

# Gelation of Amphiphilic Lipopolymers at the Air–Water Interface: 2D Analogue to 3D Gelation of Colloidal Systems with Grafted Polymer Chains?

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Amphiphilic lipopolymers are known to form 2D thermoreversible gels at the air–water interface. Recently, we have reported surface rheology and film balance experiments on poly(ethylene glycol) (PEG) lipopolymers of different molecular weights, which indicated that a sufficient cross-sectional area mismatch between polymer and lipid moieties is necessary to form stable 2D gels (J. Coffman and C. Naumann, *Macromolecules* **2002**, *35*, 1835). In the current studies, we have investigated the influence of the hydrophobic anchor on the gelation properties by surface rheology and film balance technique. Experiments on PEG lipopolymers carrying saturated and partially unsaturated alkyl chains and on poly[(2-*n*-nonyl-2-oxazoline)<sub>*x*</sub>-*b*-(2-methyl- or 2-ethyl-2-oxazoline)<sub>*y*</sub>] (N<sub>*x*</sub>M<sub>*y*</sub> or N<sub>*x*</sub>E<sub>*y*</sub>) diblock copolymers of different block length show that the gel formation is not merely the result of the area mismatch between hydrophilic and hydrophobic moieties of the amphiphile (cone shape), but that a sufficient strength of van der Waals interaction within the hydrophobic moiety is necessary for the 2D gel to form, thus verifying earlier predictions that an alkyl chain condensation is a necessary precursor for the gelation process to occur. We also present neutron reflectometry data on PEG lipopolymers above and below the alkyl chain condensation and gelation transitions, which, in agreement to previous neutron and X-ray scattering experiments, reveal that both transitions occur after surface micelles of lipopolymers are formed at the air–water interface. On the basis of these findings, we assume that the gelation process of lipopolymers at the air–water interface is caused by a surface micellization of lipopolymers, which can be seen as the 2D analogue to the 3D gel formation observed for polymeric colloids with grafted polymer chains, such as copolymers and star polymers.

## Introduction

Crystallizable polymers that can form physical gels after cooling of the polymeric solution represent an important class of polymeric materials.<sup>1</sup> A remarkable feature of these polymeric systems is the heat reversibility of the gelation process, which led to their name thermoreversible gels. Unlike chemical gels, which are characterized by permanent network junctions, the reversibility of physical gels is a result of the weakness of their network junctions either based on transient network junctions or quasi permanent ones that are breakable upon applied stress. Several microstructures of physical junction zones in thermoreversible networks have been reported, such as crystallites connecting neighboring polymer chains or fibrillar structures.<sup>1–3</sup> Gellike behavior has also been reported on 3D aqueous solutions of diblock copolymers,<sup>4–6</sup>

triblock copolymers,<sup>7–9</sup> and star polymers.<sup>10–12</sup> It has been shown that these polymeric systems show no attractive interaction potential.<sup>13,14</sup> Using dynamic light scattering, three different relaxation modes have been found for interacting spherical polymer brushes: (1) fast cooperative diffusion of entangled polymer arms; (2) slow self-diffusion mode of stars; and (3) intermediate structural mode due to the local ordering of the cores.<sup>15</sup>

Unlike the 3D gels discussed above, 2D thermoreversible gels represent a new class of materials discovered recently.<sup>16–18</sup> The first reports on 2D physical gelation were based on surface rheology experiments on monolayers

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of amphiphilic poly(ethylene glycol) (PEG) lipopolymers at the air–water interface. Lipopolymers are amphiphiles consisting of a single, hydrophilic polymer chain terminally attached to a lipidlike hydrophobic moiety.<sup>16,18</sup> Oscillating stress–strain experiments on monolayers of these amphiphiles at the air–water interface revealed a rheological transition if the PEG chains are confined in a brush conformation with the storage modulus,  $G_s'$ , being larger than the loss modulus,  $G_s''$ , above the transition and with  $G_s' < G_s''$  below. It was found that the gelation is sensitive to the strength of hydrogen bonding ( $H_2O$  was replaced with  $D_2O$ ; pH was changed)<sup>16</sup> and that the pressure–area isotherms of PEG lipopolymers are characterized by a small plateau in the region of the rheological transition shown to be related to an alkyl chain condensation.<sup>17–20</sup> These findings lead to the model that a 2D physical gel is formed among these network-forming amphiphiles, which is held together by two different types of junction zones located within their hydrophilic and hydrophobic moieties—alkyl chain condensation forming small clusters of lipopolymers and formation of attractive H-bond bridges between polymer moieties of neighboring clusters mediated by water molecules.<sup>16,18</sup>

Previous surface rheology and film balance studies on PEG lipopolymers of different polymer molecular weights ( $M_n$ : 750, 1000, 2000, 3000, 5000 g/mol) but identical lipid moiety (DSPE) at the air–water interface have provided additional insight into the effect of important molecular parameters of network-forming amphiphiles on the gelation process, including the amphiphilic balance and the molecular area mismatch between hydrophilic and hydrophobic moieties.<sup>21</sup> An interesting result of this study was that the surface rheology data showed a qualitative difference between longer ( $M_n$ : 2000, 3000, 5000 g/mol) and shorter polymers ( $M_n$ : 750, 1000 g/mol). While no network breakdown could be observed in the first case, a partial breakdown of the network was found in the latter if the monolayer was compressed beyond an area per molecule of about 90–100 Å<sup>2</sup>. By comparing  $G_s'$  plotted versus reduced film pressure,  $\pi_r = (\pi - \pi_{r,theo})/\pi_{r,theo}$ , with  $\pi$  being the film pressure and  $\pi_{r,theo}$  being the film pressure at the gel point, it was also discovered that the relative strength of the physical network among lipopolymers of different  $M_n$  (low  $M_n$  species considered before network collapse) follows an interesting trend with  $G_s'$  ( $M_n$ : 750 g/mol)  $<$   $G_s'$  ( $M_n$ : 1000 g/mol)  $<$   $G_s'$  ( $M_n$ : 2000 g/mol)  $>$   $G_s'$  ( $M_n$ : 3000 g/mol)  $>$   $G_s'$  ( $M_n$ : 5000 g/mol).<sup>21</sup>

One of the remarkable features of network-forming amphiphiles at the air–water interface is the obvious correlation between the alkyl chain condensation at the high-film pressure transition and the rheological transition. Recent surface rheology and film balance experiments on lipopolymer–phospholipid mixtures at the air–water interface revealed that both transitions describe correlated but different phenomena.<sup>17</sup> This indicates that the formation of small clusters of lipopolymers (with or without phospholipids) is necessary for the rheological transition to occur. Interestingly, Helm and co-workers verified the formation of a molecular superstructure using grazing incidence X-ray diffraction for lipopolymer monolayers at

the air–water interface and those on solid substrates above the alkyl chain condensation.<sup>22,23</sup> Furthermore, Wurlitzer et al.<sup>24</sup> reported X-ray and neutron reflectometry experiments on poly(2-oxazoline) lipopolymers below and above the alkyl chain condensation, which showed that the lipid moieties of these lipopolymers partially immerse into the water phase during the condensation transition, whereas no significant change within the polymer moiety was detected. Previously, we showed that these poly(2-oxazoline) lipopolymers show rheological properties similar to PEG lipopolymers.<sup>18</sup> These findings indicate that the alkyl chain condensation transition is linked to the formation of surface micelles at the air–water interface. Interestingly, Merkel and Nuyken also reported the formation of surface micelles on monolayers of poly(2-nonyl)-poly(2-oxazoline) diblock copolymers at the air–water interface.<sup>25</sup>

It is the purpose of this study to explore whether the 2D gelation is a direct result of the surface micellization process similar to the behavior of diblocks, triblocks, and star polymers in 3D. First, we will present experiments exploring the effect of the hydrophobic anchor of network-forming amphiphiles on film balance and surface rheology properties. Particularly, we will show how the 2D gelation process depends on the molecular shape and the strength of van der Waals interaction among hydrophobic anchors of lipopolymers. The effect of the following molecular properties is studied on both the  $\pi$ - $A$  isothermal and rheological transitions: (1) varying the alkyl chain length of the lipid moiety for PEG lipopolymers, (2) comparison between saturated and unsaturated alkyl chains of the lipid moiety for PEG lipopolymers, and (3) studying the impact of the degree of asymmetry between hydrophobic and hydrophilic blocks of poly[(2- $n$ -nonyl-2-oxazoline) <sub>$x$</sub> - $b$ -(2-methyl- or 2-ethyl-2-oxazoline) <sub>$y$</sub> ] ( $N_xM_y$  or  $N_xE_y$ ) diblock copolymers. Second, neutron reflectometry experiments are presented which provide structural information about monolayers of PEG lipopolymers at the air–water interface below and above the gelation point. In the latter case, the argument is that micellization has to lead to a roughening of the monolayer, as observed recently for poly(2-alkyl-2-oxazoline) lipopolymers.<sup>24</sup>

## Materials and Methods

All amphiphilic molecules investigated are illustrated in Figure 1. The lipopolymers studied were 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[poly(ethylene glycol) 2000] (DMPE-PEG2000), 1,2-dipalmytoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[poly(ethylene glycol) 2000] (DPPE-PEG2000), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[poly(ethylene glycol) 2000] (DSPE-PEG2000), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[poly(ethylene glycol) 2000] (DOPE-PEG2000), 1,2-*O*-dioctadecyl-*sn*-glyceryl-3-poly(2-methyl-2-oxazoline) 3640] (DC<sub>18</sub>Gly-M<sub>35</sub>), and dioctadecanoylglycerol-[poly((2-ethyl-2-oxazoline) 3710] (DC<sub>18</sub>Gly-E<sub>31</sub>). All PEG lipopolymers were purchased from Avanti Polar Lipids (>99% pure, Alabaster, AL). Lipopolymers based on poly(2-alkyl-2-oxazoline), such as DC<sub>18</sub>Gly-M<sub>35</sub> and DC<sub>18</sub>Gly-E<sub>31</sub>, were synthesized by following the procedure described recently.<sup>18</sup>

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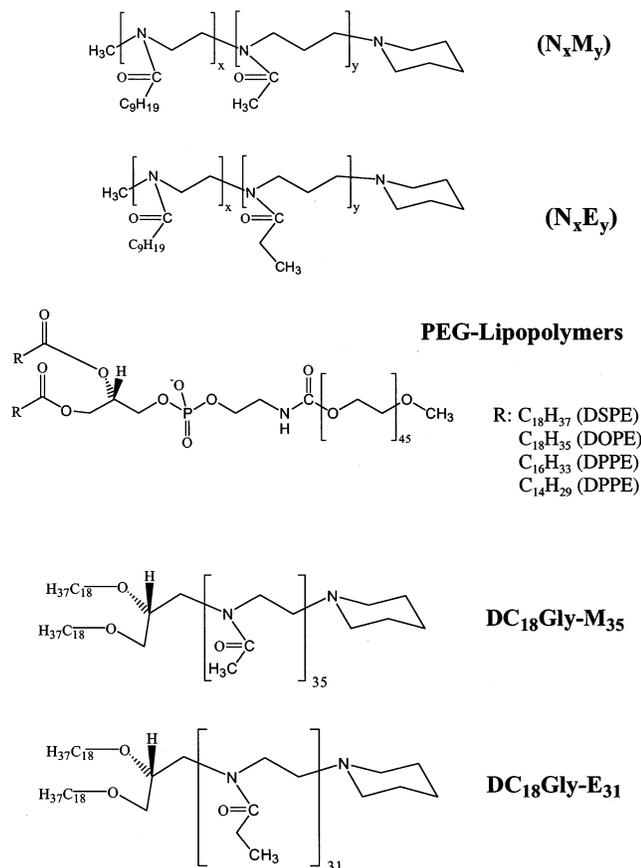
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**Figure 1.** Molecular structures of diblock copolymers and lipopolymers used in this study. Experiments on diblock copolymers used poly(2-*n*-nonyl)-poly(2-methyl or 2-ethyl-2-oxazoline) ( $N_xE_y$  and  $N_xM_y$ ), where  $x$  and  $y$  denote the block sizes of the hydrophobic nonyl and hydrophilic oxazoline blocks. The PEG lipopolymers include 1,2-*x-n*-glycero-3-phosphoethanolamine-*N*-[poly(ethylene glycol)2000] ( $x$ -PEG2000), where  $x$  symbolizes the different lipid anchors used. The lipid anchors include  $x =$  DMPE (C<sub>14</sub>, saturated); DPPE (C<sub>16</sub>, saturated); DSPE (C<sub>18</sub>, saturated); and DOPE (C<sub>18</sub>, one *cis*-double bond at the position 9 of each alkyl chain). The poly(2-oxazoline) lipopolymers presented are dioctadecanoylglycerol-[poly(2-ethyl-2-oxazoline) 3710] (DC<sub>18</sub>Gly-E<sub>31</sub>) and dioctadecanoylglycerol-[poly(2-methyl-2-oxazoline) 3640] (DC<sub>18</sub>Gly-M<sub>35</sub>).

Poly(2-oxazoline) block copolymers were synthesized as previously reported.<sup>26</sup> All the polymerization reactions were carried out in a Schlenk-type reactor under inert nitrogen atmosphere using freshly distilled and dried solvents. 2-ethyl-2-oxazoline and 2-methyl-2-oxazoline were obtained from Aldrich, whereas 2-*n*-nonyl-2-oxazoline was obtained from Henkel KGaA, Duesseldorf, Germany as a gift. All monomers were distilled under nitrogen atmosphere and purified and stored under nitrogen atmosphere with molecular sieve (A4).

In short, the general procedure for the polymerization was as follows: To a solution of 0.976 g (4.95 mmol) 2-*n*-nonyl-2-oxazoline in a mixture of acetonitrile (3.3 mL) and chlorobenzene (3.3 mL), trifluoromethanesulfonate (0.082 g, 0.33 mmol) was added at room temperature. The solution was heated in a prepared oil bath to 110 °C and stirred for 24 h. At room temperature, 0.461 g (4.95 mmol) of 2-ethyl-2-oxazoline was added to the solution and stirred for 15 h at 80 °C. At room temperature, 0.052 g of piperidine (0.99 mmol) was added and stirred at 40 °C for 3 h. After removal of the solvent, the polymer was redissolved in 15

mL of methylene chloride. Potassium carbonate (dry and finely grounded) was added and the mixture was stirred for 2 days. After filtration, the polymer was purified by precipitation (CHCl<sub>3</sub>/heptane) and freeze-drying (benzene) to give a colorless powder.

The synthesized polymers were poly[(2-*n*-nonyl-oxazoline)<sub>*x*</sub>-*b*-(2-ethyl-2-oxazoline)<sub>*y*</sub>], ( $N_xE_y$ ), and poly[(2-*n*-nonyl-2-oxazoline)<sub>*x*</sub>-*b*-(2-methyl-2-oxazoline)<sub>*y*</sub>], ( $N_xM_y$ ), with the following degrees of polymerization ( $x$ ,  $y$ ) and molecular weight ( $M_n$ ) (as calculated by <sup>1</sup>H NMR spectroscopy) and polydispersity index (PDI) (as obtained by gel permeation chromatography).  $N_{15}E_{14}$ :  $x = 15.5$ ,  $y = 13.6$ ,  $M_n = 4504.68$  g/mol, PDI = 1.09, yield = 65%;  $N_{16}M_{13}$ :  $x = 16.2$ ,  $y = 13.2$ ,  $M_n = 4304.63$  g/mol, PDI = 1.12, yield = 60%;  $N_8E_{24}$ :  $x = 8.2$ ,  $y = 23.8$ ,  $M_n = 3969.89$  g/mol, PDI = 1.08, yield = 58%;  $N_8M_{26}$ :  $x = 8.3$ ,  $y = 26.1$ ,  $M_n = 3857.46$  g/mol, PDI = 1.09, yield = 58%.

$N_xE_y$ . IR (Film).  $\nu$  (cm<sup>-1</sup>) 2954, 2924, 2971, 2853, 1646, 1464, 1425.

<sup>1</sup>H NMR (CDCl<sub>3</sub>).  $\delta$  (ppm)  $\delta$  0.8–0.9 (CH<sub>3</sub>-(CH<sub>2</sub>)<sub>8</sub>-CO-N, 3H);  $\delta$  1–1.2 (CH<sub>3</sub>-CH<sub>2</sub>-CO-N, 3H);  $\delta$  1.2–1.4 (CH<sub>3</sub>-(CH<sub>2</sub>)<sub>6</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CO-N, 12H);  $\delta$  1.4–1.6 (CH<sub>3</sub>-(CH<sub>2</sub>)<sub>6</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CO-N, 2H, CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-N, 2H; CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-N, 2H);  $\delta$  2.2–2.5 (CH<sub>2</sub>-CO-N, 2H, CH<sub>2</sub>-N-CH<sub>2</sub>, 4H);  $\delta$  3.0 (CH<sub>3</sub>-N, 3H);  $\delta$  3.3–3.6 (CH<sub>2</sub>-N(CO)-CH<sub>2</sub>, 4H).

$N_xM_y$ . IR (Film).  $\nu$  (cm<sup>-1</sup>) 2954, 2924, 2971, 2853, 1646, 1464, 1425.

<sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  (ppm)  $\delta$  0.8–0.9 (CH<sub>3</sub>-(CH<sub>2</sub>)<sub>8</sub>-CO-N, 3H);  $\delta$  1.2–1.4 (CH<sub>3</sub>-(CH<sub>2</sub>)<sub>6</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CO-N, 12H);  $\delta$  1.4–1.6 (CH<sub>3</sub>-(CH<sub>2</sub>)<sub>6</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CO-N, 2H, CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-N, 2H; CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-N, 2H);  $\delta$  2.0–2.4 (CH<sub>2</sub>-CH<sub>2</sub>-CO-N, 2H, CH<sub>2</sub>-N-CH<sub>2</sub>, 4H, CH<sub>3</sub>-CO-N, 3H);  $\delta$  3.0 (CH<sub>3</sub>-N, 3H);  $\delta$  3.3–3.6 (CH<sub>2</sub>-N(CO)-CH<sub>2</sub>, 4H).

The purity of all synthesized polymers used in this study was checked by NMR spectroscopy and MALDI-TOF mass spectrometry as well as chromatography (GPC). No impurities, other than the molecular weight distribution, as stated above for the individual polymers, could be detected.

Monolayers were formed by dissolving the amphiphiles in chloroform followed by the spreading of the chloroform solution over milli-Q water (pH = 5.5, 18.2 M $\Omega$ -cm resistivity).

A more detailed description of the surface rheology experiments was given recently.<sup>21</sup> In short, a Camtel CIR-100 Interfacial Rheometer (Camtel, U.K.) was used that operates on the basis of an oscillating Pt/Ir De Noüy ring attached to a virtually frictionless suspension wire. The system's drive unit is controlled by the drive unit coil, which operates similarly to a taut band galvanometer. The movement of the ring is detected by a sensor that reflects light off a target that rests on the saddle of the ring. All experiments were conducted in normalized resonance mode where the feedback control system forces the system into phase resonance. Working in this mode,  $G'_s$  and  $G''_s$  can be calculated independent of instrumental factors.<sup>27</sup> The CIR-100 was operated with a small Labcon Molecular Photonics 700 Series LB film balance (Labcon, U.K.), thus allowing control of the surface pressure and the area per molecule of the monolayer. The amphiphiles were spread at the air-water interface of the small LB trough in the rheometer at 19 °C. The film pressure was set to the desired pressure with an accuracy of  $\pm 0.06$  mN/m and the film was presented to the Pt/Ir De Noüy ring of

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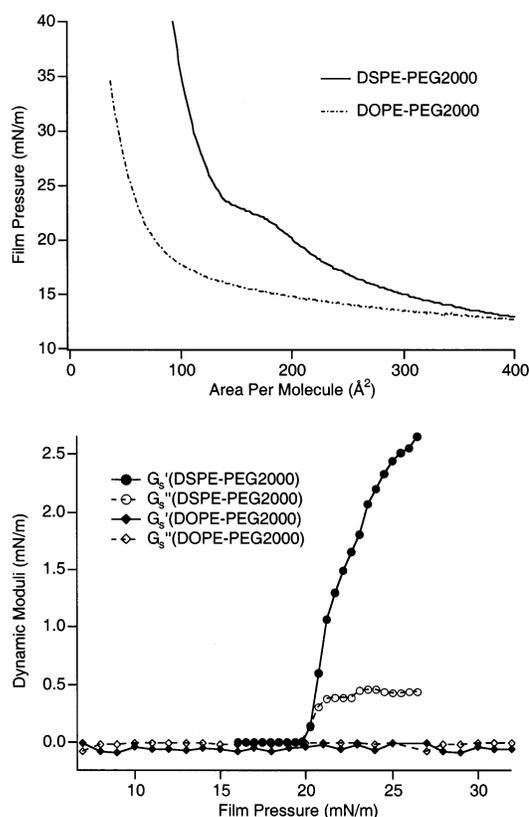
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the rheometer. Following a 5-min incubation period, the dynamic moduli were measured at a frequency of 2 Hz and an amplitude of 1500  $\mu\text{rad}$ . All experimental results reported for surface rheology measurements involved 240-min long experiments with 24 data points collected. In this case, each data point was averaged over eight cycles. During the time of the surface rheology experiment, film pressure data were taken every minute.

Neutron reflectometry experiments were conducted at the Surface Profile Analysis Reflectometer (SPEAR) at the Los Alamos Neutron Science Center (LANSCE) of the Los Alamos National Laboratory. LANSCE is equipped with a spallation source that provides neutrons over a wavelength range of 1–16 Å. To conduct neutron reflectometry experiments of PEG lipopolymers at the air–water interface, a film balance (Molecular Photonics 700 series, U.K.) was placed inside of the neutron reflectometer. The temperature was controlled via water circulator at 19 °C. The film pressure was set to the respective values using a movable barrier, controlled by an active feedback loop. For each film pressure, neutron reflectometry experiments were conducted at two different angles of 0.75° and 1.2°. To ensure a good signal-to-noise ratio, data were collected over a time period of 3 (low angle) and 6 h (high angle). The scattering contrast used was PEG2000 (protonated) on  $\text{D}_2\text{O}$ . The fitting analysis was done on the basis of a simple two-box model using the fitting program Parratt32 version 1.5.2 (Berlin Neutron Scattering Center (BENSCE) at the Hahn-Meitner-Institute, Berlin, Germany).

## Results and Discussion

To verify whether the 2D gelation of lipopolymer monolayers is directly linked to the formation of micellelike structures at the air–water interface, we first studied the effect of the molecular geometry of PEG lipopolymers on the rheological and alkyl chain condensation transitions. The rationale behind these experiments is that an increasing area mismatch between hydrophilic and hydrophobic moieties of gel-forming amphiphiles will lead to a more conelike shape of these molecules, thus promoting the formation of self-assembled structures of higher surface curvature such as micelles. PEG lipopolymers with lipid anchors carrying saturated alkyl chains are in general characterized by a pronounced cross-sectional area mismatch between the polymer and lipid segments, with the molecular area of the polymer moiety being significantly larger than that of the lipid. Figure 2 illustrates the  $\pi$ – $A$  isotherms (A) and  $G'_s/G''_s$  versus  $\pi$  plots (B) for DSPE-PEG2000 and DOPE-PEG2000. The rationale is that the saturation within the alkyl chain region if the lipid anchor results in a more pronounced conelike shape for DSPE-PEG2000 than for its counterpart carrying unsaturated chains (DOPE-PEG2000). Figure 2A shows that DSPE-PEG2000 exhibits an alkyl chain condensation whereas DOPE-PEG2000 lacks such a transition. Figure 2B reveals that unlike in DSPE-PEG2000 no rheological transition can be found for DOPE-PEG2000. This result leaves two possible explanations. First, the lack of a sufficient geometrical mismatch (cone shape) of DOPE-PEG2000 prevents a micellization transition. Second, the unsaturation of the DOPE anchor prevents the alkyl chain condensation as a necessary precursor for gelation at the film pressures observed. The latter argument is, for example, supported by the fact that the chain-melting transition of lipids is strongly dependent on the degree of unsaturation of their alkyl chains and the position of the double bond within the alkyl chains.<sup>28</sup> The existence of



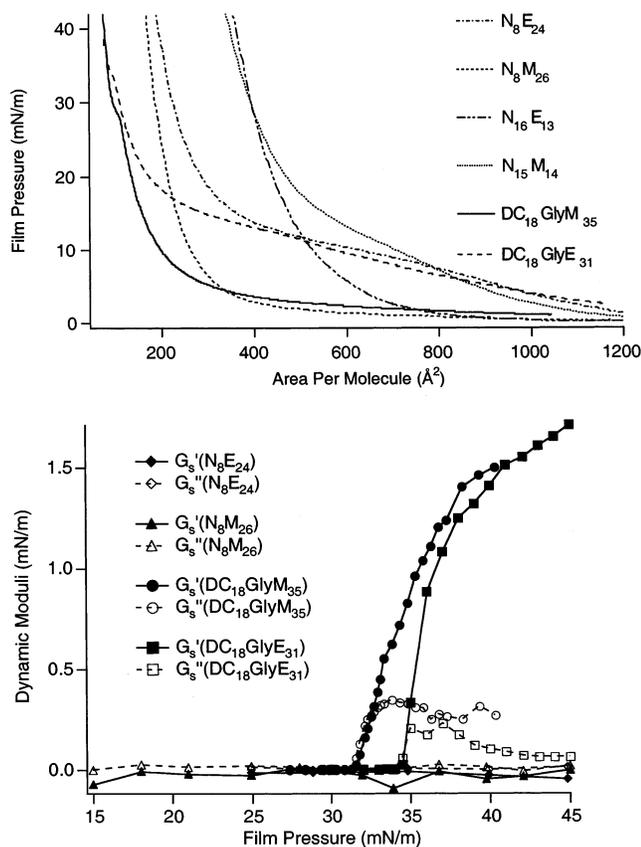
**Figure 2.**  $\pi$ – $A$  isotherms (A) and  $G'_s/G''_s$  vs pressure plots (B) of DOPE-PEG2000 and DSPE-PEG2000 around the alkyl chain condensation transition (plateau region of the  $\pi$ – $A$  isotherm).

the cis-double bond at the two cis-9 positions of alkyl chains of DOPE, for example, dramatically lowers the chain-melting transition to  $T = -16$  °C from  $T = 74$  °C of DSPE without double bonds.<sup>29</sup>

To investigate the impact of the molecular geometry on  $\pi$ – $A$  isothermal and rheological transitions, we conducted film balance and surface rheology experiments on  $\text{N}_x\text{E}_y$  and  $\text{N}_x\text{M}_y$  diblock copolymers at the air–water interface. Studies on diblocks are helpful because appropriate block sizes of their hydrophilic and hydrophobic blocks facilitate micelle formation, without the possibility of an alkyl chain condensation for short side chains. Figure 3 shows the  $\pi$ – $A$  isotherms (A) and  $G'_s/G''_s$  versus  $\pi$  plots (B) for  $\text{N}_8\text{E}_{24}$ ,  $\text{N}_{15}\text{E}_{14}$ ,  $\text{N}_8\text{M}_{26}$ , and  $\text{N}_{16}\text{M}_{13}$ . While  $\text{N}_{15}\text{E}_{14}$  and  $\text{N}_{16}\text{M}_{13}$  represent diblocks of similar block size,  $\text{N}_8\text{E}_{24}$  and  $\text{N}_8\text{M}_{26}$  are characterized by a hydrophobic/hydrophilic block size ratio of 1:3. Figure 3A shows that all diblocks studied lack the high film pressure transition plateau interpreted as alkyl chain condensation in the lipopolymers. Notably, these diblocks do not show a rheological transition, as depicted in Figure 3B. To illustrate the qualitative difference between nonyl block and lipid anchor, Figure 3A/B also contains the corresponding transition behavior of the poly(ethyl oxazoline) and poly(methyl oxazoline) lipopolymers ( $\text{DC}_{18}\text{Gly-E}_{31}$  and  $\text{DC}_{18}\text{Gly-M}_{35}$ ). As reported previously, these lipopolymers exhibit both alkyl chain condensation and 2D gelation transitions.<sup>18</sup> It can be directly studied that there is no direct correlation between the molecular area mismatch between hydrophobic and hydrophilic moieties of amphiphiles and their ability to form 2D gels. No 2D gelation could be found for the

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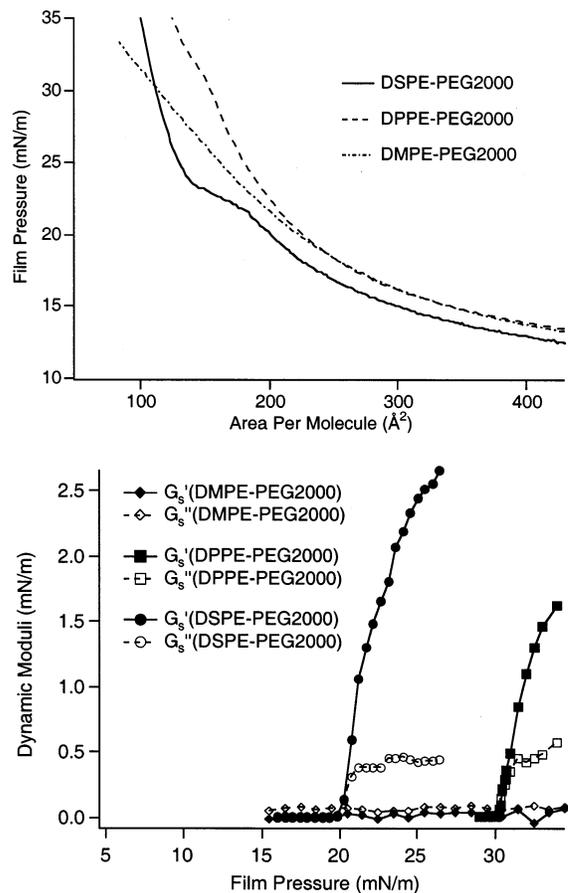
(29) The Lipid Data Bank. <http://www.ldb.chemistry.ohio-state.edu>, July 2002.



**Figure 3.**  $\pi$ - $A$  isotherms (A) and  $G'_s/G''_s$  vs pressure plots (B) of diblock copolymers N<sub>15</sub>E<sub>14</sub>, N<sub>8</sub>E<sub>24</sub>, N<sub>16</sub>M<sub>13</sub>, and N<sub>8</sub>M<sub>26</sub>. Unlike the lipopolymers DC<sub>18</sub>-Gly-E<sub>31</sub> and DC<sub>18</sub>-Gly-M<sub>35</sub>, which are also shown for comparison, the diblocks investigated show no  $\pi$ - $A$  isothermal and rheological transitions. All diblocks show the same area per molecule at high surface pressure (not shown).

asymmetric diblock copolymers, N<sub>8</sub>E<sub>24</sub> and N<sub>8</sub>M<sub>26</sub>, nor for the symmetric counterparts, N<sub>15</sub>E<sub>14</sub> and N<sub>16</sub>M<sub>13</sub>.

Let us now consider the second possible explanation for the missing alkyl chain condensation and gelation transitions in DOPE-PEG2000 shown in Figure 2A/B, which links both transitions to the strength of the van der Waals interaction between interacting lipid moieties. To study the influence of this parameter, we compare both the  $\pi$ - $A$  isotherms and the  $G'_s/G''_s$  versus  $\pi$  plots comparing PEG lipopolymers (PEG  $M_n$ : 2000 g/mol) of different lipid anchor lengths, as shown in Figure 4 A/B. Alkyl chain lengths considered are C<sub>14</sub> (DMPE-PEG2000), C<sub>16</sub> (DPPE-PEG2000), and C<sub>18</sub> (DSPE-PEG2000). As shown previously,<sup>16</sup> the transition pressures at the alkyl chain condensation transition for DPPE-PEG2000 and DSPE-PEG2000 vary by about 10mN/m (Figure 4A). Nevertheless, both systems show a qualitatively similar rheological transition behavior (Figure 4B).<sup>16</sup> Figure 4A also shows that the  $\pi$ - $A$  isotherm of DMPE-PEG2000 lacks the alkyl chain condensation transition within the observed pressure range. Supporting earlier assumptions that no rheological transition occurs without prior alkyl chain condensation transition,<sup>17</sup> Figure 4B exhibits that DMPE-PEG2000 lacks a rheological transition too. The observed behavior would be counterintuitive from the perspective of the molecular geometry because in this case the micellization transition would be more likely for shorter alkyl chain lengths at a given  $M_n$  of the PEG. Obviously, the data in Figure 4A/B can be understood if one considers the different strengths of van der Waals interaction among



**Figure 4.**  $\pi$ - $A$  isotherms (A) and  $G'_s/G''_s$  vs pressure plots (B) of DMPE-PEG2000, DPPE-PEG2000, and DSPE-PEG2000 around the alkyl chain condensation transition (plateau region of the  $\pi$ - $A$  isotherm). While DPPE-PEG2000 and DSPE-PEG2000 show both the alkyl chain condensation and the gelation transitions, DMPE-PEG2000 lacks those transitions. The slope of the  $\pi$ - $A$  isotherm of DMPE-PEG2000 above 20mN/m indicates some instability of the monolayer at high film pressures.

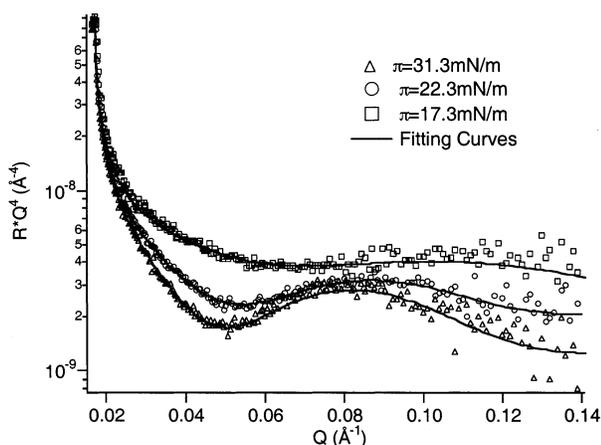
lipopolymers of different alkyl chain lengths. Thus, one can explain why the film pressure values of film balance and rheological transitions increase with increasing alkyl chain length, as observed for DPPE-PEG2000 and DSPE-PEG2000 and why DMPE-PEG2000 lacks both transitions. Notably, the surface rheology and film balance data in Figure 4A/B show that a first-order alkyl chain condensation transition is required for the 2D gelation to occur, thus agreeing with earlier predictions from surface rheology and film balance experiments on phospholipid-lipopolymer mixtures.<sup>17</sup>

The next question is how the structural properties of the monolayer of PEG lipopolymers change at the alkyl chain condensation and rheological transitions. Particularly, it is of interest to know whether the alkyl chain condensation is linked to a surface micellization of the monolayer. Neutron reflectometry has been proven to be a very powerful technique to explore structural properties of monolayers of amphiphiles at the air-water interface because it provides a refractive index profile perpendicular to the interface with Å resolution.<sup>30-34</sup> Recent X-ray and

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**Figure 5.** Neutron reflectometry data obtained from DSPE-PEG2000 below ( $\pi = 17.3$  mN/m) and above ( $\pi = 22.3$  mN/m and  $\pi = 31.3$  mN/m) the alkyl chain condensation transition. Neutron data are shown as  $RQ^4$  vs  $Q$  plots, with  $R$  being the reflectivity and  $Q$  being the momentum transfer. Corresponding fitting results using a two-box model are listed in Table 1.

**Table 1. Fitted Parameters of Layer Thicknesses,  $d_i$ , Scattering Length Densities,  $\rho_i$ , Interfacial Roughnesses,  $\sigma_i$ , of Monolayers of PEG Lipopolymers at the Air–Water Interface<sup>a</sup>**

film pressure (mN/m)	$d_1$ (Å)	$\rho_1$ ( $\times 10^6 \text{ \AA}^{-2}$ )	$\sigma_1$ (Å)	$d_2$ (Å)	$\rho_2$ ( $\times 10^6 \text{ \AA}^{-2}$ )	$\sigma_2$ (Å)	$\chi^2$ ( $\times 10^{-2}$ )
17.3	17.1	5.52	4.7	31.8	5.46	18.3	1.09
22.3	32.8	4.51	4.2	36.9	5.30	17.4	0.54
25.5	33.6	4.39	4.7	38.0	5.22	18.3	0.60
31.3	31.6	4.18	6.0	40.2	5.05	27.2	1.17

<sup>a</sup> The data were fitted using a two-box model, with boxes 1 and 2 describing the lipid and polymer moieties, respectively. The indices 1 and 2 denote boxes 1 and 2. The parameter  $\chi^2$  provides information about the quality of the fits.

neutron reflectivity experiments on poly(2-oxazoline) lipopolymers at the air–water interface showed, for example, that the alkyl chain condensation is linked to the formation of surface micelles.<sup>24</sup>

Figure 5 shows the neutron reflectometry curves of DSPE-PEG2000 in  $D_2O$  recorded below (17.3 mN/m) and above the alkyl chain condensation transition (22.3 mN/m; 31.3 mN/m). Table 1 summarizes the fitting parameters for these measurements using a two-box model of the optical matrix method (also included experiment at 25.5 mN/m).<sup>35</sup> Here, layers 1 and 2 represent lipid and polymer moieties, respectively. As the protonated alkyl chains have a very low scattering contrast versus air, layer 1 primarily involves the lipid headgroup. The thickness of this layer of  $d_1 = 17.1$  Å can be understood on the basis of a pronounced protrusion among lipopolymers also found recently on mixed PEG lipopolymer–phospholipid monolayers at the air–water interface.<sup>33</sup> The most notable result from Table 1 is the significant increase of the thickness of layer 1 from  $d_1 = 17.1$  Å at  $\pi = 17.3$  mN/m to  $d_1 = 32.8$  Å at  $\pi = 22.3$  mN/m and the fairly constant values of this parameter above the transition. This is accompanied by a similar qualitative behavior of the scattering length density,  $\rho_1$ . While a significant decrease of  $\rho_1$  can be observed from  $\rho_1 = 5.51 \times 10^{-6} \text{ \AA}^{-2}$  at  $\pi = 17.1$  mN/m to

$\rho_1 = 4.51 \times 10^{-6} \text{ \AA}^{-2}$  at  $\pi = 22.3$  mN/m, only a moderate gradual decrease was found above  $\pi = 17.1$  mN/m with  $\rho_1$  being  $\rho_1 = 4.51 \times 10^{-6} \text{ \AA}^{-2}$  at  $\pi = 22.3$  mN/m,  $\rho_1 = 4.39 \times 10^{-6} \text{ \AA}^{-2}$  at  $\pi = 25.5$  mN/m, and  $\rho_1 = 4.18 \times 10^{-6} \text{ \AA}^{-2}$  at  $\pi = 31.3$  mN/m. If one considers the behavior of layer 2 below and above the alkyl chain condensation transition, which is primarily linked to the polymer moiety, there is only a moderate increase in the film thickness,  $d_2$ , and a moderate decrease in the scattering length density,  $\rho_2$ . The fitting data can be understood if one assumes a roughening of the monolayer as a result of a surface micellization of PEG lipopolymers which is in good qualitative agreement to similar experiments on poly(2-oxazoline) lipopolymers reported recently.<sup>24</sup> In the latter case, a partial immersion of the lipid moieties into the aqueous subphase was observed across the plateau region of the alkyl chain condensation transition.<sup>24</sup> Such a partial immersion was also proposed on the basis of recent grazing incidence X-ray diffraction studies on PEG lipopolymers at the air–water interface.<sup>23</sup> Interestingly, the grazing incidence X-ray diffraction studies also found clear experimental evidence for the existence of a 2D superstructure above the studied transition. Furthermore, in agreement to our findings, Wurlitzer et al. found no indication for a significant structural change of the polymer moiety at the lipid chain condensation.<sup>24</sup> Obviously, the neutron and X-ray scattering data on network-forming lipopolymers at the air–water interface support the idea that the alkyl chain condensation is associated with a nanocluster formation leading either to spherical nanoclusters resembling surface micelles or nanostripes, as observed previously.<sup>23</sup> In contrast to our earlier assumptions that lipopolymer clusters only consist of a few (three to four) molecules, where an attractive bridging force seemed to be necessary to explain the elastic behavior of the monolayer in the gel regime,<sup>16–18,21</sup> the recent neutron and X-ray data discussed above make bigger clusters with “colloidal” properties more likely. The moderate decrease of  $\rho_2$  with increasing film pressure, furthermore, indicates that the water content within this layer decreases as the monolayer gets more compressed, though the data shows no indication for a collapse. Previously, similar effects have been reported concerning the hydration of phospholipids.<sup>34</sup> Again our data on PEG lipopolymers are in good qualitative agreement with the findings from Wurlitzer et al. on poly(2-oxazoline) lipopolymers.<sup>24</sup>

So far, the experimental data discussed clearly show that the alkyl chain condensation among hydrophobic anchors of lipopolymers is a prerequisite for the formation of 2D gels. At the same time, surface rheology experiments on DPPC and DMPE show no 2D gelation ( $G_s' > G_s''$ ) occurring for amphiphilic phospholipids at the transition from the liquid-expanded to the liquid-condensed phase.<sup>16</sup> The latter finding illustrates the importance of the polymer moiety of lipopolymers for the 2D gelation process. Furthermore, there is no direct correlation between the strength of alkyl chain condensation and the relative strength of the 2D gel, as recent film balance and surface rheology experiments on PEG lipopolymers of different  $M_n$  have shown.<sup>21</sup> A comparison between the surface rheology data on PEG lipopolymers of different PEG molecular weight presented previously and the surface rheology results presented in this study indicates that the strength of the network is dependent on the strength of molecular interaction within the hydrophilic polymer moiety of the monolayer.<sup>16,21</sup>

The above experimental findings indicate that the phenomenon of 2D gelation of lipopolymers at the air–

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water interface is linked to formation of surface micelles among these amphiphiles. This result gives rise to the hypothesis that the gelation process observed for lipopolymers at the air–water interface can be seen as a 2D analogue to the 3D gelation described for colloidal systems with grafted polymers, such as star polymers or diblock and triblock copolymers.<sup>4–12</sup> An important aspect of the 2D gelation process is that it requires the existence of surface micelles of lipopolymers held together by alkyl chains in the liquid-condensed phase. Interestingly, interaction potentials on colloidal systems with grafted polymer chains in good and athermal solvents were strictly repulsive,<sup>13,14</sup> whereas attractive polymer–polymer bridging forces mediated by water molecules via hydrogen bonding have been discussed in the case of 2D gels of lipopolymers.<sup>16</sup> In the latter case, it has been argued that the 2D gel formation of lipopolymers is caused by two parallel processes, the steric frustration and the partial dehydration of hydrophilic polymer chains. While the first process may lead to the formation of surface micelles, the second one could result in attractive polymer–polymer bridges, as discussed earlier.<sup>16</sup>

Meanwhile, it is well established that the 3D gelation of copolymers is related to the formation of liquid-crystalline mesophases.<sup>4–9,36</sup> Recent reports on the thermoreversible gelation of star polymers suggest, on the other hand, gelation on the basis of the cluster formation of jammed stars.<sup>11,12</sup> In the latter case, where SANS experiments did not show any indication for solidification, the gelation is explained by a dynamic frustration of the system because of a mixture of trapped and free spheres.<sup>12</sup> On the basis of our current knowledge of the 2D gelation process of lipopolymers, it is not quite clear whether the 2D gelation could be the result of the formation of liquid-crystalline mesophases or whether this peculiar phenomenon rather resembles the formation of jammed clusters predicted for 3D gels of star polymers. Though the 2D gelation based on a jamming transition seems to be very attractive to explain the  $\pi$ - $A$  isothermal and surface rheology behavior of lipopolymers at the air–water interface, the verification of a superlattice structure in lipopolymer monolayers at the air–water interface above the alkyl chain condensation transition leaves open questions.<sup>23</sup>

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## Conclusion

In this study, we have presented surface rheology and film balance data, which unambiguously show that the alkyl chain condensation is necessary for the 2D gelation to occur, whereas a molecular area mismatch between hydrophobic and hydrophilic moieties of amphiphiles (asymmetric shape) does not automatically lead to this peculiar phenomenon. Instead, the 2D gelation process is more likely related to the existence of micellelike structures formed at the alkyl chain condensation transition. In this case, the rationale is that the gelation phenomenon observed for lipopolymers at the air–water interface represents a 2D analogue of the 3D gelation phenomena observed in the star polymers and copolymers. Still unanswered remains the question whether the 2D gelation resembles the formation of a liquid-crystalline mesophase, as observed for copolymers in 3D,<sup>4–9</sup> or whether it shows more similarities to a jamming transition resulting in a mixture of trapped and free clusters, as found for star polymers.<sup>12</sup> To obtain more insight into this open topic, we are currently conducting single-particle tracking experiments on monolayers of lipopolymers at the air–water interface below and above the gelation transition.<sup>37,38</sup> Furthermore, it seems possible that 2D gels of lipopolymers may form which are not held together by attractive forces among their hydrophilic polymer chains. To explore this opportunity in more detail, we are also studying the surface rheology properties of several lipopolymers at the air–water interface, which are characterized by different polymer side chains.

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