

pH-Dependent Aggregation Properties of Mixtures of Sugar-Based Gemini Surfactants with Phospholipids and Single-Tailed Surfactants

Marco Scarzello,[†] Jaap E. Klijn,[†] Anno Wagenaar,[†] Marc C. A. Stuart,[†] Ron Hulst,[‡] and Jan B. F. N. Engberts^{*,†}

Physical Organic Chemistry Unit, Stratingh Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands, and Kiadis BV, Zernikepark 6-8, 9747 AN Groningen, The Netherlands

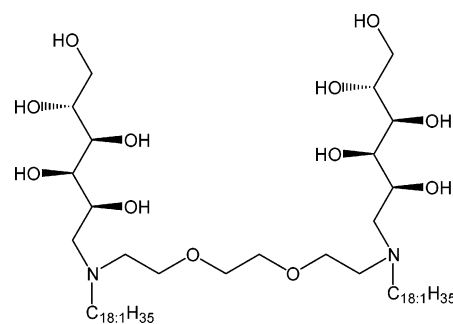
Received November 28, 2005. In Final Form: January 13, 2006

Sugar-based gemini surfactants (GSs) display rich pH-dependent phase diagrams and are considered to be promising candidates as gene- and drug-delivery vehicles for biomedical applications. Several sugar-based GSs form vesicles around neutral pH. The vesicular dispersions undergo transitions toward wormlike micelles and spherical micelles at acidic pH, whereas flocculation followed by redispersion upon charge reversal is observed at basic pH. The influence of various amounts of the double-tailed phospholipids DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) and DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine) and of the single-tailed surfactants lyso-PC (1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine) and OTAC (octadecyltrimethylammonium chloride) on the phase behavior of GS1 (1,8-bis(*N*-octadec-9-yl-1-deoxy-*D*-glucitol-1-ylamino)3,6-dioxaoctane) was determined as a function of pH, in water and in water at physiological ionic strength. The pH corresponding to the phase transitions and the characteristics of the aggregates were determined by means of a combination of physical techniques: static and dynamic light scattering (SLS and DLS), fluorescence spectroscopy, cryo-TEM and diffusion- and ³¹P NMR. The results show that the additives affect the phase behavior of the GS1 dispersions in a pH-dependent fashion. In the presence of double-tailed phospholipids, a higher degree of protonation of GS1 must be reached to observe micelle formation, whereas single-tailed surfactants affect these transitions only slightly. In the presence of increasing amounts of lyso-PC, the pH range of flocculation becomes more narrow, indicating the increased hydration of the vesicles. The pH of redispersion after charge reversal is particularly sensitive to the presence of positively charged additives. It is suggested that the cationic headgroups disturb the hydrogen-bond structure of water at the vesicular surface, hampering OH[−] binding. The effect of an increase in ionic strength to physiological values is found to be modest, except for the dispersions containing the positively charged additives.

Introduction

A gemini surfactant (GS) is obtained by connecting two single-tailed surfactants via a spacer at the level of the headgroups. GSs generally present a lower cmc and a greater ability to decrease the surface tension of water with respect to the parent surfactants,^{1,2} which are advantageous characteristics for practical applications. Recently, sugar-based GSs with pH-dependent aggregation properties were synthesized in our group and studied as nonviral gene-delivery agents for eukaryotic cell lines;^{3,4} promising results have been obtained with these compounds also in in vivo experiments (manuscript submitted). The excellent transfection efficiencies displayed by these compounds have been associated with morphological transitions of the lipid–DNA complex (lipoplex).⁴ The pH dependence of the phase behavior of a pure sugar-based GS comes from the presence in the headgroup of two amino moieties (estimated p*K*_a values are 6.0 and 8.1),⁵ whose degree of protonation is a function of pH.^{5–7} Upon decreasing the pH of a vesicular dispersion of the sugar-based

Scheme 1. Structure of the Sugar-Based Gemini Surfactant GS1



GS1, (1,8-bis(*N*-octadec-9-yl-1-deoxy-*D*-glucitol-1-ylamino)3,6-dioxaoctane, Scheme 1) prepared around neutral pH, the system undergoes transitions from a lamellar phase to wormlike micelles and ultimately to spherical micelles. By increasing the pH, the colloidal stability of the vesicular suspension decreases when the surface charge approaches neutrality (ζ potential $< +15$ mV),⁵ resulting in rapid flocculation. Interestingly, a further increase in pH leads to charge reversal and, for ζ potentials < -15 mV,⁵ to redispersion of the flocculated material as negatively charged vesicles. The negative charge appears to be due to selective hydroxide adsorption at the vesicular surface. Dynamic light scattering experiments, performed before and after charge reversal, showed that flocculation is not associated with vesicle fusion because the size distribution of the negatively charged

* Corresponding author. E-mail: j.b.f.n.engberts@rug.nl.

[†] University of Groningen.

[‡] Kiadis BV.

(1) Esumi, K.; Ueno, M. *Structure-Performance Relationships in Surfactants*; Marcel Dekker: New York, 1997; Vol. 70.

(2) Menger, F. M.; Keiper, J. S. *Angew. Chem., Int. Ed.* **2000**, *39*, 1907–1920.

(3) Fielden, M. L.; Perrin, C.; Kremer, A.; Bergsma, M.; Stuart, M. C.; Camilleri, P.; Engberts, J. B. F. N. *Eur. J. Biochem.* **2001**, *268*, 1269–1279.

(4) Bell, P. C.; Bergsma, M.; Dolbnya, I. P.; Bras, W.; Stuart, M. C. A.; Rowan, A. E.; Feiters, M. C.; Engberts, J. B. F. N. *J. Am. Chem. Soc.* **2003**, *125*, 1551–1558.

(5) Johnsson, M.; Wagenaar, A.; Stuart, M. C. A.; Engberts, J. B. F. N. *Langmuir* **2003**, *19*, 4609–4618.

(6) Johnsson, M.; Wagenaar, A.; Engberts, J. B. F. N. *J. Am. Chem. Soc.* **2003**, *125*, 757–760.

(7) Johnsson, M.; Engberts, J. B. F. N. *J. Phys. Org. Chem.* **2004**, *17*, 934–944.

vesicles after redispersal is comparable to that of the positively charged ones.

In this study, we use a combination of static and dynamic light scattering (SLS and DLS), Nile red fluorescence, ^{31}P NMR, and diffusion ^1H NMR to determine the effects of physiological ionic strength conditions (NaCl 150 mM) and double- (DOPC and DOPE) and single-tailed (lyso-PC and OTAC) amphiphilic additives on the phase behavior of GS1. (The molecular structures of the additives are reported in the Supporting Information, Figure S1.)

The data obtained demonstrate that in the presence of double-tailed phospholipids the formation of highly curved aggregates requires a higher degree of protonation of GS1 (i.e., lower pH), whereas single-tailed surfactants affect these transitions only slightly. Lyso-PC increases the colloidal stability of the vesicles, most likely increasing the hydration with respect to that of pure GS1 dispersions. The results further suggest that positively charged additives might hamper OH^- binding, disturbing the water structure at the surface. Finally, the effect of an increase in ionic strength to physiological values is found to be modest, except for dispersions containing positively charged additives.

These results are expected to be helpful in the optimization of the GS preparations, in particular, for biomedical applications, providing possibilities for modulating the morphologies of the aggregates under physiologically relevant conditions.

Experimental Procedures

GS1 was synthesized according to a new procedure that will be reported elsewhere (manuscript in preparation). ^1H NMR (CD_3OD , 300 MHz) δ : 5.36–5.33 (m, 4H) 3.82–3.59 (m, 20H) 2.78–2.58 (m, 12H) 2.03 (m, 8H) 1.50 (m, 4H) 1.31 (chain, 44H) 0.91 (t, 6H); ^{13}C NMR (CD_3OD , 50.3 MHz) δ : 131.5, 130.9, 74.0, 72.9, 72.4, 71.9, 71.0, 69.9, 64.8, 58.7, 56.4, 54.9, 33.7, 33.1, 30.8, 30.6, 30.5, 30.4, 28.7, 28.2, 27.4, 23.8, 14.5; Anal. Calcd. for $\text{C}_{54}\text{H}_{108}\text{N}_2\text{O}_{12}$: C, 66.36; H, 11.14; N, 2.87. Found: C, 66.3; H, 11.22; N, 2.82.

DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine), DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine), and lyso-PC (1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). OTAC (octadecyltrimethylammonium chloride) was purchased from Fluka (Neu-Ulm, Switzerland).

N-Hexadecyl-*N*-octadecene-9-yl-*N*-dimethylammonium chloride (HOAC) was synthesized according to a literature procedure.⁸ ^1H NMR (CDCl_3 , 300 MHz) δ : 5.31–5.25 (m, 2H), 3.44–3.38 (m, 4H), 3.35 (s, 6H) 1.95–1.90 (m, 4H) 1.27–1.18 (chain, 52H), 0.81 (t, 6H); ^{13}C NMR (CDCl_3 , 75.4 MHz) δ : 129.9, 129.5, 63.4, 51.1, 32.5, 31.8, 29.6, 29.52, 29.44, 29.38, 29.31, 29.22, 29.17, 29.07, 28.96, 28.81, 27.08, 27.00, 26.1, 22.65, 22.5, 14.0; Anal. Calcd. for $\text{C}_{36}\text{H}_{74}\text{NCl}$: C, 77.71; H, 13.40; N, 2.52. Found: C, 77.6; H, 13.64; N, 2.47.

Structural formula of the additives are given in the Supporting Information.

The solvatochromic probe Nile red was obtained from Acros (Landsmeer, The Netherlands). All chemicals were of the highest available grades.

Sample Preparation. Solutions of GS1 with the appropriate amount of additive in chloroform were dried under a stream of nitrogen. Traces of residual solvent were removed under high vacuum. To obtain small unilamellar vesicles (SUVs) with a narrow and reproducible size distribution, the lipid films were hydrated at room temperature in bidistilled water containing 5 mM each of the buffer substances Hepes, Mes (4-morpholinoethanesulfonic acid), APS (3-amino-1-propanesulfonic acid), and Taurine (2-aminoethanesulfonic acid) (pH 7.00–7.30), vortexed for several minutes, briefly tip

sonicated (<1 min), freeze–thawed [$\text{N}_2(\text{l}) \leftrightarrow$ water bath (50 °C)] 5 times, and extruded 15 times through 200-nm-pore-size polycarbonate filters.

The samples for light scattering and Nile red fluorescence measurements were prepared by diluting the 5 mM (total lipid concentration) vesicular stock solutions to a final concentration of 0.25 mM and adding the appropriate amount of $\text{HCl}(\text{aq})$ or $\text{NaOH}(\text{aq})$ to the desired pH. The dispersions were equilibrated overnight before measuring.

The dispersions for the diffusion NMR experiments were prepared to a final total lipid concentration of 30 mM, and 10 vol % D_2O was added.

The multilamellar vesicles (MLVs) studied by ^{31}P NMR were prepared by hydrating the lipid films to a final concentration of 100 mM (total lipid concentration), vortexing, and by carrying out five freeze–thaw cycles to ensure full hydration and mixing of the lipids.

The samples were equilibrated overnight before measuring.

Static and Dynamic Light Scattering. Static and dynamic light scattering (DLS) measurements were performed at 25 °C on a Zetasizer 5000 instrument (Malvern Instruments, U.K.) at $\lambda = 633$ nm. To obtain the hydrodynamic radii, the intensity autocorrelation functions were analyzed using CONTIN.⁹

^{31}P NMR Spectroscopy. ^{31}P NMR spectra were recorded on a Varian Unity-Plus 500 spectrometer operating at 202.653 MHz for the phosphorus channel. Data were acquired with single-pulse excitation applying high-power proton decoupling. The 90° pulse length was 28 μs , the recycle delay was 1.3 s, the spectral width was 40.588 kHz, and the data size was 16.236 K. Typically, 4000 transients were signal averaged and exponentially multiplied with 50 or 100 Hz line-broadening functions.

Pulsed Field Gradient (PFG) NMR Self-Diffusion Measurements. The relative self-diffusion coefficient of water was determined using the BPPSTE (bipolar pulse pair stimulated echo) pulse sequence¹⁰ using a Varian Unity-Plus 500 spectrometer operating at 499.85 MHz for the ^1H channel. In this experiment, spins are spatially encoded with a bipolar gradient pulse pair and then decoded after a certain delay by a second bipolar pulse pair, thus producing an echo of the original signal. The echo attenuation is described by the Stejskal–Tanner equation¹¹

$$\ln\left(\frac{I}{I_0}\right) = -(\gamma g \delta)^2 \left(\Delta - \frac{\delta}{3} - \frac{\tau}{2} \right) D$$

which can be rewritten as

$$\ln\left(\frac{I}{I_0}\right) = -k g^2 \quad (1)$$

where I_0 is the signal intensity in the absence of a magnetic field gradient, γ is the gyromagnetic ratio, and g and δ are the strength and length of the gradient pulses, respectively (τ is the 90 – 180° pulse distance in the BPPSTE sequence). D is the diffusion constant of the spin system under study, Δ is the diffusion time between the encoding and decoding bipolar pulse pairs, and k is $-(\gamma \delta)^2 (\Delta - \delta/3 - \tau/2) D$. The BPPSTE experiments were recorded at 298 K, with 8000 data points in the FID, $\Delta = 2.15$ ms, and $\delta = 1$ ms, and gradient levels ranging between 0.6 and 30 G cm^{-1} were used. Before Fourier transformation, FIDs were exponentially multiplied with 10 Hz line broadening, and zero filling of 64 K was used. Typically, 90° pulse angles were 13 μs .

The variations in the diffusion coefficients of the dispersions were expressed as

$$D_n = \frac{k_{\text{ves}} - k_n}{k_{\text{ves}} - k_0}$$

(9) Provencher, S. W. *Comput. Phys. Commun.* **1982**, 27, 229–242.

(10) Wu, D. H.; Chen, A. D.; Johnson, C. S. *J. Magn. Res. Ser. A* **1995**, 115, 260–264.

(11) Stejskal, E. O.; Tanner, J. E. *J. Chem. Phys.* **1965**, 42, 288–292.

(8) March, J. *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, 2nd ed.; McGraw-Hill: Tokyo, 1977; p 377.

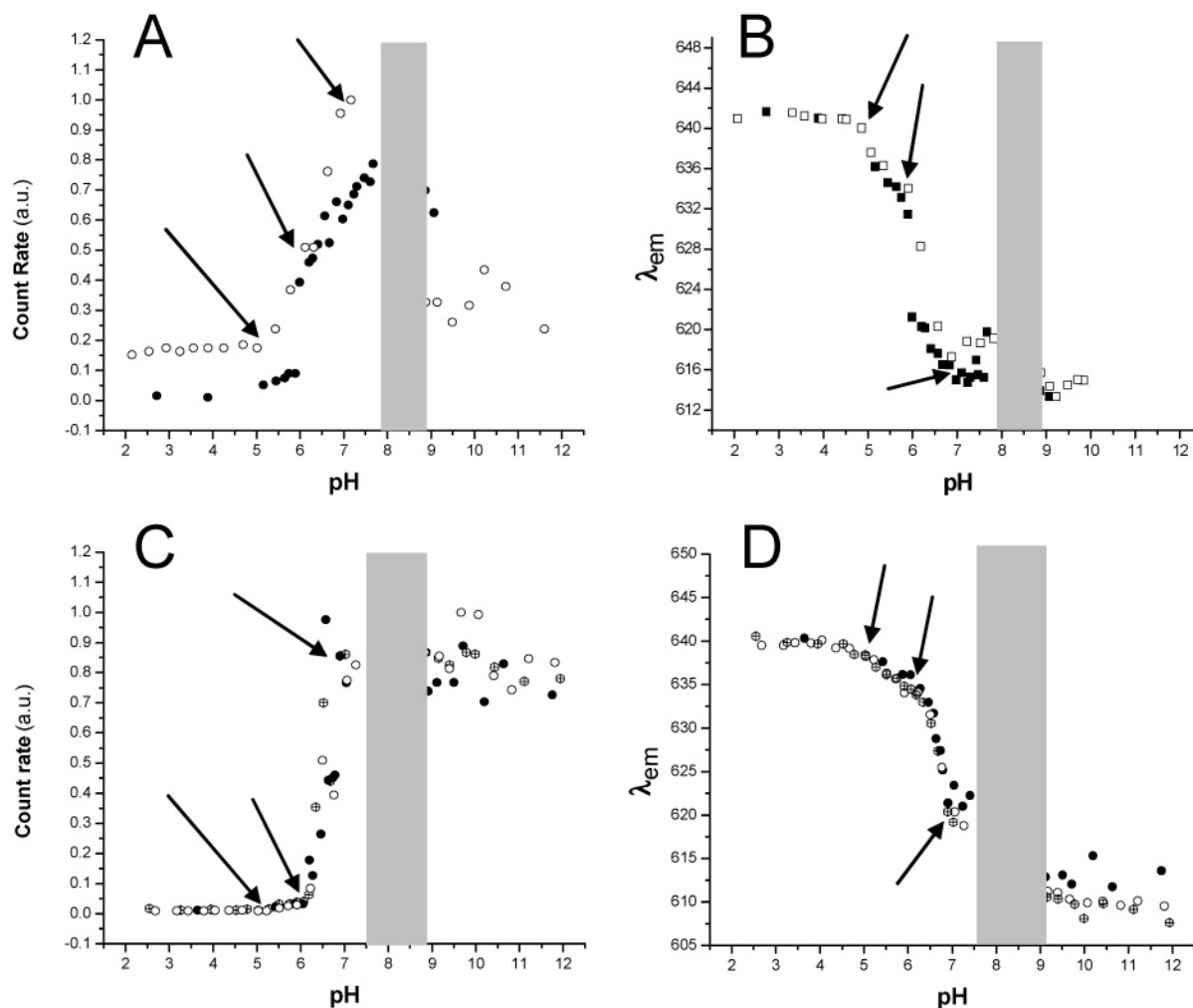


Figure 1. Normalized count rates from SLS experiments and wavelength of maximum Nile red emission in water (A, B) and in NaCl 150 mM (C, D). Open and closed symbols refer to independent measurements. Crossed symbols refer to vesicles prepared in water and, subsequently, diluted to physiological ionic strength (as opposed to hydration of the film with saline buffer). The shaded areas indicate the pH regions where rapid vesicle flocculation is observed. Arrows indicate the onset of the different transitions. (See the text for explanation.)

where k_{ves} , k_n , k_0 are the angular coefficients of eq 1, obtained at the pH at which the maximum number of vesicles is present, at the pH under investigation, and for the buffer in the absence of surfactant, respectively. Using this equation, the data are normalized both with respect to the diffusion coefficient of the buffer in the absence of surfactant ($D_n = 0$) and with respect to the maximum amount of water entrapped in the vesicles ($D_n = 1$).

Cryo-Transmission Electron Microscopy. A drop of the lipid suspension was deposited on a glow discharged holey carbon-coated grid. After blotting away the excess of lipid, the grids were rapidly plunged into liquid ethane. The frozen specimen were mounted in a Gatan (model 626) cryo-stage and examined in a Philips CM 120 cryo-electron microscope operating at 120 kV. Micrographs were recorded under low-dose conditions.

Fluorescence Spectroscopy. Nile red fluorescence was measured at room temperature, on a SPF-500c spectrofluorometer (SLM Aminco), using an excitation wavelength of 550 nm. The signal was recorded between 570 and 700 nm with a 1 nm step size. Nile red was added to the colloidal dispersions from a stock in acetonitrile to a final lipid/probe molar ratio of about 100. The Nile red emission maxima (λ_{max}) were deduced from the log-normal fittings of the emission bands.¹²

Results

GS1. Aggregation Behavior of GS1 in Water. The phase behavior of dilute aqueous dispersions of GS1 as a function of pH has already been reported.⁷ We could qualitatively reproduce

the DLS data reported therein (Figure 1A). The pH at which the count rate is the highest (around 7.5), which corresponds to the maximum number of vesicles in the suspension, and the pH of flocculation (7.7), which indicates proximity to the pH corresponding to charge neutrality (ζ potential $< +15$ mV), are higher than the ones previously reported.

The phase behavior of GS1 has been further investigated using the fluorescent probe Nile red. The plot of the wavelength as a function of pH corresponding to the Nile red emission maxima in an aqueous solution of GS1 is reported in Figure 1B. Because of the higher hydration of the surfactant headgroups in the micellar phase with respect to the more compact L_α phase, the fluorescent probe senses a transition from the L_α phase to micelles as a bathochromic shift.¹²

The wavelength of the Nile red maximum emission in a vesicular suspension of GS1 is about 615 nm and is constant between pH 7.0 and 7.7. Upon decreasing the pH, the wavelength increases rapidly with the formation of wormlike micelles, consistent with the trend in the intensity of scattered light, which indicates a decrease in the size of the aggregates. A second, less-pronounced break at pH 5.9 is associated with the onset of the formation of spherical micelles. At pH values lower than 5.2, both the Nile red emission maxima and the count rates of the

(12) Stuart, M. C. A.; van de Pas, J. C.; Engberts, J. B. F. *N. J. Phys. Org. Chem.* **2005**, *18*, 929–934.

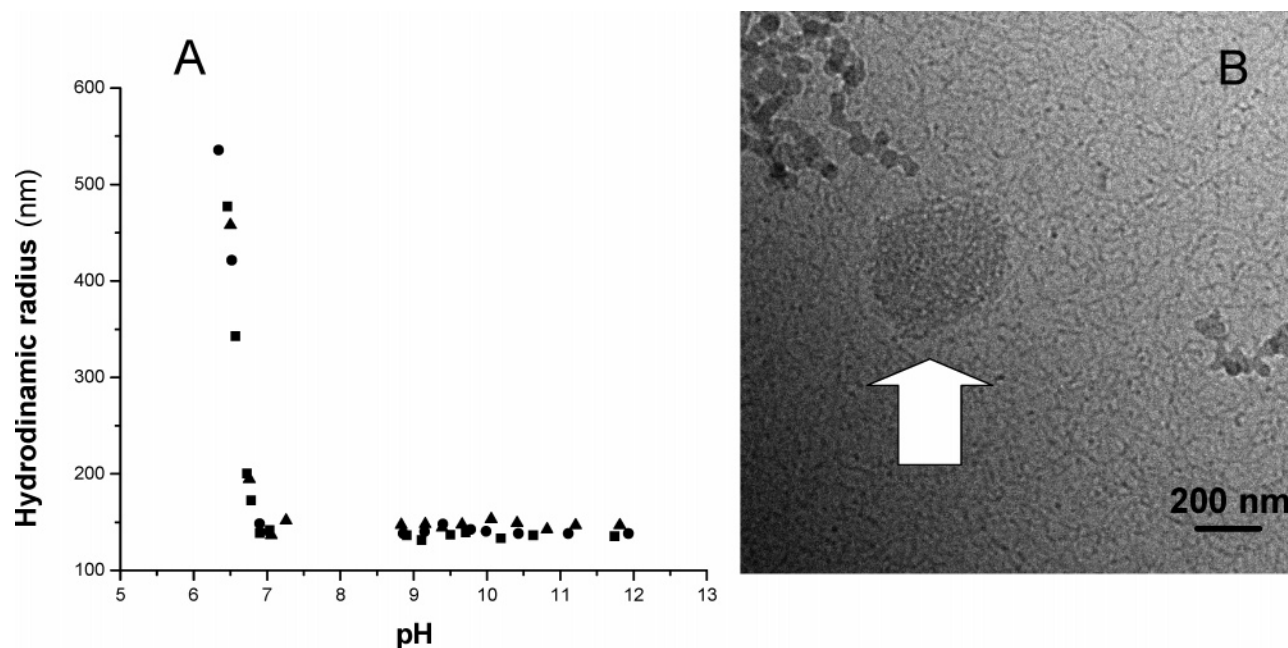


Figure 2. (A) Hydrodynamic radius of a dispersion of GS1 in 150 mM NaCl. The different symbols refer to two independent measurements. (B) Cryo-TEM picture of GS1 in 150 mM NaCl recorded at pH 6.5. The arrow indicates an aggregate of wormlike micelles. (See the text for explanation.)

scattered light remain constant, indicating that the transition to spherical micelles is complete.

In the pH range corresponding to flocculation, the quality and the intensity of the fluorescence spectra decrease dramatically. Typically, the values observed are in the range expected for the L_α phase.

A control experiment was performed starting from micelles at pH 2 by increasing the pH (data not shown). The fluorescence experiments confirmed the transitions observed in the previous experiments (e.g., from micelles to the L_α phase, subsequent flocculation, and finally redispersion). The pH of the transitions did not vary with respect to the experiments carried out starting from vesicles. The low values of the count rates measured for the control experiments at pH 7 suggest that, upon raising the pH, bilayer fragments are formed instead of vesicles, consistent with the thermodynamic instability of cationic vesicular aggregates.¹³

Aggregation Behavior of GS1 at Physiological Ionic Strength

Analogous measurements performed on dispersions prepared in aqueous NaCl (150 mM, Figure 1C and D) revealed that the pH of the transitions is only slightly affected by the increase in ionic strength and, different from other transfection systems,^{14,15} the increase in ionic strength does not lead to the formation of different phases with respect to those observed in H_2O .

Flocculation occurs at a lower pH with respect to the one observed in water, consistent with the charge-screening effect of the salt, which decreases the colloidal stability of the aggregates.

The pH at which spherical micelle formation starts is higher in water than at physiological ionic strength.

The most remarkable difference between the phase behavior of GS1 in water and in 150 mM NaCl is an increase in the size of the aggregates (up to about 500 nm) upon decreasing the pH

from 6.9 to 6.2 (Figure 2A). In water, in this pH range, wormlike micelles are formed from vesicles (vide supra). We attribute the increase in size at high ionic strength conditions to the formation of aggregates of wormlike micelles, favored by the decrease in the electrostatic repulsion between the wormlike micelles induced by the salt and confirmed by cryo-TEM pictures recorded at pH 6.5. In Figure 2B, a large, disordered aggregate of wormlike micelles is recognizable with a diameter of approximately 400 nm. The increase in size ends suddenly at the pH corresponding to the transition to spherical micelles. Another cryo-TEM picture taken at pH 6.8 (not shown) reveals the presence of vesicles together with wormlike micelle aggregates.

Measurements performed on dispersions prepared by the hydration of the lipid films with a buffer at physiological ionic strength or on vesicles prepared in water and subsequently diluted 1–1 with a 300 mM buffer (to the same final concentration) gave an identical outcome.

Mixtures of GS1 with Phospholipids in Water. The effect of DOPC on the pH-dependent transitions of GS1 was studied in a broad range of compositions. SLS, DLS, and Nile red fluorescence were recorded as a function of pH at each composition. Up to DOPC contents of 30 mol %, analogous morphological transitions as for the dispersions of the pure GS1 are recognizable (Figure 3).

However, even DOPC contents as low as 12 mol % (about 7 molecules of GS per molecule of phospholipid) require a lower pH (higher degree of protonation of the GS headgroup) to obtain highly curved structures, such as spherical and wormlike micelles from vesicles. The pH associated with the transitions to micelles decreases linearly with the DOPC content. This observation is an indication of the homogeneous mixing of the two components.

As expected on the basis of the colloidal stability of DOPC vesicles in the pH range under examination, the pH of flocculation increases when DOPC is included in the GS1 bilayer. At DOPC contents higher than 40 mol %, the behavior of the mixtures becomes more complex. In Figure 4, the intensities of the scattered light as a function of pH are reported for mixtures of GS1/DOPC, with DOPC contents equal to or higher than 30 mol %.

(13) Laughlin, R. G. *Colloids Surf., A* **1997**, *128*, 27–38.

(14) Scarzello, M.; Smisterova, J.; Wagenaar, A.; Stuart, M. C. A.; Hoekstra, D.; Engberts, J. B. F. N.; Hulst, R. J. *Am. Chem. Soc.* **2005**, *127*, 10420–10429.

(15) Scarzello, M.; Chupin, V.; Wagenaar, A.; Stuart, M. C. A.; Engberts, J. B. F. N.; Hulst, R. *Biophys. J.* **2005**, *88*, 2104–2113.

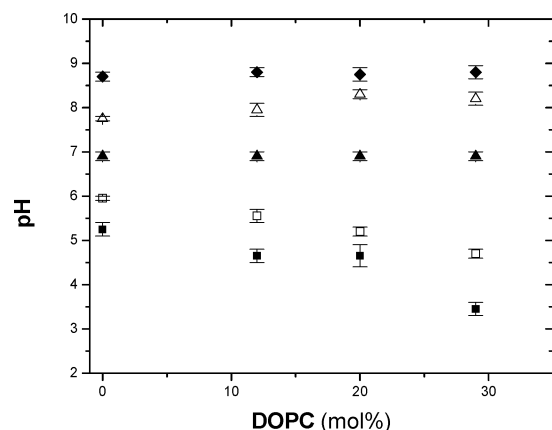


Figure 3. Phase transitions of mixtures of GS1/DOPC in water. \blacktriangle indicates the pH at which wormlike micelle formation from vesicles starts, \square indicates the pH at which the formation of spherical micelles from wormlike micelles starts, \blacksquare indicates the highest pH at which only spherical micelles are observed, \triangle indicates the pH of flocculation, and \blacklozenge indicates the pH of redispersion. (See the text for explanation.)

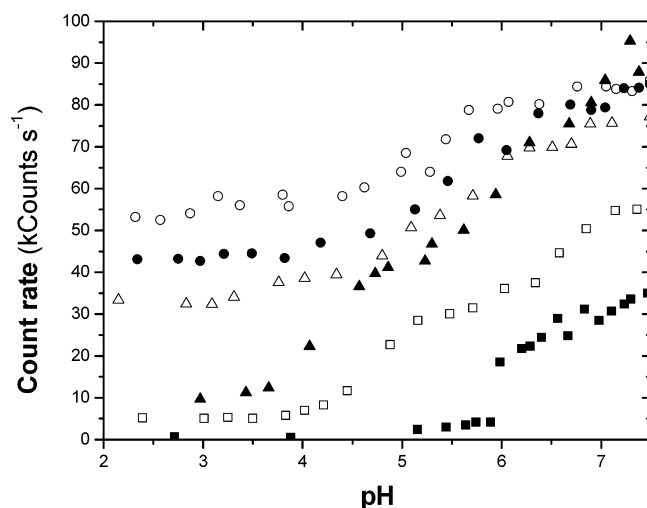


Figure 4. Intensity of the scattered light of GS1/DOPC dispersions as a function of pH. The symbols refer to DOPC contents of \blacksquare , 0 mol %; \square , 40 mol %; \blacktriangle , 50 mol %; \triangle , 60 mol %; \bullet , 66 mol %; and \circ , 75 mol %.

The analogous plot for a pure GS1 dispersion is also shown as a reference.

For DOPC contents between 40 and 60 mol %, the transitions to wormlike micelles and to spherical micelles occur at steadily more acidic pH values for increasing DOPC contents, consistent with the results for lower DOPC contents. Different from observations for DOPC contents lower than 40 mol %, the residual count rate measured after the transition to spherical micelles is higher than the one for the spherical micelles of GS1. For DOPC contents higher than 60 mol %, the pH values of the transitions do not longer vary with the DOPC content (i.e., the compositions of the wormlike micelles and of the spherical micelles remain constant). Consistently, the count rates at the lowest pH indicate the presence of residual vesicles and their number increases for increasing DOPC contents. The hydrodynamic radius measured under these conditions (data not shown) confirms the presence of large aggregates (about 100 nm) at acidic pH.

The different behavior of the GS1/DOPC mixtures for DOPC contents of 40 and 60 mol % was further investigated by monitoring the change in the ratios (D_n) of the diffusion coefficients of water in the dispersions (D) relative to the diffusion

coefficient of the pure solvent (D_0), determined as a function of pH (Supporting Information, Figure S2). The data demonstrate that the amount of trapped water in the vesicles of GS1/DOPC = 60/40 mol % varies as a function of pH in an analogous way as for the pure GS1 dispersions. For DOPC contents of 60 mol %, the variation of the self-diffusion coefficient of water going from neutral to acidic pH is small, confirming that most of the water trapped in the vesicles at pH 7 is still trapped at acidic pH.

These conclusions are supported by cryo-TEM pictures recorded for mixtures with DOPC contents of 40 and 60 mol % (Figure 5). Parts A and B of Figure 5 show two pictures recorded for a mixture of GS1/DOPC = 40/60 mol % at pH 7. In Figure 5A, vesicles with sizes consistent with the hydrodynamic radii measured by DLS can be detected. However, ellipsoidal aggregates were also present (Figure 5B), which are likely lamellar intermediates in the transition from vesicles to wormlike micelles. After lowering the pH to 4.5 (Figure 5C), wormlike micelles are observed together with some vesicles. Cryo-TEM pictures recorded at pH 3.5 do not show wormlike micelles (Figure 5D); only a few small vesicles (about 30 nm) are present. Figure 5E shows that at high(er) DOPC contents, vesicles with a diameter of about 100 nm are present even at very acidic pH, consistent with the SLS and NMR results (vide supra).

Up to DOPC contents of 60 mol %, the high-pH transitions are little affected by the presence of DOPC. The pH of flocculation increases slightly with increasing phospholipid content, consistent with the stability of DOPC vesicles over the whole interval of pH values under investigation. The pH of redispersion after flocculation is not affected by the presence of the phospholipids although DOPC vesicles do not absorb OH^- ions (the ζ potential of DOPC vesicles at pH 10 is close to 0 mV).⁵ DOPC vesicles containing less than 35 mol % GS1 do not flocculate upon increasing the pH.

The effects of DOPE, a frequently used helper lipid in gene transfection, on the colloidal properties of its mixtures with GS1 are similar to those of DOPC, although the effects of DOPE on the low-pH transitions are larger (Supporting Information, Figure S3). The slope of the linear fitting of the pH of transitions from vesicles to wormlike micelles as a function of phospholipid content is about 30% higher for DOPE than for DOPC. This observation is consistent with the higher packing parameter of DOPE with respect to that of DOPC. The presence of DOPE of up to 50 mol % does not affect the pH of redispersion after flocculation, in accord with the results for DOPC.

Mixtures of GS1 with Single-Tailed Surfactants in Water.

Mixtures with Lyso-PC. The effects of lyso-PC contents of up to 50 mol % on the transitions of its mixtures with GS1 are shown in Figure 6.

Visual inspection during sample preparation revealed that the aggregates obtained after freeze and thaw are smaller for the mixtures containing the single-tailed phospholipid compared to those containing double-tailed phospholipids. This is supported by the much higher turbidities of the 5 mM dispersions of GS1 with DOPC and DOPE (milky) with respect to those containing lyso-PC or OTAC (bluish and transparent), at the same total lipid concentration.

It is immediately clear from Figure 6 that the influence of lyso-PC on the phase behavior of GS1 is modest, in particular, at acidic pH. The transitions that lead to the formation of wormlike and spherical micelles remain nearly constant for lyso-PC contents up to 50 mol %.

The pH of flocculation increases slightly for lyso-PC contents up to 25 mol %. For still higher lyso-PC contents, no pH range

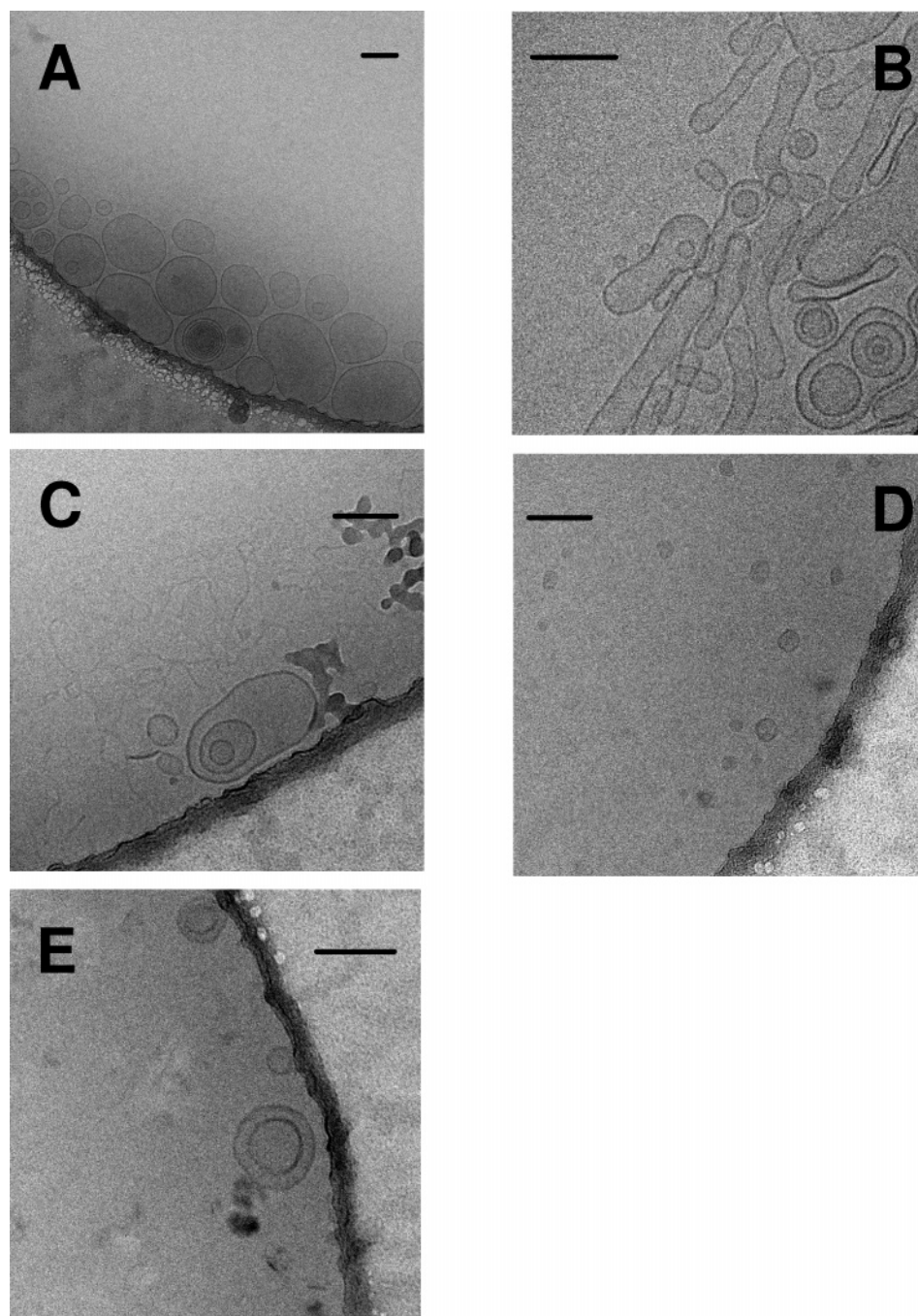


Figure 5. Cryo-TEM pictures of GS1/DOPC = 60/40 mol % at pH 7 (A and B), pH 4.5 (C), and pH 3.5 (D), and GS1/DOPC = 40/60 mol % at pH 2 (E). Bars indicate 100 nm.

of flocculation was detected, indicating that the neutral mixed vesicles are now colloidally stable. Even when flocculation does not occur, the aggregates apparently acquire a negative charge as indicated by the fact that the addition of Ca^{2+} ions (5 mM) leads to the aggregation of the vesicles at basic pH (data not shown).

As observed for the double-tailed phospholipids, the effect of lyso-PC on the pH of redispersion is only modest.

From the perspective of diffusion NMR (Supporting Information, Figure S2), the behavior of the mixtures with single-tailed surfactants is similar to that of pure GS1 dispersions: the inner aqueous compartments of the vesicles are disrupted in accord with wormlike micelle formation, as shown by the increase in the diffusion coefficients of water to a value similar to that for the bulk solvent.

Mixtures with OTAC

We finally investigated the effect of a single-tailed cationic surfactant on the phase behavior of GS1 (Figure 7).

As observed for the lyso-PC-containing mixtures, the effect of OTAC on the low-pH transitions is small. Interestingly, in case OTAC is mixed with GS1, the transitions from vesicles to wormlike micelles occur at higher pH (i.e., at lower degrees of protonation of GS1).

The pH of flocculation increases strongly with the OTAC content, consistent with the increase in positive charge at the surface of the aggregates, which results in an increase in the colloidal stability of the vesicular aggregates. Flocculation is observed only for an OTAC content of 15 mol %. For this composition, redispersion does not occur in the range of pH values under investigation. Control experiments were performed

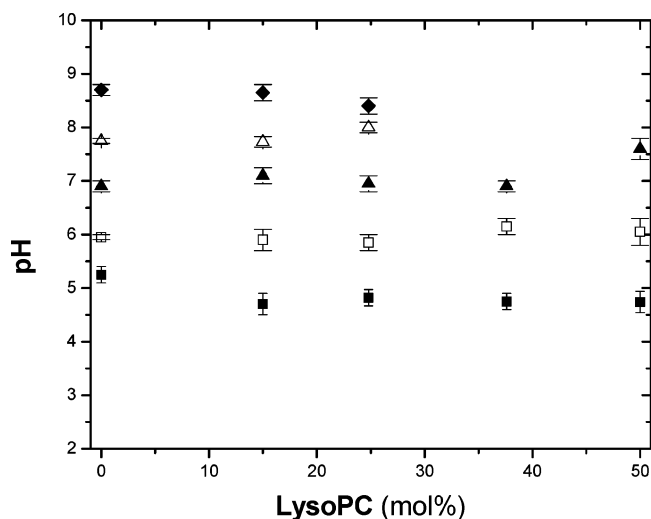


Figure 6. Phase transitions of mixtures of GS1/lyso-PC in water. See Figure 3 for the legend.

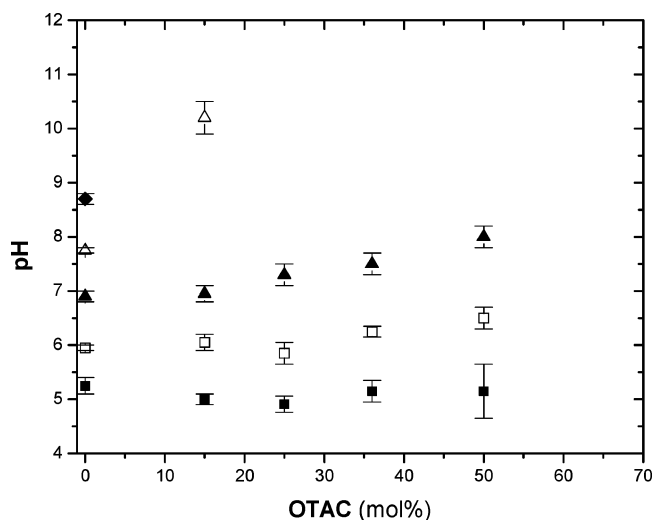


Figure 7. Phase transitions of mixtures of GS1/OTAC in water. See Figure 3 for the legend.

by mixing GS1 with the double-tailed cationic surfactant *N*-hexadecyl-*N*-octadecene-9-yl-*N*-dimethylammonium chloride (data not shown), which carries the same positive charge as OTAC but is expected to have a lower tendency than the single-tailed surfactant to partition into the aqueous phase. However, the results obtained for the double-tailed cationic amphiphile at basic pH are identical to those obtained for OTAC, indicating that the amount of OTAC not included in the vesicular bilayer is negligible.

Effect of Ionic Strength on Mixtures of GS1 with Phospholipids and Single-Tailed Surfactants. The effect of an increase in ionic strength to physiological values (NaCl(aq) 150 mM) on the morphology of the mixed systems has been examined. (See Supporting Information, Figure S4, for a comparison between the pH of transition of the mixtures (75 mol % GS1) in water and in saline aqueous solution.)

For all of the systems under investigation, flocculation of the vesicles upon charge neutralization corresponds to the transition in which the effect of the screening of the electrostatic interheadgroup repulsion by the salt is the most evident. GS1/OTAC mixtures are most strongly affected, in accordance with their higher surface charge with respect to the systems containing zwitterionic lipids. The narrow flocculation range observed for GS1/lyso-PC in water is no longer detected in the presence of

NaCl. Only for OTAC mixtures is the pH of redispersion sensitive to a variation in ionic strength.

A small effect on the pH at which spherical micelles start to form is present in all of the mixed systems except for the mixtures with DOPC. At high ionic strength, the formation of spherical micelles from wormlike micelles occurs at higher pH than in water. This finding is counterintuitive on the basis of the expected effect of the screening of the electrostatic interheadgroup repulsion by the salt.

In the mixtures containing the double-tailed phospholipids, a quantitative transition toward spherical micelles is not observed at physiological ionic strength. Cryo-TEM experiments (data not shown) performed on the dispersions containing 25 mol % DOPC at pH 3.1 revealed the presence of aggregates with irregular shapes and structures, which are most likely composed of branched micelles.

When GS1 is mixed with DOPC, DOPE, or lyso-PC, the increase in size attributed to the aggregation of wormlike micelles in NaCl solution is not observed or strongly reduced with respect to pure GS1 (vide supra). In the presence of OTAC, aggregation occurs to a similar extent as for pure GS1.

The differences between the morphologies in water and in 150 mM NaCl of the equimolar mixtures of GS1 with the double-tailed phospholipids were investigated by ^{31}P NMR (Figure 8).

The line-shape characteristic of the L_α phase¹⁶ is clearly recognizable under all of the conditions examined. Consistent with the DLS results, no quantitative transitions to the (isotropic) micellar phase were observed. The DOPC mixtures show the coexistence of isotropic and lamellar phases under all conditions. The contribution of the isotropic peak increases with decreasing pH and decreases with increasing ionic strength. The effects of pH and ionic strength are similar for DOPE-containing mixtures. As anticipated on the basis of the higher packing parameter of DOPE relative to that of DOPC, the contribution of the isotropic peak under the same conditions is lower for DOPE mixtures. Even if it is likely that isotropic and bilayer phases have different compositions, the absence of the spectral features characteristic of the H_{II} phase¹⁶ indicates the absence of domains of pure DOPE even at high ionic strength and low pH.

Discussion

The influence of single- and double-tailed surfactants on the pH-dependent aggregation behavior of GS1 has been determined in water and at physiological ionic strength by employing a variety of physical techniques.

The dependence of the morphology of the aggregates on the molecular shape of an amphiphile can be described by the dimensionless packing parameter, $P = V/a_0 \times l$, which is defined as the hydrophobic chain volume (V) divided by the optimal cross-sectional headgroup area (a_0) and the length of the all-trans hydrophobic tails (l). It can be shown that for values of $P = 1, 0.5$, and 0.33 , planar bilayers, infinite cylinders, and spherical micelles, respectively, show optimal stability.¹⁷

There are several possible ways to affect the packing parameter of a sugar-based GS without varying its molecular structure. The degree of protonation of the amine headgroups is pH-dependent, and acid/base titration is the obvious way to modify the packing parameter⁶ (vide supra). A different way to modify the aggregate morphology, mainly acting on the surface contribution to the Gibbs energy of self-assembly, consists of varying the ionic strength of the solution.

(16) Cullis, P. R.; De Kruffy, B. *Biochim. Biophys. Acta* **1979**, 559, 399–420.

(17) Fennell Evans, D.; Wennerstrom, H. *The Colloidal Domain*; 2nd ed.; Wiley-VCH: New York, 1999.

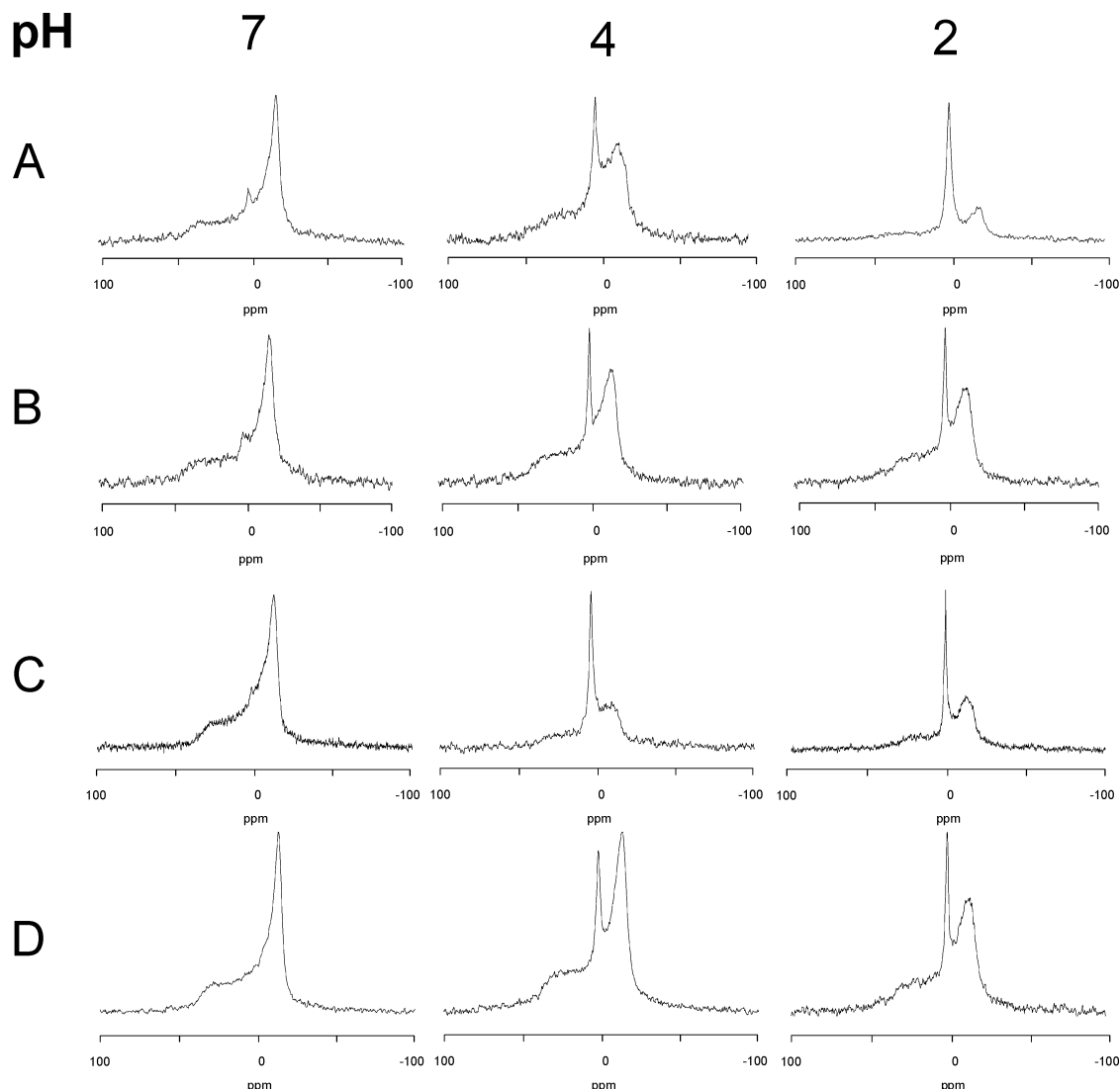


Figure 8. ^{31}P NMR spectra of equimolar mixtures of GS1/DOPC and GS1/DOPE in water (A and C, respectively) and 150 mM NaCl (B and D, respectively).

Table 1. Summary of the Effects of the Additives on the pH of the Morphological Transitions for Mixtures of GS1 with DOPC, Lyso-PC, and OTAC Using the Pure GS1 Dispersions as Reference

additive	micelle formation	vesicle flocculation	vesicle redispersion
DOPC	occurs at more acidic pH	occurs at slightly more basic pH (for DOPC contents higher than 65 mol %, not observed)	almost unaffected
lyso-PC	almost unaffected	occurs at more basic pH (for lyso-PC contents higher than 38 mol %, not observed)	occurs at more acidic pH
OTAC	occurs at slightly more basic pH	occurs at much more acidic pH (for OTAC contents higher than 25 mol %, not observed)	not observed

Finally, in the simplest approximation of ideal mixing, the packing parameter of a mixture of surfactants corresponds to the weighted average of the packing parameters of the individual components.

The main effects of DOPC, lyso-PC, and OTAC on the transition pH of GS1 are summarized in Table 1.

Mixtures of GS1 with DOPC and DOPE. DOPC is a double-tailed amphiphile commonly used as a model for biological membranes. In water, in the range of pH explored, pure DOPC dispersions are in the L_α phase. Because the packing parameter of the GS is pH-dependent whereas the packing parameter of

DOPC is not, the phase diagram of GS1 is affected to a different extent at different pH values.

Around neutral pH, both of the packing parameters of GS1 and DOPC are close to 1, and the phase behaviors of the mixtures are similar to those of the pure amphiphiles, even at high DOPC content.

In the cryo-TEM pictures recorded for the dispersions containing 40 mol % DOPC at pH 7, ellipsoidal vesicles are observed together with spherical vesicles. Similar ellipsoidal aggregates have been observed as intermediate morphologies in the transition from spherical egg-phosphatidylcholine vesicles

to micelles, induced by a cationic surfactant.¹⁸ We suggest that in the GS1/DOPC mixed vesicles the presence of small amounts of double-protonated GS1 in the bilayer induces the local increase in curvature associated with the formation of the ellipsoidal vesicles. The packing parameter of the double-charged GS1 is, in fact, expected to be similar to that of a single-tailed cationic surfactant.

The packing parameter of GS1 diminishes upon increasing the degree of protonation of the amine headgroup whereas that of DOPC does not vary as a function of pH. Because DOPC is present in mixed bilayers with GS1, lower pH values (i.e., higher degrees of protonation of GS1) are required to obtain the average packing parameters associated with micelle stability.

As previously mentioned (*vide infra*), the linearity between the DOPC content in the mixtures and the pH values associated with the transitions to micelles is an indication of homogeneous mixing of the phospholipid with GS1.

The dilution of GS1 with the zwitterionic phospholipid is also expected to favor the second protonation step of the GS headgroup; however, the decrease in the pH of the transitions to micelles indicates that the dilution of the charge is not the dominant effect.

For DOPC contents higher than 40 mol %, a quantitative transition to spherical micelles is no longer observed. Cryo-TEM proved the presence of residual closed vesicles for this and higher DOPC contents, indicating that DOPC can be included only in mixed micelles with GS1 at DOPC contents lower than 40 mol %. Further evidence of the presence of closed vesicles in the mixtures with high DOPC contents comes from the reduced self-diffusion coefficient of H₂O measured by ¹H NMR. This technique has been employed to detect the presence of enclosed aqueous pockets in colloidal dispersions.¹⁹ The values obtained for GS1 clearly show that at pH 4.5 the amount of entrapped water is negligible because the diffusion coefficient is close to that of bulk water. For mixtures with DOPC contents of 40 mol %, the reduced diffusion coefficient at pH 2 is slightly smaller than that of the solvent, consistent with the presence of some small vesicles as observed by cryo-TEM pictures (*vide supra*). The increase in the diffusion coefficient of H₂O at pH 2, with respect to that at the pH at which the vesicles are prepared, is even smaller for higher DOPC content (60 mol %), indicating that most of the water enclosed in the vesicular aqueous pockets remains isolated from the bulk solvent. The result is again consistent with the presence of relatively large, closed vesicles as observed by cryo-TEM.

When considering the transitions at basic pH, we must remember that DOPC vesicles are stable over the whole range of pH values explored. The stability of zwitterionic phospholipid vesicles is governed by the Gibbs energy of molecular dehydration.²⁰ Vesicle aggregation requires partial dehydration of the approaching lipid bilayers at the sites of contact.²¹ Our results indicate that the hydration shell of DOPC membranes at the sites of closest approach is strongly perturbed by the presence of GS1 so that the aggregation properties of GS1-containing vesicles, for DOPC contents up to 60 mol %, tend to be more similar to those of pure GS1 vesicles than to those of pure DOPC vesicles. Analogous conclusions can be deduced from the fact that the pH of redispersion after flocculation seems to be unaffected by the

presence of DOPC in this range of composition, despite the fact that DOPC vesicles do not adsorb hydroxide ions.⁵ It has been argued that the mechanism of the selective OH[−] adsorption to GS1 vesicles could be related to that of hydroxide absorption to water/hydrophobic interfaces and not to a specific interaction with the sugar headgroups.⁶ This suggestion was based on the fact that, for surfaces covered with nonionic surfactants, OH[−] binding does not depend on the identity of the surfactant headgroup and, moreover, an inverse correlation is observed between the surfactant surface excess and the surface charge density. Molecular dynamics simulations indicate that OH[−] binding to hydrophobic surfaces is determined by the interaction of the dipole moment of the hydroxide ion with the electrical potential generated by the preferential orientation of the water molecules in the first two water layers away from the hydrophobic surface.²² The independence of the OH[−] adsorption to the flocculated vesicles with respect to DOPC content suggests that the hydration at the vesicular surface is dominated by the GS1 surfactant for DOPC content up to 60 mol %.

The second phospholipid tested, DOPE, is one of the most commonly used helper lipids for gene transfection,^{15,23,24} as a consequence of its propensity to form inverted structures and in particular inverted hexagonal phases (H_{II}), which are considered to be beneficial for *in vitro* transfection. The behavior of the GS1/DOPE system is similar to that observed for GS1/DOPC mixtures. The amount of GS1 mixed with the phospholipid is sufficient to prevent the formation of an H_{II} phase (the morphology adopted by pure DOPE at temperatures higher than 10 °C), even for mixtures with 50 mol % DOPE, as proven by ³¹P NMR.

Mixtures of GS1 with Lyso-PC and OTAC. The binding of surfactants to neutral lipid bilayers is inversely proportional to their cmc.²⁵ The cmc values of lyso-PC and OTAC are 4.0×10^{-5} M²⁶ and 3.5×10^{-4} M,²⁷ respectively. Thus, we expect a higher miscibility with GS1 for lyso-PC than for OTAC, a difference that increases with increasing positive charge of the aggregates. Although we did not perform binding experiments, the results indicate that, at least in the high-pH region, mixing with GS1 is achieved to a certain extent for both of the single-tailed surfactants. The identity of the results obtained for the GS1 bilayers containing OTAC or *N*-hexadecyl-*N*-octadecene-9-yl-*N*-dimethylammonium chloride further supports this conclusion for the single-tailed cationic surfactant.

Lyso-PC is a natural single-tailed phospholipid generated in living organisms by the enzymatic hydrolysis of phosphatidylcholine at the *sn*-2 position and is known to play an important role in diverse biochemical processes.^{28–30} The dispersions of mixtures of GS1 and lyso-PC are less turbid than the analogous dispersions of mixtures of GS1 with DOPC. The presence of smaller particles in the sample containing the single-tailed surfactant is confirmed by the hydrodynamic radius obtained by DLS; these data can be rationalized considering the low(er) packing parameter of lyso-PC that favors the formation of aggregates with high(er) curvature. The effect of lyso-PC on the

(18) De Carlo, S.; Fiaux, H.; Marca-Martin, C. A. *J. Lipid Res.* **2004**, *14*, 61–76.

(19) Söderman, O.; Herrington, K. L.; Kaler, E. W.; Miller, D. D. *Langmuir* **1997**, *13*, 5531–5538.

(20) Ceve, G. *J. Chem. Soc., Faraday Trans.* **1991**, *87*, 2733–2739.

(21) Zellmer, S.; Ceve, G.; Risse, P. *Biochim. Biophys. Acta* **1994**, *1196*, 101–113.

(22) Zangi, R.; Engberts, J. B. F. *N. J. Am. Chem. Soc.* **2005**, *127*, 2272–2276.

(23) Koltov, I.; Salditt, T.; Radler, J. O.; Safinya, C. R. *Science* **1998**, *281*, 78–81.

(24) Zuhorn, I. S.; Bakowsky, U.; Polushkin, E.; Visser, W. H.; Stuart, M. C. A.; Engberts, J. B. F. N.; Hoekstra, D. *Mol. Ther.* **2005**, *11*, 801–810.

(25) Heerklotz, H.; Seelig, J. *Biochim. Biophys. Acta* **2000**, *1508*, 69–85.

(26) Hoyrup, P.; Davidsen, J.; Jorgensen, K. *J. Phys. Chem. B* **2001**, *105*, 2649–2657.

(27) Kang, K. H.; Kim, H. U.; Lim, K. H. *Colloids Surf., A* **2001**, *189*, 113–121.

(28) Yuan, Y. P.; Schoenwaelder, S. M.; Salem, H. H.; Jackson, S. P. *J. Biol. Chem.* **1996**, *271*, 27090–27098.

(29) Mori, S.; Nakata, Y.; Endo, H. *Cell. Mol. Biol.* **1991**, *37*, 421–431.

(30) Gopfert, M. S.; Siedler, F.; Siess, W.; Sellmayer, A. *J. Vas. Res.* **2005**, *42*, 120–132.

colloidal stability of the mixed vesicles with GS1 is stronger than that induced by DOPC. The data clearly show that the colloidal stability is increased by amounts of lyso-PC higher than 15 mol % and that with 36 mol % lyso-PC aggregation is no longer observed. We conclude that lyso-PC strengthens the hydration of GS1-containing vesicles so that the vesicles do not aggregate even when they are electrically neutral. This effect probably arises from the hydrogen-bonding capability of the hydroxyl group at the *sn*-2 position of the glycerol backbone of lyso-PC compared to that of the ester function present in DOPC.

The lower pH of redispersion of the mixture containing 25 mol % lyso-PC with respect to the pure GS1 dispersion indicates either that lyso-PC-containing vesicles bind OH⁻ more strongly than pure GS1 vesicles or that a lower electrostatic contribution is necessary to induce redispersion (i.e., higher hydration energy).

The effect of lyso-PC on the transition to micelles is modest, as expected on the basis of its small packing parameter.

As anticipated, the inclusion of a positively charged surfactant (OTAC) in the GS1 bilayer increases the pH at which charge neutrality is approached; consequently, flocculation is observed at high(er) pH (pH 10.2) and only for OTAC contents smaller than 15 mol %. For mixed vesicles of this composition, redispersion is not observed in the interval of pH values under investigation (up to pH 12). This result indicates that the hydrogen-bond structure of the water as induced by GS1 in proximity to the vesicular surface is disturbed by the presence of the cationic single-tailed surfactant to such an extent that OH⁻ binding does not occur. We cannot exclude the fact that charge reversal can take place only at extremely high pH (> 12). Flocculation of GS1 vesicles containing 25 mol % OTAC is observed at physiological ionic strength because of the screening of the electrostatic repulsion between the positively charged vesicles.

Effect of Ionic Strength. The main effects expected from an increase in ionic strength on ionic surfactant dispersions result from the screening of the electrostatic interactions between the charged headgroups. The consequent decrease in the cross-sectional headgroup area generally leads to a destabilization of normal, highly curved phases (i.e., increase in *P*). Salt-induced transitions toward structures characterized by a higher packing parameter have been observed for several transfection cocktails.^{14,31}

Comparing the behavior in water and at physiological ionic strength, it appears that the differences in the pH values of the transitions are modest. The mixture that shows a higher sensitivity to the ionic strength conditions is the one containing OTAC, in agreement with the high(er) surface charge of the aggregates.

For all systems investigated except for the lyso-PC-containing one, the pH of flocculation is reduced by the addition of NaCl, consistent with the decrease in the ζ potential of the aggregates upon increasing ionic strength. Flocculation is no longer observed in the mixture GS1/lyso-PC. We tentatively assign this effect to a conformational change at the aggregate surface, which brings more polar residues to the surface upon an increase in ionic strength, enhancing surface hydration. The presence of lyso-PC disturbs the packing of the GS bilayer and seems to decrease the barrier for such a conformational change with respect to additives with a higher packing parameter.

At acidic pH, the mixtures containing lyso-PC and OTAC present rather similar behavior compared to that of the pure GS1 dispersions. Without aiming to present a detailed description of the behavior of these complex systems, some considerations can

be made. The expected similarity between the packing parameter of GS1 at acidic pH and that of the single-tailed surfactants is consistent with the small effect of these additives on the transitions to micelles. Moreover, the mixing of OTAC with GS1 is expected to decrease with increasing charge of GS1 because of increased electrostatic repulsion; the mixing of lyso-PC with GS1 is expected to decrease with increasing ionic strength because of the decrease in electrostatic repulsion between the GS1 headgroups.

As mentioned above, a decrease in the pH of the transitions at which spherical micelle formation starts was observed at physiological ionic strength for all of the systems except for the mixture of GS1 with DOPC. This result is unexpected on the basis of simple electrostatic considerations because the decrease in the headgroup area expected upon an increase in ionic strength should favor the lamellar morphology with respect to wormlike micelles.

Large aggregates with a diameter of several hundreds nanometers were observed at high ionic strength for pure GS1 dispersions and for dispersions containing GS1 and a cationic additive. Phospholipid additives (DOPC, DOPE, and lyso-PC) hamper the formation of these aggregates, probably as a consequence of the high hydration energy of the zwitterionic phospholipids.

Conclusions

The effects of double- and single-tailed surfactants on the aggregation behavior of a sugar-based gemini surfactant (GS) were determined. The results open up new possibilities for modulating the phase behavior of these surfactants to optimize their use in biomedical applications.

The selected additives affect the phase behavior of GS1 in a pH-dependent fashion, and in general, the results can be rationalized on the basis of the relative packing parameters of the components. The double-tailed phospholipids show a stronger influence on the transitions toward highly curved aggregates (low-pH transitions), which occur only for high(er) degrees of protonation of the GS1 headgroup. Interestingly, when the content of the bilayer-forming phospholipid DOPC is higher than 40 mol %, a lamellar phase is also present at pH 2, together with a micellar phase, and for DOPC contents of 60 mol %, a considerable amount of water is still trapped in the vesicles even at pH 2.

Flocculation and redispersion of the vesicles was observed even for DOPC contents up to 60 mol %. For lower DOPC contents, the pH of redispersion is almost constant, suggesting that the hydration of the mixed vesicles is dominated by the GS1 surfactant in this range of compositions.

On the contrary, single-tailed surfactants lyso-PC and OTAC have a very modest influence on the low-pH transitions. The zwitterionic phospholipid lyso-PC narrows the interval of pH values at which flocculation is observed, and for lyso-PC contents of 36 mol %, the vesicles are colloidally stable over the whole range of pH investigated. The positive charge of the single-tailed cationic amphiphile OTAC strongly increases the colloidal stability of the mixed vesicles at basic pH, and flocculation is observed only for OTAC contents lower than 15 mol %. For this system, redispersion is not observed, indicating that the presence of the positive charge perturbs the ordering of the water molecules at the vesicular surface, hampering the OH⁻ binding. Anyway, we cannot exclude that OH⁻ binding may still occur at extremely basic pH (> 12.5).

The main effect of physiological ionic strength conditions on the phase behavior of pure GS1 dispersions is observed in the region where wormlike micelles are expected. Upon screening

(31) Šmisterová, J.; Wagenaar, A.; Stuart, M. C. A.; Polushkin, E.; ten Brinke, G.; Hulst, R.; Engberts, J. B. F. N.; Hoekstra, D. *J. Biol. Chem.* **2001**, 276, 47615–47622.

of the headgroup electrostatic repulsion at high salt concentration, large aggregates with a diameter of several hundreds nanometers are observed, resulting from the aggregation of the wormlike micelles. Similar aggregates are observed with the cationic single- and double-tailed additives but not with the zwitterionic additives (DOPC, DOPE, and lyso-PC). The ionic strength does not influence the pH of redispersion of the pure GS1 and of the mixtures of GS1 with double-tailed phospholipids. The behavior of the mixtures with the single-tailed phospholipid is weakly affected by the salt, and the most remarkable difference with respect to the behavior at low ionic strength is the absence of the narrow flocculation region at physiological ionic strength. On the contrary, the colloidal stability of the mixtures of GS1

with OTAC is strongly affected by physiological salt concentrations, consistent with their high(er) surface charge.

Supporting Information Available: Molecular structures of DOPC, DOPE, lyso-PC, OTAC, and HOAC. Plot of the reduced self-diffusion coefficients (D_r/D_0) of GS1, GS1/DOPC = 60/40 mol %, GS1/DOPC = 40/60 mol %, GS1/lyso-PC = 75/25 mol %, and GS1/OTAC = 75/25 mol % as a function of pH. Plot of the pH of phase transitions for mixtures of GS1/DOPE in water as a function of DOPE content. Graph presenting a comparison between the pH of the transitions in water and in 150 mM aqueous NaCl for GS1 and its mixtures with DOPC, DOPE, lyso-PC, OTAC, and HOAC (additive 25 mol %). This material is available free of charge via the Internet at <http://pubs.acs.org>.

LA053211W