Synthesis and Properties of Di-n-dodecyl α,ω-Alkyl Bisphosphate Surfactants

Francis L. Duivenvoorde,† Martinus C. Feiters,‡ S. J. van der Gaast,§ and Jan B. F. N. Engberts*†

Department of Organic and Molecular Inorganic Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands, Department of Organic Chemistry, NSR Center, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands, and Netherlands Institute for Sea Research (NIOZ), 1709 AB Den Burg, Texel, The Netherlands

Received November 25, 1996. In Final Form: April 7, 1997

Three gemini and two bolaform bisphosphate surfactants of the type 12s-12, with s = 6, 8, 12, 18, and 24 carbon atoms, have been synthesized and their aggregation behavior has been studied. The bolaform surfactants 12-18-12 and 12-24-12 were found to form vesicles in aqueous solution, as indicated by electron microscopy. The geminis 12-6-12, 12-8-12, and 12-12-12 form micellar structures. The cmc's of the geminis, obtained from conductivity measurements, spectroscopic methods, and microcalorimetry, are very low, on the order of 10−4 to 10−5 M. The cmc decreases with increasing spacer length. For the bolaform amphiphiles 12-12-18 and 12-24-12 noncooperative phase transitions are detected using fluorescence depolarization and DSC. NMR line-broadening studies display unusual behavior. The spacer within the 12-24-12 vesicles has been found to be membrane spanning, as confirmed by X-ray powder diffraction.

Introduction

Bolaform-type and gemini-type surfactants have gained much interest over the last years, because of their unusual aggregation properties,1−10 compared to those of the corresponding conventional surfactants. Bolaform amphiphiles are found in natural membranes of archae bacteria, a class of organisms that live in habitats with extreme circumstances, such as high temperatures, low pH values, and saturated salt environments.11 When there is a single spacer, this spacer is longer than the other chains and is therefore membrane spanning in such membranes. Other types of bolaform membranes consist of two membrane-spanning spacers. As a result, these membranes possess an unusual thermodynamic stability. They display a very slow lateral and transversal diffusion, and they resist fusion. Several synthetic bolaform amphiphiles have been studied.10,12−21 They were found to form monolayer structures with a membrane-spanning spacer as well. In a few cases, backfolding of the spacer has been observed.3,22−24

Gemini surfactants have much shorter spacer lengths. They form micellar structures in aqueous solution due to considerable backfolding of the spacer.3,6,10 Their properties are different from those of single-chained micelle-forming amphiphiles. The most pronounced differences are the extremely low cmc's and low Krafft points.4 Zana et al.5,6,10 have reported studies on m-s-m type geminis with two tetraalkylammonium headgroups. They found that geminis with short spacers (C2 and C3 chains) form threadlike micelles and that those with spacers of medium length (C5−C12) form spherical micelles.10 Geminis with rigid spacers, which cannot fold back, display unusual properties.3 For these geminis, premicellar aggregates are believed to be formed at concentrations just below the cmc. Despite rather extensive studies, their exact aggregation behavior in aqueous solution is still not fully understood.

We became highly interested in these new types of amphiphiles, because of several potential applications, such as, for instance, drug targeting. For this reason, we wanted to synthesize bolaform- and gemini-type surfactants which would have a membrane spanning spacer within a vesicular aggregate. In this paper, we report the synthesis and some physical properties of three gemini-type and two bolaform-type amphiphiles with two phosphate headgroups, two dodecyl chains, and one connecting spacer of variable length s (Figure 1).

Figure 1. Synthetic sequence for the preparation of the 12s-12 surfactants. s = 6, 8, 12, 18, and 24.
Following the nomenclature used by Zana et al., the amphiphiles will be called m-s with m = 12. As no clear distinction has been made so far in the literature between bolaform and gemini surfactants, we propose to define bolaform surfactants as those having a spacer longer than the other chains (s > 12 in the present work) and gemini surfactants as those with shorter spacers. This is a useful definition in light of our results (see Results and Discussion), which prove that clear relationships between molecular and aggregate structure exist.

Results and Discussion

Synthesis. The novel amphiphiles were synthesized according to the method described by Bauman, as outlined in Figure 1. In all cases, the chemical yields were excellent. The surfactants were all converted into their disodium salts and checked using IR spectroscopy prior to the experiments.

Electron Microscopy. For the geminis 12-6-12, 12-8-12, and 12-12-12, no vesicular structures were found with either transmission electron microscopy (TEM) or freeze fracture electron microscopy (FFEM). Similar results were obtained by Zana et al. for the corresponding dimeric ammonium amphiphiles.

For the bolaform amphiphiles 12-18-12 and 12-24-12 unilamellar and multilamellar vesicles were observed with both TEM and FFEM. Some micrographs are shown in Figure 2. The vesicles varied in size between 150 and 700 nm. Vesicles prepared with the ethanol injection method (Experimental Section) were smaller than those prepared by simply stirring the surfactant solution. This is a common phenomenon. The vesicular solutions were stable above their Tc for several months, as monitored by TEM and by visual inspection of the slightly turbid solutions. No differences could be detected between the samples prepared in double-distilled water and those prepared in aqueous NaAc/HEPES buffers (pH = 7.4).

Phase Behavior. The phase transition temperatures (Tc) were determined by fluorescence depolarization, DSC, and NMR. The results are summarized in Table 1.

The method of vesicle preparation (Experimental Section) had no effect on the results. With fluorescence depolarization, a gradual, noncooperative transition was found for 12-18-12 and 12-24-12, as is shown in Figure 3. The transition range was 22–75 °C for 12-18-12 and 26–64 °C for 12-24-12.

Noncooperativity has been observed previously for bolaform amphiphiles, having a membrane-spanning spacer, probably due to the presence of gaps in the outer leaflets of the vesicles. Due to this noncooperativity, it is difficult to define a precise phase transition temperature. We have, therefore, also determined the Tc of 12-24-12 with DSC, a method which does not depend on the use of a probe molecule. The DSC curves are shown in Figure 4.

The result of the analysis of the curves is listed in Table 2.

A fairly cooperative transition at 60.5 °C is observed, with a transition enthalpy of 32.7 ± 0.8 kJ (mol...
Table 1. Main Phase Transition Temperatures of the 12-s-12 Surfactants*

<table>
<thead>
<tr>
<th>compound</th>
<th>fluorescence depolarization</th>
<th>¹H-NMR</th>
<th>³¹P-NMR</th>
<th>DSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-6-12</td>
<td>c</td>
<td>g</td>
<td>g</td>
<td>g</td>
</tr>
<tr>
<td>12-8-12</td>
<td>c</td>
<td>g</td>
<td>g</td>
<td>g</td>
</tr>
<tr>
<td>12-12-12</td>
<td>47.5 (35–60)×d</td>
<td>g</td>
<td>g</td>
<td>g</td>
</tr>
<tr>
<td>12-18-12</td>
<td>48.5 (22–75)×b</td>
<td>g</td>
<td>c</td>
<td>g</td>
</tr>
<tr>
<td>12-24-12</td>
<td>45 (26–64)×b</td>
<td>59 (48–70)×c</td>
<td>69 (63–75)×e</td>
<td>60.5×e</td>
</tr>
</tbody>
</table>
|               | 44 (30–58)×c                | 67 (62–72)×f | 62×f   | 64 °C, which is in better agreement with the 1H- and 31P-NMR experiments for 12-24-12.

Fluorescence depolarization studies with the geminis gave rather capricious results. This is shown in Figure 3 as well.

For 12-6-12, a nearly straight line was obtained, characteristic for micelles. The polarization P at low temperatures, however, is rather high for micellar structures. The Krafft point lies below 0 °C, because no precipitation of 12-6-12 crystals at the lowest temperature was observed. Thus, the micelles of 12-6-12 appear to be less dynamic than conventional micelles. Similar results were obtained for 12-8-12 (not shown) and 12-12-12 at concentrations well above the cmc (vide infra). At concentrations slightly above the cmc, however, P gradually reached values > 0.4 at low temperatures, apparently indicating that the micelles become less dynamic at lower concentrations. This may be ascribed to probe-induced aggregation of the amphiphiles at low concentrations.

Attempts to determine the Tc of 12-24-12 with NMR line-broadening experiments were not successful. As is shown in Figure 5, line broadening occurs at high temperatures, which is opposite to the normal behavior. This result is reproducible for both ¹H and ³¹P NMR experiments. It looks as if the alkyl chains and the headgroups become more flexible below Tc, which, of course, conflicts with expectation.

**Critical Aggregation Concentrations.** The critical aggregation concentrations were determined using conductivity measurements, microcalorimetry, and spectroscopic methods (pyrene fluorescence and pinacyanol chloride absorption). The results are summarized in Table 3. It was not possible to determine critical vesicle concentrations (cvc's) for the bolaform amphiphiles 12-18-12 and 12-24-12 with either method, because they are probably too low for accurate experimental determination.

For the geminis, the conductivity plots show one clear break at the cmc. The obtained cmc's are 0.35, 0.48, and 0.06 mM for 12-6-12, 12-8-12, and 12-12-12, respectively. Figure 6 shows the conductivity plots for 12-6-12. The counterion binding constants β, estimated from the ratio of the slopes after and before the cmc, are small, 0.16 for 12-6-12, 0.38 for 12-8-12, and 0.12 for 12-12-12. Apparently, the headgroup repulsion within the micelle is small, probably due to the spacer, keeping the headgroups far apart from each other.

Spectroscopic determination of the cmc gave cmc values that are somewhat lower than those obtained from

---

* Listed values are the midpoints of the transition range, listed between brackets. † EtOH injection method in buffer. ‡ No Tc measurable. § At 6.6 × 10⁻⁵ M. ‖ Stirring method in double-distilled water. ‡‡ Stirring method in D₂O. †† Not determined.

---


solutions, giving rise to an endothermic peak. This discrepancy may be attributed to probe-induced micellar aggregation, causing a local change in medium polarity,16 at concentrations just below the actual cmc. It has been argued before that conductometry indicates the onset of the micellization process,16 because micellization occurs over a concentration range rather than at a fixed concentration.

The plots of the microcalorimetry experiments are shown in Figure 7. The cmc’s obtained from the enthalpograms agree well with those obtained by the previous methods. The geminis display nonideal behavior below the cmc, which increases with the spacer length. It can be ascribed to the thermodynamic nonideality of the solutions, giving rise to an endothermic peak.

The microcalorimetry experiments are summarized in Table 2 and Figure 8. The nonideality at the onset of the plots.

X-ray Powder Diffraction. In order to determine whether or not the spacer is membrane spanning in the vesicles, X-ray powder diffraction (XRD) studies were performed on dried amphiphile solutions. The reflections were interpreted as due to ordering of the material in stacked layers or membranes, with a d-spacing identical to the membrane thickness. From this value, a molecular picture of the packing of the membranes is proposed. The results are summarized in Table 4 and Figure 8.

Table 2. DSC Measurements for 12-24-12

<table>
<thead>
<tr>
<th>T in °C</th>
<th>H^2 in kJ (mol patch)^{-1}</th>
<th>∑H</th>
<th>∑H_{int}</th>
<th>T_c</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>53</td>
<td>372</td>
<td>60.1</td>
<td>62.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>57.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>411</td>
<td>1113</td>
<td>1142</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>75.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>53.4</td>
<td>213</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>57.9</td>
<td>577</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60.5</td>
<td>887</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62.1</td>
<td>552</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Analysis of scans 2, 3, 4, and 5. b Analysis of the first scan.

Table 3. Cmc’s (mM) of the 12-s-12 Surfactants Obtained by Different Methods

<table>
<thead>
<tr>
<th>s</th>
<th>conductivity (μS cm⁻¹)</th>
<th>pyrene (°C)</th>
<th>pinacyanol chloride (°C)</th>
<th>microcalorimetry (kJ mol⁻¹)</th>
<th>cmc ammonium (kJ mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.35 ± 0.05</td>
<td>b</td>
<td>0.19 ± 0.03b</td>
<td>0.36 (0.17–0.53)</td>
<td>1.03b</td>
</tr>
<tr>
<td>8</td>
<td>0.48 ± 0.04</td>
<td>0.15 ± 0.03c</td>
<td>0.10 ± 0.01c</td>
<td>0.17 (0.08–0.25)</td>
<td>0.83c</td>
</tr>
<tr>
<td>12</td>
<td>0.06 ± 0.01</td>
<td>0.044 ± 0.003c</td>
<td>0.035 ± 0.002c</td>
<td>0.04 (0.02–0.05)</td>
<td>0.37c</td>
</tr>
</tbody>
</table>

Values between parentheses indicate the concentration range between the plateaus. a Micellization enthalpy at 30 °C in kJ mol⁻¹. b Cmc of the corresponding 12-s-12 bisammonium gemini surfactant. c Conductivity measurements. d Surface tension measurements.

Figure 6. Typical plot of the conductivity vs surfactant concentration.

Figure 7. Result of the microcalorimetric study of the micellization of 12-6-12 (□), 12-8-12 (○) and 12-12-12 (○). Note the nonideality at the onset of the plots.

Figure 8. X-ray Powder Diffraction.

Table 4. XRD Results Related to the Geometry of the 12-5-12 Surfactants As Derived from CPK Models

<table>
<thead>
<tr>
<th>s</th>
<th>geometry in Å</th>
<th>d in Å</th>
<th>alkyl tail cross section in Å²</th>
<th>proposed packing*</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>25.5 (c), 23–25 (Z), 49 (ext)</td>
<td>23.9, 47.9</td>
<td>19.3</td>
<td>C₁₂ mono- or bilayer</td>
</tr>
<tr>
<td>12</td>
<td>22–23 (Z), 55 (ext)</td>
<td>34.7, 30.1, 24.0</td>
<td>22.3</td>
<td>intercalated bilayer</td>
</tr>
<tr>
<td>18</td>
<td>20 (Z), 31–32 (Z), 63 (ext)</td>
<td>20.7</td>
<td>20.0</td>
<td>30° tilted C₁₂ monolayer</td>
</tr>
<tr>
<td>24</td>
<td>37 (n), 70 (ext)</td>
<td>40.9, 27.2</td>
<td>19.7</td>
<td>hydrated C₁₂ bilayer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>41.4, 27.5</td>
<td></td>
<td>hydrated 30° tilted monolayer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40.8, 26.3d</td>
<td></td>
<td>C₁₂ bilayer, tilted monolayer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38.6</td>
<td></td>
<td>hydrated C₂₄ monolayer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38.8</td>
<td></td>
<td>C₂₄ monolayer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36.3d</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Underlined values have been obtained from low-angle measurements. † 50% relative humidity. ‡ 0% relative humidity. See Figure 8.

Figure 8. (a) Possible conformations of gemini/bolaform surfactants. (b) Possible modes of packing in bilayers. Arrows indicate the periodicity observed in an XRD experiment on stacked bilayers.

Figure 9. XRD (CoKα radiation) of dried samples of 12-24-12 with controlled relative humidities (—, 50% RH; ---, 0% RH). Additional 'shadow' peaks appeared at 40.8 and 26.3 Å, indicating slow dehydration.

For 12-24-12, the second-, third-, fourth-, and sixth-order reflections, corresponding to d being either 23.9 or 47.9 Å, depended on their assignments. This points to a packing of non-intercalated monolayers or bilayers. Measurements on the low-angle diffractometer confirmed the d of 24.0 Å but did not give more positive evidence regarding the question whether this was a first- or second-order reflection, as no first-order reflection corresponding to a d of 48 Å was observed. The sample also showed polymorphism, with weak additional reflections at 34.7 (corresponding to an intercalated bilayer), 30.1, and 24.0 Å.

A dried sample of 12-12-12 showed a d of 20.7 Å, on the basis of the first-, second-, third-, and fourth-order reflections. This d is consistent with a packing in tilted C₁₂ monolayers, with a membrane-spanning spacer. The presence of a tilt is corroborated by the slightly larger alkyl tail cross section, perpendicular to the bilayer normal, of 22.3 Å² (calculated from the d, reflection at approximately 4.0–4.2 Å, using the relation 40.41 alkyl tail cross section = 2d²/√3.

Conclusions

We have synthesized five new bolaform-type and gemini-type bisphosphatet surfactants and studied their aggregation in aqueous solution. The geminis 12-6-12, 12-8-12, and 12-12-12 form micellar aggregates. The c.m.c.'s are very low, which is characteristic for geminis. The counterion binding constants β are low as well, indicating a small headgroup repulsion in the micelle. This has been attributed to the effect of the spacer, which keeps the headgroups far apart.

The bolaform amphiphiles 12-18-12 and 12-24-12 form vesicles in aqueous solution. Both surfactants display a noncooperative transition around 48.5 °C for 12-18-12 and 60.5 °C for 12-24-12. 1H and 31P NMR line-broadening studies gave unexpected results: linebroadening occurred above the Tc.

XRD studies revealed that the spacer of 12-24-12 is indeed membrane spanning within the vesicular structure. For 12-18-12, the spacer is not membrane spanning.

It is clear that gemini and bolaform bisphosphate surfactants are an interesting class of amphiphiles with unusual properties. Further studies of their aggregation in aqueous solutions will be reported in due course.

Experimental Section

Materials. n-Dodecyl phosphate was prepared according to a standard literature procedure. 1,6-Dibromohexane, 1,8-dibromooctane, 1,12-dibromododecane, and 1,12-dibromododecane.

References:


can be purchased from J. Ansen Chimica and used as received. Pyrene, pinacolyl chloride, NaAc, and HEPES were purchased from Aldrich and were used as received. The water used throughout the experiments was distilled twice in an all-quartz distillation apparatus. NaAc/HEPES buffer (pH 7.4) was prepared by dissolving NaAc and HEPES in double-distilled water to give final concentrations of 5 mM NaAc and HEPES. NMR spectra were recorded on a Varian Gemini 200 or a Varian VXR-300, using the chemical shifts of the solvents as internal standards. Infrared spectra were recorded on a Perkin-Elmer 841 infrared spectrophotometer as KBr pellets. Melting points were determined on a Mettler FP1 melting point apparatus equipped with an Ernst Leitz Wetzlar microscope 411657. Mass spectra were recorded using an AEI-MS 59 mass spectrometer.

Vesicle Preparation. Vesicles were prepared at 60 °C, either in double-distilled water or in NaAc/HEPES buffers by means of two different methods: the ethanol injection method

27,28 or a stirring method. For the ethanol injection method, 5 mg of surfactant was dissolved in ca. 50 μL of 96% ethanol and 40 μL of this solution was injected rapidly with a preheated 100 μL Exmix syringe into 1 g of double-distilled water or NaAc/HEPES buffer, which was placed in a water bath of 60 °C. Stirring was continued for a few minutes.

For the stirring method, 5 mg of surfactant was added to 1 g of double-distilled water or NaAc/HEPES buffers and this suspension was stirred vigorously for a few minutes in a water bath at 60 °C. In some cases the solutions were placed in an ultrasonic water bath (at 60 °C) for 15–30 min afterward.

Electron Microscopy. For transmission electron microscopy (TEM), the two droplet techniques were used for the preparation of the samples. A droplet of the surfactant solution was placed on a Formvar/carbon copper grid. After ca. 10 s, the grid was blotted off and a droplet of a 1% uranyl acetate solution was placed on the grid. This was blotted off again after ca. 10 s. The grids were air-dried before examination. For freeze fracture electron microscopy (FFEM), replicas were prepared using a Balzers EVM 052A electron beam evaporation instrument. Both TEM and FFEM samples were examined in a Philips EM 2001 instrument, operating at 80 kV.

Fluorescence Depolarization. Into 5 mL of double-distilled water or NaAc/HEPES buffers, which was brought to the initial temperature of the experiment (> 60 °C) was injected 50 μL of a surfactant solution, which was injected using a preheated 100 μL Exmix syringe. trans,trans,trans-1,6-Diphenyl-1,3,5-hexatriene (DPh) (5 μL of a 5 x 10⁻⁴ M solution in THF) was injected into the surfactant solution to yield a final [DPh] of 5 x 10⁻⁵ M. The experiments were started immediately after the preparation, using a quartz cuvet (1 cm). Samples of 12-6-12 and 12-8-12 were prepared by the stirring method in double-distilled water; samples of 12-12-12 and 12-18-12 were prepared by the ethanol injection method in NaAc/HEPES buffer; samples of 12-24-12 were prepared by both methods. Measurements were performed with an SLM-AMINCO SPF-500C spectrofluorometer. The emission and excitation wavelengths were 428 and 360 nm, respectively. A band-pass of 5 nm was used. The samples were thermostated by means of a water bath, and the samples were allowed to equilibrate for 10 min. Measurements involved cooling scans, with 3 °C intervals. P was calculated from the emitted light parallel and perpendicular to the direction of the excitation light, according to P = (I₁ - 1)/I₁ + 1. Reported values for P are the average values of five independent scans.

NMR. The vesicle solutions were prepared at 60 °C by the stirring method in D₂O or in double-distilled water with 10% D₂O, followed by sonication for 15–30 min. Experiments involved cooling experiments. For the ¹H-NMR experiments, the line widths of the resonance peaks at 1.2–1.3 ppm were examined.

Differential Scanning Calorimetry (DSC). DSC measurements were performed at the University of Leicester with a Microcal calorimeter. A 4.35 x 10⁻¹ M solution of 12-24-12 in water was heated to 60 °C with stirring for 15–20 min. After it was cooled to room temperature, the cloudy solution was degassed and scanned four times by immediately heating the solution after cooling to 20 °C. The fifth scan was performed after the solution was kept at 20 °C for 11 h. Analyses of the scans were carried out with the ORIGIN software.

Conductivity. Inverse resistivities were measured using a Wayne-Kerr Autobalance Universal Bridge 6842 fitted with a Philips electrode PW9512/00 (cell constant C = 0.654 cm⁻¹) or PW9512/00 (cell constant C = 0.654 cm⁻¹). Solutions were thermostated for at least 20 min prior to the experiment. Small aliquots (typically 10–50 μL) of a concentrated stock solution were injected into the cell with a 100 μL Exmix syringe, while stirring magnetically. Concentrations were corrected for volume changes. Conductivities were calculated from the equation ε = C/R = CG.

Pyrene Fluorescence. A saturated solution of pyrene in water was prepared by adding ca. 2 mg of pyrene to ca. 50 mL of double-distilled water. The aqueous solution was then vigorously stirred for 3 h at room temperature, and it was then allowed to stand overnight with gentle stirring.

A quartz cuvette was filled with the pyrene solution and...
equilibrated at 25 °C for at least 15 min. Small aliquots (typically 10–50 µL) of a concentrated stock solution were injected into the cell with a 100 µL Exmire syringe, while stirring magnetically.

Fluorescence measurements were performed with an SLM-AMINCO SPF-500C spectrofluorometer, with an excitation wavelength of 335 nm (band-pass 5 nm). Spectra were recorded in the ratio mode from 360–390 nm (band-pass 1 nm), by 0.1 nm steps. The first peak was taken at 372–373 nm, and the third one was taken at 383 nm. $I_1/I_3$ ratios were determined graphically.

**Pinacyanol Chloride Absorption.** A glass cuvette was filled with ca. 2.5 g of a 1.03 × 10^{-5} M pinacyanol chloride solution in double-distilled water. Into this solution, small volumes (typically 10–25 µL) were injected with a 100 µL Exmire syringe. The solution was stirred magnetically. Absorption was measured at 610 nm (bandwidth 1 nm). The temperature was controlled with a Haake water bath. Time intervals of 2 min were taken between the successive injections. Measurements were performed using a Philips PU 8740 UV/vis scanning spectrophotometer.

**Titration Microcalorimetry.** Microcalorimetric experiments were performed with an OMEGA Microcal titration microcalorimeter. Small aliquots (typically 5–10 µL) of a concentrated surfactant solution (ca. 10 (cmc)) were injected into water. The heat effects were recorded and analyzed with a computer. Enthalpies were calculated using the ORIGIN software.

**XRD Studies.** Samples for the powder diffraction studies were prepared by dispersing the surfactant in water above the $T_g$ (final concentration ca. 10 mg/mL) and putting a few drops of this suspension on a silica wafer, followed by lyophilization in a desiccator over P₂O₅. For the low-angle measurements, special Si single-crystal wafers, cut along the (501) plane, were used. Powder diffraction measurements were carried out on a commercial Philips PW 1710 X-ray powder diffractometer equipped with a Cu LFF X-ray tube operating at 40 kV and 55 mA. For low-angle measurements two Bragg–Brentano diffractometers were used, viz. (a) an optimized home-built (NIOZ, Texel) high-accuracy θ–θ diffractometer equipped with a Cu LFF tube, a variable divergence and anticrass slit, and an energy dispersive Si/Li detector (Kevex), which enables a high peak to background ratio, and (b) a commercially available θ–θ diffractometer (Philips), slightly adapted for measuring at low angles, equipped with a Co LFF tube, a variable divergence slit, a fixed anticrass slit, a graphite monochromator in the diffracted beam, a detector of the Peltier-cooled Si/Li type, and a specimen chamber, the relative humidity (RH) of which can be controlled by a humidity generator providing for He gas of a desired RH value.

Both instruments were operated at 40 kV and 40 mA.

**Acknowledgment.** We thank Mr. A. Wagenaar for his help with the NMR studies; Dr. A. Sein for help with the electron microscopy studies; Dr. K. Bijma for the microcalorimetry experiments; Prof. M. J. Blandamer and Dr. B. Briggs (University of Leicester, England) for the DSC measurements; and Mr. A. J. Vaars (Netherlands Institute for Sea Research (NIOZ), Den Burg, The Netherlands) for performing the XRD studies.

LA9620420

---