Two-Dimensional Polymerization of Lipid Bilayers:[†] Degree of Polymerization of Sorbyl Lipids

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ABSTRACT: The reactivity of polymerizable amphiphiles in the two-dimensional matrix of supramolecular assemblies, such as lipid bilayers, is dependent on the monomer structure, the mode of initiation, the monomer to initiator ratio, and the lateral diffusion of the monomer within the assembly. As part of a study of these factors, we examined the thermally initiated polymerization and the photopolymerization of SorbPCs. The AIBN-initiated polymerization of hydrated bilayers of 1-palmitoyl-2-[10–(2′,4′-hexadienoyloxy)decanoyl]-*sn*-glycero-3-phosphocholine (mono-SorbPC) or 1,2-bis[10-(2′,4′-hexadienoyloxy)-decanoyl]-*sn*-glycero-3-phosphocholine (bis-SorbPC) at 60 °C produced linear and cross-linked polymers, respectively. In each case the polymers were transesterified to remove the lipid head group and yield linear copolymers composed of random repeat units of methyl sorbate and methyl carboxynonanyl sorbate, which were soluble in tetrahydrofuran and analyzed by size-exclusion chromatography relative to PMMA standards. The ^{1}H-NMR spectra of the transesterified polymers indicated the structures were 1,4-polymers. The number-average relative degree of polymerization (X_n) ranged from 50 to nearly 600 and was proportional to $[I]^{-1}$. These results, which suggest that chain termination at high conversion of SorbPC to polymer is dominated by primary termination, are similar to the behavior of acryloyl-substituted lipids (AcrylPC) in bilayers. The poly(AcrylPC)s were 4 times larger than poly(SorbPC)s at a given ratio of monomer to initiator. This is likely due to greater resonance stabilization of the sorbyl radical compared to the acryloyl radical. The photoreaction of SorbPC bilayers appears to be a singlet-mediated process, which produced oligomers at temperatures $> T_m$. The rate of the photoreaction was directly proportional to the incident light intensity in a manner consistent with photoactivated addition.

Introduction

The ability of certain molecules to self-assemble into supramolecular structures is important to both biological and material sciences. Hydrated amphiphiles yield lamellar or nonlamellar assemblies depending upon their structure and the variables of concentration, temperature, and pressure.[1] The polymerization of these preformed assemblies may be accomplished by the use of appropriately designed reactive amphiphiles.[2] Unlike molecules in isotropic solution, the motion of amphiphilic molecules is limited by the two-dimensional local environment of the assembly. Their reactivity depends on the constraints imposed by this two-dimensional fluid composed of thousands of lipids with their hydrophilic head groups exposed to water and their hydrophobic tails associated in a manner to exclude water. At temperatures above the main phase transition, T_m, of the bilayer, the lipids rapidly diffuse within the plane of the bilayer,[3] thereby providing a highly ordered yet dynamic structure for the polymerization of monomeric lipids.

Considerable research to date has led to the successful preparation of polymerizable amphiphiles to stabilize and alter the properties of supramolecular assemblies.[2,4,5] However, most of these studies did not attempt to control the polymerization process. Since an understanding of the factors governing two-dimensional polymerizations is important for the reproducible preparation of polymerized assemblies for specific applications, we have systematically examined the variables that affect these polymerizations.[6–8] Comparisons of the degree of polymerization, $\bar{X}_n$, as well as the rates of polymerization, R_p, for a number of polymerizable amphiphilies indicate that the nature of the reactive group and initiator have strong effects on the polymerization process. The thermal initiation of dimethyldialkylammonium methacryloyl lipids in vesicles gave polymers with a $\bar{X}_n$ of 500,[9] was independently confirmed for a similar lipid by Regen and Bolikal.[10] Direct UV irradiation resulted in a smaller X_n of 135.[10] The photopolymerization of a lipid with a styrene attached to one lipid tail gave polymers with a $\bar{X}_n$ of 400,[11] while the photopolymerization of vinylbenzoylammonium lipids exhibited X_n of 10–20.[12] Polymers from thio-substituted phosphatidylcholines (PCs) had $\bar{X}_n$ values of 17–25.[13] Higashi et al. reported that the photoinitiated polymerization of a styrenesulfonate monomer, which was electrostatically associated to the surface of a cationic lipid bilayer, gave polymers with a $\bar{X}_n$ of 7×10^4.[14]

Sells and O'Brien[6,7] examined the effect of variation in initiator concentration [I] and effective monomer concentration [M] on the relative number-average degree of polymerization (X_n) of hydrated bilayers of a mono-substituted acryloyl PC (mono-AcrylPC, **1**), where the reactive acrylate was attached to the terminal end of the lipid *sn*-2 tail. The resulting linear polymers were characterized by size-exclusion chromatography (SEC) after polymer methanolysis to yield copolymers composed of random repeat units of methyl acrylate and methyl (carboxylundecyl)acrylate. The X_n at high conversion ranged from 200 to 2×10^3 and was proportional to $[M]^2$ and $[I]^{-1}$. These results indicate that the propagating chains in the lipid bilayer were preferentially terminated by initiator fragments, i.e., primary termination, rather than the usual bimolecular chain termination found in solution polymerizations. In addition, Lei and O'Brien found that at high conversion the rate of polymerization, R_p, depended on [M] and [I]

[†] Previous papers in this series: Sells, T. D.; O'Brien, D. F. *Macromolecules* **1994**, *27*, 226–233; Lei, J.; O'Brien, D. F. *Macromolecules* **1994**, *27*, 1381–1388.

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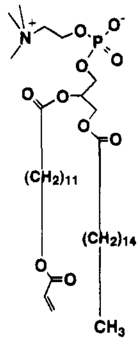
in a manner consistent with primary termination of the growing polymer chains.[8]

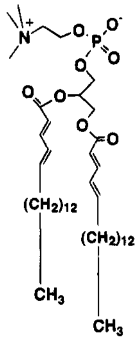
Tsuchida and co-workers reported that the polymer size for bilayer polymerizations of dienoyl-substituted PCs depended upon the method of initiation.[15] Photopolymerization with UV light of bis-DenPC, **2**, at temperatures > T_m resulted in oligomers ($\bar{X}_n = 6$), whereas photopolymerization of the mono-DenPC at temperatures < T_m gave somewhat longer polymers ($\bar{X}_n = 18$). Polymerization of mono-DenPC at 8 °C with redox initiators was reported to give polymers with a $\bar{X}_n$ of 28, whereas redox polymerization at 35 °C produced somewhat longer polymers ($\bar{X}_n = 45$). Similar or slightly longer polymer chains were prepared by radical polymerization with AAPD (either photochemical or thermal decomposition) or by γ-irradiation. While larger polymers were obtained at temperatures below the T_m for photopolymerization of mono-DenPC, the opposite was observed for the photochemical initiation with AAPD; i.e., the $\bar{X}_n$ was greater at temperatures above the T_m. If the AAPD concentration, [I], was decreased, then the $\bar{X}_n$ increased.

Here we report a systematic study of the effect of variation of [I] at constant [M] on thermal-initiated polymerization, as well as the direct photopolymerization, of lipid bilayers composed of either mono- or bis-substituted SorbPCs (**3** and **4**). The polymerization of these diene-substituted PCs provides an instructive comparison to our previous studies of AcrylPC bilayers[6-8] as well as an indirect comparison to the reported behavior of the DenPCs.[15] These studies demonstrate the importance of careful attention to the [M]/[I] ratio in order to reproducibly control the size and properties of polymers formed in bilayers and other supramolecular assemblies.



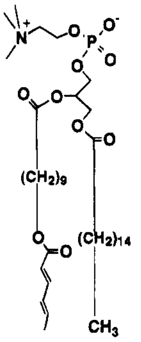
1

mono-AcrylPC



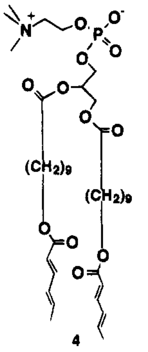
2

bis-DenPC



3

mono-SorbPC



4

bis-SorbPC

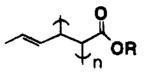
Results

The polymerizations of AcrylPC and methacryloylPC (MethPC) in hydrated lipid bilayers were characterized previously.[6-8] The corresponding SorbPCs are an important type of chain-substituted lipid, which may be polymerized in a bilayer by either radical initiation or direct photoactivation. Thus characterization of the degree of polymerization of SorbPC in bilayers offers new insights into the two-dimensional polymerization process and how it is affected by the structure of the monomer and the mode of initiation. The reactive sorbyl group(s) were incorporated into the lipid in a manner that favored the formation of linear polymers (one sorbyl per lipid, **3**) or cross-linked polymers (one sorbyl per lipid tail, **4**).

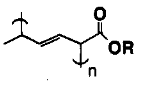
Polymerization of Lipid Bilayers. The appropriate amounts of lipid and AIBN were dissolved in solution and dried under vacuum to give a thin film, which was hydrated as described in the Experimental Section. The sample ampules were heated at 60 °C, a temperature significantly greater than the T_m of the SorbPCs. The multilamellar vesicles varied in size but were generally greater than 100 nm in diameter (100 nm unilamellar vesicles consist of ~8 × 10^4 lipids/vesicle). The photopolymerization of mono-SorbPC bilayers was performed by exposure of a suspension of 100 nm unilamellar vesicles to light from a low-pressure mercury lamp (predominantly 254 nm light).

Samples were lyophilized and their solubility tested in organic solvents. The thermal polymerizations were considered to be successful if the dried lipid film was insoluble in chloroform or benzene; photopolymerized vesicles of mono-SorbPC were only slightly soluble. Partially soluble and insoluble lipid polymers were transesterified to yield soluble modified polymers for NMR and SEC.

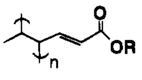
Characterization of Poly(SorbPC). The radical polymerization of SorbPC could proceed by either 1,2-, 1,4-, and/or a 3,4-polymerization. Polymerization of the bis-SorbPC vesicles in H_2O diminished the absorption peak at 254 nm with a corresponding increase in the absorption at 195 nm (Figure 1). The absorption characteristics of the polymer product indicate the formation of an isolated double bond (1,4- and/or 1,2-polymerization) rather than an acryloyl group (3,4-polymerization), which would absorb at ca. 210 nm. After 2 h the polymerization was more than 75% complete, and ca. 2% of the monomer was detectable after 18 h (one half-life of AIBN at 60 °C). Subsequent UV light exposure of poly(SorbPC) vesicle suspensions with a low-pressure Hg lamp for 30 min caused the absorption peak at 195 nm to disappear, apparently due to further reaction of the residual double bond (see Figure 1 insert).



1,2-Polymer

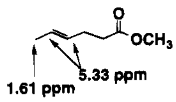


1,4-Polymer

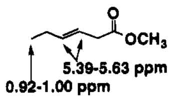


3,4-Polymer

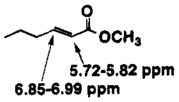
Analysis of the ^1H NMR spectrum of transesterified poly(SorbPC) showed the loss of signals corresponding to the diene moiety. The signals originally associated with the methyl protons adjacent to the diene moiety are shifted to higher field upon polymerization, indicating formation of the 1,4-polymer, whereas a 1,2-polymer would not lead to any significant changes in chemical shift of the methyl protons. Examination of the model molecules shown below provides support for this conclusion. ^1H NMR of the 3,4-polymer model shows the residual double bond split into two peaks at 5.72–5.82 ppm and 6.85–6.99 ppm, which is different from that observed for the transesterified polymer at 5.25–5.60 ppm (Figure 2). A comparison of the ^1H NMR spectra for the 1,2- and 1,4-polymer models (the chemical shifts for the 1,2-polymer model were obtained from Naf and Degen[16] to that of transesterified polymer shows the residual double bond in all three cases occurring at similar) chemical shifts −5.33 and 5.39–5.63 ppm,



1,2-Model



1,4-Model



3,4-Model

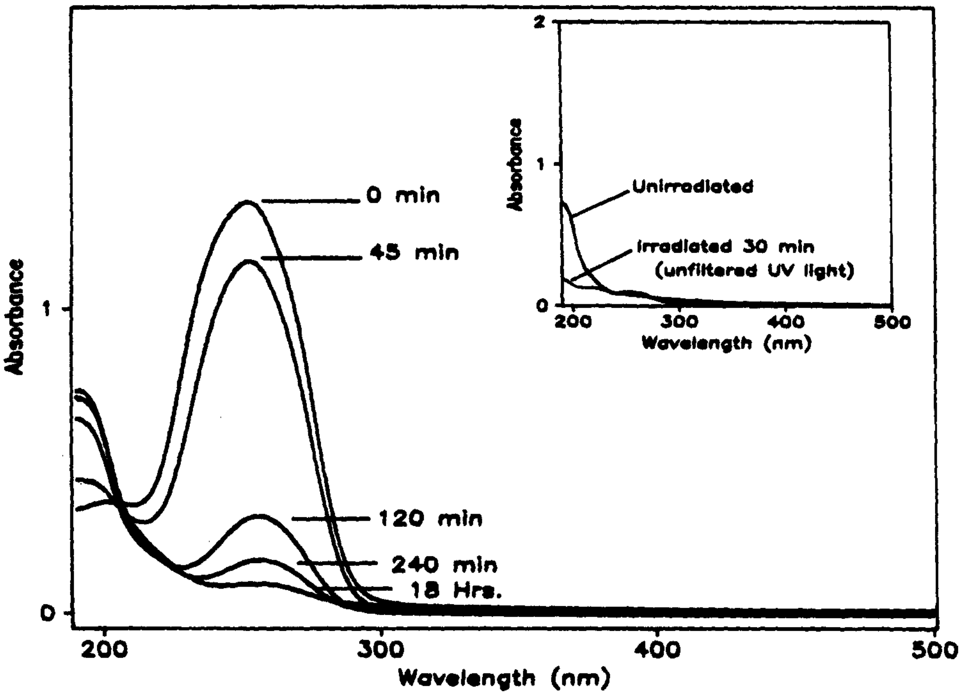


Figure 1. Absorption spectra of SorbPC vesicles in water at different polymerization times. AIBN-initiated polymerization ([M]/[I] of 20) at 60 °C for the indicated times. Inset: Absorption spectra of poly(SorbPC) vesicles before and after 30 min exposure to UV light from a low-pressure mercury lamp.

respectively. These two compounds can be distinguished from one another by examination of the chemical shift of the terminal methyl protons. The terminal methyl protons in the model 1,2-polymer at 1.61 ppm were upfield from the value of unpolymerized lipid, 1.78–1.86 ppm, and appeared at 0.92–1.00 ppm for the model 1,4-polymer. The chemical shift of the latter is nearly identical to that of the transesterified poly(SorbPC) (0.80–1.00 ppm, broad peak).

Transesterification of Polylipids. The insolubility of polymerized lipids was assumed to result from the presence of the polar zwitterionic head group and/or from extensive cross-linking in the case of the bis-substituted PCs. Tsuchida et al. modified a polymerized styrene-substituted PC by acetolysis as well as by methanolysis.[11] Methanolysis of the lipid polymers cleaved the hydrophobic chains from the glycerol backbone, whereas acetolysis removed the phosphocholine moiety and retained the glycerol backbone–acyl chain structure. The methanolized lipid polymers were soluble in THF and CHCl$_3$.

Two methanolysis methods were examined for the cleavage of poly(SorbPC)s: BF$_3$–MeOH (12%) and MeOH–HCl (5%). The former reagent was previously used for the transesterification of poly(AcrylPC)s.[6,7] This method is less satisfactory for polymers with isolated double bonds because the reagent can react with polyunsaturated fatty acids and lipids.[17] Therefore the MeOH–HCl procedure was used to render the poly(SorbPC)s soluble for SEC. The effect of acid addition to the residual double bond in the polymer backbone was examined with model compounds. Heating unsaturated model compounds, methyl 2-hexenoate and methyl 3-hexenoate, with MeOH–HCl between 45 and 50 °C for 24 h did not alter the NMR spectra of the compounds.

Transesterification of the unpolymerized **4** was performed to test whether the lipid head group could be successfully removed to leave only the hydrophobic tails and the reactive sorbyl moiety. ^{1}H NMR analysis of the extracted organic products showed that the phosphorylcholine was completely removed. In addition, the sorbyl group was unaffected by the reaction conditions. Polymerized **3** and **4** were transesterified to methyl esters in a similar manner. While methanolic-HCl cleavage of the unpolymerized material occurred rapidly, the polymeric cleavage reaction was slower as a result of the solubility problems associated with the polymer in methanol.[7] Benzene was used as a cosolvent to improve polymer solubility. In methanol the polymer should have a compact globular shape, which limits the reagent's ability to penetrate into the polymer matrix, whereas the addition of a cosolvent relaxes the polymer conformation.

The transesterification of polymerized **3** or **4** yielded a copolymer composed of random repeat units of methyl sorbate and methyl carboxynonanyl sorbate (Figure 2). The relative amounts of short and long side chain esters (copolymer composition) was determined by comparing the ^{1}H NMR integration of a peak associated with the long side chain repeat unit to a peak whose relative intensity did not change during the course of the transesterification. The variable peak chosen for this analysis occurs at 3.88–4.12 ppm and corresponds to the methylene α to the polymer backbone, referred to as the polymer α-methylene sorbate ester (p-MSE) peak. The peak shifted upfield from its original position at 4.12 ppm (MSE) in the unpolymerized sample due to the loss of the electron-withdrawing sorbyl group upon polymerization. The triplet at 2.28 ppm corresponds to the α-methylene glycerol ester (MGE) peak, i.e., two methylene groups α to the carbonyls of the ester groups attached to the *sn*-1 and *sn*-2 glycerol portion of the lipid, which remain constant during the transesterification. The MGE peak integrated to 4 protons, compared to the p-MSE peak in poly(bis-SorbPC) which varied from an intensity of 4 protons for a polymer consisting exclusively of the long side chain ester to zero protons for the short side chain polyester, poly(methyl

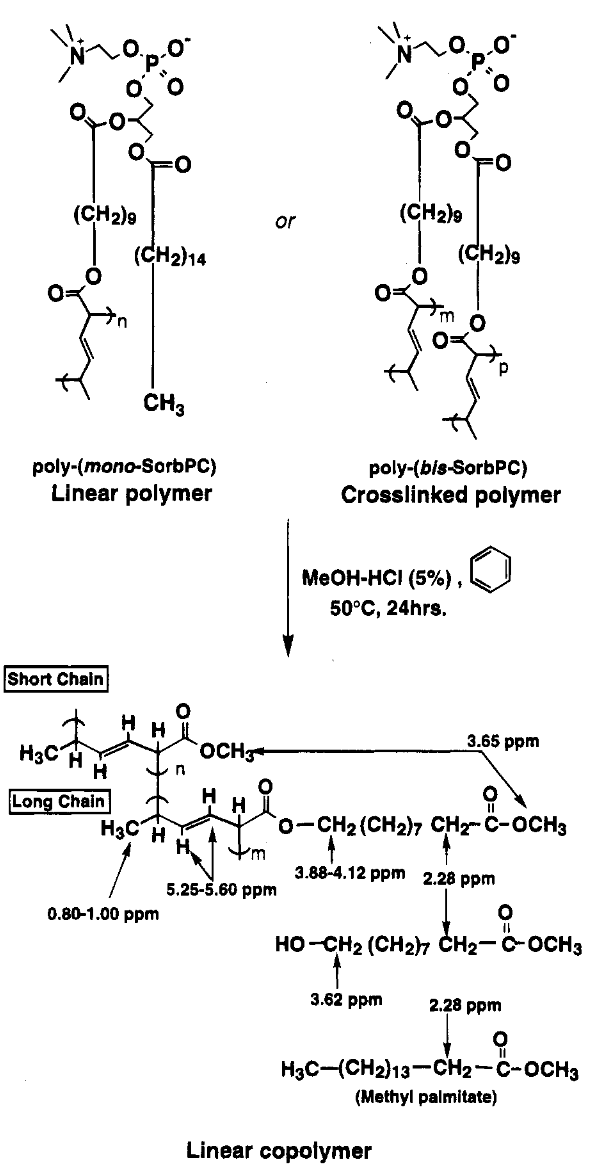


Figure 2. Methanolic-HCl (5%) cleavage of polymerized bilayers composed of either mono-SorbPC or bis-SorbPC and ^1H NMR analysis of the transesterified poly(SorbPC). The reaction of poly(mono-SorbPC) resulted in the polysorbate copolymer as well as methyl palmitate, whereas the transesterification of poly(bis-SorbPC) gave only the copolymer.

sorbate). In the case of poly-**3** the intensity of the p-MSE would integrate to 2 protons for a polymer consisting only of the long side chain ester.

Transesterification of samples of polymerized **3** or **4** resulted in copolymers composed predominantly of the long side chain ester, which varied from 61 to 93% of the total. The copolymer composition did not appear to depend on the initiator concentration and therefore was not related to the molecular weight of the polymer. The effective molecular weight of the copolymer repeat unit was determined from the experimentally determined copolymer composition in the manner described previously, where it was demonstrated that the experimentally determined X_n did not depend on the relative proportion of long vs short side chain ester in the copolymer.[7]

Thermal Polymerization of SorbPCs. Degree of Polymerization. After transesterification of polymerized SorbPCs, the molecular weights of samples were determined by SEC in order to estimate the X_n. The effect of [I] on the X_n was determined while the monomer concentration was held constant. Note that although the initiator concentration is expressed in millimoles of AIBN per milliliter of H_2O, the AIBN is an oil-soluble initiator and is present almost exclusively in the lipid bilayer; therefore the ratio of [M] to [I] is the important variable.[7] The X_n was examined at [M]/[I] of 5, 10, 20, and 30 for mono-SorbPC and 10, 20, 30, and 60 for bis-SorbPC. At least two samples were polymerized at each [M]/[I], and each transesterified polymer was analyzed a minimum of three times relative to PMMA standards by SEC. The SEC analysis of the modified poly(SorbPC)s are summarized in Tables 1 and 2. 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 effective molecular weight of the repeat unit in the copolymer sample.

Table 1. Number-Average Relative Degree of Polymerization of mono-SorbPC as a Function of [M]/[I] at Constant [M]

[M]/[I][a]	X_n	PDI range
5	54 ± 1	1.46–1.84
10	102 ± 22	1.81–2.34
20	191 ± 13	2.39–3.82
30	279 ± 41	2.07–3.43

[a] [M] was 10 μmol/mL mono-SorbPC.

Table 2. Number-Average Relative Degree of Polymerization of bis-SorbPC as a Function of [M]/[I] at Constant [M]

[M]/[I][a]	X_n	PDI range
10	112 ± 15	1.92–2.35
20	190 ± 18	2.03–2.48
30	295 ± 3	1.72–2.21
60	578 ± 61	1.91–2.27

[a] [M] was 10 μmol/mL bis-SorbPC.

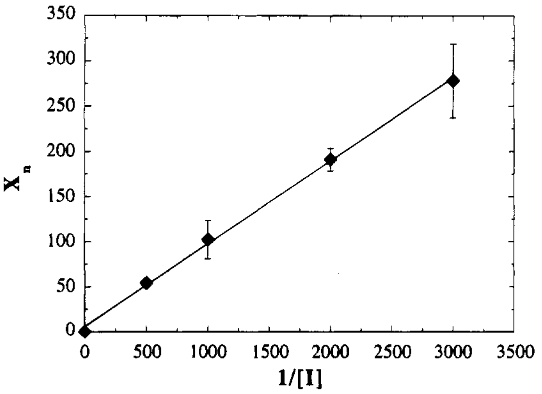


Figure 3. Number-average relative degree of polymerization (X_n) of mono-SorbPC vs the inverse of the initiator concentration, [I].

Poly(mono-SorbPC). The experimentally determined X_n values, which ranged from 50 to nearly 300 (Table 1), were plotted as a function of the inverse square root of the initiator concentration (not shown). A linear least squares regression of the data gave a correlation coefficient of 0.964. In contrast, a plot of X_n versus the inverse of [I] showed a linear fit with a correlation coefficient of 0.999 (Figure 3). A further test of the data is depicted in Figure 4, where log X_n is plotted versus log [I]. The order of the polymerization with respect to [I] is given by the slope of the plot, −0.96 (correlation coefficient 0.999), which indicates an inverse first order dependence of X_n on [I].

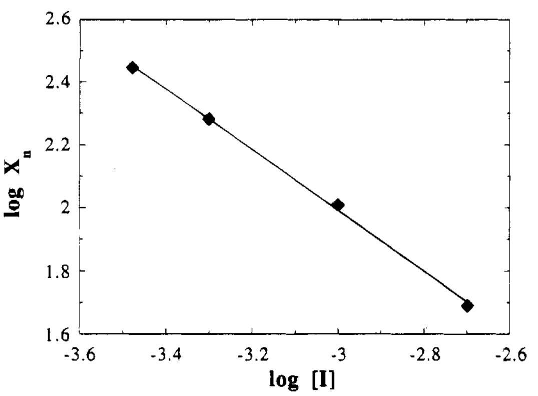


Figure 4. Logarithm of the number-average relative degree of polymerization (X_n) of mono-SorbPC vs the logarithm of the initiator concentration.

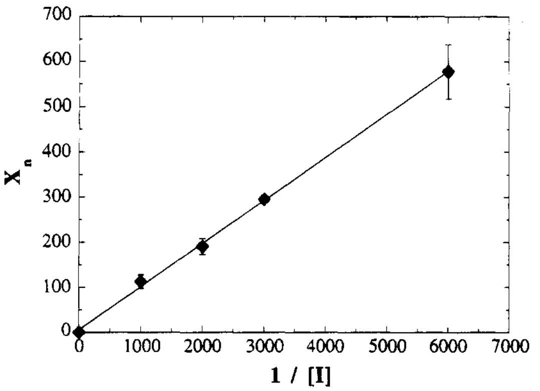


Figure 5. Number-average relative degree of polymerization (X_n) of bis-SorbPC vs the inverse of the initiator concentration, [I].

Poly(bis-SorbPC). The polymerization of bis-SorbPC was analyzed in an identical fashion to that described for mono-SorbPC. Unlike mono-substituted PC, bis-substituted PCs can form cross-linked polymer networks. The analysis of cross-linked systems is generally difficult because polymer networks are insoluble in organic solvents. However, transesterification of the polymerized bis-substituted PCs renders the polymers soluble because the reaction removes the glycerol backbone that connects the cross-linked network. The resulting linear polymers were soluble in THF and could be characterized by SEC.

The effect of varying the initiator concentration on X_n for bis-SorbPC bilayers is reported in Table 2. A comparison of X_n between mono- and bis-SorbPCs reveals there is little or no change in X_n for identical ratios of sorbyl group to initiator. The experimentally determined X_n values for bis-SorbPC were plotted as a function of the inverse [I] (Figure 5). A linear fit to the data gave a correlation coefficient of 0.999. Therefore, as in the case of the mono-SorbPC, the X_n for bis-SorbPC is inversely proportional to the first order of the initiator concentration. A log−log plot of the data showed a slope of −0.92 with a correlation coefficient of 0.997 (not shown).

Photopolymerization of SorbPCs. *Rate of Reaction.* The relationship between light intensity, I_{int}, and the initial rate of reaction, R_p, was investigated for UV-irradiated vesicles of both mono- and bis-SorbPC. Photolysis of the sorbyl moiety caused a decrease in the monomer absorbance at 254 nm (H_2O) with an absorbance increase at ≈195 nm. The well-defined isosbestic region at 222 nm suggests that the photolysis primarily yields one photoproduct. Prolonged UV irradiation of the sample eventually caused the disappearance of the peak at 195 nm, which suggests the occurrence of a second slower reaction of the nonconjugated double bond.

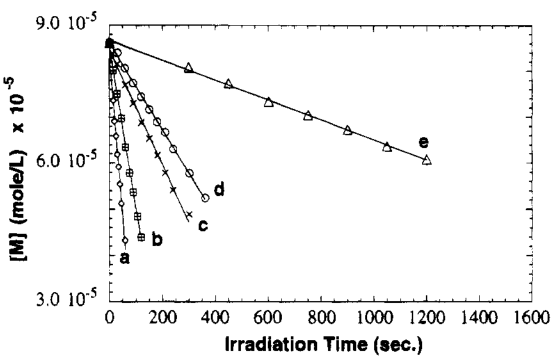


Figure 6. Rate of reaction of mono-SorbPC vesicles as a function of the time of UV exposure. Vesicles were photopolymerized with a low-pressure mercury lamp (254 nm) at 40 °C. Intensity of UV light: (a) 5.0 × 10^{14}, (b) 2.64 × 10^{14}, (c) 9.95 × 10^{13}, (d) 6.60 × 10^{13}, and (e) 1.40 × 10^{13} photons/s.

Table 3. Initial Rate of Photoreaction (R_p) of UV-Irradiated mono- and bis-SorbPC Vesicles[a]

I_{int} (photons/s × 10^{-14})	% rel transmittance	R_p (mol L^{-1} s^{-1} × 10^8) mono-SorbPC 40 °C	mono-SorbPC 30 °C	bis-SorbPC 30 °C
5.00	100	71.8	59.4	44.1
2.64	52.7	35.5	33.4	24.2
0.99	19.9	12.6		8.1
0.66	13.2	9.5		6.0
0.14	2.8	2.2		1.7

[a] The photolyses were performed at either 40 or 30 °C, as indicated, at different 254 nm light intensities (I_{int}).

The loss of monomeric mono- and bis-SorbPC was determined for each sample and expressed in mol/L. Vesicles were irradiated for times of between 10 s and 20 min depending upon the light intensity. The light intensity was modulated by a series of neutral-density filters, resulting in the following light fluxes (photons/s (relative percent transmittance of UV light at 254 nm)): 5.0 × 10^{14} (100%), 2.6 × 10^{14} (52.7%), 9.9 × 10^{13} (19.9%), 6.6 × 10^{13} (13.2%), and 1.4 × 10^{13} (2.8%). A minimum of two trials were performed and averaged for each irradiation time and light flux. Vesicles of mono- and bis-SorbPC were irradiated at temperatures above the main phase transition, T_m, of each hydrated lipid.[18]

The experimentally determined concentration of mono-SorbPC was plotted as a function of UV irradiation time for each I_{int} examined (Figure 6). The initial R_p for each I_{int} was determined from the slope (linear least squares regression) and are reported in Table 3. The dependence of the R_p on I_{int} for the photopolymerization of mono-SorbPC vesicles was first order. Figure 7 shows the log R_p values versus log I_{int} have a slope of 0.97 and correlation coefficient of 0.999.

The initial rate of reaction for UV-irradiated bis-SorbPC vesicles was evaluated in a similar fashion to that described for the mono-SorbPC vesicles. The loss of bis-SorbPC, [M], was examined as a function of UV irradiation time for each I_{int}. At the outset all the plots show a linear decrease in monomer concentrations. However, at high conversion of monomer the decrease deviates from linearity with time because of the decreased light absorption of the sample as the reaction

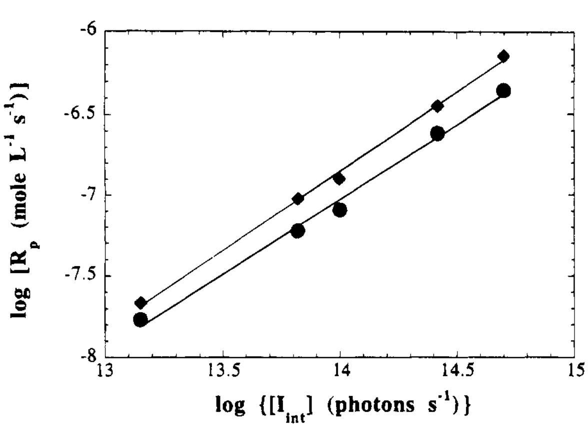


Figure 7. Dependence of the initial rate of photoreaction, R_p, of mono-SorbPC vesicles (squares) and bis-SorbPC vesicles (circles) on light intensity, I_{int}.

proceeds.[19] The R_p for each I_{int} was determined via a linear least squares regression of the data from the initial slope of each plot (correlaiton coefficients of at least 0.996 were obtained for each fit). The calculated values of R_p are reported in Table 3. The dependence of R_p on I_{int} of bis-SorbPC vesicles was also found to be first order by plotting the log R_p values versus log I_{int}, which has a slope of 0.89 and correlation coefficient of 0.997 (Figure 7). The greater R_p values found for mono-SorbPC compared to bis-SorbPC are only due partly to the higher experimental temperature of 40 versus 30 °C, respectively. The R_p for the photopolymerization of mono-SorbPC vesicles was observed to be temperature dependent in a manner that shows maximum conversion per unit time at temperatures near the T_m of mono-SorbPC (36 °C).[20] The R_p at 30 °C for mono-SorbPC bilayers is greater than that of bis-SorbPC bilayers (Table 3) even though at 30 °C mono-SorbPC is in the gel phase and bis-SorbPC is in the liquid crystalline (L_α) phase.[18] Thus the rate of photoreaction is not especially sensitive to the lateral mobility of the monomeric lipids in the bilayer. This is probably a consequence of the low degree of polymerization of this reaction.

Degree of Polymerization of mono-SorbPC Vesicles. The X_n for UV-photolyzed mono-SorbPC vesicles was determined after sample exposure in a Rayonet photoreactor (254 nm lamps). Attempts to dissolve the photoproducts in chloroform or benzene showed the material to be only partially soluble, and hence they were transesterified in the same manner described for the AIBN polymers. In contrast to the polymers formed from AIBN initiation, this UV exposure of mono-SorbPC gave oligomers with X_n of 3–10.

Discussion

The polymerization of lipid bilayers has been accomplished by a variety of methods.[4,5] The focus of this study is the radical chain polymerization, as well as the photopolymerization, of a reactive sorbyl group attached to the chain terminus of the lipid, which residues in the most mobile region of the lipid bilayer. In contrast to three-dimensional or solution polymerizations where the monomers are isotropically mixed, the two-dimensional environment of a bilayer contrains the motion of the monomeric lipid. However, polymerized and nonreactive phospholipids in the liquid-analogous phase laterally diffuse at ca. 1 μm s^{-1}.[3,21] Therefore in this fast diffusion regime the monomeric lipids have ample opportunity to diffuse to the growing polymer chain ends. Furthermore, lipids in the liquid-analogous phase exhibit significant chain disorder in the middle of the bilayer which facilitates the adoption of the appropriate conformations for radical chain reaction.

The thermal-initiated radical polymerization of SorbPCs was examined using the radical initiator AIBN. The polymerization was carried out in extended bilayers, i.e., large multilamellar vesicles, in order to preclude the possibility of the vesicle size limiting the length of the polymer chains. The bilayer nature of these hydrated SorbPC assemblies was previously indicated by differential scanning calorimetry[18] as well as electron microscopy. For reference, note that a 100 nm diameter unilamellar vesicle has ca. 4 × 10^4 lipids per leaflet of the bilayer. Therefore the propagation process should not be limited by the available lipids per vesicle. Tsuchida et al. described a vesicle size effect on the thermal AAPD-initiated polymerization of mono-DenPC, which in small unilamellar vesicles (20 nm diameter) produced a weight-average degree of polymerization, $\bar{X}_w$, that was considerably smaller than with 40 nm diameter vesicles; e.g., $\bar{X}_w$ varied from 28 to 255.[22] It was suggested that the lower $\bar{X}_w$ was due to a decreased freedom of motion of the polymerizable group, which is located at the hydrocarbon/water interphase, in the more highly curved smaller vesicles. The magnitude of the difference is significantly less if the $\bar{X}_n$ values are used for comparison. Calculation of $\bar{X}_n$ from the published data reveals values of 21 and 58 for 20 and 40 nm diameter vesicles, respectively. The number of lipids in the outer leaflet of 20 and 40 nm diameter vesicles is approximately 1500 and 6000, respectively.

The kinetic chain length, ν, for thermally initiated free-radical solution polymerization is proportional to [M] and $[I]^{-0.5}$.[23] The relationship is derived from the steady-state approximation, where the rate of initiation, R_i, and the rate of termination, R_t, are equal. At low concentrations of initiating radicals, and excluding solvent or impurity chain transfer reactions, the growing radical chain predominantly terminates by chain–chain interactions such as coupling and/or disproportionation. The number-average degree of polymerization, $\bar{X}_n$, is equal to the kinetic chain length when the radical polymerization reaction is terminated by disproportionation, or twice the kinetic chain length when two radicals couple. Both modes of termination are diffusion controlled and require pairing of their electron spins. The termination of diene monomers occurs by a combination of both coupling and disproportionation, with the extent depending upon the method and conditions of the polymerization.[24] The method of termination for the related sorbyl monomers remains to be described. The availability of β-hydrogens on the terminal methyl suggests that a relatively high degree of disproportionation could occur.

The degree of polymerization of SorbPC bilayers is inversely proportional to [I]. This same type of dependence of $\bar{X}_n$ on [I] was reported for the bilayer polymerization of AcrylPC,[6,7] and it was proposed that at high conversions to polymer in bilayers and reaction was terminated by primary termination, i.e., the reaction of a primary radical fragment generated from the initiator with the growing radical chain.[25-27] Under these conditions the $\bar{X}_n$ for thermally initiated polymerization is

$$\bar{X}_n = k_p k_i [M]^2 / 2 f k_d k_{tp} [I] \qquad (1)$$

where k_p is the rate constant for propagation, k_d is the rate constant for initiator homolysis, k_{tp} is the rate constant for primary termination, and f is the initiator efficiency. Thus, in the case of primary radical termination, X_n is expected to be proportional to $[I]^{-1}$ as observed for the radical polymerization of both mono- and bis-SorbPCs bilayers.

Primary radical termination of two-dimensional bilayer assemblies could result from either the high [I] and/or the constrained motion of the polymer chains in the bilayer. Since both monomer and polymer are constrained to the confines of their two-dimensional environment, their mobility should decrease as the polymerization proceeds. Utilizing fluorescence after photobleaching techniques, Kölchens et al. demonstrated a moderate decrease in the lateral diffusion coefficient (D) of nonpolymeric lipid probes as the size of polylipids in the bilayer was increased.[21] The D of an NBD−PE fluorescent probe in bilayers composed of poly(AcrylPC) decreased from 1.4 to 0.24 $\mu m^2\ s^{-1}$ as the X_n of the polymer increased from 230 to 1900. These data indicate that polymerization increases the bilayer viscosity, thereby decreasing the translational and/or segmental motion of the growing polymer chains and making bimolecular termination processes more difficult. The longer lifetime of the radical chain ends and slower monomer diffusion should enhance the probability of a small radical fragment, e.g., isobutyronitrile radical, diffusing through the bilayer and terminating the reaction.

Although the polymerizations of bilayers composed of either SorbPCs or AcrylPCs appear to terminate by primary radical termination, there are at least two notable differences between these classes of reactive lipids. (1) The polymerization of mono-AcrylPC bilayers gave larger polymers than observed with mono-SorbPC bilayers. (2) The polydispersities of the poly(SorbPC)s were generally smaller than those of the poly(Acryl)-PCs. A comparison of the X_ns for poly(mono-AcrylPC) and poly(mono-SorbPC) which werre polymerized at identical [M]/[I] ratios shows the former to be about 4 times larger, e.g., [M]/[I] = 5: poly(mono-AcrylPC), $X_n = 233 \pm 29$; poly(mono-SorbPC), $X_n = 54 \pm 1$. The different X_ns of the two monomers are probably due to the relative resonance stabilization of the radicals formed from the monomers. In general, resonance-stabilized radicals exhibit a lower reactivity with monomers than nonstabilized radicals. Since the sorbyl moiety is a conjugated diene, the resulting sorbyl radical is more resonance stabilized and less reactive than the acryloyl radical.

The calculated polydispersities (Tables 1 and 2) for the transesterified polymers of SorbPC are somewhat narrower than those reported for poly(AcrylPC).[7] Competition between radical chain termination mechanisms can result in large polydispersities. Kinetic studies of the mono-AcrylPC bilayer polymerization indicate that bimolecular termination (most probably coupling) dominates the polymerization at low conversion, whereas primary termination is favored at high conversion. These circumstances will produce relatively large polydispersities because coupling yields polymers that are double the size of those formed by primary termination. At the outset of the SorbPC bilayer polymerization the growing chains are likely to terminate by disproportionation as well as coupling. Consequently, there will be fewer double-length polymers due to coupling, and a narrower polydispersity.

The similar X_ns of the transesterified polymers formed from either mono- or bis-SorbPCs at the same [M]/[I] are notable. A cross-linking reaction, which limits monomer diffusion to the propagating chain end, should yield X_n values smaller for the bis-substituted lipid than the mono-substituted lipid. This result suggests that cross-linking of the SorbPC bilayer either does not significantly limit the diffusion of monomers to the propagating chain end or the lifetime of the radical chain end is increased. Inhibition of the rate of diffusion of monomers to the chain end may be compensated by an increased lifetime of the radical.

Tsuchida et al. demonstrated that the UV photopolymerization (254 nm) of mono-DenPC vesicles in the liquid-analogous phase results in oligomer formation ($X_n \approx 6$).[15] In a similar manner the photopolymerization of mono-SorbPC vesicles at temperatures > T_m also resulted in the oligomer formation. When the I_{int} was reduced by half, the R_p was reduced by a similar extent, but no significant change in X_n was observed. The photoformation of oligomers of SorbPC clearly demonstrates this reaction proceeds by a different propagation mechanism than associated with the radical chain polymerization of SorbPC.

The R_p for the photoreaction of mono-SorbPC vesicles was examined as a function of light intensity. Direct photoexcitation of the SorbPC monomer initiated the polymerization. Excitation of the sorbyl moiety from the ground state to an excited state may result in the formation of diradicals which can propagate, or the reaction may proceed by photoactivated addition of monomer.[28,29] Both the absorption characteristics of the sorbyl lipids and the insensitivity of the photoreaction to oxygen suggest that the excited state is singlet in character. The R_p for the photopolymerization of both mono-SorbPC and bis-SorbPC was observed to be proportional to the first order of the light intensity, I_{int}. Similar observations were reported by Fendler et al. for the UV photopolymerization of styrene-containing amphiphiles.[30–32]

Conclusions

We have previously reported that at high conversion the radical polymerization of lipid bilayers composed of either mono- or bis-AcrylPC does not preferentially terminate by coupling or disproportionation.[6–8] The growing polymer chains are terminated by reaction with an initiator fragment, i.e., primary radical. Although the kinetic chain length of mono-AcrylPC is ca. 4 times larger than that of either mono- or bis-SorbPCs, the motion of the growing poly(SorbPC) chains is also constrained in the bilayer, thereby reducing the probability of interaction between the propagating chain ends, even though there are hundreds of polymer chains per bilayer vesicle. The smaller observed X_n of mono-SorbPC vs. mono-AcrylPC at identical [M]/[I] ratios is most likely the result of the greater resonance stabilization of the sorbyl radical. Furthermore, the similar kinetic chain length for both mono- and bis-substituted SorbPCs suggests any reduction of monomer diffusion by cross-linking the bilayer is compensated by an increased lifetime of the propagating chain end.

The relatively large values of X_n for poly(SorbPC) as well as poly(AcrylPC) are in marked constrast to the polymer sizes expected if the propagating polymer chain coils in upon itself and reduces the ability of monomers to diffuse to the living chain end.[33] Sells and O'Brien suggested that since the covalent links between repeat units of poly(AcrylPC) are at the end of the lipid tails, monomeric mono-AcrylPC is able to diffuse over a low barrier presented by the polymer chains.[7] This should

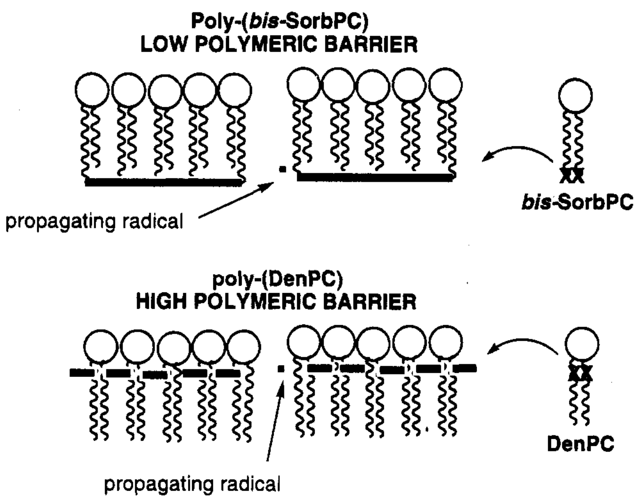


Figure 8. Schematic of low and high barriers to monomer diffusion presented by poly(SorbPC) and poly(DenPC) in the lipid bilayer, where the X denotes the location of the polymerizable group.

facilitate the continued chain growth beyond that suggested for coiled polymers. The longer kinetic chain length observed for SorbPCs compared to the related DenPC described by Tsuchida et al.[15] may be explained in terms of the ability of the monomers to diffuse past a barrier presented by the polymer. At an [M]/[I] ratio of 20 the X_n of DenPC was 21, compared to ca. 200 for SorbPC. Since the reactive group of DenPC is adjacent to the glycerol backbone, the barrier preventing diffusion of monomers to the propagating chain end is significantly higher than that of the poly(SorbPC)s (Figure 8). Thus the location as well as the nature of the polymerizable group in the lipid chain critically affects the size of the polymer chains.

Experimental Section

Materials. The polymerizable lipids mono-SorbPC and bis-SorbPC were synthesized as described previously.[34] Lipid purity was confirmed by thin-layer chromatography (TLC) with chloroform/methanol/water (65:25:4 by volume). Pure lipids exhibited a single spot (R_f of 0.35–0.40).

Azobis(isobutyronitrile) (AIBN) was purchased from Eastman Kodak Co. (Rochester, NY) and purified by recrystallization three times from methanol. Acetyl chloride and anhydrous methanol were purchased from Aldrich (Milwaukee, WI) and used as received. All solvents were purified by standard techniques.

Methods. Thermal Polymerization. Large multilamellar vesicles of polymerizable lipid were prepared as follows: approximately 20–25 mg of lipid was evaporated from a stock solution (10 mg/mL in benzene) by passing a gentle stream of argon over the sample and drying under high vacuum (0.1 mmHg) for a minimum of 4 h. The lipid weight was determined and the lipid film redissolved in 2 mL of benzene; then the appropriate amount of initiator from a freshly prepared AIBN stock solution (1–1.5 mg/mL of benzene) was added to yield monomer to initiator ratios ([M]/[I]) of 5, 10, 20, 30, and 60. AIBN in benzene was added by weight as opposed to volume (the density of the AIBN stock solution was assumed to be 0.8787 g/mL) to avoid errors associated with pipetting organic solutions. The solvent was evaporated as above the dried under high vacuum for 3 h. All steps involving AIBN and lipid were performed in subdued light. The dried lipid/AIBN film was hydrated with deoxygenated MilliQ water to a final concentration of 10 μmol/mL. Samples were vortexed to uniformity then (1) bubbled with argon for 10 min, (2) warmed to above the lipid T_m for 45 s ($T_m = 35$ °C), (3) vortexed for 1 min, (4) cooled in ice water for 90 s, (5) with repetition of steps 2–4 twice, and (6) flushed with argon for 10 min. Ampules were immediately sealed with a rubber septum. Lipids were dried and weighed individually before being combined with AIBN. The lipid vesicles were polymerized at 60 ± 2 °C in an oil bath for 3 days under an argon atmosphere; all polymerizations were carried out in the dark. In order to prevent the sample from aggregating, the ampules were occasionally shaken by hand. The loss of monomeric SorbPC was monitored by the decrease in the chromophore absorption at 258 nm.

UV Photopolymerization. Approximately 25 mg of mono-SorbPC was evaporated from a stock solution as described above. The dried lipid film was hydrated with deoxygenated MilliQ water to a final concentration of 10 μmol/mL and then vortexed to uniformity, followed by 10 freeze–thaw cycles (dry ice/2-propanal → 30 °C). The lipid suspension was extruded 10 times through two stacked 0.1 μm pore size Nuclepore polycarbonate filters at 250 psi and 40 °C using a stainless steel extruder (Lipex Biomembranes; Vancouver, BC). After the last extrusion, 2–3 mL of MilliQ water was pushed through to remove any residual lipid. The extruded vesicles were diluted to a concentration of 0.08 mM ($OD_{258\ nm} \approx 1.65$).

UV irradiations of mono-SorbPC vesicles were carried out in quartz reaction tubes (path length 2 cm, volume 60 mL) using a Rayonet photochemical reactor (Model RPR-100, Southern New England Ultraviolet Co.) equipped with 254 nm lamps. The photoreactor was equilibrated for 30 min prior to use, reaching a constant temperature of 40 ± 2 °C; the reaction vessel and sample were equilibrated at this same temperature prior to photolysis. The intensity I of UV light was controlled by the number of lamps in the photoreactor and by light attenuation with a fine-mesh screen. The loss of monomeric mono-SorbPC was monitored by the decrease in UV absorption at 254 nm. Aliquots of the vesicle suspension were pipetted at different time intervals and the absorption spectra recorded. After 30 min of irradiation the photoreaction was more than 96% complete. The sample was concentrated by rotary evaporation to a small volume (≈3 mL) and lyophilized to remove water.

In separate experiments, the effect of light intensity, I_{int}, on the initial rate of polymerization (R_p) was evaluated. The vesicle suspensions were diluted to an optical density of 2.00 ± 0.05 at 258 nm, bubbled with argon for 5 min, and placed in a thermostated cell holder 4 cm from a low-pressure mercury lamp (incident flux of ca. 5×10^{14} photons/s in the absence of a neutral-density filter). Samples were equilibrated in the cell holder for approximately 5 min to a constant temperature (30 or 40 °C) which did not vary more than ±0.2 °C during the course of the experiment. Photopolymerization was carried out continuously for each new sample while stirring by Ar bubbling. The light intensity was controlled with neutral-density filters. The extent of monomer remaining after photolysis was calculated from the decrease in the sorbyl absorbance at 258 nm. The sample absorbance did not change after the UV lamp was shut off. A minimum of three trials were performed per experiment. The optical density at 254 nm of the neutral-density filters was determined spectrophotometrically.

Transesterification Reaction. After polymerization, samples were lyophilized to a fine powder and transesterified to enhance their solubility prior to analysis by size-exclusion chromatography (SEC). Polymeric lipids as well as model compounds were cleaved by methanolic HCl (5%) which was freshly prepared for each sample by slowly adding (3 min) 5 mL of acetyl chloride to 50 mL of cold anhydrous methanol (ice bath) with constant stirring.

Monomeric bis-SorbPC. Twenty-five milligrams of bis-SorbPC was dried to a thin film. The lipid was cleaved by adding 2 mL of methanolic HCl (5%) and 4 mL of benzene and heating at 50 °C for 24 h. The acidic solution was neutralized with solid $NaHCO_3$ until the evolution of CO_2 stopped. The solid was filtered and the filtrate reduced to a chalky oil. The residue was taken up in 5 mL of water and extracted with 5 × 5 mL of $CHCl_3$. The organic phase was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the ^1H NMR taken. TLC: 2 spots, $R_f^1 = 0.35–0.2$; $R_f^2 = 0.15$ ($CHCl_3/CH_3OH$, 95:5). ^1H NMR ($CDCl_3$; note: v = variable integration depending on the extent of the cleavage reaction): 1.20−1.40 (br s, 10H, $\mathrm{C}H_2$), 1.52−1.70 (m, 4H, $\mathrm{C}H_2\mathrm{CH_2R}$), 1.78−1.86 (d, 3H, $\mathrm{C{=}CHC}H_3$), 2.28−2.37 (t, 2H, $\mathrm{C}H_2\mathrm{CO_2H}$), 3.58−3.76 (t, v, $\mathrm{HOC}H_2\mathrm{CH_2}$), 3.65 (s, 3H, $\mathrm{OC}H_3$), 4.05−4.15 (t, v, $\mathrm{CO_2C}H_2$), 5.70−5.79 (d, 1H, $\mathrm{C{=}C}H\mathrm{CO_2}$), 6.05−6.22 (m, 2H, $\mathrm{{=}C}H\mathrm{C}H\mathrm{{=}}$), 7.15−7.27 ppm (m, 1H, $\mathrm{CH{=}C}H\mathrm{CH_3}$).

Polymeric mono-SorbPC and bis-SorbPC. Cleavage reactions were performed by adding 2 mL of methanolic HCl (5%) along with 4 mL of benzene to the lipid polymer and refluxing at 50 °C for 24 h. Typically, the polymeric lipid went into solution after 15 min of refluxing. The solution was neutralized with solid $\mathrm{NaHCO_3}$ until the evolution of $\mathrm{CO_2}$ ceased. The solid was filtered, evaporated to an oily precipitate (rotary evaporation), and extracted with 5 × 8 mL of $\mathrm{CHCl_3}$ from water (10 mL). The organic phase was dried over $\mathrm{Na_2SO_4}$, filtered, and evaporated to a viscous oil. ^{1}H-NMR ($\mathrm{CDCl_3}$; note: v = variable integration depending on the extent of the cleavage reaction): (a) poly-**3**, 0.81−0.88 (t, 3H, $\mathrm{CH_2C}H_3$), 0.75−1.00 (br s, 3H, $\mathrm{{=}CHCHC}H_3$), 1.18−1.40 (br s, 38H, $\mathrm{C}H_2$), 1.50−1.69 (m, 2H, $\mathrm{C}H_2\mathrm{CH_2R}$), 2.22−2.33 (t, 2H, $\mathrm{C}H_2\mathrm{CO_2CH_3}$), 2.50−2.88 (br weak s, 2H, $\mathrm{C}H\mathrm{CH{=}CHC}H$), 3.65 (s, 3H, $\mathrm{OC}H_3$), 3.58−3.65 (t, v, $\mathrm{HOC}H_2\mathrm{CH_2}$), 3.84−4.10 (br s, v, $\mathrm{CO_2C}H_2\mathrm{CH_2}$), 5.19−5.50 ppm (br s, 2H, $\mathrm{C}H\mathrm{{=}C}H$); (b) poly-**4**, 0.75−1.00 (br s, 3H, $\mathrm{{=}CHCHC}H_3$), 1.18−1.40 (br s, 24H, $\mathrm{C}H_2$), 1.50−1.69 (m, 4H, $\mathrm{C}H_2\mathrm{CH_2R}$), 2.22−2.33 (t, 2H, $\mathrm{C}H_2\mathrm{CO_2CH_3}$), 2.50−2.88 (br weak s, 2H, $\mathrm{C}H\mathrm{C}H\mathrm{{=}CHC}H$), 3.65 (s, 3H, $\mathrm{OC}H_3$), 3.58−3.65 (t, v, $\mathrm{HOC}H_2\mathrm{CH_2}$), 3.84−4.10 (br s, v, $\mathrm{CO_2C}H_2\mathrm{CH_2}$), 5.19−5.50 ppm (br s, 4H, $\mathrm{C}H\mathrm{{=}C}H$).

Size-Exclusion Chromatography. The average molecular weights for all transesterified polymers were determined by SEC with a Waters Maxima 820 chromatography workstation (Millford, MA). Lipids polymerized by AIBN were examined using an Ultrastyragel linear column having an effective molecular weight range of 2×10^3 to 10^7 and the photopolymerized samples were examined using an Ultrastyragel 10^3 Å column with an effective molecular weight range of 200 to 3×10^4. The mobile phase, tetrahydrofuran (THF), was filtered through 0.45 μm Waters nylon filters and purged with helium. The detector was a Waters Model R401 differential refractometer. The instrument was interfaced to a NEC Powermate 1 computer with Maxima 820 version 3.31 software. The calibration and analysis were performed as described by Sells and O'Brien.[7]

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