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Molecular Weight Dependence of Viscoelastic Properties in Two-Dimensional Physical Polymer Networks: Amphiphilic Lipopolymer Monolayers at the Air−Water Interface

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ABSTRACT: Lipopolymers are known to form a two-dimensional physical network (physical gel) at the air−water interface that is stabilized by two different types of associative interactions: microcondensation of alkyl chains of lipopolymers to form small clusters and physical junction zones linking neighboring polymer chains stabilized by hydrogen bonding of water molecules. In this study we present surface rheology and film balance experiments on amphiphilic PEG lipopolymers of different molecular weights of their polymer moiety (MW: 750, 1000, 2000, 3000, 5000) at the air−water interface. Our experimental data show that the gelation transition shifts within the MW range of 1000−5000 to smaller areas per molecule as the polymer chain length gets shorter until it reaches a specific minimum area where no further change is observed (MW = 1000). Surface rheology data of the storage modulus indicate a qualitative difference between higher MW species (MW: 2000, 3000, 5000) and lower MW species (MW: 750, 1000). While the well-known power law dependence above the gelation point can be observed in the first case, a breakdown of the network after an initial power law-like behavior is found in the latter one. Our data suggest that a sufficient area mismatch between polymer and lipid moieties of these peculiar amphiphiles is essential for a stable two-dimensional physical network to form.

Introduction

Lipopolymers are amphiphiles consisting of a single, hydrophilic polymer chain covalently attached to the headgroup of a phospholipid or phospholipid-like molecule. Their peculiar molecular structure has led to several important applications: (1) as building blocks in artificial biomembranes based on tethered polymer−lipid composites,[1−5] (2) as components in site-specific, drug delivery liposomes,[6] (3) as model systems for studies on thin films of end-grafted polymers with mobile grafting points,[7−11] and (4) for the study of two-dimensional physical network phenomena.[12−14] The latter two aspects take advantage of the fact that many lipopolymers can form a stable monolayer at the air−water interface, thereby allowing for an adjustment of the polymer conformation by compression or expansion of the monolayer.[7]

Baekmark et al. first reported that monolayers of lipopolymers at the air−water interface show two characteristic transition regions in their π−A isotherms:[7] the low-film-pressure transition, π_{low}, at which the polymer chains are forced from the air−water interface into the subphase (found only if polymer chains show amphiphilic character) and the high-film-pressure transition, π_{high}, caused by the condensation of alkyl chains of lipopolymers.[9] Helm and co-workers showed recently, on the basis of grazing incidence diffraction and X-ray reflectivity, that the latter transition is accompanied by the formation of nanostripes[11] which could be visualized via AFM after transfer from the air−water to the solid−water interface.[10] Interestingly, similar structures have been proposed and observed in the case of diblock copolymer systems.[15]

Recent surface rheology studies on poly(ethylene glycol) and poly(oxazoline) lipopolymers conducted in the oscillating stress−strain mode revealed a viscoelastic transition in the region of π_{high} with the storage modulus, G_s', being larger than the loss modulus, G_s'', above the transition and with $G_s' < G_s''$ below, which has been interpreted as the formation of a two-dimensional physical polymeric network.[12,14] In this case, it is assumed that the network is held together by two different kinds of junction zones: (1) alkyl chain condensations leading to small spherical clusters or nanostripes of lipopolymers and (2) physical junction zones between polymer chains of neighboring polymer clusters which are stabilized by water intercalates via hydrogen bonding. The polymer moiety of lipopolymers seems to be crucial for the gelation phenomenon to occur because phospholipids (without grafted polymer chains) lack the ability to form physical networks.[12] The stabilization of the network by water intercalates was derived not only from the fact that PEG is known to form small crystals and microgels[16,17] and is more ordered in aqueous solution than in organic solvents[18] but also because the gelation was found to be sensitive to the strength of hydrogen bonding in the system (H_2O was replaced with D_2O) and to changes in pH.[12] Such a stabilization of physical gels by polymer−solvent complexes is already known and was first reported on atactic polystyrene−carbon disulfide systems by Guenet and co-workers.[19,20] It should be mentioned, however, that recent surface rheology experiments on end-tethered polymer monolayers at the air−water interface showed a similar rubberlike behavior within a specific frequency range, even though the polymer chains were exposed to air.[21] Since solvent effects can be excluded in this case, the interpretation of rubberlike behavior has been based on chain overlap among neighboring polymer chains. As surface rheology studies on lipopoly-

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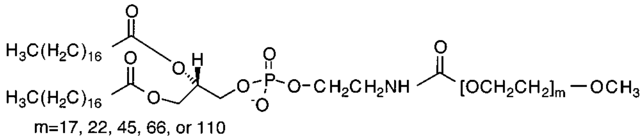

Figure 1. Molecular structure of 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[poly(ethylene glycol)*x*] (DSPE-PEG*x*), where *x* symbolizes the various molecular weights of the PEG chain—5000, 3000, 2000, 1000, and 750. These correspond to total numbers of monomer segments, *m*, of $m = 113$, 67, 44, 22, and 16.

mer monolayers with different hydrophobic moieties (dioctadecylglycerol vs dioctadecylamine) have shown, not only the polymer moiety but also the hydrophobic anchor seems to be crucial for the physical gelation to occur. Thus, it was found that the network would form if the hydrophobic anchor was dioctadecylglycerol but not if it was dioctadecylamine.[14] This supports the idea that the physical gelation of network-forming amphiphiles at the air—water interface is caused by two separate but equally important physical interactions involving both the hydrophilic and hydrophobic moieties of these molecules. As recent surface rheology and film balance experiments on mixtures of phospholipids (DMPC) and PEG lipopolymers have verified, both the high-film-pressure transition in the π–A isotherm and the rheological transition describe two correlated but different phenomena because the latter one occurred sometimes well above the high-pressure plateau of the corresponding π–A isotherm.[13]

The purpose of the work presented herein was to address another important aspect of these peculiar polymeric networks—the relationship between the rheological properties and the molecular weight of the polymer chain of lipopolymers. Such studies are of great importance for the understanding of the two-dimensional physical gelation phenomenon among network-forming amphiphiles because they allow for the modification of important molecular parameters, such as the amphiphilic balance and the area mismatch between hydrophilic and hydrophobic moieties, thereby providing insight into the role of these parameters for the gelation process. Previous studies comparing DSPE-PEG2000 and DSPE-PEG5000 already indicated the importance of the polymer chain MW on both the high-pressure and rheological transitions.[12] Most notably, it was found that G_s' of the higher MW species showed a smaller value on a reduced area scale, $A_r = (A_{rheo} - A)/A_{rheo}$, than its low MW counterpart with G_s'(DSPE-PEG2000)/G_s'(DSPE-PEG5000) ~ 1.8 above the gel point. In the current study, the following MW's have been investigated: 750, 1000, 2000, 3000, and 5000. Of particular interest is the question of whether or not a variation of the molecular weight of the polymer moiety will result in qualitative or quantitative changes in the two-dimensional physical network.

Materials and Methods

The lipopolymers studied were 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[poly(ethylene glycol)*x*] (DSPE-PEG*x*), where *x* symbolizes the various molecular weights of the PEG chain—5000, 3000, 2000, 1000, and 750 (Figure 1). These lipopolymers were purchased from Avanti Polar Lipids (>99% pure, Alabaster, AL). Each compound was dissolved in chloroform and spread as a monolayer over Milli-Q water (pH = 5.5, 18.2 MΩ·cm resistivity). The pressure—area isotherms were run for each lipopolymer at 15 °C on a large Labcon Molecular Photonics 700 series LB trough (Labcon, UK).

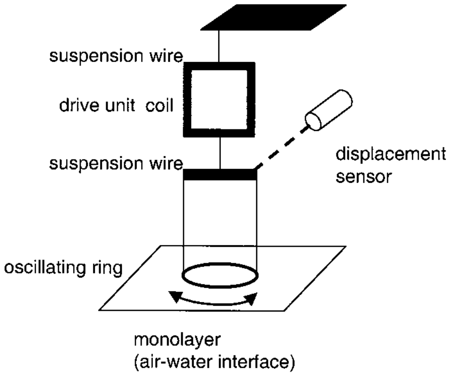

Figure 2. Schematic of the interfacial rheometer Camtel CIR-100 (Camtel, UK) designed for oscillating stress—strain experiments of monolayers at the air—water interface.

The rheometer used in all applications was a Camtel CIR-100 interfacial rheometer (Camtel, UK). As illustrated in Figure 2, it uses 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. Working in normalized resonance mode (>2 Hz), the feedback control system forces the system into phase resonance. In this case, the experiments are conducted under the condition that the input stress leads the output strain by 90°. Thus, the storage modulus, G_s', and loss modulus, G_s'', can be calculated independent of instrumental factors.[22] The CIR-100 was operated with a small Labcon Molecular Photonics 700 series LB film balance (Labcon, UK), thus allowing control of the surface pressure and the area per molecule of the monolayer. Each lipopolymer was spread at the air—water interface of the small LB trough in the rheometer at 15 °C. The film pressure was set to the desired pressure with an accuracy of ±0.06 mN/m, and the film was presented to the Pt/Ir De Noüy ring of the rheometer. Following a 5 min incubation period, the dynamic moduli were measured at a frequency of 2 Hz and an amplitude of 1500 μrad. All experimental results reported in this study involved 200 s long experiments with five data points collected. Each data point was averaged over eight cycles. After the experiment, the five data points were in turn averaged. The above procedure was repeated at various film pressures in order to obtain an adequate number of data points for analysis of each of the lipopolymers.

Results and Discussion

Unlike chemical polymeric networks with covalent cross-links, physical polymeric networks exhibit cross-linking zones stabilized by physical interactions such as hydrogen bonding or hydrophobic interactions. Usually, physical polymeric networks can be found in synthetic polymers or biopolymers, which have the ability to form crystals. Often called thermoreversible networks, these gels are heat-reversible with regard to their gelation transition. In this case, the reversibility is provided by the weakness of the cross-links involving either transient network junctions or quasi-permanent ones such as crystallites that are breakable due to applied stress. The literature reported on thermoreversible gels reveals a variety of microstructures of physical junction zones including, for example, crystallites linking adjacent polymer chains or fibrillar structures.[23,24,26] In contrast, there exists only limited information on the structural properties of network-forming amphiphiles below and above the gelation point.[12–14,21] Particularly, not much is known concerning how changes in the amphiphilic character affect structural and viscoelastic

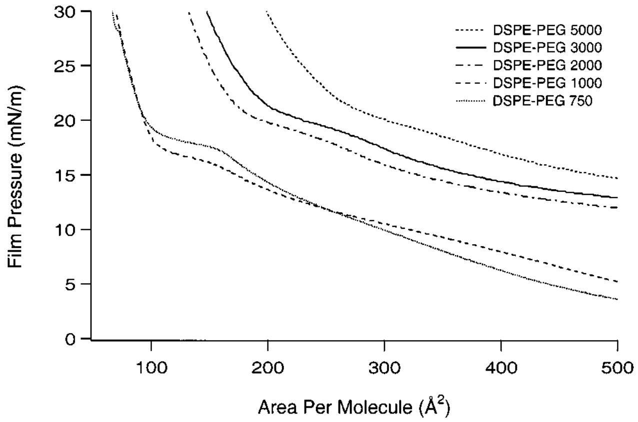

Figure 3. $\pi-A$ isotherms of DSPE-PEG750, DSPE-PEG1000, DSPE-PEG2000, DSPE-PEG3000, and DSPE-PEG5000 around the high-pressure transition.

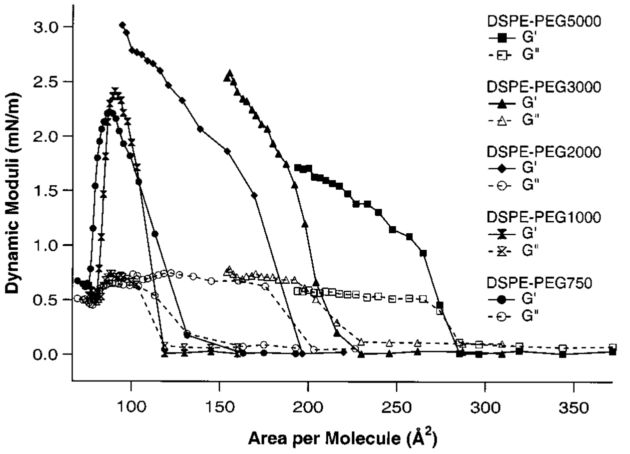

Figure 4. Storage modulus, G_s', and loss modulus, G_s'', of DSPE-PEG750, DSPE-PEG1000, DSPE-PEG2000, DSPE-PEG3000, and DSPE-PEG5000 plotted vs area per molecule. All lipopolymers show a rheological transition, which has been associated with the formation of a physical polymer network.[12]

properties within the monolayer of these amphiphiles at the air–water interface. The main purpose of the current study was to evaluate this topic on the basis of MW-dependent surface rheology and film balance experiments.

One important, but still unanswered, question, is: Do polymer chains of lipopolymers at the rheological transition resemble fibrils stabilized by water molecules or elongated polymer coils? A first indirect insight can be obtained from the pressure–area ($\pi-A$) isotherms of all lipopolymers as illustrated in Figure 3; $\pi-A$ isotherms provide information about the area per molecule at the high-pressure transition described by the area per molecule at the center of this transition, A_{fb}, which was reported to be close to the area per molecule of the physical network formation, A_{rheo}.[12,14] Notably, the $\pi-A$ isotherms in Figure 3 reveal a shift of A_{fb} to smaller values if the MW is decreased over the range of 1000–5000, while this area parameter remains constant for MW's 750 and 1000. Additional, more direct insight is provided by corresponding surface rheology experiments. The result of these experiments is illustrated in Figure 4, which shows the surface storage modulus, G_s', and the surface loss modulus, G_s'', of all lipopolymers plotted vs the area per molecule of the monolayer. It is found that each MW system exhibited a rheological transition with G_s' being larger than G_s'' above the gel point and with G_s' being smaller than G_s'' below (gel point: $G_s' = G_s''$). In agreement with the behavior of the $\pi-A$ isotherms in Figure 3, the rheological transition shifts to smaller area per molecule values if the MW is decreased within the range 1000–5000 but remains unchanged for MW's of 750 and 1000. Thus, film balance and surface rheology experiments strongly indicate that the polymer chains of lipopolymers of MW's 2000–5000 show, in the region of the physical network formation, the conformation of an elongated coil, whereas those of MW's 750 and 1000 resemble a rodlike fibrillar structure. While the behavior of A_{fb} and A_{rheo} leads to interesting insight into the polymer conformation, Figures 3 and 4 provide no conclusive answer as to how changes in MW of the polymer moiety of lipopolymers affect properties of the two-dimensional physical network. To obtain a better understanding about such network properties, it would be helpful to analyze the effects of the polymer and lipid moieties of lipopolymers on the network formation separately.

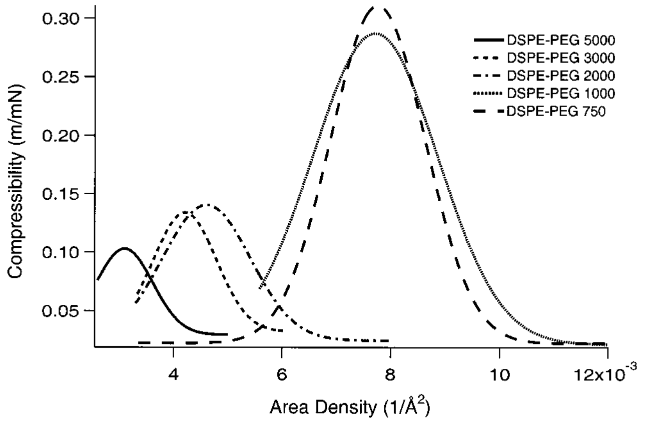

Figure 5. Compressibility as a function of area density for DSPE-PEG750, DSPE-PEG1000, DSPE-PEG2000, DSPE-PEG3000, and DSPE-PEG5000 around the high-pressure transition. The peaks represent the plateau region of the $\pi-A$ isotherms in Figure 3.

To understand the contribution of the lipid moiety on the network formation of lipopolymers, it is helpful to look at the strength of alkyl chain condensation for the different MW systems studied. Corresponding information can be obtained from the compressibility, $C = A^{-1}(\mathrm{d}A/\mathrm{d}\pi)$, of each lipopolymer plotted vs the area density, A^{-1} (Figure 5), because the size of the peaks is correlated to the strength of alkyl chain condensation. Figure 5 shows an increase in peak size (alkyl chain condensation) if the MW of the lipopolymer decreases. While the smallest peak can be found for DSPE-PEG5000, DSPE-PEG2000 and DSPE-PEG3000 show larger peaks, both of similar size. Still larger peaks are observed for DSPE-PEG750 and DSPE-PEG1000. This finding is not surprising if one considers that the value of A_{fb} in Figure 3 decreases as a result of a lower MW, thus allowing for a stronger coupling between alkyl chains of adjacent molecules at the high-pressure transition.

To study the contribution of the polymer moiety of lipopolymers on the physical network formation, results from surface rheology studies would be beneficial, as shown in Figure 4. The most striking result shown in Figure 4 is that there seems to be a marked qualitative difference with respect to the surface rheology data when the high molecular weight lipopolymers (5000, 3000, 2000) are compared to the low molecular weight

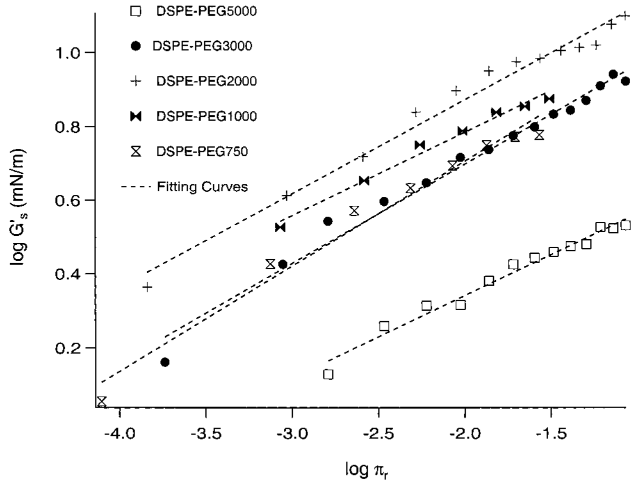

Figure 6. Log–log plots of G_s' vs reduced pressure $\pi_r = (\pi - \pi_{rheo})/\pi_{rheo}$ in the "power law region" (gel regime) for all lipopolymers investigated (data for MW: 750 and 1000 represent network before collapse at 100 Å$^2 < A < A_{rheo}$).

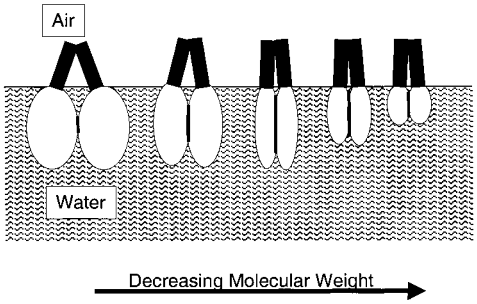

Figure 7. Schematic of a possible model to describe the observed MW-dependent surface rheology and film balance data. It assumes that the tethered polymer chains behave like entropic springs, thus showing interaction zones of different size between adjacent chains at different MW.

ones (1000, 750). While the high molecular weight species show the well-known power law dependence of G_s' above the gel point, their low molecular weight counterparts reveal a much more complex behavior. The most remarkable feature of the latter polymer system is that we observe the formation of a physical network, but the network breaks down at $A \sim 90-100$ Å^2. After the collapse, both G_s' and G_s'' are still significantly higher than their corresponding values below the gel point, though they are not different from each other. Since this network failure occurs at a film pressure well below the collapse pressure of the $\pi-A$ isotherm of the monolayer, some other phenomenon must be responsible for the network collapse. Unfortunately, we cannot exclude the possibility that the high MW species show similar unstable properties observed in the case of their low MW counterparts around $A \sim 90-100$ Å^2 because the first systems could not be compressed to such a small area per molecule values.

The observed difference between low and high molecular weight species of lipopolymers in Figure 4 leads to the question: What is the relative strength and stability of the physical network of lipopolymers if different MW's of low and high molecular weight species of lipopolymers are compared? While stability-related properties are not readily available from the experiments presented in this study, some interesting information can be obtained concerning the relative strength of the physical network. The $\pi-A$ isotherms in Figure 3 show that the film pressure of the high-film pressure transition, π_{high}, is independent of MW of the lipopolymer, thus indicating that a specific lateral stress (film pressure) must build up for the high-pressure and rheological transitions to occur. The independence of film balance and rheological transitions on film pressure makes it reasonable to compare the surface rheology data on a reduced pressure scale, $\pi_r = (\pi - \pi_{rheo})/\pi_{rheo}$. This is done in Figure 6, which illustrates an analysis of log–log plots of G_s' vs the reduced pressure, π_r, in the "power law region" (gel regime) for all MW's investigated (data for MW: 750 and 1000 represent network before collapse at 100 Å$^2 < A < A_{rheo}$). G_s' has been chosen because it should provide a direct measure of the strength of the network. As Figure 6 shows, G_s' follows a power law behavior if plotted vs the reduced pressure, $\pi_r = (\pi - \pi_{rheo})/\pi_{rheo}$. Here π_{rheo} represents the film pressure at the center of the rheological transition. Using the power law

$$G_s' \propto \pi_r^{\kappa}$$

the scaling exponents κ can be determined. A remarkable result illustrated in Figure 6 is that the same scaling exponent of $\kappa = 0.25 \pm 0.03$ can be found for all MW's studied. This indicates a similar response of the physical networks for different MW systems with respect to applied stress. By comparing G_s' for different MW's at a given value of π_r, one can see that the molecular weight has a remarkable effect on the magnitude of G_s', thereby following the trend G_s'(DSPE-PEG750) < G_s'(DSPE-PEG1000) < G_s'(DSPE-PEG2000) > G_s'(DSPE-PEG3000) > G_s'(DSPE-PEG5000). This trend shows that the strongest physical network (largest value of G_s') occurs at MW 2000, whereas a weaker network can be found if MW > 2000 and MW < 2000. It should be mentioned that the loss modulus, G_s'', exhibits a less pronounced but similar behavior (data not shown). Notably, there is no direct correlation between the strength of alkyl chain condensation and the magnitude of G_s' at a given π_r. At the same time, there is experimental evidence that the alkyl chain condensation is a necessary requirement for the physical gelation to occur.[13,14]

On the basis of the results related to the strength of alkyl chain condensation shown in Figure 5 and to those of the relative magnitude of G_s' illustrated in Figure 6, we have developed a phenomenological model presented in Figure 7, which is primarily based on steric considerations. It shows the lipopolymers as they would appear in a monolayer at a given reduced pressure. The MW dependences of A_{fb} and A_{rheo} shown in Figures 3 and 4 indicate that the high MW species exist in a less stretched polymer conformation at a given reduced pressure than their low MW counterparts. It is reasonable to assume that a more stretched polymer conformation results in a larger interaction zone between neighboring polymer chains. A plausible argument would be that the size of these zones of interaction determines the strength of the polymeric network. Thus, by lowering the molecular weight from DSPE-PEG5000 to DSPE-PEG2000, an increase in the strength of the physical network is expected and is observed experimentally (see Figure 6). This model would also allow for an explanation of the behavior of the low molecular weight species DSPE-PEG750 and DSPE-PEG1000. If one assumes that maximal stretching occurs somewhere in the range between PEG 1000 and PEG 2000, the surface area of interaction at a lower MW (DSPE-PEG750 and DSPE-PEG1000) is no longer dependent upon the stretching of the polymer and merely depends on the chain length. This would explain why the strength of the physical network becomes smaller as one lowers the MW from 2000 to 750. Most notably, it suggests that a specific area mismatch between hydrophilic and hydrophobic moieties of lipopolymers is required to form a stable two-dimensional physical network.

Conclusion

The current study has shed light on the polymer conformation of lipopolymers at the rheological transition. The experimental data support a polymer conformation based on an elongated coil (for PEG lipopolymers of MW 2000−5000) and for those of MW 750−1000 a rodlike, fibrillar structure. Most notably, our current study improved the understanding of how the amphiphilic character of lipopolymers affects the two-dimensional physical network formation. Our surface rheology data can be described by a phenomenological model of changing interaction zones between neighboring polymer chains, as illustrated in Figure 7. An important result of this study is that the relative strength of the two-dimensional physical network is dependent on the area mismatch between polymer and lipid moieties of lipopolymers. In addition, there is some indication of differences in network stability between low and high MW species of lipopolymers. Further information about the stability of these two-dimensional physical networks can be expected from time-dependent surface rheology experiments, which are currently in progress in our laboratory.[27]

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References and Notes

(1) Spinke, J.; Yang, J.; Wolf, J.; Liley, M.; Ringsdorf, H.; Knoll, W. *Biophys. J.* **1992**, *63*, 167.
(2) Seitz, M.; Wong, J. Y.; Park, C. K.; Alcantar, N. A.; Israelachvili, J. *Thin Solid Films* **1998**, *327−329*, 767.
(3) Wagner, M. L.; Tamm, L. K. *Biophys. J.* **2000**, *79*, 1400.
(4) Naumann, C. A.; Prucker, O.; Lehmann, T.; Ruehe, J.; Knoll, W.; Frank, C. W. The polymer-supported phospholipid bilayer: Tethering as a new approach toward substrate-membrane stabilization. *Biomacromolecules* **2002**, *3*, 27.
(5) Ke, P. C.; Naumann, C. A. *Langmuir* **2001**, *17*, 5076.
(6) Lasic, D. D. *Science* **1995**, *267*, 1275.
(7) Baekmark, T. R.; Elender, G.; Lasics, D. D.; Sackmann, E. *Langmuir* **1995**, *11*, 3975.
(8) Baekmark, T. R.; Wiesenthal, T.; Kuhn, P.; Albersdoerfer, A.; Nuyken, O.; Merkel, R. *Langmuir* **1999**, *15*, 3616.
(9) Wiesenthal, T.; Baekmark, T. R.; Merkel, R. *Langmuir* **1999**, *15*, 6837.
(10) Ahrens, H.; Baekmark, T. R.; Merkel, R.; Schmitt, J.; Graf, K.; Raiteri, R.; Helm, C. A. CHEMPHYSCHEM copublished with *Angew. Chem., Int. Ed.* **2000**, *1*, 101.
(11) Ahrens, H.; Graf, K.; Helm, C. A. *Langmuir* **2001**, *17*, 3113.
(12) Naumann, C. A.; Brooks, C. F.; Fuller, G. G.; Knoll, W.; Frank, C. W. *Langmuir* **1999**, *15*, 7752.
(13) Naumann, C. A.; Brooks, C. F.; Wiyatno, W.; Knoll, W.; Fuller, G. G.; Frank, C. W. *Macromolecules* **2001**, *34*, 3024.
(14) Naumann, C. A.; Brooks, C. F.; Fuller, G. G.; Lehmann, T.; Ruehe, J.; Knoll, W.; Kuhn, P.; Nuyken, O.; Frank, C. W. *Langmuir* **2001**, *17*, 2801.
(15) Bates, F. S.; Fredrickson, G. H. *Annu. Rev. Phys. Chem.* **1990**, *41*, 525.
(16) Polik, W. F.; Burchard, W. *Macromolecules* **1983**, *16*, 978.
(17) Daoust, H.; St-Cyr, D. *Macromolecules* **1984**, *17*, 596.
(18) Liu, K.-J.; Parsons, J. L. *Macromolecules* **1969**, *2*, 529.
(19) François, J.; Gan, J. Y. S.; Guenet, J.-M. *Macromolecules* **1986**, *19*, 2755.
(20) Gan, J. Y. S.; François, J.; Guenet, J.-M. *Macromolecules* **1986**, *19*, 173.
(21) Luap, C.; Goedel, W. A. *Macromolecules* **2001**, *34*, 1343.
(22) Camtel, *CIR 100 Interfacial Rheometer: Operating Manual,* 1999.
(23) Te Nijenhuis, K. Thermoreversible Networks. *Adv. Polym. Sci.* **1997**, *130*, 1.
(24) Guenet, J.-M. In *Thermoreversible Gelation of Polymers and Biopolymers;* Academic Press: London, 1992.
(25) Stauffer, D.; Aharony, A. *Introduction to Percolation Theory*; Taylor & Francis: Boston, 1991.
(26) Guenet, J.-M. *J. Rheol.* **2000**, *44*, 947.
(27) Coffman, J. P.; Naumann, C. A. Time dependence of viscoelastic properties in two-dimensional physical polymer networks of amphiphilic lipopolymer monolayers at the air−water interface. Manuscript in preparation.

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