particular, the capability to form hydrogen bonds seems to be of central importance [28].

Recent studies confirm that the number of water molecules held around the different components of the headgroup strictly depends on the directional hydrogen bonding ability in the headgroup. In the second solvation shell, the water molecules are weakly bonded even if they are absolutely critical for determining the physical properties of the system [30].

Indeed the hydrophilicity and wetting of bilayer surface are essentially attributed to the attractive forces between surface groups and water via hydrogen bonds. In this sense, the nature of the headgroup may be the rationale to govern the level of hydration [31,32].

In conclusion, our results demonstrate that EDXD can be successfully applied to retrieve detailed structural informations from multilamellar liposomes at full hydration. We have also shown that **SS** molecules in aqueous solution self-assemble into multilamellar vesicles with a well-defined d-spacing which decreases as temperature increases between 290 and 320 K. We have applied the full q refinement model to this gemini surfactant for the first time characterizing the structural features, packing properties and level of hydration of the aggregates in aqueous solution. Further experimental efforts to investigate the physical properties of binary mixtures containing cationic surfactant **SS** as DNA condensing agent are currently in progress in our laboratory.

References

[1] F.M. Menger, J.S. Keiper, Angew. Chem., Int. Ed. 39 (2000) 1906, and references therein.
[2] K. Kwetkat, WO 9731890, 1997.
[3] L. Perez, J.L. Torres, A. Manrese, C. Solans, M.R. Infante, Langmuir 12 (1996) 5296;
F.M. Menger, N.A. Mbadugha, J. Am. Chem. Soc. 123 (2001) 875.
[4] C. McGregor, C. Perrin, M. Monck, P. Camilleri, A.J. Kirby, J. Am. Chem. Soc. 123 (2001) 6215.
[5] V. Matti, J. Säily, S.J. Ryhänen, J.M. Holopainen, S. Borocci, G. Mancini, P. Kinnunen, Biophys. J. 81 (2001) 2135.
[6] K.H. Jennings, I.C.B. Marshall, M.J. Wilkinson, A. Kremer, A.J. Kirby, P. Camilleri, Langmuir 18 (2002) 2426.
[7] S.J. Ryhänen, M.J. Säily, T. Paukku, S. Borocci, G. Mancini, J.M. Holopainen, P.K.J. Kinnunen, Biophys. J. 84 (2003) 578.
[8] P.C. Bell, M. Bergmsa, I.P. Dolbnya, W. Bras, M.C.A. Stuart, A.E. Rowan, M.C. Feiters, J.B.F.N. Engberts, J. Am. Chem. Soc. 125 (2003) 1551.
[9] L. Luchetti, G. Mancini, Langmuir 16 (2000) 161.
[10] J. Chlotier, S.J. Tomellini, Chromatogr. A 723 (1996) 179.
[11] Y. Mechref, Z. Elrassi, Chirality 8 (1996) 518.
[12] P. Desbene, C. Fulchic, J. Chromatogr. A 749 (1996) 257.
[13] G. Pabst, M. Rappolt, H. Amenitsch, P. Laggner, Phys. Rev. E 62 (3) (2000) 4000.
[14] M.J. Hope, M.B. Bally, G. Webb, P.R. Cullis, Biochim. Biophys. Acta 812 (1985) 55.
[15] G. Caracciolo, G. Mancini, C. Bombelli, P. Luciani, R. Caminiti, J. Phys. Chem. B 107 (44) (2003) 12268.
[16] R. Caminiti, V. Rossi Alberini, Int. Rev. Phys. Chem. 18 (2) (1999) 263.
[17] G. Caracciolo, G. Amiconi, L. Bencivenni, G. Boumis, R. Caminiti, E. Finocchiaro, B. Maras, C. Paolinelli, A. Congiu Castellano, Eur. Biophys. J. 30 (2001) 163.
[18] H.L. Casal, R.N. McElhaney, Biochemistry 29 (1990) 5423.
[19] G. Pabst, J. Katsaras, V.A. Raghunathan, M. Rappolt, Langmuir 19 (2003) 1716.
[20] J. Lemmich, Phys. Rev. E 53 (1996) 5169.
[21] J.F. Nagle, S. Tristram-Nagle, Biochim. Biophys. Acta 159 (2000) 1469.
[22] R. Zhang, W. Sun, S. Tristram-Nagle, R.L. Headrick, R.M. Suter, J. Nagle, Phys. Rev. Lett. 74 (14) (1995) 2832.
[23] G. Pabst, J. Katsaras, V.A. Raghunathan, Phys. Rev. Lett. 88 (12) (2002) 128101.
[24] T. Hongher, K. Mortensen, J.H. Ipsen, J. Lemmich, E. Bauer, O.G. Mouritsen, Phys. Rev. Lett. 72 (24) (1994) 3911.
[25] J. Lemmich, K. Mortensen, J.H.. Ipsen, T. Hongher, E. Bauer, O.G. Mouritsen, Phys. Rev. Lett. 75 (21) (1995) 3958.
[26] F.Y. Chen, W.C. Hung, H.W. Huang, Phys. Rev. Lett. 79 (20) (1997) 4026.

[27] L. Perera, U. Essmann, M.L. Berkowitz, Langmuir 12 (1996) 2625.
[28] H. Binder, B. Kohlstrunk, W. Pohle, J. Phys. Chem. B 104 (2000) 12049.
[29] A. Guinier, X-ray Diffraction, Freeman, San Francisco, 1963.
[30] M. Pasenkiewicz-Gierula, T. Rog, K. Kitamure, A. Kusumi, Biophys. J. 78 (2000) 1376.
[31] C. Ho, C.D. Stubbs, Biophys. J. 63 (4) (1992) 897.
[32] C. Ho, S.J. Slater, C.D. Stubbs, Biochemistry 34 (18) (1995) 6188.