Two-Dimensional Polymerization of Lipid Bilayers: Rate of Polymerization of Acryloyl and Methacryloyl Lipids

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*Received August 9, 1993; Revised Manuscript Received December 10, 1993**

ABSTRACT: A systematic study of the effect of monomer [M] and initiator [I] concentration on the rate of polymerization, R_p, for bilayers of acryloyl- and methacryloyl-substituted lipids is described. In a companion paper (*Macromolecules* 1994, *27*, 226) Sells and O'Brien describe the effect of these variables on the degree of polymerization for the thermally initiated radical polymerization of an acryloyl-substituted phosphatidylcholine (mono-AcrylPC) in bilayer assemblies. Interestingly, the relative degree of polymerization (X_n), for the polymerization was proportional to $[M]^2$ and $[I]^{-1}$, which indicates that chain termination at high conversion to polymer was dominated by primary termination. Both the polymerizations of mono-AcrylPC and mono-MethPC bilayers exhibit a R_p dependence on [M] and $[I]^{0.5}$ at the start of the polymerization, which indicates the bilayer-constrained polymer chains terminate by chain coupling and/or chain disproportionation. As the polymerization approaches high conversion, the kinetic behavior changes. Both the R_p of mono-AcrylPC and the mono-MethPC bilayer polymerizations at high conversions show an increased dependence on [M], 1.86 and 1.6, respectively, and a diminished dependence on [I]. The course of the polymerization appears to progressively modify the bilayer in a manner that reduces the probability of bimolecular chain termination reactions and favors increased domination by primary termination.

Introduction

The polymerization of supramolecular assemblies of noncovalently associated molecules, e.g., monolayers, bilayers, and multilayers, provides an opportunity to prepare novel materials which are of interest in both biological and materials sciences. Many conventional polymerization methods can be used to polymerize lipid assemblies by thermal, redox, and photochemical initiation.[1-3] Optimum utilization of these chemistries relies on a clear understanding of the variables that effect these polymerizations. Obviously, the nature of the reactive group has an important effect on both the degree of polymerization, X_n, and the rate of polymerization, R_p. Although there are several reports of supramolecular assembly polymerization that describe the X_n and/or R_p for specific circumstances, there have been few systematic studies of these polymerizations. The highly ordered yet dynamic nature of bilayer assemblies, which is manifested by the rapid lateral diffusion of lipids within the bilayer, indicates that the polymerization reactions may differ from conventional bulk, solution, or emulsion polymerizations.

We have undertaken a systematic study of the effect of monomer [M] and initiator [I] concentration on the X_n and R_p for various polymerizable lipids. In a companion paper we describe the effect of these variables on the X_n for the thermally initiated radical polymerization of an acryloyl-substituted phosphatidylcholine (mono-AcrylPC) in bilayer assemblies.[4] Interestingly, the number-average relative degree of polymerization (X_n) for polymerization of this monomer was proportional to $[M]^2$ and $[I]^{-1}$. These data indicate that the chain termination at high conversion of mono-AcrylPC to polymer is dominated by primary termination. The reduced mobility of long polymer chains in the constrained environment of the bilayer interior is the probable cause of the lack of biomolecular chain termination in this polymerization. In order to further probe the nature of the acryloyl polymerization in the bilayer, we have investigated the kinetics of the two-dimensional polymerization process in lipid bilayers. Here we report these kinetic studies of the thermally initiated polymerization of mono-AcrylPC and mono-methacryloylPC (mono-MethPC) lipid bilayers.

Results

Monomers. The synthesis of both the mono-AcrylPC, 1-palmitoyl-2-[12-[(acryloyl)oxy]dodecanoyl]-*sn*-glycero-3-phosphocholine, and the mono-MethPC, 1-palmitoyl-2-[12-[(methacryloyl)oxy]dodecanoyl]-*sn*-glycero-3-phosphocholine, were described by Sells.[4,5] Hydration of the individual lipids results in the formation of bilayer structures, which may be manipulated by the usual techniques to give a uniform population of vesicles or extended bilayers. The thermotropic behavior of extended bilayers of mono-AcrylPC and mono-MethPC has been reported, and the main phase transitions, T_m, were 31.8 and 11.4 °C, respectively.[6]

Polymerization of Lipid Monomer Assemblies. It should be pointed out that to polymerize a small amount of lipid vesicles, the lipid film must be completely dried before hydration and the container, ampule, must be very clean. This is important because the presence of any solvent or grease on the wall of the container will cause the initiator to concentrate in the organic phase and undermine the polymerization of the vesicles. The success of polymerization is also greatly dependent on degassing the sample. Oxygen is a very powerful inhibitor for the polymerization of methyl methacrylate.[7] Since the amount of the polymerizable lipid is very small, even a trace of oxygen can stop the polymerization or make the measurements of the kinetic parameter unreliable. The polymerizations were performed at 61 °C, which is comfortably above the T_m of the lipid assemblies; therefore, the lipids are in the rapid lateral diffusion regime. The following polymerization scheme illustrates the change in

* Abstract published in *Advance ACS Abstracts*, February 15, 1994.

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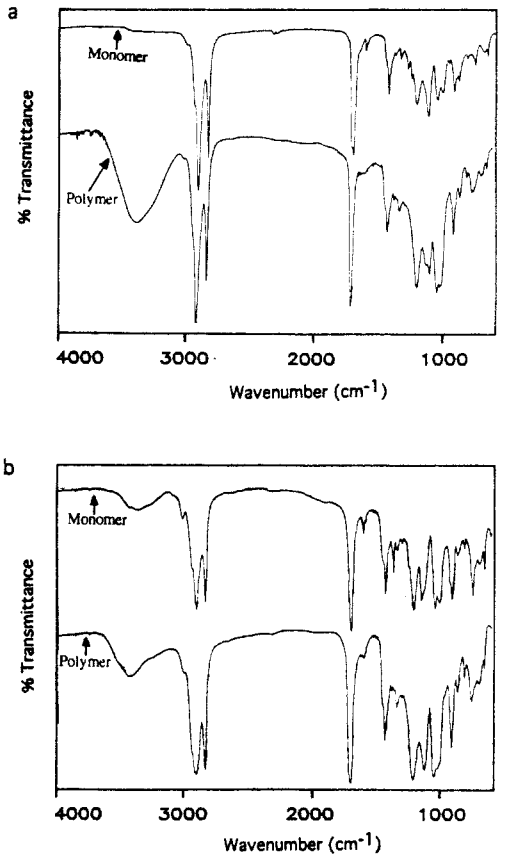
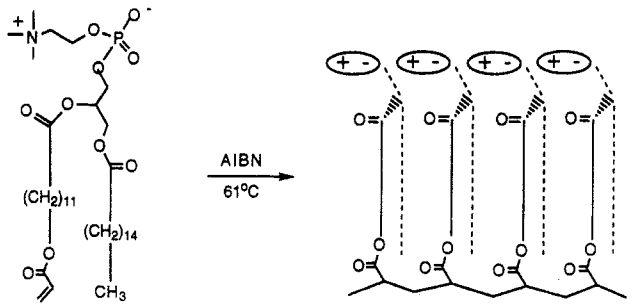


Figure 1. (a) FTIR spectra of mono-MethPC monomer film prepared on a ZnSe surface and mono-MethPC polymer prepared in a KBr pellet. (b) FTIR spectra of mono-AcrylPC monomer film prepared on a ZnSe surface and mono-AcrylPC polymer prepared in a KBr pellet.

the sample structure upon conversion of mono-AcrylPC to polymer:



IR Spectra of Monomer and Polymer Scheme. FTIR spectra of the mono-MethPC monomer film and the poly(mono-MethPC) are each shown in Figure 1a. The most distinct differences between the spectra upon polymerization of the mono-MethPC were the disappearance of the C=C band at 1640 cm^{-1} and the C=O band at 1727 cm^{-1}. The C=C band disappears upon conversion to a new C–C bond. The two monomer carbonyl bands centered at 1727 and 1740 cm^{-1} were assigned to the conjugated carbonyl of the methacrylate and the saturated ester carbonyls in each alkyl chain, respectively.[8,9] The polymerization reaction removes the conjugation with a concomitant disappearance of the lower energy band at 1727 cm^{-1}.

FTIR spectra of the mono-AcrylPC film and the poly(mono-AcrylPC) are shown in Figure 1b. Comparison of these spectra shows the disappearance of the C=C band at 1640 cm^{-1} and the alkene CH stretch at 3030 cm^{-1} upon polymerization. However, the difference between the saturated ester carbonyls and the acryloyl carbonyl band in the mono-AcrylPC spectrum is not as distinct as that of mono-MethPC, where the ester and methacryloyl carbonyls are well resolved. The lower stretching frequency of the methacryloyl carbonyl (1727 cm^{-1}) compared to that of the acryloyl carbonyl (1733 cm^{-1}) has been attributed to the effect of the neighboring methyl group.[10] The acryloyl carbonyl band shifts from 1733 to 1738 cm^{-1} upon polymerization.

Many spectral features of each monomer remain in the spectra of each polymer even though there are some differences in the bandwidths and the relative intensities between the spectra of the monomer and that of the polymer. This contrasts with the solution polymerization of small monomers, where there is usually a large change in several spectral features from monomer to polymer.[11] The similarity of the IR spectral features of monomeric and polymeric lipids is probably due to the dominant features of the nonpolymerizable portion of these relatively large molecules.

Characteristic IR Band for Kinetic Studies. A potentially useful characteristic band for determining the rate of polymerization was the C=C stretching band because the intensity of this band decreases upon polymerization. Quantitative measurements of the changes in band intensity, however, proved to be experimentally difficult because of the small sample size and the low absorption coefficient of the band. Consequently, after the polymerization proceeds for a period of time, a significant fraction of the unreacted monomer signal was below the detection limit.

Another band that can be used to measure the proportion of the unreacted monomer is the carbonyl stretching band of the methacrylate. Although this band overlaps the saturated ester carbonyls, it is free from interference from solvent and has a large absorption coefficient. The intensity of the carbonyl band can be quantitatively related to the unreacted monomer provided that the unreacted lipid is completely extracted from the polymer by $CHCl_3$; i.e., the polymer is insoluble in the solvent.

The requirements for the quantitation by the intensity of the carbonyl band in a polymerized sample were indeed fulfilled. A freeze-dried sample of polymerized mono-MethPC/dimyristoylphosphatidylcholine (DMPC) (1/1) was extracted with chloroform, and the IR spectra were recorded after the first, second, and third extractions, respectively. Comparison of the peak areas of the spectra for the first and second extractions from the sample indicates that >95% of the unreacted lipid and DMPC in the polymerized sample was solubilized in the first extraction. There was no detectable carbonyl band in the third extracted solution. These data demonstrate that the unreacted lipid monomer was nearly quantitatively removed from the polymer by the first extraction with $CHCl_3$. Furthermore, there was no detectable polymer dissolved in $CHCl_3$ since the carbonyl band in the third extracted solution was not resolvable. Therefore, the intensity of the carbonyl band could be directly related to the concentration of the unreacted lipid.

Additional evidence to support the use of the carbonyl band as a characteristic band for quantitation of the fraction of the extracted unreacted lipid was obtained from the IR intensity ratio of $I_{C=C}/I_{C=O}$. Table 1 gives the IR intensity ratios of these two bands for one set of experimental data (taken from samples with [M]/[I] = 50, polymerized for 0, 10, 30, and 60 min). The consistency of the ratios indicates that the intensity of the carbonyl band is proportional to that of the unreacted monomer.

Table 1. Ratios of Infrared Bands as a Function of Polymerization Time for [mono-MethPC]/[I] = 50 at 61 °C

time (min)	$I_{C=O}$	I_{C-C}	$I_{C=O}/I_{C-C}$
0	4.586	0.369	12.4
10	3.284	0.263	12.5
30	2.296	0.204	11.3
60	1.487	0.118	12.6

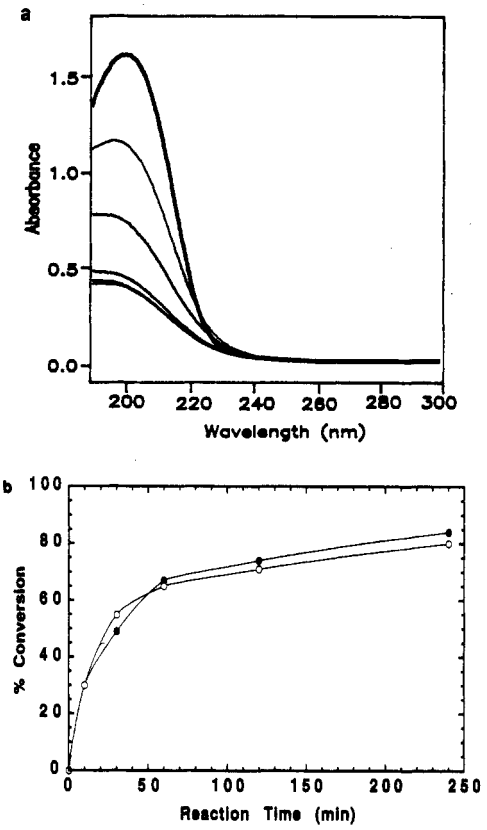


Figure 2. (a) Change of monomer concentration followed by UV absorption during the AIBN polymerization of mono-MethPC at 61 °C. (b) Conversion of mono-MethPC determined by UV and FTIR measurements during the AIBN-initiated polymerization at 61 °C: UV (●); FTIR (○).

UV–vis absorption spectroscopy was also used to determine the decrease in the monomer concentration during the polymerization reaction. Figure 2a shows a representative set of UV spectra obtained by sampling mono-MethPC vesicles in a dispersion during polymerization (the light scattering from the vesicles was corrected by baseline subtraction in this set of spectra; the molar absorptivity calculated for mono-MethPC at 200 nm, λ_{max}, was 1.52×10^4). The loss of the monomer during the polymerization reaction was derived from the spectra in Figure 2a and replotted as shown in Figure 2b. The loss of the monomer during the polymerization reaction (identical reaction conditions) derived from FTIR measurements is also plotted in Figure 2b to show the good agreement between the two methods.

The correspondence of the UV–vis and FTIR spectroscopic methods not only verifies the validity of the FTIR method but also indicates that the polymer formed during the reaction remains as a homogeneous dispersion. This observation suggested that the UV–vis spectroscopic method can be conveniently used to follow the polymerization reaction. This was of practical importance because the UV–vis method was simpler, was less time consuming, and required less sample.

Operational Definition of Concentration. The term concentration needs to be operationally defined for the polymerization of lipid vesicles before proceeding to kinetic analyses. In conventional solution polymerization, the concentrations of monomer and initiator are expressed as bulk concentrations. In lipid vesicles, the monomers are highly ordered within the bilayer and the initiator, AIBN, is localized in the lipid bilayer wall.[4,5] The polymerization occurs within each individual vesicle, which are essentially isolated from each other during the time scale of the experiment. The concentrations of monomer and initiator in individual vesicles cannot be diluted by simply adding aqueous buffer or water to the reaction mixture. In order to dilute the concentration of the monomer in a vesicle, a nonpolymerizable lipid, e.g., DMPC, must be incorporated into the vesicles to reduce the surface concentration of the polymerizable lipid. The phase behavior of mono-AcrylPC was reported by Sells and O'Brien.[4] At the polymerization temperature the lipids are in the fluid phase and appear to be randomly mixed. As a result, we use molar ratios, nonpolymerizable lipid to polymerizable lipid and the lipid to initiator, rather than bulk concentrations in the kinetic analyses in this paper.

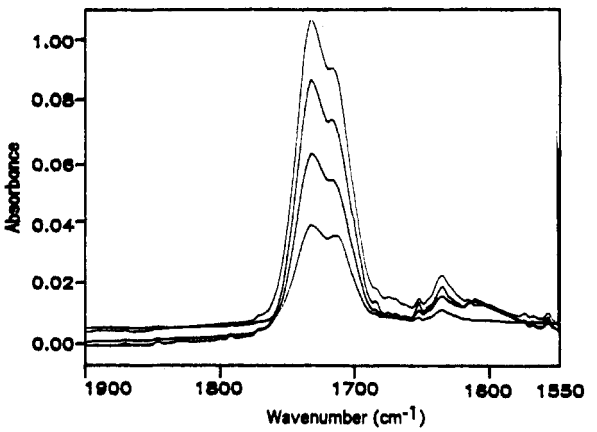


Figure 3. Typical FTIR spectra of the extracted monomer from hydrated bilayers of mono-MethPC that were polymerized at 61 °C for 0, 10, 30, and 60 min (top to bottom) at a [M]/[I] of 50.

Kinetic Analyses. Typical FTIR spectra for mono-MethPC extracted from polymerized samples by $CHCl_3$ are shown in Figure 3. The unextracted lipid in these samples corresponds to the poly(mono-MethPC) in the vesicle bilayers which formed during the polymerization reaction for 0, 10, 30, and 60 min, respectively. The decrease in the intensities of the carbonyl band is directly related to the decrease of lipid monomer during the polymerization. The loss of the monomer during the polymerization reaction was obtained according to eq 1,

$$\% \text{ conversion } (X) = \frac{I_0 - I_t}{I_0} \times 100 \qquad (1)$$

where I_0 is the intensity of the carbonyl band for the sample before polymerization and I_t is the calculated intensity of the same band after reaction for a specified time, t. Figure 4a shows the conversions of mono-MethPC to polymer at several initial concentrations of the monomer: [DMPC]/[mono-MethPC] = 1, 0.5, 0.2, and 0 at a constant [M]/[I] = 50. Figure 4b shows the conversion for mono-MethPC samples polymerized at a variety of monomer to initiator ratios for pure mono-MethPC: [M]/[I] = 150, 100, 50, 30.

UV spectra of mono-AcrylPC recorded during a typical AIBN-initiated polymerization reaction are shown in Figure 5. The loss of the monomer during polymerization reaction under different conditions was calculated according to eq 1, where I_0 is the intensity of the band at 195 nm for the sample before polymerization and I_t is the

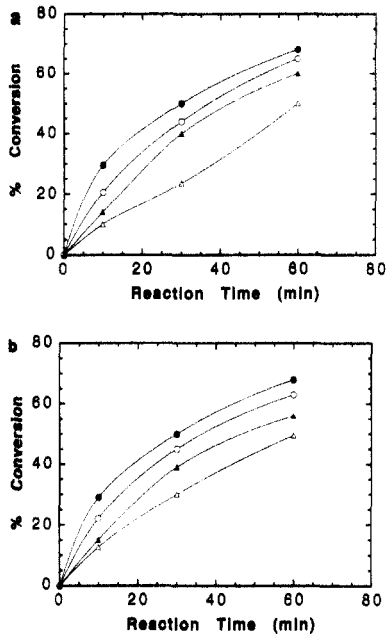


Figure 4. (a) Effect of the dilution of mono-MethPC with DMPC on the rate of conversion to polymer at 61 °C, where the [DMPC]/[mono-MethPC] was 1 (▲), 0.5 (▲), 0.2 (○), and 0 (●) and [M]/[I] was 50. (b) Effect of variation in the ratio of mono-MethPC to AIBN on the rate of conversion to polymer at 61 °C, where the [M]/[I] was 30 (●), 50 (○), 100 (▲), and 150 (△).

intensity of the same band after reaction for time t. Figure 6a shows loss of the mono-AcrylPC monomer during polymerization for the monomer samples diluted with DMPC in the following ratios: [DMPC]/[mono-AcrylPC] = 1, 0.5, 0.2, and 0 at a [M]/[I] = 50. Figure 6b shows the conversion for the pure mono-AcrylPC bilayers polymerized at 61 °C with various initial ratios: [M]/[I] = 100, 50, and 30.

The slope at any point of the percent conversion vs t curves gives the rate of polymerization under specific conditions. The kinetic parameters, reaction orders, can be derived from the general kinetic expression

$$R_p = k[M]^{\alpha}[I]^{\beta} \tag{2}$$

where k is a rate constant, [M] and [I] are the concentrations of monomer and initiator, respectively, and α and β are reaction orders of [M] and [I], respectively. R_p is the rate of polymerization, which is defined as the consumption of the monomer at unit volume and unit time

$$\begin{aligned} R_p &= -\frac{dc}{dt} \\ &= -\frac{1}{V}\frac{dn}{dt} \\ &= \frac{n_0}{V}\frac{d\chi}{dt} \end{aligned} \tag{3}$$

where c is the bulk concentration, V is the volume of the suspension, n_0 is the initial moles of the monomer, and χ is the percent conversion.

Rewriting eq 2 as

$$\log R_p = \log k + \alpha \log [M] + \beta \log [I] \tag{4}$$

yields a linear equation if [M] or [I] is constant. The kinetic parameters, k, α, and β, can be obtained by solving eq 4 with the data from the percent conversion vs t curves.

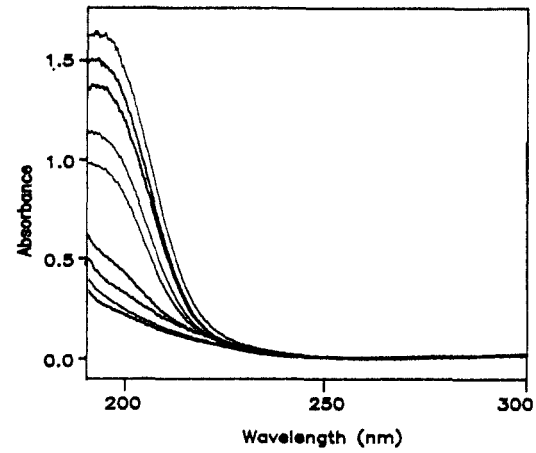


Figure 5. Change of mono-AcrylPC UV absorption during AIBN polymerization at 61 °C.

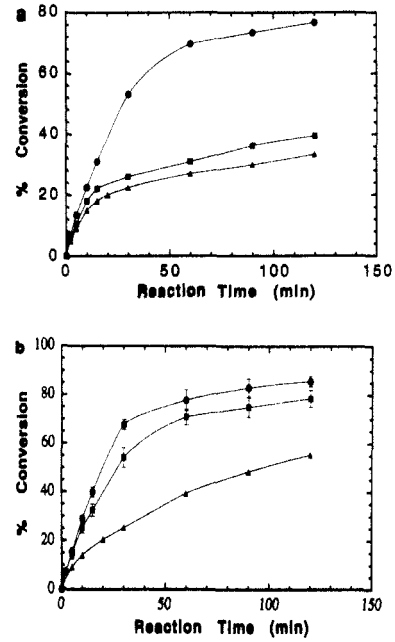


Figure 6. (a) Effect of the dilution of mono-AcrylPC with DMPC on the rate of monomer conversion at 61 °C, where the [DMPC]/[mono-AcrylPC] was 1 (▲), 0.5 (■), 0.2 (●) and [M]/[I] was 50. (b) Effect of variation in the ratio of mono-AcrylPC to AIBN on the rate of monomer conversion at 61 °C, where the [M]/[I] was 30 (●), 50 (■), and 100 (▲).

The slopes of the log k vs log [M] plots at constant [I] yield α, whereas the log k vs log [I] plots at constant [M] give β.

Initial Rate of Polymerization. In order to minimize the effect of the products on the kinetic measurements, it was desirable to measure the reaction orders at low conversion to polymer. This was accomplished at a series of initial concentrations of monomer/initiator and a constant initiator/monomer ratio, so that eq 4 can be written as

$$\log R_p = \log k_I + \alpha \log [M] \tag{5}$$

$$\log R_p = \log k_M + \beta \log [I] \tag{6}$$

where $\log k_I = \log k + \beta \log [I]_0$ in eq 5 and $\log k_M = \log k + \alpha \log [M]_0$ in eq 6. $[M]_0$ and $[I]_0$ are the initial concentrations of the monomer and initiator, respectively. By measuring R_p at different [M] or [I], the slope of the log R_p vs [M] plot gives α and the slope of the log R_p vs log [I] plot yields β. The reaction orders α and β for the 61 °C AIBN-initiated polymerization of mono-MethPC

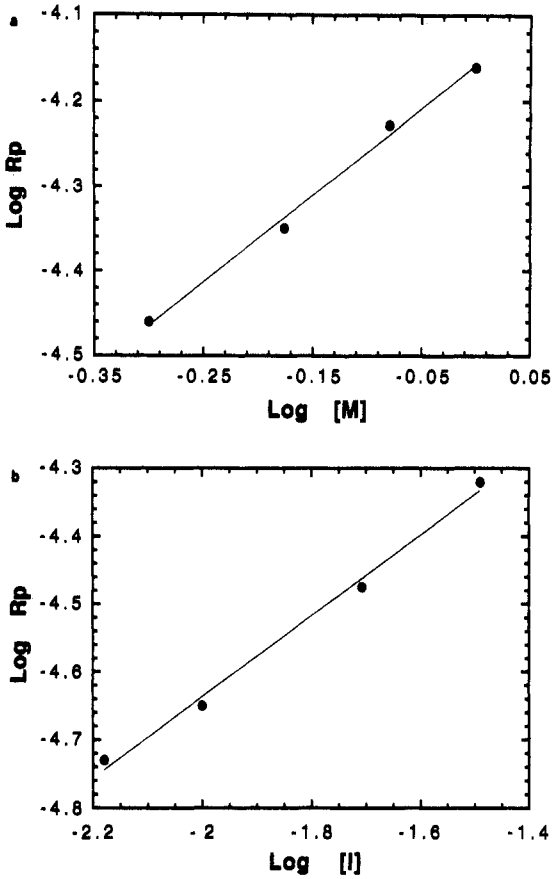


Figure 7. (a) log R_p log $[M]_0$ for the polymerization of mono-MethPC at constant $[I]_0$. (b) log R_p vs log $[I]_0$ at constant $[M]_0$.

were 1.0 and 0.6, respectively (Figure 7). The reaction orders α and β for the AIBN-initiated polymerization of mono-AcrylPC were 1.1 and 0.5, respectively.

Kinetics at High Conversion. The kinetics derived from the initial polymerization of mono-MethPC shows that the initial rate of polymerization is dependent on the first order of the monomer concentration and approximately the square root of the initiator concentration. This dependence is the same as that observed for many monomer–initiator combinations over a wide range of concentrations for conventional solution radical-chain polymerizations.[12-14] However, our comparison study of the degree of polymerization of mono-AcrylPC in lipid bilayers found that X_n was proportional to $[M]^2$ and $[I]^{-1}$.[4] These results suggest that the R_p should depend on $[M]^2$ and be independent of the initiator. Since the degree of polymerization study was performed at high conversion of monomer to polymer, it appeared likely that the initial kinetics differ from those at high conversion.

We estimated the kinetics at high conversion by utilizing eq 4 and generating experimental data for lipid bilayers of mono-MethPC for longer times to reach the high conversion conditions shown in Figure 8. The dependence of the rate of polymerization on the concentrations of the monomer and initiator can be determined if the rate of polymerization, R_p, and the concentrations of the monomer, [M], and initiator, [I], at high conversion are known.

The concentrations of the monomer, [M], at a given time during the polymerization reaction can be derived from the conversion curves in Figure 8. The concentration of the initiator, [I], at any time during the polymerization reaction can be calculated from the kinetics of the decomposition of the initiator, which is a first-order reaction, and the decomposition rate constant, which is relatively independent of solvents.[7] The half-life time,

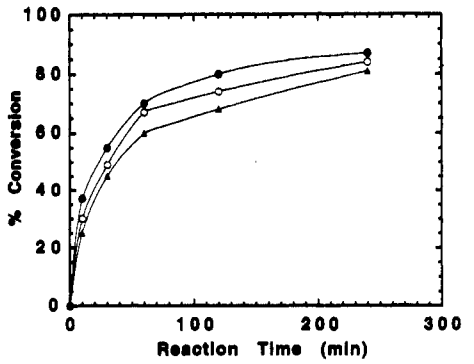


Figure 8. Extended polymerization of mono-MethPC at 61 °C to derive the kinetics at high conversion where the [M]/[I] was 30 (●), 50 (○), and 100 (▲).

$t_{1/2}$, of AIBN at 61 °C obtained from a plot of $t_{1/2}$ vs T was 17 h.[7] The concentration of AIBN at any time during the polymerization reaction was calculated by eq 7:

$$C = C_0 e^{-kt} \tag{7}$$

where k is 6.8×10^{-4} s^{-1}.

The rate of polymerization at the end of the fourth hour was derived from the slopes of the C vs t plot (Figure 8), and the concentrations of the monomer and initiator were obtained from the general concept:

$$\text{molar fraction} = n/n_0$$

where n_0 is the total moles of monomer *in a vesicle* and n is the moles of the monomer after polymerization for time t. The value of n_0 is therefore not changed in the course of polymerization even though some monomers are converted into polymer. The amount of the unreacted monomer, n, can be calculated from conversion.

$$n = n_0(1 - \chi)$$

Here, χ is the fraction conversion at time t. Therefore, the molar fraction of the monomer time t is $1 - \chi$.

$$\text{molar fraction of monomer} = 1 - \chi\%$$

Substitution of the values of R_p, $[m]_{(4)}$, and $[i]_{(4)}$ into eq 4 gives three independent equations

$$-5.58 = \log k - 0.886\alpha - 1.55\beta \tag{8}$$

$$-5.45 = \log k - 0.796\alpha - 1.77\beta \tag{9}$$

$$-5.31 = \log k - 0.699\alpha - 2.07\beta \tag{10}$$

which upon solution yield an α of 1.6 and a β of 0.06. Thus at high conversion the R_p is dependent on $[M]^{1.6}$ and is almost independent of the initiator concentration.

The same method was applied to determine the reaction orders at high conversion of mono-AcrylPC in lipid bilayers to polymer. The following three independent equations

$$-7.13 = \log k - 0.65\alpha - 1.51\beta \tag{11}$$

$$-6.98 = \log k - 0.54\alpha - 1.74\beta \tag{12}$$

$$-6.49 = \log k - 0.24\alpha - 2.03\beta \tag{13}$$

were solved to give an α of 1.86 and β of 0.23.

Discussion

We previously reported that the relative number-average degree of polymerization of mono-AcrylPC bilayers was

dependent on the $[M]^2$ and $[I]^{-1}$. These polymerizations were initiated by the thermal decomposition of AIBN, and the polymer sizes were analyzed after high conversion to polymer. These results suggest that termination of the growing mono-AcrylPC chain is dominated by primary termination under high conversion conditions. Primary termination is a result of interaction of the primary radicals from the initiator with the growing polymer. The expected rate of polymerization under these conditions is given by eq 14.

$$R_p = k_p k_i [M]^2 / k_{tp} \quad (14)$$

where k_{tp} is the rate constant for primary radical termination, k_p is the rate constant for propagation, and k_i is the rate constant for initiation. This rate expression is readily distinguished from that observed (eq 15) in a conventional biomolecular chain termination process, i.e., termination by chain coupling and/or chain disproportionation.

$$R_p = k_p [M] (f k_d [I] / k_t)^{0.5} \quad (15)$$

The rate constants in this expression designate the initiator decomposition, k_d, and chain termination, k_t, where f is the initiator efficiency.

Both the AIBN-initiated polymerization of mono-AcrylPC and mono-MethPC bilayers exhibit a kinetic dependence on [M] and $[I]^{0.5}$ at the start of the polymerization which is consistent with eq 15. Thus in the early stages of the polymerization the bilayer-constrained polymer chains terminate by chain coupling and/or chain disproportionation. Since alkyl acrylates tend to terminate exclusively by coupling, it is likely that this is the dominant termination process at the outset of the mono-AcrylPC polymerization. In contrast, it is expected that the mono-MethPC will behave like other alkyl methacrylates, which terminate by coupling and disproportionation.

As the polymerization approaches high conversion, the kinetic behavior changes. Both the mono-AcrylPC and the mono-MethPC bilayer polymerizations show an increased dependence on [M] and a diminished dependence on [I] at high conversions. The order of dependence, α, on [M] was observed to be 1.86 and 1.6, respectively, at conversions of 70–80%. The order of dependence, β, on [I] was nearly zero at these high conversions to polymer. These data are more consistent with eq 14 rather than eq 15. The course of the bilayer polymerization appears to progressively modify the bilayer in a manner that reduces the probability of bimolecular chain termination reactions and favors increased domination by primary termination. In other words, as more of the lipid monomers are converted to polymer chains, the freedom of polymer chain motion is increasingly constrained by the two-dimensional nature of the bilayer membrane. This is expected to reduce the translation mobility of the growing polymer chains and eventually effect the polymer segmental mobility as well. Decreased polymer mobility in the bilayer should increase the lifetime of polymer radicals, which will favor the diffusion of AIBN fragments through the bilayer and primary termination of the radical chain.

The kinetic analysis provides a valuable complement to the degree of polymerization study of mono-AcrylPC. It reveals the transition in polymer chain growth and termination as the polymerization proceeds from low to high conversion, whereas the number-average degree of polymerization data was obtained only at high conversion. Polymer size is frequently independent of the percent conversion to polymer. However, in the case of the bilayer polymerization of mono-AcrylPC and mono-MethPC, the initial polymer chains produced by coupling will be about twice the size of those formed by primary termination. Therefore, at high conversion the smaller chains resulting from primary termination should dominate the number-average molecular weight and X_n. Consequently, analysis of the dependence of X_n on [M] and [I] will emphasize the contribution of primary termination as observed by Sells and O'Brien.[4]

Comparative evaluation of the rates of polymerization between mono-AcrylPC/mono-MethPC and those of methyl methacrylate/methyl acrylate can be accomplished using the rate constants for the polymerizations. This is possible since the rate of polymerization of methyl methacrylate at low conversion and the initial rate of polymerization of mono-MethPC follow the same kinetics (eq 15).[15-17] A rate constant for polymerization, k, can be defined as

$$k = k_p (k_d / k_t)^{1/2} \quad (16)$$

The value of k can be evaluated by substitution of k_p, k_t, and k_d, if these parameters are known. It can also be determined experimentally as in this study. The kinetic parameters for the radical-chain polymerization of methyl methacrylate and methyl acrylate and the rate constant for dissociation of AIBN at 60 °C are listed in Table 2.[7,12,18]

As defined in eqs 5 and 6, the rate constants for the initial polymerization of the mono-MethPC can be derived from the intercept in Figure 7. Thus from Figure 7a we find

$$\log k = -4.15 - b \log [I]_0$$
$$= -4.15 - 0.5 \log(1/51)$$
$$= -3.3$$
$$k = 5.0 \times 10^{-4}$$

and from Figure 7b we find

$$\log k = -3.45 - a \log [M]_0$$
$$= -3.45 - \log(50/51)$$
$$= -3.4$$
$$k = 3.6 \times 10^{-4}$$

We take the average of these two values to estimate the initial rate constant for polymerization of mono-MethPC: $k_{av} = 4.25 \times 10^{-4}$. A similar approach for mono-AcrylPC gives an average rate constant of $k_{av} = 6.7 \times 10^{-4}$. These rate constants for polymerization of monomers in the lipid bilayer are similar to the rate constants for simple monomers, which were calculated from the data in Table 2 and eq 16. The data in Table 3 show that the difference in the reactivity between methyl methacrylate and methyl acrylate is almost 7-fold. When the polymerizable moieties in lipid molecules are polymerized in lipid bilayers, the

Table 2. Literature Values for the Polymerization Rate Constants at 60 °C

monomer	$k_p \times 10^{-3}$ (M^{-1} s^{-1})	$k_t \times 10^{-7}$ (M^{-1} s^{-1})	$k_d \times 10^5$ (s^{-1})
methyl methacrylate	0.515	2.55	0.845
methyl acrylate	2.09	0.95	0.845

Table 3. Rate Constants for Polymerization

monomer	$k \times 10^{-4}$ ($M^{-1/2}$ s^{-1})	monomer	$k \times 10^{-4}$ ($M^{-1/2}$ s^{-1})
methyl methacrylate	2.96	mono-MethPC	4.25
methyl acrylate	19.7	mono-AcrylPC	6.7

difference in reactivity between the polymerizable moieties is less than a factor of 2. The highly ordered nature of bilayer assemblies appears to dominate the reactivity of the monomers, thereby minimizing the differences between these monomers.

Furthermore, the rate of polymerization of the monomers in the bilayer is similar to solution polymerization. Finally, it should be noted that at high concentrations some radical-chain polymerizations are notable for the presence of an autoacceleration in the polymerization rate, known as the gel effect. That is, the rate of polymerization increases, rather than decreases, with time as the reaction proceeds.[19] The gel effect has been observed for methyl methacrylate, vinyl acetate, styrene, and other monomers in solution and bulk polymerizations.[17,19-23] The onset of marked autoacceleration in the rate of polymerization of methyl methacrylate occurs when the concentration of polymer approximates a condition of macromolecular close packing.[20] Interestingly the gel effect was not observed in polymerization of mono-MethPC or mono-AcrylPC bilayers which again indicates the strong effect of the bilayer assembly on the process of polymerization. Whether these effects are entropic or enthalpic in nature must await future studies on the effect of temperature on the rate of polymerization of these supramolecular assemblies.

Experimental Section

Materials. The synthesis of polymerizable lipids, mono-MethPC and mono-AcrylPC, was described in detail by Sells.[4,5] The nonpolymerizable lipid, L-α-dimyristoylphosphatidylcholine (DMPC), was obtained from Avanti Polar Lipids, Inc. The purity of each lipid, mono-MethPC, mono-AcrylPC, and DMPC, was greater than 99% (TLC). Azobis(isobutyronitrile) (AIBN; Eastman Chemicals) was recrystallized twice from methanol and was used as initiator. Chloroform and benzene were purified by distillation and were used as solvents to prepare stock solutions. The chloroform used for extraction and in FTIR measurements was purified by distillation from P_2O_5 and then passed through a column of activated Al_2O_3. MilliQ water (Millipore) was used to hydrate the lipids. The ampules used as containers for polymerization were cleaned by soaking in concentrated sodium hydroxide overnight and then extensively rinsed with tap water and MilliQ water successively.

Extruded Vesicles. A desired weight of lipid was transferred from stock solution(s) into a vial, and the solvent was evaporated under a stream of argon to form a lipid film on the wall of the vial. The exact weight of the lipid was obtained after the lipid film was completely dried under vacuum. The lipid was then mixed with an appropriate amount of AIBN stock solution and was redried as before. Since AIBN was found to decompose on prolonged evacuation, the lipid and AIBN mixture was only placed under vacuum for less than 4 h.

MilliQ water was added to the lipid to adjust the total concentration of the lipids to 5.26×10^{-3} mM (4 mg/mL) for mono-MethPC and 6.69×10^{-3} mM (5 mg/mL) for mono-AcrylPC. In the case of mixtures of each polymerizable PC and DMPC, the total concentration of the lipids was either 5.26×10^{-3} or 6.69×10^{-3} mM. The lipid dispersion was frozen in a dry ice/propanol bath and then slowly thawed in a water bath at room temperature, followed by vortexing. This freeze–thaw–vortexed cycle was repeated 10 times. The dispersion was then extruded 10 times through two 0.2-mm Nuclepore polycarbonate filters at room temperature using a stainless steel extruder (Lipex Biomembranes). This procedure produced a clear, translucent dispersion. The vesicle sizes were determined by quasi-elastic light scattering (QELS) measured at 60, 90, and 120° as described by Kölchens et al.[24] The average vesicle diameter from the QELS was 140 ± 5 nm.

Polymerization. The lipid dispersions were each divided into four to six equal parts and placed into sample ampules after extrusion. Each ampule contained either 4 (mono-MethPC) or 5 mg (mono-AcrylPC) of lipids. The ampules were sealed with rubber septums, which had two inserted syringe needles. One long syringe needle was connected to an ultrapure argon line directing a stream of the Ar to flush the dispersion in the ampule. Another short syringe needle was used as an outlet for the purged gas. Outgassing proceeded for 1 h or longer before the ampules were placed into a thermostated water bath to initiate the polymerization at 61 ± 0.02 °C. The polymerization was stopped immediately at the desired polymerization time, either by taking the ampules out of the bath and placing them into a dry ice/acetone bath (mono-MethPC) for subsequent FTIR analysis or by withdrawing a small portion of the dispersion and diluting 1/40 with cold water followed by immediate UV absorption analysis.

FTIR Sample Analyses. After quenching of the polymerization, the reaction mixture sample was freeze-dried. The unreacted monomer was then extracted with 0.14 mL of purified $CHCl_3$. The insoluble polymer was recovered for FTIR measurement. The extracted monomer and the collected polymer were analyzed by a Nicolet 510P FTIR spectrometer equipped with a DTGS detector. The liquid cell used for the measurements of the unreacted monomer after subtraction of $CHCl_3$ was a Sealed Precision Pathlength Cell (Spectra Tech) with KBr windows. The cell path length was 0.1 mm with a volume of 0.11 mL. The cell was cleaned with purified $CHCl_3$ and dried by a N_2 stream after each measurement. The collected polymer was washed three to four times by $CHCl_3$ and then dried under dynamic vacuum for 12 h. The polymer was then prepared as a KBr pellet for FTIR measurement.

Mono-MethPC and mono-AcrylPC were each prepared as a lipid film to examine by FTIR. The film was prepared by spreading the lipid/$CHCl_3$ solution on a ZnSe IR window. All spectra were 200 coadded interferograms with a resolution of 4 cm^{-1}.

UV Absorption Sample Analyses. A mono-MethPC vesicle dispersion was polymerized in an ampule as above. The polymerization process was followed spectrophotometrically with the aid of a Varian DMS 200 UV–vis spectrophotometer. This dispersion was sampled with a 1-mL syringe at desired intervals during the reaction. To prevent the possibility of the introduction of oxygen into the reaction vessel during the sampling, an empty ampule provided with a septum was also connected to the ultrapure Ar line and thus the ampule was kept under positive Ar pressure. The syringe used for sampling was inserted through the septum and allowed the Ar to purge and replace the air in the syringe before sampling.

Poly(mono-AcrylPC) was obtained by collecting the polymerized dispersion. After the sample was lyophilized, the unreacted monomer was removed from the polymer by extraction with $CHCl_3$. The polymer was further cleaned by $CHCl_3$ three times and then dried under dynamic vacuum for 12 h. The polymer was then prepared as a KBr pellet for FTIR measurement as above.

Acknowledgment. This research was supported by a grant from the Division of Materials Research of the National Science Foundation.

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