Two-Dimensional Polymerization of Lipid Bilayers: Degree of Polymerization of Acryloyl Lipids

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ABSTRACT: The chemical reactivity of the amphiphiles in supramolecular assemblies is dependent on the constraints imposed by the two-dimensional matrix of the assembly. As part of a systematic study of the variables that control the polymerization of reactive lipids in lipid bilayers, the effect of both monomer [M] and initiator [I] concentrations on the degree of polymerization of acryloyl-substituted phospholipids was analyzed. The AIBN-initiated polymerization of hydrated bilayers of 1-palmitoyl-2-[12-(acryloyloxy)-dodecanoyl]-*sn*-glycero-3-phosphocholine (mono-AcrylPC) at 70 °C produced linear polymers, which were characterized after transesterification to remove the lipid head group. The resulting copolymers, which were composed of random repeat units of methyl acrylate and methyl carboxyundecylacrylate, were soluble in chloroform and analyzed by size-exclusion chromatography relative to PMMA standards. The number-average relative degree of polymerization (X_n) ranged from 50 to nearly 2000 and was proportional to $[M]^2$ and $[I]^{-1}$. These results suggest that chain termination at high conversion of mono-AcrylPC to polymer is dominated by primary termination. The observed effects are probably due to reduced mobility of the long polymer chains in the constrained environment of the bilayer interior, which diminishes the opportunity for bimolecular chain termination.

Introduction

Supramolecular assemblies are arrays of noncovalently associated molecules. In many types of assemblies, e.g., monolayers, bilayers, multilayers, etc., the amphiphilic molecules are free to move in a two-dimensional environment. The chemical reactivities of the amphiphiles are dependent on the constraints imposed by the two-dimensional matrix. In recent years several methods to polymerize these assemblies have been introduced.[1-3] Three strategies may be identified: (a) the formation of a supramolecular assembly from a polymerizable amphiphile(s) and the subsequent polymerization of the assembly; (b) the polymerization of a suitably designed amphiphile in isotropic media, followed by assembly formation from the prepolymerized amphiphile; and (c) the hydrophobic or electrostatic association of polymers with supramolecular assemblies. The first approach, polymerization of a preformed supramolecular assembly, relies both on the design and synthesis of reactive amphiphiles and on the physical characteristics of the assembly. The polymerizable group(s) may be incorporated into one or both of the hydrophobic tails or may be either covalently or electrostatically associated with the hydrophilic head group.

These polymerization provides opportunities to prepare novel materials with particular properties of interest in both biological and materials sciences. For example, it is already known that bilayer vesicle permeability[4-6] and chemical stability[5,7] are affected by polymerization of the vesicles. The formation of linear polymers results in moderate decreases in permeability as well as increases in vesicle stability to ethanol or surfactant, whereas the polymerization of bis-substituted lipids leads to more complex cross-linked polymer networks with corresponding large changes in vesicle properties. These early studies determined the size of the polymers but did not attempt to control the polymerization. Future success in the design of polymerized assemblies for particular uses requires a clearer understanding of the two-dimensional polymerization processes. However, until recently little systematic effort has been devoted to the characterization of these polymerizations of supramolecular assemblies. Clearly, the nature of the reactive group has a strong effect on the degree of polymerization ($\bar{X}_n$) as well as the rate of polymerization (R_p). Dorn et al. estimated the $\bar{X}_n$ to be about 500 for the thermal polymerization of dialkylammonium *mono*methacryloyl lipids in vesicles,[8] which was independently confirmed for a similar lipid by Bolikal and Regen.[9] Photopolymerization of chain-substituted styrene phospholipids gave polymers with a $\bar{X}_n$ of 400.[10] In contrast, photopolymerization of vinylbenzoylammonium halide lipids produced short polymers with $\bar{X}_n$ of 10-20.[11] Samuel et al. reported that polymerization of thio-substituted phosphatidylcholines (PC's) gave $\bar{X}_n$ of 17-25.[12] The $\bar{X}_n$ of a styrene-substituted quaternary ammonium lipid polymerized in monolayers was shown to be dependent on light intensity:[13] pulsed Nd-YAG laser light gave $\bar{X}_n$ ranging from 11 to 27, whereas low-intensity continuous-wave irradiations produced polymers an order of magnitude larger. Higashi et al. reported that the photoinitiated polymerization of styrenesulfonate electrostatically associated with the surface of cationic lipid bilayers gave high molecular weight polymers ($\bar{X}_n = 7 \times 10^4$).[14] The formation of two-dimensional polymers from smectic liquid crystals has also been described recently.[15]

To date there have been no reports examining the effect of other variables on the size of the polymers formed in supramolecular assemblies. The lack of systematic studies has made it difficult to clearly define the fundamental principles that underlie this important new class of polymerization. Accordingly, we have undertaken to determine the effect of monomer [M] and initiator [I] concentrations on the $\bar{X}_n$ for different classes of lipids. Here we describe the behavior of acryloyl-substituted PCs in bilayer membranes.[16] The lipid bilayer is a two-dimensional fluid composed of thousands of lipids with their hydrophilic head groups exposed to water and their hydrophobic tails aggregated in a manner to exclude water. Prior to polymerization the monomeric lipids rapidly diffuse within the plane of the bilayer.[17] Thus the lipid bilayer provides a highly ordered yet dynamic structure for the polymerization of the acryloyl lipids.

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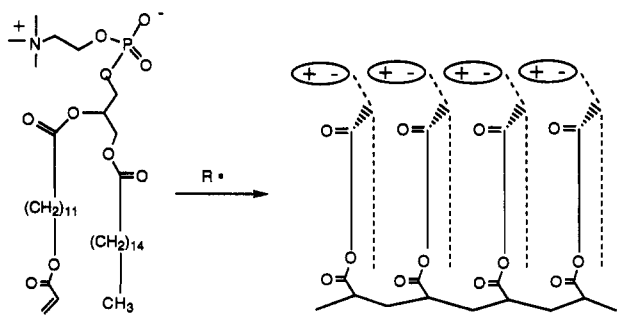
Results

Lipid Synthesis. Key considerations in the design of a polymerizable lipid are the choice and location of the polymerizable group within the lipid molecule. In this study *the effect of initiator and monomer concentrations on the degree of polymerization* was examined to test whether two-dimensional polymerizations occur in a manner similar to conventional three-dimensional polymerizations. The acryloyl polymerizable group was chosen since there is ample information concerning its polymerization behavior in isotropic media. The reactive groups were incorporated into the lipid in a manner that favored either the formation of linear polymers (one acryloyl per lipid) or cross-linked polymers (one acryloyl per each lipid chain).[17] Since these groups were located in the interior of the bilayer, an oil-soluble initiator, azobis(isobutyronitrile) (AIBN), was selected.

The lipids were synthesized by condensation of the appropriate fatty acid with either 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LPC) or L-α-glycero-*sn*-phosphocholine (GPC). The starting point for the fatty acid, 12-acryloyl-1-dodecanoic acid, was the monoesterification of excess 1,12-dodecanediol with freshly distilled acryloyl chloride. After workup and flash chromatography, the purified colorless 12-(acryloyloxy)-1-dodecanol was oxidized with excess pyridinium dichromate in DMF to yield 12-acryloyl-1-dodecanoic acid. The mono-AcrylPC, 1-palmitoyl-2-[12-(acryloyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine (**1**), was synthesized by reaction of LPC with 3 equiv of 12-acryloyl-1-dodecanoic acid and 1 equiv each of (dimethylamino)pyridine (DMAP) and dicyclohexylcarbodiimide (DCC) in chloroform. The bis-AcrylPC, 1,2-bis[12-(acryloyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine (**2**), was synthesized by condensation of GPC with 5 equiv of 12-acryloyl-1-dodecanoic acid with DCC using DMAP as catalyst.[18]

Polymerization of Lipid Bilayers. The appropriate amounts of lipid and AIBN were mixed in solution and then dried under vacuum to give a thin film. The sample was hydrated with argon-saturated Milli-Q to the desired lipid concentration, then heated above the lipid phase transition temperature (T_m), vortexed, and cooled in an ice bath. The procedure was repeated several times to ensure lipid hydration and mixing. The ampules were bubbled with argon again before sealing with a rubber septum and then placed in an oil bath at 70 °C for 18 h (four $t_{1/2}$ of AIBN),[19] which is significantly greater than the T_m of mono-AcrylPC (31.8 °C).[20] Although the extended bilayers would occasionally settle after polymerization overnight, the samples were always homogeneous for the first 5–8 h. The vesicles varied in size but were greater than 100 nm in diameter. Since a 100-nm-diameter vesicle consists of ~80 000 lipids in the bilayer, it will be seen that the growth of the polymer chains was not limited by the availability of lipids.



The molar ratio of lipid to initiator was varied from a high of 40 to a low of 5. A test of AIBN solubility in the bilayer was performed by demonstrating the complete solubility of 60 mg of AIBN (0.36 mmol) in 3.5 mmol of 1-heptanol at 55 °C. We assumed that the solubility at a molar ratio of 10 with a single-chain molecule was comparable to a molar ratio of 5 with a double-chain amphiphile, e.g., a phospholipid. Homolysis of AIBN generates two isobutyronitrile radicals plus nitrogen. Isobutyronitrile, $(CH_3)_2CHCN$ (IBN), was used as a model for the radical solubility. At room temperature at least 0.10 mL of IBN (1.1 mmol) was soluble in 1.0 mL of hexadecane (3.2 mmol). When the sample was warmed to 55 °C, 3.3 mmol of IBN could be dissolved in 1.0 mL of hexadecane. These experiments indicate that at the highest initiator concentrations, [M]/[I] of 5, used in the following studies both the AIBN and the isobutyronitrile radicals were soluble in the lipid phase at the experimental temperatures. It should be noted that, even though the solubility of AIBN in solvent models for the bilayer surpasses the requirements of the experiments, the partition coefficient for AIBN between the bilayer and aqueous buffer is unknown. Although the value of the partition coefficient is expected to be quite large, its uncertainty means that the values used for the initiator concentration in the bilayer are uncorrected.

After polymerization, the hydrated bilayers were lyophilized. A protocol was developed to enhance the polymer solubility for size-exclusion chromatography (SEC), because the dried polymers were insoluble in hexane, ether, chloroform, benzene, acetonitrile, tetrahydrofuran, dimethylformamide, and dimethyl sulfoxide and only sparingly soluble in dry hexafluoroisopropyl alcohol. BF_3–MeOH is a useful reagent for the methylation of fatty acids as well as transesterification of phospholipid chains; e.g., the overnight reaction of BF_3–MeOH with dioleoylphosphocholine gives methyl palmitate. Matsushita et al. utilized HCl/MeOH to increase the solubility of phospholipid polystyrene.[10] The reaction of BF_3–MeOH with mono-AcrylPC was examined to ensure the reaction proceeded in a satisfactory manner without effecting the polymerizable groups and to establish which lipid ester groups were transesterified. Reaction of BF_3–MeOH with mono-AcrylPC gave an isolable product, which was analyzed by ^1H NMR. The original lipid head group was completely absent, whereas signals due to a methyl ester peak and the acryloyl polymerizable group were both present in the spectrum. Therefore, the reactive group was unaffected by the reaction conditions, and both lipid tails were cleaved in the reaction. A potential complicating factor is the possibility of transesterification at two different positions in the polymerizable tail, either at the ester associated with the polymerizable group to give the short-chain methyl acrylate or at the ester linkage at the secondary glycerol position to give a long-chain methyl ester with a terminal acrylate group.

Transesterification of Polymerized Mono-AcrylPC. The most significant differences between the BF_3–MeOH transesterification of monomeric mono-AcrylPC (**1**) and of poly-**1** are the length of time necessary for the reaction and the solubility problems associated with the polymer in methanol. Consequently, the polymerized lipids were cleaved with BF_3 in a MeOH/benzene cosolvent. The polymer in methanol is thought to have a compact globular shape, which limits reagent penetration into the polymer network, whereas the addition of the cosolvent relaxes the polymer conformation. In some instances polymerizations were performed on bilayers composed of two lipids, mono-

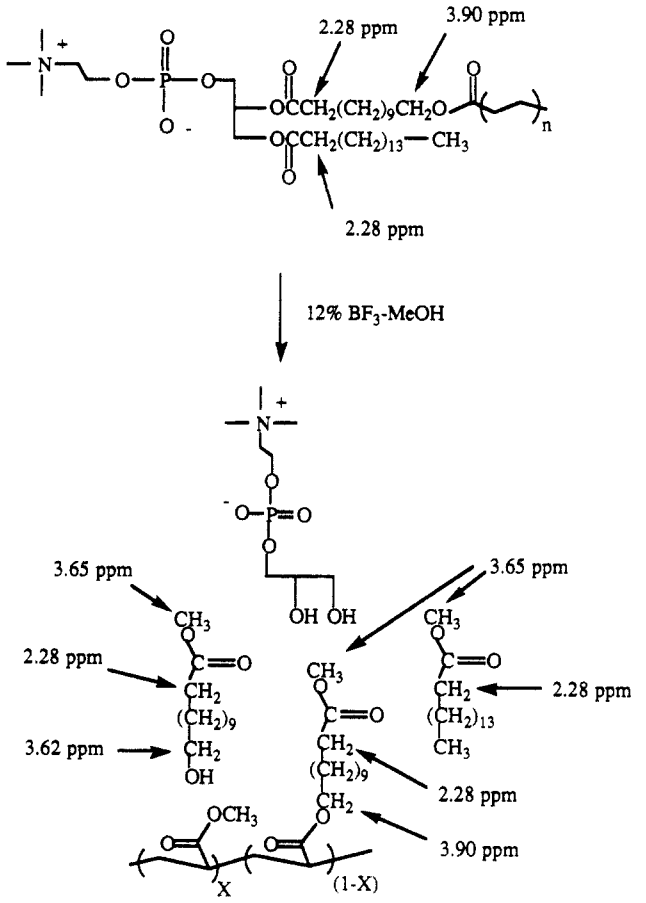


Figure 1. Products associated with the transesterification of polymerized mono-AcrylPC and the ^{1}H NMR resonances used to calculate the ratio between long- and short-side-chain-substituted repeat units. The peak integral at δ 2.28 (MGE protons) was a constant 4 protons, and the peak integral at δ 3.90 (*p*MVE protons) varied between 0 and 2 protons.

AcrylPC and the nonpolymerizable DMPC. In these cases the DMPC was extracted from the polymerized sample prior to reaction with BF_3–MeOH in order to simplify the polymer analysis by ^{1}H NMR and SEC.

The transesterification of poly-1 yields a copolymer composed of random repeat units of methyl acrylate and methyl carboxyundecylacrylate (Figure 1). The relative amounts of long- and short-side-chain esters (copolymer composition) was determined by comparing the integration of a peak associated with the long-side-chain repeat units with a peak whose relative intensity remained constant during the course of the transesterification reaction. The peak chosen for this analysis is at δ 3.90, which is the methylene α to the polymer backbone and is referred to as the polymer α-methylene vinyl ester (*p*MVE) peak. This peak has shifted upfield from its position at δ 4.12 (MVE) in the unpolymerized sample due to the loss of an electron-withdrawing acryloyl group upon polymerization. The triplet at δ 2.28 is referred to as the α-methylene glycerol ester (MGE) peak since it is due to the two methylene groups α to the carbonyls of the ester groups attached to the glycerol portion of the lipid. The MGE peak was constant and integrated for 4 protons, compared to the *p*MVE peak which varied from an intensity of 2 protons for a polymer consisting exclusively of the long-side-chain ester to 0 protons in the case of 100% short-side-chain ester, poly(methyl acrylate).

Degree of Polymerization of Mono-AcrylPC. Effect of Initiator Concentration. Following polymer modification via transesterification, the samples were analyzed by SEC to estimate the degree of polymerization. The

Table 1. Relative Degree of Polymerization of Mono-AcrylPC as a Function of [M]/[I] at Constant [M]

[M]/[I][a]	X_n	PDI range
5	233 ± 29	2.6–3.9
10	413 ± 37	3.7–4.8
20	695 ± 100	1.7–3.3
30	1476 ± 105	1.6–4.7
40	1936 ● 168	3.0–4.4

[a] The [M] was 5 mg/mL of mono-AcrylPC.

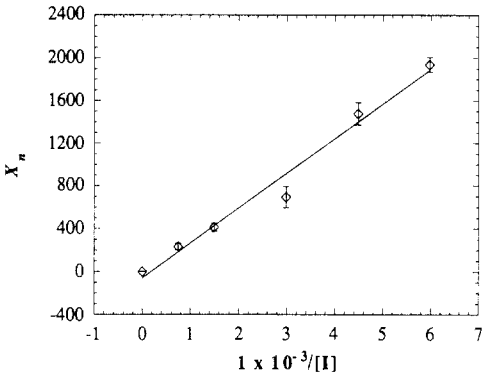


Figure 2. Number-average relative degree of polymerization (X_n) of mono-AcrylPC vs the inverse of the initiator concentration, [I].

effect of initiator concentration on the relative degree of polymerization of mono-AcrylPC was examined at monomer to initiator ([M]/[I]) ratios of 5, 10, 20, 30, and 40, where the [M] was held constant. The initiator concentration is expressed in millimoles of AIBN per milliliters of H_2O, but it is important to note that AIBN is present almost exclusively in the bilayer. The results of the SEC analysis of the modified polymers including the polydispersity indices (PDI) are found in Table 1. These data are a result of at least two polymerizations at each [M]/[I] ratio, followed by the BF_3–MeOH transesterifications on each polymer sample, and duplicate or triplicate SEC analysis. All the reported molecular weights are relative to PMMA standards. The number-average relative degree of polymerization (X_n) was calculated by dividing the number-average molecular weight (M_n) obtained from SEC analysis by the average repeat unit molecular weight of the copolymer sample, which was calculated from the ^{1}H NMR of each sample.

These experimentally determined X_n values which range from 200 to nearly 2000 are plotted as a function of the inverse of the molar initiator concentration in Figure 2. The linear fit to the data shown in the figure has a correlation coefficient of 0.989. In contrast, a plot of the data versus the inverse of the square root of the initiator concentration (not shown) gave a linear fit with a correlation coefficient of only 0.924. Therefore, the X_n for polymerized mono-AcrylPC appears to be inversely proportional to [I]. A further test of the data is shown in Figure 3, where log X_n is plotted vs the log [I]. The slope of the plot is −1.002, which is the order of the polymerization with respect to [I].

Since chemical modification of the original polymer was necessary for SEC analysis and the BF_3–MeOH chemistry produced a copolymer of variable proportions of the two repeat units, it was necessary to test whether the copolymer composition had a significant effect on the measured X_n. In the first test, samples were polymerized as described earlier and then divided in half. Each half was then subjected to different transesterification conditions to examine whether two samples with identical molecular weight distributions, but different copolymer compositions

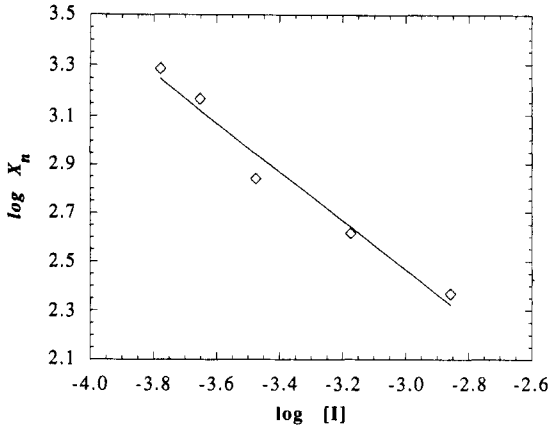


Figure 3. Log of the number-average relative degree of polymerization (log X_n) of mono-AcrylPC vs log of the initiator concentration, log [I].

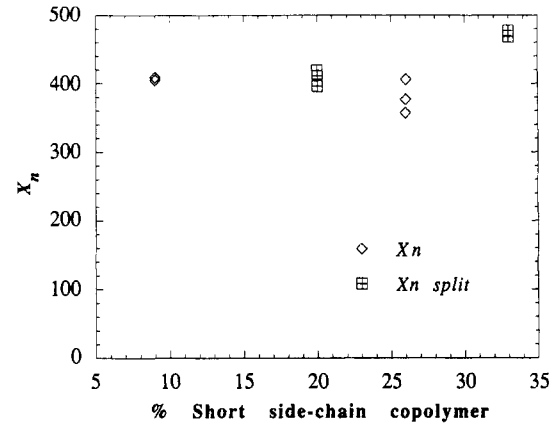


Figure 4. Plot of the number-average relative degree of polymerization (X_n) for poly-1 at [M]/[I] of 10 vs the composition of the copolymer formed by the transesterification of the polymer, including those samples which were divided (X_n split) prior to the transesterification reaction.

due to the transesterification reaction, gave similar X_n values. Experiments were run at [M]/[I] of 10 and 30, in order to examine polymer samples with either relatively low or high X_n. In both cases the modified polymers composed of high percentages of short-side-chain repeat units gave only moderately higher apparent degrees of polymerization (SEC), than found for the modified polymers composed of low percentages of the short-side-chain repeat units. The observed values in each case were within the range of error of those found in other polymerizations with the same [M]/[I] ratios.

In separate data analyses, all of the X_n data for [M]/[I] = 10 or 30 were plotted as a function of their percentage of short-side-chain composition (Figures 4 and 5). Examination of the graphs does not show a trend of either increasing or decreasing X_n values as the copolymer composition of the modified poly-1 is varied. Further analysis was performed by plotting the measured degree of polymerization vs $[I]^{-1}$ either using only the data where the copolymer composition was greater than 50% short-side-chain copolymer or the data from copolymer compositions less than 50% short-side-chain polymer. The two plots (not shown) were essentially superimposable. These data show that variation in the percentage of short- vs long-side-chain copolymer does not significantly effect the measured X_n of these polymers.

Degree of Polymerization of Mono-AcrylPC. Effect of Monomer Concentration. In order to characterize the dependence of X_n on the [M], it is obviously necessary to vary [M]. However, simply increasing the [M] only

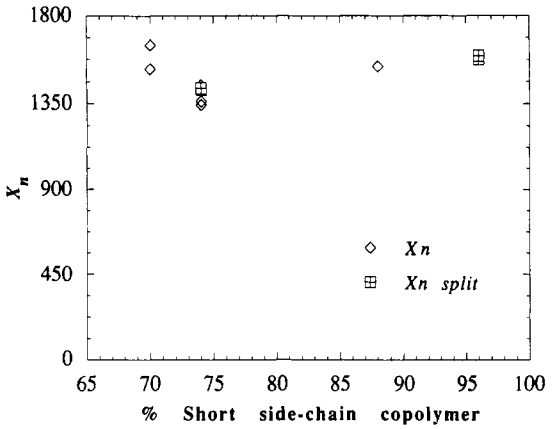


Figure 5. Plot of the number-average relative degree of polymerization (X_n) for poly-1 at [M]/[I] of 30 vs the composition of the copolymer formed by the transesterification of the polymer, including those samples which were divided (X_n split) prior to the transesterification reaction.

increases the number of bilayer vesicles in the suspension without modifying the local effective concentration of the monomer, because the critical micelle concentration of the lipids is low ($<10^{-6}$ M). This difficulty was circumvented by changing the mole fraction of monomer in the bilayer by mixing the mono-AcrylPC with a second nonpolymerizable lipid. The choice of the colipid is guided by the following considerations: first, it should be neither polymerizable nor reactive under the reaction conditions; second, it should mix uniformly with the polymerizable lipid to avoid the possibility of localized domain formation; and third, it should be readily available in a pure form. Dimyristoylphosphocholine (DMPC) was selected as the colipid for mono-AcrylPC, because it is a well-studied saturated PC which is widely available in high purity. Furthermore, the T_m of pure DMPC bilayers is 24 °C, which is similar to that of mono-AcrylPC (31.8 °C).[20] The two lipids vary in chain length by 2 atoms (14 carbons for DMPC; 16 atoms for mono-AcrylPC). These circumstances favor homogeneous mixing of the lipids at temperatures above the T_m of each lipid.[21] However, nonideal mixing will occur with evidence of lateral phase separation of the phospholipids when the chain lengths differ by 4 or more methylene groups.[22] Since the polymerization temperature of 70 °C is considerably greater than the T_m of either lipid, it was expected that the mixtures of the two lipids would be a homogeneous liquid crystalline phase at the start of the polymerization. This assumption was tested by differential scanning calorimetry of several mixtures of DMPC and 1. The data are shown in Figure 6, where the onset and completion temperatures of the endotherm for each mixture were used to construct the liquid–solidus lines by the procedure of Mabrey and Sturtevant.[22] Each of the DMPC/1 mixtures is in the liquid crystalline phase at the polymerization temperature.

The experimental polymerizations were performed with samples of mono-AcrylPC diluted with DMPC at a total lipid concentration of 5 mg/mL per sample. The ratio [M]/[I] was 30. The SEC data shown in Table 2 was obtained for the following molar ratios of mono-AcrylPC to DMPC: 1/0, 1/0.1, 1/0.5, and 1/1. The pure mono-AcrylPC gave a X_n of almost 1500, whereas the addition of DMPC to the bilayer substantially decreased the X_n as the mole fraction of DMPC was increased. These data were analyzed by plotting X_n versus [M]. In this instance a linear fit to the data could only be obtained with a correlation coefficient of 0.936. A much better correlation was found for X_n versus $[M]^2$, which is shown in Figure 7 (correlation coefficient 0.996); thus, the relative degree

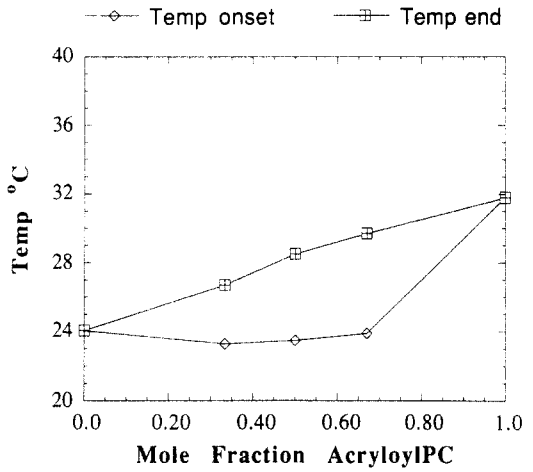


Figure 6. Phase diagram for DMPC and mono-AcrylPC constructed from the onset and completion temperatures of the thermotropic transitions of the indicated hydrated lipid mixtures.

Table 2. Relative Degree of Polymerization of Mono-AcrylPC as a Function of the Monomer to DMPC Ratio at Constant [I]

[M]/[DMPC][a]	X_n	PDI range
1/0	1476 ± 105	1.6–4.7
1/0.1	1300 ± 95	2.3–2.9
1/0.5	578 ± 39	1.5–2.8
1/1	299 ± 64	2.3–3.1

[a] The total lipid, monomer and DMPC, concentration was 5 mg/mL, and the molar ratio of lipid to initiator was 30.

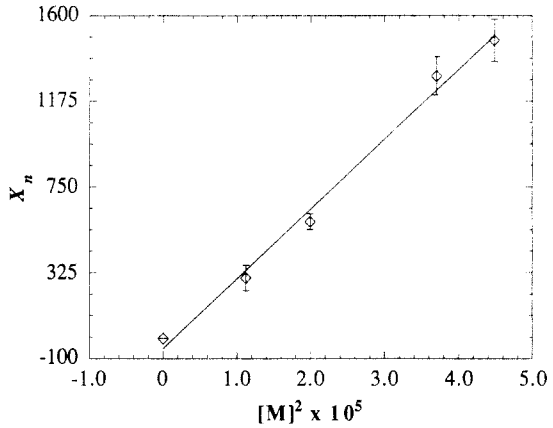


Figure 7. Number-average relative degree of polymerization (X_n) vs the square of the monomer concentration for mono-AcrylPC.

of polymerization of mono-AcrylPC appears to be proportional to the square of the monomer concentration.

Discussion

Lipid bilayers may be polymerized in a variety of ways using lipids with reactive groups located in distinct locations in the lipid and the bilayer. Here we are concerned with radical chain polymerization of reactive groups located at the chain terminal of the lipid, as exemplified by the monomer, mono-AcrylPC. Consequently, the acryloyl group is buried in the hydrophobic region of the bilayer at the most mobile region of the lipid chain. Although the monomers are constrained in the two dimensions of the bilayer, the lipids laterally diffuse past one another,[17] rather like a milling crowd, and the lipid chain ends are free to wag in the center of the bilayer. This affords ample opportunity for the monomers to diffuse to the growing polymer chain end with sufficient freedom of motion for the acryloyl group to adopt the proper conformation for reaction.

In this study the ratio of monomer to initiator was varied to reveal the relationship between the degree of polymerization and [M] and [I]. Usually the effect of variations in the monomer and initiator in solution polymerizations is analyzed at relatively low conversions to polymer. The data reported here were obtained at high conversions for the following reasons: first, many researchers who utilize the polymerization of lipids in bilayers or other supramolecular morphologies are interested in the polymer size at the completion of the reaction; second, it is experimentally easier to obtain reliable polymer molecular weights after transesterification of the original polymer, if the conversion is high rather than ~10%; third, our contemporary studies of the rate of polymerization, R_p, of the same lipid permit analysis of the relationship between [M] and [I] at both high and low conversions.[23]

Initiation with AIBN involves homolytic cleavage to produce two primary radicals which react with monomers to yield chain-initiating species. The chain propagates by successive addition of large numbers (hundreds or perhaps thousands) of monomer molecules. Each addition creates a new radical with the same chain end. Termination with annihilation of the radical centers frequently occurs by bimolecular reaction between radical chains by either coupling or disproportionation.

Thermally initiated free-radical polymerizations in solution usually have a kinetic chain length, ν, which is proportional to the first power of [M] and inversely dependent on the square root of [I]. This relationship shown in (1) is derived via the steady-state assumption; i.e., the rate of initiation, R_i, and the rate of termination, R_t, are equal. The rate of change of the concentration of radicals quickly becomes and remains zero during the course of the polymerization. The kinetic chain length is therefore the ratio of R_p, the rate of polymerization, to either R_i or R_t. The expression for the kinetic chain length is

$$\nu = R_p/R_i = k_p[M]/2(fk_dk_t[I])^{0.5} \quad (1)$$

where k_p is the rate constant for propagation, k_d is the rate constant for initiator homolysis, k_t is the rate constant for termination, and f is the initiator efficiency. The form of the expression is a consequence of the production of two initiator fragments per initiator molecule.

The number-average degree of polymerization, $\bar{X}_n$, is either equal to the kinetic chain length when termination occurs by disproportionation or is twice the kinetic chain length when termination results from coupling by two living polymer chains. Both coupling and disproportionation are diffusion controlled and require pairing of the electron spins. Many polymer radicals terminate predominately or entirely by coupling. However, certain features of the monomer structure promote the probability of disproportionation. Steric hindrance to bimolecular chain interactions will interfere with coupling, and the presence of β-hydrogens on the radical will facilitate H-transfer. Therefore, methyl acrylate polymerizations tend to terminate exclusively by coupling, whereas methyl methacrylate polymerizations may terminate by disproportionation as well.[24] An increase in the polymerization temperature increases the extent of disproportionation.[25]

The number-average degree of polymerization is first order in [M] and proportional to $[I]^{-0.5}$ in standard solution polymerizations. In contrast, the degree of polymerization of mono-AcrylPC in bilayers is proportional to $[M]^2$ and $[I]^{-1}$. Deviations from the normal bimolecular chain termination model for polymer chain growth are found at high initiator to monomer ratios or at high viscosity of the

medium. Under these conditions some primary radicals formed from the initiator may react with the growing polymer chain and provide a third termination mechanism, i.e., primary radical termination.[26-28] The rate of polymerization under these conditions is given by (2)

$$R_p = k_p k_i [M]^2 / k_{tp} \quad (2)$$

where k_{tp} is the rate constant for primary radical termination. This translates into the following expression for the $\bar{X}_n$, for a thermally initiated polymerization.

$$\bar{X}_n = k_p k_i [M]^2 / 2f k_d k_{tp} [I] \quad (3)$$

In the case of primary radical termination, $\bar{X}_n$ is proportional $[I]^{-1}$ as observed for mono-AcrylPC.

Primary termination could be a consequence of high [I] and/or low diffusional behavior of the polymer chains in the bilayer. Since a linear dependence on $[I]^{-1}$ was observed over the complete concentration range investigated, it is likely that the observed effects are due to the reduced mobility of the long polymer chains in the constrained environment of the bilayer interior. Either the translational mobility and/or the segmental mobility of the growing polymer chains may be limited by the bilayer. These circumstances tend to increase the lifetime of the polymer chains, thereby allowing the smaller primary radical fragments sufficient time to diffuse through the bilayer and terminate the growing polymer chain.

The calculated polydispersities (PDI) for the modified polymers (Tables 1 and 2) reveal a wide variation in polymer molecular weights in the samples. Frequently, high PDIs suggest a competition between radical chain termination mechanisms, i.e., coupling, disproportionation, and/or reaction with primary radicals. Exclusive primary termination should yield relatively small PDI values. The experimentally observed PDIs suggest the occurrence of another mode of termination, in competition with primary termination. This is likely to the coupling since acrylates do not usually disproportionate. If some fraction of the polymer chains terminate via coupling, the polymer would have a higher molecular weight fraction and a large PDI. This supposition is also consistent with the rate of polymerization data for mono-AcrylPC bilayers,[23] which revealed that at low conversion to polymer the reaction followed the conventional bimolecular termination rate expression and at higher conversion the rate expression was consistent with primary termination.

Primary termination requires the diffusional freedom of the isobutyronitrile radical regardless of the polymer size. Kölchens et al. have recently shown that the lateral diffusion coefficient (D) of a single lipid probe is only moderately decreased as the size of poly-1 is increased.[17] The D at 40 °C for the NBD–PE probe in poly-1 bilayers was $1.4 \pm 0.4\ \mu m^2\ s^{-1}$ when X_n was 2.3×10^2 and still $0.24\ \mu m^2\ s^{-1}$ for X_n of 1.9×10^3. The smaller hydrocarbon-soluble IBN radicals are expected to diffuse more rapidly than the axially oriented lipid, NBD–PE. Consequently, the available data indicate that IBN radicals should be able to diffuse to the growing polymer chain end and terminate the reaction.

Finally a word about the relatively large size of X_n found in these studies. The largest X_n measured here ($\sim 2 \times 10^3$) was considerably less than the number of lipids per vesicle; therefore, there were many polymer chains per bilayer vesicle at high conversions. On the other hand, a value of 2×10^3 is almost an order of magnitude larger than that suggested for a collapsed two-dimensional coiled polymer.[29] If the propagating polymer chain coils in upon itself, it can eventually be starved for monomers, unless of course additional monomers can diffuse to the living chain end. The covalent links between repeat units in poly-1 are at the end of the lipid tails. This arrangement may allow mono-AcrylPC molecules to diffuse over the low barrier presented by the polymer chain. Such a process would facilitate continued chain growth beyond that previously suggested for coiled polymers. With this in mind it will be interesting to evaluate the X_n for monomers with reactive groups at other locations in the lipid, which will yield polymer barriers at different depths in the bilayer.

Experimental Section

Materials. 4-(*N*,*N*-Dimethylamino)pyridine, 4-pyrrolidinopyridine, and 1,12-dodecanediol were obtained from Aldrich Chemical Co. and were recrystallized prior to use. Acryloyl chloride and pyridine were obtained from Aldrich Chemical Co. and were vacuum distilled prior to use. L-α-Glycero-*sn*-phosphocholine–cadmium chloride (GPC) and 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LPC) were obtained from Avanti Polar Lipids. Azobis(isobutyronitrile) (AIBN) was obtained from Eastman Chemicals and purified by recrystallization twice from methanol. Tetrahydrofuran (THF) and benzene were dried by distillation from sodium benzophenone. Chloroform was distilled from P_2O_5. *N*,*N*-Dimethylformamide was vacuum distilled. Water was purified using a Millipore Milli-Q system.

Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker WM-250 spectrophotometer. All samples were run in $CDCl_3$. Chemicals shifts are reported in ppm downfield from tetramethylsilane (TMS).

Syntheses. 12-(Acryloyloxy)-1-dodecanol. 1,12-Dodecanediol (25 g, 120 mmol) was dissolved in 250 mL of freshly distilled THF by gentle heating, and dry pyridine (4.0 mL, 50 mmol) was added. Freshly distilled acryloyl chloride (4.8 mL, 50 mmol) was dissolved in 50 mL of dry THF and added dropwise for 0.5 h under argon. One crystal of 2,6-di-*tert*-butyl-*p*-cresol was added as an inhibitor. The solution was allowed to stir overnight at room temperature. The mixture was filtered to remove the pyridine hydrochloride, and the THF was evaporated. The residue was taken up in 100 mL of CCl_4, cooled to −10 °C for 1 h, and then filtered to recover excess unreacted diol. The filtrate was evaporated and purified by flash chromatography on silica gel with $CHCl_3$ and then $CHCl_3/CH_3OH$ (95/5). The product, which was recovered from the $CHCl_3$/MeOH fractions by rotoevaporation, was a colorless oil. It was stored at −10 °C for use in subsequent reactions. Yield: 7.0 g (56%). TLC: R_f = 0.3 ($CHCl_3/CH_3OH$, 98/2). ^{1}H NMR: δ 6.36 (d, 1 H, vinyl), 6.20 (q, 1 H, vinyl), 5.78 (d, 1 H, vinyl), 4.12 (t, J = 6.6 Hz, 2 H, O=OCH_2), 3.62 (t, J = 6.6 Hz, 2 H, CH_2OH), 1.63 (br m, 4 H, CH_2), 1.24 (br s, 16 H).

12-(Acryloyloxy)-1-dodecanoic Acid. 12-(Acryloyloxy)-1-dodecanol (2.2 g, 8 mmol) was dissolved in 5.0 mL of DMF, and the resulting solution was added dropwise to a solution of pyridinium dichromate (10.6 g, 28 mmol) in 15.0 mL of DMF with one crystal of 2,6-di-*tert*-butyl-*p*-cresol. The reaction was performed with an ice bath and then allowed to reach room temperature with stirring for 1 day. The reaction mixture was rotoevaporated, and the residue was extracted first with water and then with ether. The ether layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel (200–425 mesh) with $CHCl_3$ followed by $CHCl_3/CH_3OH$ (97/3). The product was recovered from the $CHCl_3/CH_3OH$ fractions by rotoevaporation. The purified product was a white solid (mp 30–32 °C) which weighed 1.0 g (44%). TLC: R_f = 0.2 $CHCl_3/CH_3OH$ (99/1). ^{1}H NMR: δ 6.36 (d, 1 H, vinyl), 6.20 (q, 1 H, vinyl), 5.78 (d, 1 H, vinyl), 4.12 (t, J = 6.6 Hz, 2 H, O=COCH_2), 2.31 (t, J = 7.4 Hz, 2 H, CH_2CO_2H), 1.63 (br m, 4 H, CH_2), 1.24 (br s, 16 H).

1-Palmitoyl-2-[12-(acryloyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine. Dried LPC (400 mg, 0.82 mmol) was mixed with the acryloyl fatty acid (660 mg, 2.5 mmol) and (dimethyl-

amino)pyridine (100 mg, 0.82 mmol). One crystal of 2,6-di-*tert*-butyl-*p*-cresol was added along with 1,3-dicyclohexylcarbodiimide (200 mg, 0.98 mmol) and 5.0 mL of freshly distilled $CHCl_3$. The suspension was stirred at room temperature in the dark under argon for 2 days. The dicyclohexylurea was filtered and washed with $CHCl_3$, and then the filtrate was evaporated and the residue taken up in 20 mL of MeOH. Five grams of Bio-Rad AG 501-8X ion-exchange resin was added to the MeOH solution and stirred for 1 h. The resin was filtered and washed, and the filtrate was dried with anhydrous sodium sulfate. The mixture was gravity filtered, and the filtrate was evaporated. The residue was dissolved in $CHCl_3$ and purified by flash chromatography using a gradient of $CHCl_3/CH_3OH$ (9/1) followed by $CHCl_3/CH_3OH/H_2O$ (65/25/1). Yield: 300 mg (49%) of clear oil. TLC: $R_f = 0.28$ $CHCl_3/CH_3OH/H_2O$ (65/25/4). ^1H NMR ($CDCl_3$): δ 6.36 (d, 1 H, vinyl), 6.20 (q, 1 H, vinyl), 5.78 (d, 1 H, vinyl), 5.18 (br m, 1 H, $POCH_2CH$), 4.42–4.20 (br m, 3 H, $CHCH_2OP$, $CHOC{=}O$) 4.12 (m, 3 H, $CHOC{=}O$, $CH_2OC{=}O$), 3.90 (m, 2 H, NCH_2CH_2O), 3.78 (m, 2 H, NCH_2CH_2O), 3.32 (s, 9 H, $(CH_3)_3N^+$), 2.25 (m, 4 H, $CH_2C{=}O$), 1.6 (br m, 6 H, CH_2CH_2R), 1.28 (br s, CH_2, 38 H), 0.85 (t, 3 H, CH_2CH_3).

1,2-Bis[12-(acryloyloxy)dodecanol]-*sn*-glycero-3-phosphocholine. Dried GPC–cadmium chlorine complex (380 mg, 0.95 mmol) was mixed with fatty acid (500 mg, 1.9 mmol) and 4-pyrrolidinopyridine (136 mg, 0.92 mmol). One crystal of 2,6-di-*tert*-butyl-*p*-cresol was added along with 1,3-dicyclohexylcarbodiimide (430 mg, 2.09 mmol) and 5.0 mL of freshly distilled $CHCl_3$. The suspension was stirred at room temperature in the dark under argon for 6 days. The dicyclohexylurea was filtered and washed with $CHCl_3$. The filtrate was evaporated and the residue taken up in 20 mL of MeOH. Five grams of Bio-Rad AG 501-8X ion-exchange resin was added to the MeOH solution and stirred for 1 h. The resin was filtered and washed with 20 mL of $CHCl_3$ followed by 20 mL of MeOH. The filtrate was dried with anhydrous sodium sulfate. The mixture was gravity filtered, and the filtrate was evaporated. The residue was purified by flash chromatography using a gradient of $CHCl_3/CH_3OH$ (9/1) followed by $CHCl_3/CH_3OH/H_2O$ (65/25/1). Yield: 280 mg (40%) of clear oil. TLC: $R_f = 0.30$ $CHCl_3/CH_3OH/H_2O$ (65/25/4). ^1H NMR ($CDCl_3$): δ 6.36 (d, 2 H, vinyl), 6.20 (q, 2 H, vinyl), 5.78 (d, 2 H, vinyl), 5.18 (br m, 1 H, $POCH_2CH$), 4.42–4.20 (br m, 3 H, $CHCH_2OP$, $CHOC{=}O$) 4.12 (m, 1 H, $CHOC{=}O$; 4 H, $CH_2OC{=}O$), 3.90 (m, 2 H, NCH_2CH_2O), 3.78 (m, 2 H, NCH_2CH_2O), 3.32 (s, 9 H, $(CH_3)_3N^+$), 2.25 (m, 4 H, $CH_2C{=}O$), 1.6 (br m, 8 H, CH_2CH_2R), 1.28 (br s, CH_2, 28 H).

Methods. Polymerization of Supramolecular Assemblies. The desired lipid (15 mg) was evaporated from a stock solution (25 mg/mL in benzene) and mixed with the appropriate amount of initiator from a stock AIBN solution (1 mg/mL in benzene). The mixture was dried by passing a stream of argon over the sample in a glass ampule and then placed under high vacuum (0.1 mmHg) for 2 h in the dark. Each sample was protected from light to prevent AIBN photodecomposition. Milli-Q water was stirred under vacuum (50 mmHg) for 20 min to remove dissolved oxygen. The Milli-Q water was bubbled with argon for 10 min. The appropriate amount of degassed Milli-Q water was added to the sample to adjust the lipid concentration to 5 mg/mL. The ampules were bubbled with argon for 10 min before sealing them with rubber septums. Each sample was heated to 40 °C, vortexed for 1 min, and then cooled in an ice bath below T_m. This was repeated three times to form extended bilayers. At this point, each sample was somewhat opaque. The samples were bubbled again with argon for 10 min and then placed under argon in an oil bath at 70 °C for 18 h to perform the polymerization.

Transesterification Reaction. Two hundred microliters of a stock mono-AcrylPC solution (25 mg/mL in benzene) was evaporated to dryness by a stream of nitrogen gas. The sample was placed under high vacuum overnight. Four milliliters of 12% BF_3–MeOH was added, and the solution was stirred at room temperature under N_2 for 4 h. Two millilters of Milli-Q water was added, and the solution was neutralized by solid $NaHCO_3$ until no more CO_2 evolved. The sample was extracted six times with 4 mL of $CHCl_3$ each time. The organic phase was dried with Na_2SO_4 and filtered. The filtrate was evaporated and the sample analyzed by ^1H NMR: δ 6.38 (d, 1 H, vinyl); 6.10 (q, 1 H, vinyl); 5.80 (d, 1 H, vinyl); 4.12 (t, v, $CH_2OC{=}O$); 3.65 (s, v, CH_3O); 3.62 (t, v, CH_2O); 2.28 (t, 4 H, $CH_2C{=}O$); 1.6 (m), 1.3 (br s, 44 H, CH_2). Note: v = variable integration depending on the extent of reaction.

After polymerization of 15 mg of mono-AcrylPC, the sample was lyophilized and then taken up in 3 mL of dry benzene. Successful polymerizations were insoluble in benzene, whereas unpolymerized samples were completely soluble in benzene. One milliliter of 12% BF_3–MeOH was added, and the sample was stirred for 2 days at 35 °C. Five milliliters of Milli-Q water was added, and the solution was neutralized by solid $NaHCO_3$ until no more CO_2 evolved. The sample was extracted six times with 4-mL volumes of $CHCl_3$. The organic phase was dried with Na_2SO_4 and filtered. The filtrate was evaporated and the sample analyzed by ^1H NMR: δ 6.38 (d, 1 H, vinyl); 6.10 (q, 1 H, vinyl); 5.80 (d, 1 H, vinyl); 3.90 (br s, v, $CH_2OC{=}O$); 3.65 (s, v, CH_3O); 3.62 (t, v, CH_2O); 2.28 (t, 4 H, $CH_2C{=}O$); 1.6 (m), 1.3 (br s, 44 H, CH_2). Note: v = variable integration depending on the extent of reaction. After the polymerization of mixed lipid systems, the nonpolymerizable DMPC was removed by $CHCl_3$ extraction following sample lyophilization. The remaining polymeric material was transesterified as described above.

^1H NMR Analysis of Modified Polymer. The relative amounts of long- and short-side-chain esters (copolymer distribution) were determined by ^1H NMR comparing the integration of a peak associated with the long-chain polymer with a peak whose relative intensity remained constant during the course of the transesterification reaction. The peak chosen for this analysis was at δ 3.90 due to the methylene α to the polymer backbone, the polymerized methylene vinyl ester (*p*MVE) peak. The *p*MVE peak was compared to the triplet at δ 2.28, which was due to the two methylene groups α to the ester carbonyls and will be referred to as the α-methylene glycerol ester (MGE) peak. The MGE peak was constant and integrates to 4 protons, whereas the *p*MVE peak varied from an integration of 2 protons for a polymer consisting exclusively of the long-chain ester to an integration of 0 protons for the 100% short-side-chain ester, poly(methyl acrylate).

Size-Exclusion Chromatography. The polymer samples modified by transesterification were concentrated in $CHCl_3$ to approximately 200 μL by a stream of argon and analyzed by SEC on a Waters Maxima 820 chromatography workstation (Milford, MA) with an Ultrastyragel linear mixed-bed column (M_n range: 2×10^3–4×10^6). The $CHCl_3$ was filtered through 0.45-μm Waters nylon filters and then purged with helium prior to use. The instrument was interfaced to a NEC Powermate 1 computer with Maxima 820 version 3.02 software. All samples were detected using a Waters Model R401 differential refractometer, since they lacked a UV-absorbing chromophore. The column was calibrated with PMMA standards of narrow polydispersity (typically $M_w/M_n = 1.1$) with number-average molecular weights ranging from 4.1×10^3 to 1.3×10^6. The linear calibration curve fit had a correlation coefficient of 0.998. Each polymer sample was analyzed at least three times, and the results were averaged. Most polymer fractions were found between 6 and 10 min. The data acquisition rate was set at 2 points/s. The peaks were sliced in order to obtain a minimum of 40 slices/polymer peak, which was analyzed to yield number- and weight-average molecular weights as well as the polydispersity index. The relative degree of polymerization was calculated from these data and the copolymer composition derived from the NMR analysis of the modified polymer.

Several concentrations of PMMA standards of both high ($M_n = 1.3 \times 10^6$) and low ($M_n = 6 \times 10^4$) molecular weights were examined by SEC to determine if the refractive index detector was equally sensitive to polymer fractions of different M_n. A plot of maximal peak voltage versus PMMA concentration for $M_n = 6 \times 10^4$ was linear with a slope of 0.5294 (correlation coefficient 0.997). A similar plot for $M_n = 1.3 \times 10^6$ was linear with a slope of 0.524 (correlation coefficient 0.999). Therefore, the RI detector was slightly more sensitive for low MW polymers than larger polymers. In the sample concentration range of 0.2–0.7 mg/mL used in this study this difference in sensitivity is minimal and did not effect the calculated MW distribution.

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References and Notes

(1) O'Brien, D. F.; Ramaswami, R. *Encyclopedia of Polymer Science and Engineering*, 2nd ed.; John Wiley & Sons: New York, 1989; Vol. 17, pp 108–135.
(2) Ringsdorf, H.; Schlarb, B.; Venzmer, J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 113–158.
(3) Regen, S. L. In *Liposomes: From Biophysics to Therapeutics*; Ostro, M. J., Ed.; Marcel Dekker: New York, 1987; p 73.
(4) Dorn, K.; Klingbiel, R. T.; Specht, D. P.; Tyminski, P. N.; Ringsdorf, H.; O'Brien, D. F. *J. Am. Chem. Soc.* **1984**, *106*, 1627.
(5) O'Brien, D. F. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1987**, *28*, 438.
(6) Stefely, J.; Markowitz, M. A.; Regen, S. L. *J. Am. Chem. Soc.* **1988**, *110*, 7463.
(7) Regen, S. L.; Singh, A.; Oehme, G.; Singh, M. *J. Am. Chem. Soc.* **1982**, *104*, 791.
(8) Dorn, K.; Patton, E. V.; Klingbiel, R. T.; O'Brien, D. F.; Ringsdorf, H. *Makromol. Chem., Rapid Commun.* **1983**, *4*, 513.
(9) Bolikal, D.; Regen, S. L. *Macromolecules* **1984**, *17*, 1287.
(10) Matsushita, Y.; Hasegawa, E.; Eshima, K.; Ohno, H.; Tsuchida, E. *Makromol. Chem., Rapid Commun.* **1987**, *8*, 1.
(11) Serrano, J.; Mucino, S.; Millan, S.; Reynoso, R.; Fucugauchi, L. A.; Reed, W.; Nome, F.; Tundo, P.; Fendler, J. H. *Macromolecules* **1985**, *18*, 1999.
(12) Samuel, N.; Singh, M.; Yamaguchi, K.; Regen, S. L. *J. Am. Chem. Soc.* **1985**, *107*, 42.
(13) Rolandi, R.; Paradiso, R.; Xu, S. Q.; Palmer, C.; Fendler, J. H. *J. Am. Chem. Soc.* **1989**, *111*, 5233.
(14) Higashi, N.; Adachi, T.; Niwa, M. *Macromolecules* **1990**, *23*, 1475.
(15) Stupp, S. I.; Son, S.; Lin, H. C.; Li, L. S. *Science* **1993**, *259*, 59.
(16) For a preliminary report, see: Sells, T. D.; O'Brien, D. F. *Macromolecules* **1991**, *24*, 336.
(17) Kölchens, S.; Lamparski, H.; O'Brien, D. F. *Macromolecules* **1993**, *26*, 398.
(18) Radhakrishnan, R.; Robson, R. J.; Takagaki, Y.; Khorana, H. G. *Methods Enzymol.* **1981**, *72*, 408.
(19) Odian, G. *Principles of Polymerization*, 3rd ed.; John Wiley & Sons: New York, 1991; p 215.
(20) Lamparski, H.; Sells, T. D.; Lee, Y. S.; O'Brien, D. F. *J. Am. Chem. Soc.* **1993**, *115*, 8096.
(21) Luna, E. J.; McConnell, H. M. *Biochim. Biophys. Acta* **1978**, *509*, 462.
(22) Mabrey, S.; Sturtevant, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3862.
(23) Lei, J.; O'Brien, D. F. *Macromolecules*, in press.
(24) Avery, G.; Humphrey, M. J.; Poller, R. C. *Polymer* **1977**, *18*, 840.
(25) Bamford, C. H.; Eastwood, G. C.; Whittle, D. *Polymer* **1969**, *10*, 771.
(26) Ito, K. *J. Polym. Sci., Polymn. Chem. Ed.* **1974**, *12*, 1263.
(27) Ito, K. *J. Polym. Sci., Polym. Chem. Ed.* **1980**, *18*, 701.
(28) Berger, K. C.; Deb, P. C.; Meyerhoff, G. *Macromolecules* **1977**, *10*, 1075.
(29) Eggl, C. P.; Pink, D.; Quinn, B.; Ringsdorf, H.; Sackmann, E. *Macromolecules* **1990**, *23*, 3472.