Communications to the Editor

Gelation of Two-Dimensional Assemblies

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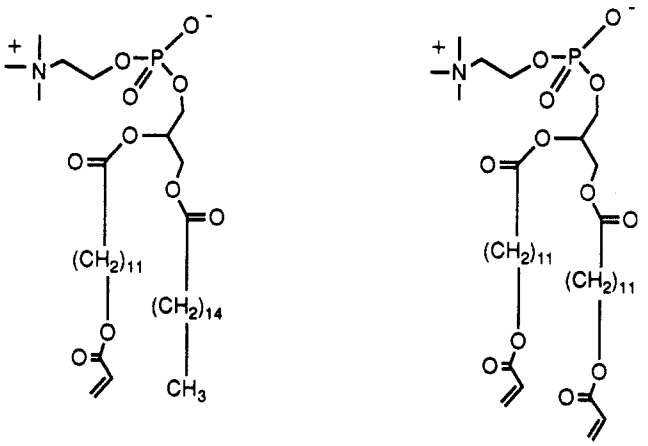
The concept of gelation of step or chain polymerizations has been fruitfully employed for more than a half century.[1,2] In three-dimensional polymerizations the gel point occurs when the polymer molecules have been cross-linked to one another to form an infinite network or macroscopic molecule. The physical properties of the polymer are dramatically altered at this point. The application of this concept to two-dimensional polymerizations is addressed here. By two-dimensional polymerizations we refer to the polymerization of supramolecular arrays of hydrated amphiphiles, e.g., monolayers, LB films, vesicles (liposomes), extended bilayers, cast multilayers, and tubules. The last decade has seen the introduction of several methods to polymerize these supramolecular assemblies (see ref 3–5 for reviews). Other approaches to two-dimensional polymeric networks have been described for polymerizations at interfaces,[6] within the hydrophilic planes of cast multilayer films[7] and of smectic liquid crystals.[8] The polymerization of hydrated amphiphiles opens opportunities to prepare new materials with particular properties of interest to both biological and material sciences. The successful design and application of two-dimensional polymers in supramolecular assemblies requires knowledge of the variables that control the size of the polymers as well as the critical composition for efficient cross-linking to form an infinite network, i.e., gelation.

It is recognized that the polymerization of double-chain amphiphiles, e.g., phospholipids, will yield linear polymer chains if the monomeric lipid contains a single reactive group (mono-substituted). The presence of two reactive groups per lipid (bis-substituted) can lead to cross-linked polymeric assemblies.[9–13] The physical properties of linearly polymerized bilayers are expected to be significantly different than those of cross-linked bilayers. Indeed that is the case judging from a limited number of permeability[9–11] and solubility[12,13] studies. Most of these studies have compared the properties of bilayers composed of a *single* lipid both before and after polymerization of the assembly. In one study, Regen and co-workers examined the effect of variation in the mole fraction of mono- and bis-lipoylphosphatidylcholine (LPC) on the sucrose permeability of the mixed lipid bilayers both before and after polymerization.[11] Increases in the mole fraction of bis-LPC, which was expected to produce a greater cross-link density in the polymerized bilayer, resulted in a progressive decrease in the bilayer permeability. An abrupt change that might be associated with a gel point was not apparent in these data.

In order to more closely examine the possibility of gelation in two-dimensional polymerizations, we have studied the dynamic properties of polymerized lipids in hydrated bilayers. The lateral diffusion of lipid molecules in bilayers or monolayers is a direct consequence of the dynamic nature of lipid assemblies. Fluorescence photobleaching recovery (FPR) is widely used to study the properties of various lipid assemblies via characterization of the lateral diffusion coefficient of a fluorescent probe molecule.[14–16] The lateral diffusion coefficient, D, for hydrated lipids above the lipid phase transition temperature (T_m) is frequently $>1\ \mu m^2 s^{-1}$.[16] FPR has also proven useful for the characterization of hydrated bilayers composed of a mixture of phospholipids exhibiting immiscibility and domain formation.[17] In addition the effect of polymerization on the lateral diffusion of a fluorescent lipid probe has been reported for mixtures of a nonpolymerizable and a polymerizable lipid, where the latter was either a bis-substituted dienoyl lipid[18] or a mono-substituted methacryloyl lipid.[19] Here we report the experimentally determined D for *N*-4-nitrobenzo-2-oxa-1,3-diazole–phosphatidylethanolamine (NBD–PE) in hydrated bilayers that were linearly polymerized or cross-linked to different extents. The lipids chosen for this study were the mono- and bis-acryloylphosphatidylcholine (MAPC and BAPC, respectively),[20] in which the polymerizable acryloyl group is located at the end of one or both alkyl chains of the phospholipid. Sells and O'Brien reported the number-average degree of polymerization for linear polymers formed in hydrated MAPC bilayers via thermal initiation with AIBN at 70 °C.[21] The observed polymer size was proportional to the [M]/[I] (over the range of 5–40) where the monomer concentration, [M], was kept constant. The same polymerization conditions were used in the present studies.



The frequently studied dimyristoylphosphatidylcholine (DMPC) bilayers were examined as a reference material. At 32 °C, which is 8 °C above the T_m of DMPC, D for NBD–PE was $3.9 \pm 0.6\ \mu m^2 s^{-1}$.[22] A similar value of D ($3.8 \pm 0.6\ \mu m^2 s^{-1}$) was measured for unpolymerized bilayers of hydrated MAPC at 40 °C (8 °C above its T_m).[23] As expected, a decrease in the sample temperature to 25 °C (below T_m) reduced D by about an order or magnitude. A comparison of the diffusion coefficients as a function of temperature permits a graphical estimation of the T_m of MAPC. This value of 32.0 ± 0.5 °C agrees well with the T_m of 31.7 °C determined by DSC.[23]

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Table I
Lateral Diffusion Coefficients of Polymerized MAPC Bilayers[a]

M/I[b]	X_n[c]	D[d] ($\mu m^2 s^{-1}$)	M/I[b]	X_n[c]	D[d] ($\mu m^2 s^{-1}$)
unpolymerized	(1)	3.8 ± 0.6	20	695 ± 100	0.28 ± 0.05
5	233 ± 29	1.4 ± 0.4	30	1476 ± 105	0.23 ± 0.04
10	413 ± 37	0.60 ± 0.16	40	1936 ± 68	0.24 ± 0.02

[a] MAPC/NBD-PE molar ratio = 1000/1. [b] M/I = monomer/initiator molar ratio. [c] Number-average degree of polymerization determined by SEC. [d] Lateral diffusion coefficient, D, for NBD-PE at 40 °C. The percent recovery was 95 ± 5% for each measurement.

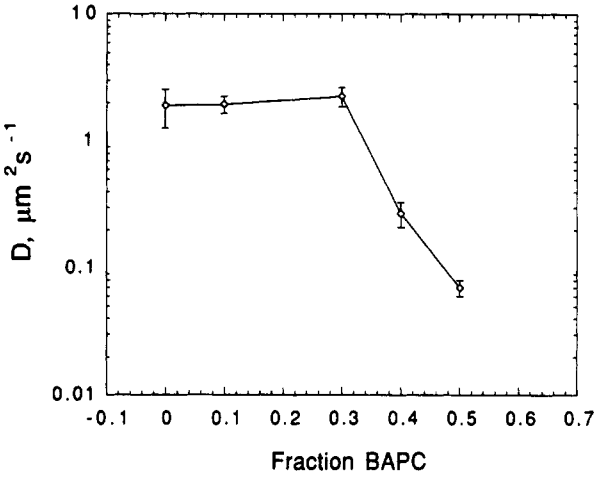

Figure 1. Measured diffusion coefficient (D, $\mu m^2 s^{-1}$) of NBD-PE in polymerized MAPC/BAPC multibilayers as a function of the mole fraction BAPC. Measurement temperature, 35 °C; molar monomer/initiator ratio, 5; lipid/NBD-PE, 1000:1.

The diffusion coefficients of NBD-PE in linearly polymerized MAPC bilayers as a function of the [M]/[I] ratio are shown in Table I. The corresponding degrees of polymerization determined by size-exclusion chromatography are taken from Sells and O'Brien.[21] The diffusion coefficients for these linearly polymerized bilayers were relatively insensitive to temperature- over an investigated range of 15–40 °C. No evidence for a phase transition was observed in polymerized MAPC bilayers. This indicates that the linear polymer chains are entangled and/or sterically arranged in a manner that prevents a cooperative lipid phase transition of the type associated with lipid bilayers, i.e., a thermotropic transition from a solid-analogous phase to a liquid-analogous phase.

The increase in the degree of polymerization of linear poly-MAPC causes a progressive decrease in D until the size of the polymers is nearly 700 repeat units. The greater than 1 order of magnitude decrease in D shows a significant inhibition of NBD-PE diffusion by the presence of the linear poly-MAPC chains. In contrast the presence of the bifunctional BAPC dramatically alters the dynamic behavior of polyacryloyl lipid bilayers. In order to maximize the observable effect of cross-linking on the D, the cross-linking experiments were performed with an M/I of 5, which yields the smallest poly-MAPC chains studied. Figure 1 shows a plot of D for NBD-PE in cross-linked bilayers formed from different compositions of MAPC and BAPC. The BAPC with one reactive group in each hydrophobic chain serves as a cross-linking agent between the linear MAPC chains. Note that the observed D remains relatively constant at mole fractions of BAPC of 0.3 or less and is comparable to that observed in linear poly-MAPC. When the fraction of BAPC was increased to 0.4, an abrupt decrease in the NBD-PE diffusion was observed. The efficient inhibition of NBD-PE mobility in these two-dimensional polymers signals a critical MAPC/BAPC molar composition of approximately 2:1.

We interpet this critical point as a manifestation of complete cross-linking of the polymers in the bilayer. The high percentage of BAPC required to reach this point is probably a consequence of the preferred conformation of BAPC and MAPC. It is known from structural studies of phospholipids that the glycerol backbone is perpendicular to the plane of the bilayer and that the *sn*-1 (α) chain penetrates more deeply into the bilayer than the *sn*-2 (β) chain.[24,25] The reactive acryloyl group in monomeric MAPC is located at the end of the β chain. The nonequivalent locations of the two acryloyl groups in BAPC suggest that it is an AB type cross-linker. This positional nonequivalence dictates that the reaction of MAPC and BAPC monomers will preferentially occur through the β chain acryloyl groups to produce linear polymers with periodic reactive α chains. The reaction of an α-acryloyl group on one polymer chain with an α-acryloyl group from another chain will link the chains. If this is the primary mode of cross-linking, it is apparent that a gel point in this two-dimensional polymer will require high mole fractions of the bis-acryloyl lipid as observed. The possibility of macrocyclization of the α- and β-acryloyl groups in BAPC, which cannot be excluded by the present data, is another potential source of cross-linking inefficiency. These observations suggest the design of other cross-linkers, which will be reported in due course.

In summary the FPR measurements reveal a significant decrease in the dynamic motion of small molecular probes in polymerized hydrated bilayers at a composition of about 2:1 mono- and bis-substituted lipids. This first observation of critical composition behavior in polymerized supramolecular assemblies provides a fresh insight into two-dimensional polymerizations which will aid in the design of new materials based on these processes.

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