NMR Study of the Early Stages of Gel Formation in the PEG/Poly(AMPS-*co*-NIPA) Semi-Interpenetrating Network System

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ABSTRACT: The rate of conversion of the monomers and crosslinker in the formation of a novel semi-interpenetrating poly(ethylene glycol)/ poly(2-acrylamido-2-methylpropane sulfonic acid-*co*-*N*-isopropylacrylamide) copolymer hydrogel was determined by using ^{1}H-NMR spectrometry. It was established that poly(ethylene glycol) does not participate in the polymerization reactions and that crosslinking by methylenebisacrylamide occurs predominantly in the early stages of copolymer chain growth. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 91: 3635–3641, 2004

Key words: radical polymerization; copolymerization; kinetics; NMR

INTRODUCTION

A semi-interpenetrating gel system comprising poly(ethylene glycol) (PEG) chains embedded in a poly(2-acrylamido-2-methylpropanesulphonic acid-*co*-*N*-isopropyl acrylamide) network was designed as a multifunctional peptide or protein drug linker and hydrogel carrier, for controlled *in vivo* administration of drugs. Incorporation of PEG in the gel increases the potential for hydrogen bond formation, because the lone pair electrons of oxygen in the repeat unit (CH_2CH_2O) of PEG serve as hydrogen bond acceptors.[1] PEG is a stable polymer which is a linker molecule for peptide or protein drugs.[2,3] PEG can be used as plasticizer for rigid polymers,[1] especially for polymer electrolytes such as sodium poly(styrene sulfonate), to increase polymer chain flexibility by reducing intermolecular attractions, increasing free volume, and hence, decreasing glass transition temperature.

Because PEG is very soluble in water, when PEG chains migrate from the semi-interpenetrating network (SIPN) to an aqueous medium, linked drug molecules will be released simultaneously to the medium. Consequently, PEG will play a key role in the physical, mechanical, and functional properties of the SIPN controlled release system, without being chemically bound to the constituent monomers or crosslinker from which the copolymer was formed.

The purpose of the present investigation was twofold. The initial aim was to confirm that when chain growth and crosslinking took place in an aqueous pregel solution containing the monomers 2-acrylamido-2-methylpropanesulfonic acid (AMPS) and *N*-isopropylacrylamide (NIPA), the crosslinker *N*,*N*′-methylenebisacrylamide (MBAA) and PEG, the expected SIPN (with PEG as the interpenetrating component), was formed. In addition, it was of interest to determine the priority (relative rates) of the crosslinking and backbone chain growth reactions in the early stages of reaction.

Because polymerization and crosslinking reactions change the chemical environment of reactants and products, NMR has been used as an effective method to investigate polymerization kinetics, crosslinking, and crosslink density.[4–7] However, work on the early stages of copolymerization and crosslinking for the PEG/poly(AMPS-*co*-NIPA) SIPN system has not been reported previously. In a related study, Pesce-Rodriguez and coworkers[8] investigated, using solid-state ^{13}C-NMR spectroscopy, a low-conversion product from the cationic polymerization of glycidyl acrylate with trioxane. They found that the acrylate group participated in the copolymerization to give a crosslinked copolymer, and a significant degree of crosslinking occurred in the early stages of copolymerization. In the present work, we have used high-field (400 MHz) ^{1}H-NMR spectroscopy to probe the early stages of polymerization and crosslinking in a solution of AMPS, NIPA, MBAA, and PEG in deuterated water.

EXPERIMENTAL

Poly(ethylene glycol) (PEG6000, with average molecular weight of about 6000 g mol^{-1}) was supplied by

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BDH (Poole, England). *N*-isopropylacrylamide (from Acros Organics, Geel, Belgium) and 2-acrylamido-2-methylpropanesulphonic acid (Merck-Schuchardt, Hohenbrunn, Germany) were both synthesis-grade (>99%) materials. *N*,*N*′-methylenebisacrylamide (Sigma-Aldrich, St. Louis, MO) was electrophoresis reagent-grade material. Ammonium persulfate (Ajax Chemicals, Auburn, NSW, Australia) used as initiator was >98% pure. All of these chemicals were used without further purification.

D_2O (99.9% deuterated) and Milli-Q water were the solvents. Pregel solutions in D_2O, containing 50 wt % PEG (based on the total mass of PEG, AMPS, and NIPA) were made by dissolving 0.113 g PEG6000, 0.22 mM AMPS, 0.60 mM NIPA, 0.040 mM MBAA, and 0.014 mM ammonium persulfate in 3.13 mL D_2O in an NMR tube at ambient temperature. The stoppered NMR tubes were then kept in an ice/water bath, avoiding exposure to light, prior to beginning the NMR experiments.

^{1}H-NMR spectra (32 scans with 1-s delay) were recorded with a Bruker Avance Dex 400 MHz spectrometer. The change in intensity of the vinyl protons of the monomers and crosslinker was determined as a function of reaction time. The methylene proton signals from PEG were also recorded.

NMR spectra were recorded at 25°C. The pregel solution in an NMR tube was then heated in a water bath at 70°C. After 2 min, the tube was transferred to an ice/water bath to stop polymerization reaction and then transferred to the NMR spectrometer after quickly removing water from the exterior of the tube, using tissue paper, and the spectrum was recorded. The heating/quenching/recording process was repeated over a total period of reaction of 10 min. For comparison, the ^{1}H-NMR spectrum was recorded for a parallel sample continuously heated at 70°C for 10 min.

For free radical polymerization of a single monomer, the rate of polymerization, r_p, is expressed by

$$r_p = -d[M]/dt \tag{1}$$

Because the initial monomer concentration is known, it is useful to work with the initial rate of polymerization given by

$$r_p^0 = -d[M]/dt|_{t=0} = \{[M]_0 - [M]\}/\Delta t|_{\Delta t \to 0} \tag{2}$$

It is assumed that all free radicals present in the system are at steady-state concentrations and that the reactivity of the propagating radicals is independent of the degree of polymerization of the radical and the kinetic chain length. In fact, the kinetic chain length may have some effect on polymerization behavior, especially for crosslinking polymerizations for which a chain length dependent termination effect has been observed.[9] The steady-state rate (r_p) of polymerization can be expressed by

$$r_p = (k_p^2/2k_t)^{1/2}(2fk_i)^{1/2}[I]^{1/2}[M] \tag{3}$$

where k_p and k_t are rate constants for propagation and termination of like radicals, f represents the fraction of initiating radicals that add to monomer to form propagating radicals, k_i is the rate constant for initiation, and $[I]$ and $[M]$ are concentrations of initiator and monomer, respectively.

For a copolymerization system of AMPS and NIPA, together with MBAA which is considered as the third monomer, there are nine distinct propagation reactions:

$$\sim M_1^{\bullet} + M_1 \rightarrow \sim M_1M_1^{\bullet} \quad \text{(with rate constant } k_{11})$$

$$\sim M_1^{\bullet} + M_2 \rightarrow \sim M_1M_2^{\bullet}\ (k_{12})$$

$$\sim M_1^{\bullet} + M_3 \rightarrow \sim M_1M_3^{\bullet}\ (k_{13})$$

$$\sim M_2^{\bullet} + M_1 \rightarrow \sim M_2M_1^{\bullet}\ (k_{21})$$

$$\sim M_2^{\bullet} + M_2 \rightarrow \sim M_2M_2^{\bullet}\ (k_{22})$$

$$\sim M_2^{\bullet} + M_3 \rightarrow \sim M_2M_3^{\bullet}\ (k_{23})$$

$$\sim M_3^{\bullet} + M_1 \rightarrow \sim M_3M_1^{\bullet}\ (k_{31})$$

$$\sim M_3^{\bullet} + M_2 \rightarrow \sim M_3M_2^{\bullet}\ (k_{32})$$

$$\sim M_3^{\bullet} + M_3 \rightarrow \sim M_3M_3^{\bullet}\ (k_{33})$$

If penultimate effects are ignored, eqs. (4)–(6) are applicable

$$[\sim M_1M_1^{\bullet}] + [\sim M_2M_1^{\bullet}] + [\sim M_3M_1^{\bullet}] = [\sim M_1^{\bullet}]_{\text{total}} = [\sim M_1^{\bullet}] \tag{4}$$

$$[\sim M_1M_2^{\bullet}] + [\sim M_2M_2^{\bullet}] + [\sim M_3M_2^{\bullet}] = [\sim M_2^{\bullet}]_{\text{total}} = [\sim M_2^{\bullet}] \tag{5}$$

$$[\sim M_1M_3^{\bullet}] + [\sim M_2M_3^{\bullet}] + [\sim M_3M_3^{\bullet}] = [\sim M_3^{\bullet}]_{\text{total}} = [\sim M_3^{\bullet}] \tag{6}$$

The rate of consumption of individual comonomers depends on the relative magnitudes of the rate constants of all possible propagation reactions. The consumption rates for M_1, M_2, and M_3 are not, in general, equal, so that

TABLE I
^{1}H-NMR Chemical Shifts and Assignments for Pregel Solution Components in D_2O at 298 K

δ/ppm[a]	Assignment	δ/ppm[b]
PEG6000		
3.76	CH_2 (—CH_2CH_2O), single peaks	3.73
MBAA		
4.78–4.82	—CH_2 (—$CONHCH_2$), 2 split peaks	4.83
5.83–5.86	—CH (—CH═CH_2), 4 split peaks	5.81–5.84
6.28–6.30	═CH_2 (—CH═CH_2), 3 split peaks	Within 6.1–6.3 (overlapping CH_2 region)
NIPA		
1.20–1.22	—CH_3, 2 split peaks	1.18–1.20
4.02–4.05	—CH (—$CH(CH_3)_2$), 7 split peaks	3.99–4.03
5.76–5.79	—CH (—CH═CH_2), 4 split peaks	Centered at 5.753
6.17–6.31	═CH_2 (—CH═CH_2), 8 split peaks	Within 6.1–6.3 (overlapping CH_2 region)
AMPS		
1.539	—CH_3, single peak	1.543
3.454	—CH_2 (—CH_2-SO_3^-), single	3.446
5.71–5.74	—CH (—CH═CH_2), 4 split peaks	Centered at 5.725
6.14–6.30	═CH_2 (—CH═CH_2), 8 split peaks	Within 6.1–6.3 (overlapping CH_2 region)

[a] In solutions of individual pure components.
[b] In mixtures of components.

$$r_p \neq -d[M_1]/dt \neq -d[M_2]/dt \neq -d[M_3]/dt \quad (7)$$

Because the comonomer concentrations are varying with time during reaction and the rate of consumption of each comonomer depends on both its reactivity and its concentration, the differential rate expressions cannot easily be correlated with the individual reaction rates. Consequently, it is helpful to use another way to study the reaction kinetics of the multi-reactant system.

RESULTS AND DISCUSSION

^{1}H signals and assignments for the components of the pregel solutions, in D_2O, are given in Table I. The basic premise of the study is that the signal from the protons linked to unsaturated carbon–carbon double bonds in MBAA, AMPS, and NIPA will decrease with reaction time, and the signal from the interpenetrating linear polymer PEG6000 will remain unchanged. The questions to be answered were (1) when polymerization and crosslinking reactions take place, are the proton signals sufficiently well defined to be useful? (2) If we stop the reaction at intervals to measure the NMR signal and then restart the reaction by heating, do the H-NMR spectra reflect the information that would have been obtained by continuous heating?

The second point is resolved by the data shown in Table II. The ^{1}H-NMR spectra in the two cases gave integrated signal intensities with differences, measured as D% defined by eq. (8), that were always smaller than 2% of the initial intensity

$$|D|\% = 100(I' - I'')/I_0 \quad (8)$$

It is clear from the spectra of the reaction mixture at various reaction times (Figs. 1 and 2) that the proton

TABLE II
Comparison of Integrated Signal Intensities (arbitrary units) for 10 min Total Reaction Time

δ/ppm	I_0	I'	I''	$\|D\|$%
1.18–1.2	34.6	34.0	34.7	1.9
~1.54	12.4	26.7	26.7	0.2
1.8–2.6	0	9.16 (new, broad)	9.76 (new, broad)	—
3.1–3.6	—	3.67 (broad)	4.71 (broad)	—
~3.44	3.94 (single)	—	—	—
~3.7 (PEG)	100.0	100.0	100.0	0
3.8–4.1	—	7.20 (broad)	6.32 (broad)	—
~4.0	5.42	—	—	—
5.7–5.77	8.15	0.56	0.40	1.96
6.16–6.29	16.1	1.02	0.75	1.68

I_0 is the integrated signal intensity before the reaction mixture is heated; I' and I'' are integrated signal intensities obtained from the heat/cool/heat procedure and continuous heating, respectively.

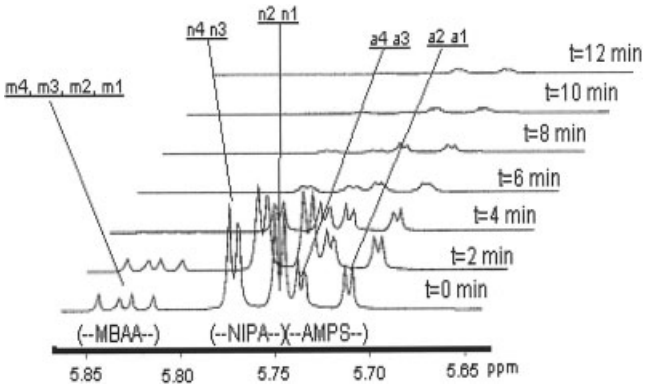

Figure 1 Reaction time dependence of ^{1}H-NMR spectra of vinyl methylene protons.

signals for partially polymerized and crosslinked reaction mixtures are sufficiently well resolved for integrated signal intensities to be evaluated for the protons of interest. In addition, the signal for PEG (at about 3.7 ppm) is invariant within experimental error with reaction time.

The signals from the vinyl protons of the monomers and the crosslinker show significant changes with reaction time. The methyl proton signals (from NIPA at ~ 1.2 ppm, and AMPS at ~ 1.54 ppm) become broader after 12 min heating, and new copolymer signals appear around 1.88–2.6 ppm (saturated CH_2 from copolymer main chain —CH_2—CH—) originating from the polymerization of vinyl CH_2 (~ 6.1–6.3 ppm in AMPS, NIPA, and MBAA). The vinyl CH proton signals from AMPS, NIPA, and MBAA are readily distinguishable. AMPS shows two resolved split peaks (a1, a2) at ~ 5.71 ppm; NIPA has two resolved split peaks (n3, n4) at ~ 5.77 ppm, and MBAA gives four resolved split peaks (m1, m2, m3, m4) at ~ 5.8 ppm. Signal integration data are summarized in Table III. From the integrated proton signal (I), % conversion for specific CH vinyl protons was calculated from

$$\% \text{ conversion} = -100(I_t - I_0)/I_0 = -100(C_t - C_0)/C_0 \quad (9)$$

In eq. (9), I_t is the integrated signal after reaction time t, and C_0 and C_t are corresponding proton concentrations at time zero and time t.

The variation of % conversion with reaction time is shown in Figure 3, from which an induction time on the order of 100 s is apparent. Moreover, MBAA is consumed much more rapidly than either NIPA or AMPS, which is not surprising if MBAA has approximately the same reactivity as NIPA and AMPS, because MBAA has two vinyl groups per molecule. In relation to NIPA and AMPS, it is apparent that NIPA is incorporated into polymer chains much more rapidly than AMPS. The % conversion ratio of NIPA to AMPS is 1.5 after 240-s reaction, which is consistent with our finding that NIPA has a larger reactivity ratio than AMPS.[9]

The values of Y, defined by

$$Y = -(1/C_0)[\{C_{t(i)} - C_{t(i-1)}\}/\{t(i) - t(i-1)\}]$$

in Table IV represent the relative rates of reactant consumption over 120-s time intervals. The graph of Y versus time in Figure 4 indicates rapid consumption of MBAA, implying that crosslinking takes place at an early stage of chain growth. Figure 4 also shows that both copolymer chain growth and

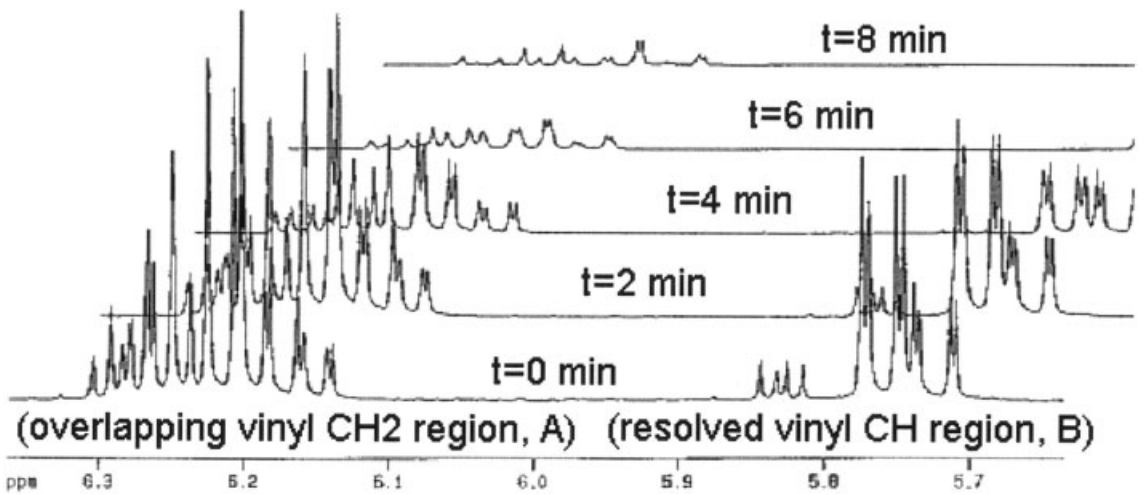

Figure 2 Reaction time dependence of ^{1}H-NMR spectra of overlapping CH_2 vinyl region (A) and resolved vinyl CH region (B) of vinyl protons from AMPS, NIPA, and MBAA.

TABLE III
Integrated Signal Intensity for Resolved CH Vinyl Protons in PEG6000/AMPS/NIPA/MBAA/D_2O Reaction Mixture as a Function of Reaction Time at 70°C

Signal assignment	δ/ppm	Reaction time (min) and integrated signal intensity for CH vinyl protons						
		0.0	2.0	4.0	6.0	8.0	10.0	12.0
AMPS (a1, a2)								
CH (—CH═CH_2)	5.71	13.1	13.1	7.25	4.40	2.63	2.21	1.97
NIPA (n3, n4)								
CH (—CH═CH_2)	5.77	35.6	35.3	11.6	3.37	1.02	0.44	0.34
MBAA (m1, m2, m3, m4)								
CH (—CH═CH_2)	5.80	10.0	9.57	0.62	0.0	0.0	0.0	0.0

Composition of reaction mixture: AMPS/NIPA ≈0.3/0.7 (mol/mol), PEG6000, 50 wt % based on total mass of comonomer.

crosslinking reactions dramatically increase to maximum values about 2 min after the start of reaction, followed by a relatively rapid decrease in rate over the next 2 to 6 min. These are typical characteristics of chain reactions.

By use of the long-chain assumption that monomer molecules are consumed solely by the propagation reactions, the rate of depletion of monomers can be expressed by

$$-d[M_1]/dt = k_{11}[M_1^{\bullet}][M_1] + k_{21}[M_2^{\bullet}][M_1] + k_{31}[M_3^{\bullet}][M_1] \quad (10)$$

$$-d[M_2]/dt = k_{22}[M_2^{\bullet}][M_2] + k_{32}[M_3^{\bullet}][M_2] + k_{12}[M_1^{\bullet}][M_2] \quad (11)$$

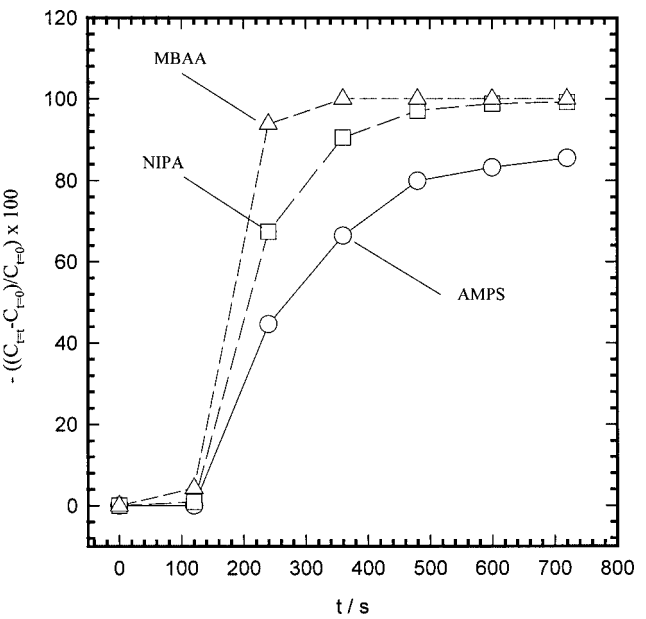

Figure 3 Vinyl proton % conversion versus heating time. The molar ratios in the pregel solution were [MBAA]/[AMPS]/[NIPA] = 1/5.5/15 and [AMPS]/[NIPA] = 0.3/0.7.

$$-d[M_3]/dt = k_{33}[M_3^{\bullet}][M_3] + k_{13}[M_1^{\bullet}][M_3] + k_{23}[M_2^{\bullet}][M_3] \quad (12)$$

Equations (10)–(12) can be converted to the relative relationships in eqs. (13), (14), and (15), respectively. For a time interval, *dt*, of 120 s, the terms on the left-hand side of eqs. (13), (14), and (15) are the values in column 5 of Table IV

$$-d[M_1]/[M_{1(0)}]dt = ([M_1]/[M_{1(0)}])(k_{11}[M_1^{\bullet}] + k_{21}[M_2^{\bullet}] + k_{31}[M_3^{\bullet}]) \quad (13)$$

$$-d[M_2]/[M_{2(0)}]dt = ([M_2]/[M_{2(0)}])(k_{22}[M_2^{\bullet}] + k_{32}[M_3^{\bullet}] + k_{12}[M_1^{\bullet}]) \quad (14)$$

$$-d[M_3]/[M_{3(0)}]dt = ([M_3]/[M_{3(0)}])(k_{33}[M_3^{\bullet}] + k_{13}[M_1^{\bullet}] + k_{23}[M_2^{\bullet}]) \quad (15)$$

The plot of $-d[M]/[M_{i0}]dt$ versus $[M]/[M_{i0}]$ shown in Figure 5 reveals that the relative reaction rate for each monomer varies with $[M]/[M_{i0}]$ and confirms that the monomers have relative rates in the order MBAA > NIPA > AMPS.

CONCLUSION

NMR spectroscopy has proven to be a useful technique for determining rates of monomer consumption in the early stages of the free radical initiated polymerization that leads to the formation of the hydrogel system formed by poly(2-acrylamido-2-methylpropanesulphonic acid-*co*-*N*-isopropylacrylamide) chains crosslinked with methylene bisacrylamide, with interpenetrating linear chains of poly(ethylene glycol). The experimental method is relatively straightforward and is capable of differentiating between the reacting species. For the PEG/poly(AMPS-*co*-NIPA) system, the significant result is that the initial rate of consumption of the

TABLE IV
Time Dependence of Kinetic Parameters in the PEG6000-AMPS/NIPA/MBAA Copolymerization System in D_2O at 70°C

t/s	$10^2 C_t$/mol L^{-1}	$10^2 (C_t - C_0)$/mol L^{-1}	$-10^2 (C_t - C_0)/C_0$	$Y \times 10^4$/s^{-1}
AMPS				
0	7.03	0	0	0
120	7.03	0	0	0
240	3.89	−3.14	44.6	37.2
360	2.36	−4.67	66.4	18.1
480	1.41	−5.62	79.9	11.3
600	1.18	−5.85	83.2	2.73
720	1.02	−6.01	85.5	1.90
NIPA				
0	19.3	0	0	0
120	19.1	−0.163	0.843	0.703
240	6.28	−13.0	67.4	55.5
360	1.82	−17.4	90.5	19.3
480	0.552	−18.7	97.1	5.50
600	0.24	−19.0	98.8	1.35
720	0.162	−19.1	99.2	0.335
MBAA				
0	1.28	0	0	0
120	1.22	−0.055	4.30	3.58
240	0.792	−0.20	93.8	74.6
360	0	−1.28	100.0	5.17
480	0	−1.28	100.0	0
600	0	−1.28	100.0	0
720	0	−1.28	100.0	0

The time interval $t(i) - t(i - 1)$ is 120 s.

crosslinking monomer is significantly greater than those of the other monomers, so that crosslinking of copolymer chains takes place predominantly in the early stages of copolymer chain growth, and the crosslink density of the network decreases with increasing monomer conversion.

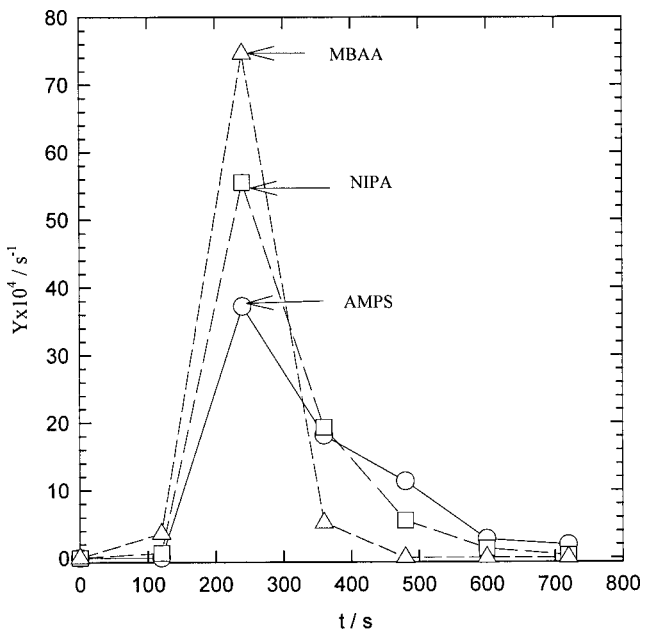

Figure 4 The time course and the relative rates, Y, of reactant consumption in the PEG6000–AMPS/NIPA/MBAA system.

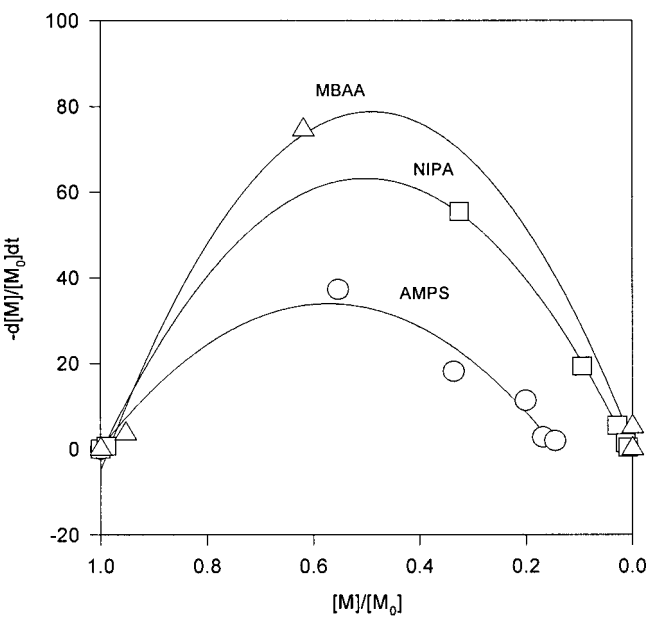

Figure 5 Relation between relative reaction rate and the relative monomer concentration. The symbols represent experimental data, and the curves are regression lines.

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