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Temperature-Responsive, Pluronic-g-poly(acrylic acid) Copolymers In Situ Gels for Ophthalmic Drug Delivery: Rheology, In Vitro Drug Release, and In Vivo Resident Property

Wen-di Ma, Hui Xu, Shu-fang Nie, and Wei-san Pan
Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, China

To prolong the precorneal resident time and improve ocular bioavailability of the drug, Pluronic-g-poly(acrylic acid) copolymers were studied as a temperature-responsive in situ gelling vehicle for an ophthalmic drug delivery system. The rheological properties and in vitro drug release of Pluronic-g-PAA copolymer gels, as well as the in vivo resident properties of such in situ gel ophthalmic formulations, were investigated. The rheogram and in vitro drug release studies indicated that the drug release rates decreased as acrylic acid/Pluronic molar ratio and copolymer solution concentration increased. It was also shown that the drug concentration had no obvious effect on drug release. The release rates of drug from such copolymer gels were mainly dependent on the gel dissolution. In vivo resident experiments showed the drug resident time and the total resident amount increased by 4-fold and 1.2-fold for in situ gel compared with eye drops. These in vivo experimental results, along with the rheological properties and in vitro drug release studies, demonstrated that in situ gels containing Pluronic-g-PAA copolymer may significantly prolong the drug resident time and thus improve bioavailability. The results showed that the Pluronic-g-PAA copolymer can be a promising in situ gelling vehicle for ophthalmic drug delivery.

Keywords temperature-responsive in situ gel; rheology; Pluronic-g-poly(acrylic acid) copolymers; ophthalmic drug delivery

INTRODUCTION

Temperature-responsive in situ gels refer to polymer solutions that can be administrated as liquid and undergo a phase transition to semisolid gel at body temperature. Due to its unique thermo-reversible gelation properties, Pluronic F127 became one of the most extensively investigated temperature-responsive materials (Bochot, Fattal, Gulik, Couarraze, & Couvreur, 1998; Desai & Blanchard, 1998). Aqueous solution of Pluronic F127 at a concentration greater than 18% formed non-chemically cross-linked hydrogel upon warming to ambient temperature. The phase transition temperature strongly depended on Pluronic F127 concentration (Edsman, Carlfors, & Petersson, 1998). Such concentration of Pluronic F127 solution had a lower phase transition temperature (< 25°C) at which Pluronic F127 solution would become gel at room temperature and became difficult to instill into the eye. Otherwise, such concentration of Pluronic F127 solution damaged the cornea (Vadnere, Amidon, Lindenbaum, & Haslam, 1984). To develop a temperature-responsive gel with suitable phase transition temperature for ophthalmic drug delivery, Wei and colleagues incorporated Pluronic F68 into Pluronic F127 solutions in order to modulate the phase transition temperature (Wei, Xu, Ding, Li, & Zheng et al., 2002). The phase transition temperature of the Pluronic mixture solutions were lower than of the individual Pluronic F127 solutions, but the concentration of Pluronic F127 (21%) in the mixture was not decreased. Carbopol was a polyacrylic acid (PAA) polymer, which showed pH-responsive properties (Davies, Farr, Hadgraft, & Kellaway, 1991). The mixture of 0.3% carbopol and 14% Pluronic F127 solutions had a suitable phase transition temperature (Lin & Sung, 2000). Mixing with PAA was more effective than with Pluronic analogs in decreasing Pluronic F127 concentration in the mixture. Those studies suggested a copolymer containing Pluronic and PAA synthesized by chemical modification might a feasible way to decrease the concentration of Pluronic F127.

Recently, various temperature-responsive materials emerged, such as chitosan-β-glycerophosphate copolymers (Ruel-Gariepy, Leclair, Hildgen, Gupta, & Leroux, 2002), poloxamer-g-hyaluronic acid copolymers (Cho et al., 2003), and PLGA-PEG-PLGA copolymers (Qiao, Chen, Ma, & Liu, 2005). Most of the applications of such materials were concerned with injection route for convenient administration and favorable local residence period. Pluronic-g-poly(acrylic acid) copolymers were investigated as novel temperature- and pH-responsive materials (Bromberg, 2001b, 2001c). These copolymers had a unique

Address correspondence to Wei-san Pan, Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, No. 103, Wen hua Road, Shenyang, Liaoning, PC 110016, PR China. E-mail: ppwwss@163.com.

graft-comb-like structure whereby PAAs were bonded to the polyether chains (primarily polypropylene oxide [PPO] segments with tertiary carbons) via C-C bonding. Because of the prominent ability of the polypropylene oxide segments to aggregate in response to temperature increase, Pluronic-g-PAA copolymer solutions exhibited reversible sol-gel transitions and presented in the form of viscoelastic gels at body temperature. When such formulation was injected into abdominal cavity or sprayed as a liquid onto the mucosal surface, it formed gels quickly. The gelation lowered the rate of diffusion and erosion of both the polymer and the entrapped drug, thereby enhancing the drug retention and bioavailability. Pluronic-g-PAA copolymers were investigated for nasal (Bromberg, 2001a), and esophageal drug delivery (Bromberg & Ron, 1998). But to date, few efforts on the application of Pluronic-g-PAA copolymers as ophthalmic temperature-responsive in situ gels were available.

An optimum ophthalmic temperature-responsive gel should have a phase transition temperature higher than room temperature (25°C) and form gel at precorneal temperature (35°C) even after diluted by tear fluid at relatively low concentration (< 5%, w/v).

In our present work, Pluronic-g-PAA copolymers were studied as in situ gelling vehicle for ophthalmic drug delivery. The rheological behaviors and in vitro drug release properties of such copolymer gels were evaluated. The resident properties of such in situ gel formulation containing vitamin B12 in the conjunctival sacs of rabbits were investigated.

MATERIALS AND METHODS

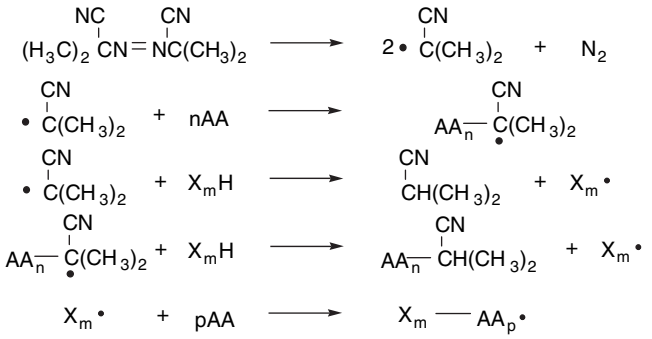
Materials

Pluronic F127 was kindly donated by BASF Co. and used without further purification. Acrylic acid (99%, monomer), dodecane (99%) and 2,2′-azobisisobutyronitrile (98%) (AIBN) were purchased from Aldrich Chemical Co. Poly(vinylpyrrolidone-co-1-hexadecane) (Antaron® V-216) (dispersion stabilizer) was obtained from International Specialty Products Co. Vitamin B12 (VB12) was purchased from Shijiazhuang Huarong Pharmaceutical Ltd. Co. (Shijiazhuang, China). All other chemicals were of reagent grade.

Synthesis and Characterization of Copolymers

Pluronic-g-PAA copolymers were synthesized by dispersion/emulsion polymerization of acrylic acid along with simultaneous grafting of poly(acrylic acid) onto Pluronic F127 backbone according to literature (Bromberg, 1998a, 1998b). In our experiments, 2,2′-azobisisobutyronitrile, instead of the initiator system composed of lauroyl peroxide and 2,2′-azobis (2,4-dimethylpentanenitrile), was used as the initiator. Synthetic scheme of Pluronic-g-PAA copolymers is shown in Figure 1.

Infrared spectra of the resulted copolymers dispersed in KBr were recorded on a Bruker FTIR Spectrometer (IFS-55, Bruker, Switzerland). The molecular weights of copolymers were determined relative to polystyrene standards by gel permeation chromatography (GPC) (Waters 410 GPC system, Waters, USA), using tetrahydrofuran as solvent. ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectra of the copolymers were obtained in CD_3OD using an NMR instrument (ARX-300, Bruker, Switzerland).



XmH is Pluronic F127 and AA is the acrylic acid monomer.

FIGURE 1. Schematic drawing of mechanism of dispersion/emulsion polymerization.

Preparation of Copolymer Solutions

Aqueous solutions of Pluronic-g-PAA copolymer (4.0%, 6.0%, 8.0%, w/v) were prepared by dispersing the copolymer in deionized water with gentle stirring at 4°C for 48 hours. The pH of the solution was then adjusted to 7.0 ± 0.1 with 1 mol·L^{-1} NaOH. Copolymer solutions containing 0.2% model drug were prepared by dissolving accurately weighed VB12 in the copolymer aqueous solutions under magnetic stirring until a homogeneous solution was obtained. All the samples were stored at 4°C until further use.

Rheological Measurements

The rheological measurements were performed with dynamic oscillation mode, on a controlled-rate rheometer (Physica MCR 300, Paar Physica, Germany) equipped with a thermostatic bath (Viscotherm VT2, Paar Physica, Germany). The measuring system was a CC17 concentric cylinder. The surface of the sample was covered by silicone oil to prevent the evaporation of water. In oscillatory shear measurements, strain amplitude was set at 0.1% so as not to destroy the three-dimensional network of the gels. The measurements of elasticity modulus G′ (storage modulus) and viscosity modulus G″ (loss modulus) were carried out over a frequency sweep between 0.1 Hz and 10 Hz at 25°C and 35°C, respectively. Complex viscosity η^*, defined as complex modulus G* divided by angular frequency (ω), was determined under the same conditions. The phase transition temperature of copolymers was performed at a fixed frequency of 0.1 Hz, and temperature range of 20~50°C. The samples were heated at a rate of 0.5°C·min^{-1}. The gelation temperature (T_{gel}) was defined as the corresponding temperature at maximal value in the differential rheological curve ($dG'/dT \sim T$, as illustrated in Figure 5).

In Vitro Release Studies

In order to study the gel erosion and drug release behavior of Pluronic-g-PAA copolymers, the in vitro release tests were performed using a flow-through cell. The design of the cell was derived from a release cell for ointments described by Loth and Holla-Benninger (1978). In our experiments, a modified device (Tardi, Brandl, & Schubert, 1998) with only an acceptor compartment and a straight channel was used for the release tests (see Figure 2).

The in vitro release apparatus consisted of an upper part and a lower part. The upper part was a 50-mm long channel with a semicircular cross section of 2.5-mm radius. Two pipelines on both sides of the channel were determined as the entrance of the release medium and the exit leading to the graduated collecting tube, by the direction of the arrow mark in the schematic drawing. The lower part was also a 50-mm long channel with rectangular cross section of 5×5 mm in order to ensure a constant contact area of 250 mm^2 between the release medium and the gels, which should not vary much even if upper layers of the gels disappear via erosion. After dealing with the gels added into the lower part to a plane, the two parts were fixed together. Then the gels were continuously rinsed with simulated tear fluid (STF) at a flow rate of 1 $ml \cdot min^{-1}$ from the entrance in order to simulate the eye blinking. The simulated tear fluid (1,000 ml) was composed of NaCl 0.67 g, $NaHCO_3$ 0.20 g, $CaCl_2 \cdot 2H_2O$ 0.008 g, and deionized water to 100 g. The volume of the solution in the graduated tube was read every 10 minutes. Then the solution was transferred to a 15-ml pyxis. The concentration of VB12 was analyzed by UV spectrophotometer (UV-2201, Shimadzu, Japan). The amount of copolymer dissolved was measured gravimetrically after freeze-drying of certain volume solutions. All experiments were done in triplicate.

In Vivo Resident Evaluation

New Zealand albino rabbits were used in the resident properties evaluation experiments. Rabbits of either sex, free of gross ocular defects and weighing 2.5 to 3 kg, were positioned into restraining boxes. 50 μL in situ gels or commercially available eye drops (Huabei Weikeda Pharmaceutical Ltd. Co., Shijiazhuang, China), both containing 0.2% VB12, were dosed by a microinjector. In order to avoid experimental bias, the control formulation (eye drops) was administered into the left eye of each rabbit, and in situ gel to the right eye. At certain time intervals, the tear fluid was absorbed with quantitative filter paper (8 mm in diameter) for 1 minute. The filter paper was then put in a 1.5-ml centrifugal tube, diluted with 0.5 ml STF, vortexed for 3 minutes and centrifuged at 4,000 rpm for 10 minutes. To determine the concentration of VB12, 20 μL of supernatant was injected into the HPLC system.

The mobile phase was a filtered and degassed mixture of acetonitrile and 0.5 M kalium dihydrogen phosphate solution (15:85) and adjusted with phosphoric acid to a pH of 3.0. The liquid chromatograph was equipped with a 361-nm detector and a 4.6-mm × 200-mm column that contained packing C_{18}. The flow rate was 1 ml per minute.

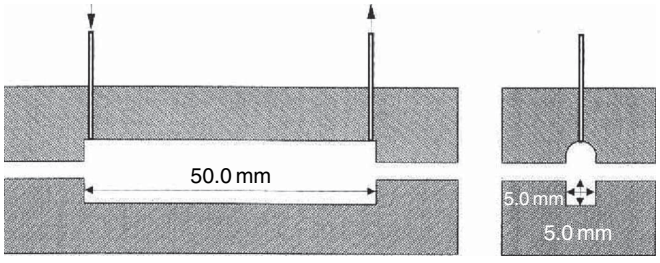


FIGURE 2. Schematic drawing of the release cell: left, longitudinal section; right, cross section (Tardi et al., 1998).

RESULTS AND DISCUSSION

Characterization of Copolymers

The FTIR spectra of Pluronic F127, PAA, and Pluronic-g-PAA copolymer are shown in Figure 3. The spectrum of Pluronic-g-PAA copolymer showed two characteristic

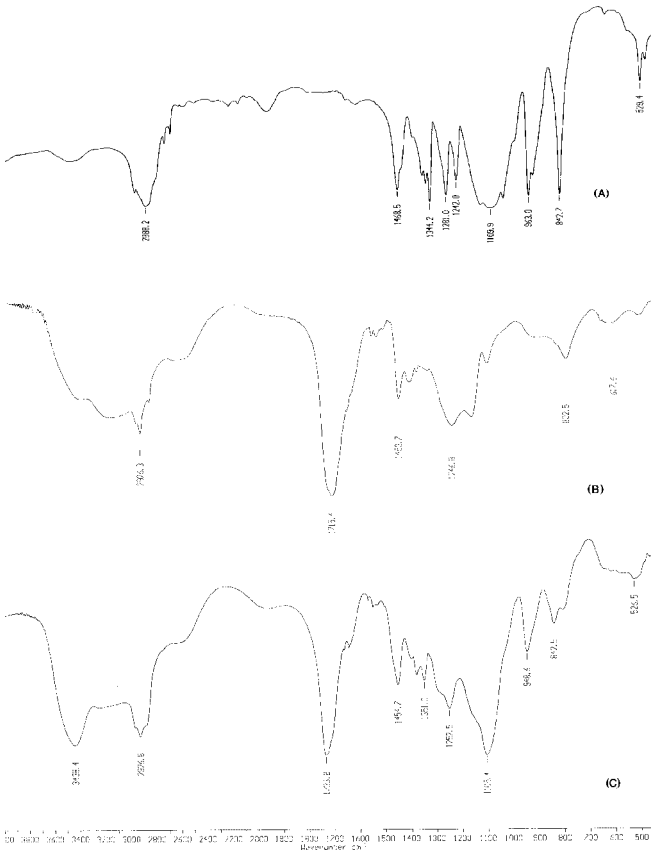


FIGURE 3. FTIR spectra of Pluronic F127 (A), PAA (B), and Pluronic-g-PAA copolymer (C).

absorption bands at about 1,733 (stretch vibration of C=O in PAA segments) and 1,105 cm^{-1} (stretch vibration of C-O-C in Pluronic F127 segments), whereas the spectrum of Pluronic F127 and PAA only showed stretch vibration band of C-O-C (1,100 cm^{-1}) and stretch vibration band of C=O (1,715 cm^{-1}), respectively. These spectral features suggest the presence of -COOH and C-O-C groups in the copolymer molecules.

^{1}H-NMR spectrum of Pluronic-g-poly(acrylic acid) copolymer is shown Figure 2. The characteristic chemical shifts at 2.4 ppm, 1.2 ppm, and 1.0 ppm are assigned to the methine hydrogen of the acrylic acid units, the methyl hydrogen of Pluronic segments grafted on poly(acrylic acid), and the methyl hydrogen of free Pluronic units, respectively. Because the ^{1}H-NMR signals of each monomer residue are distinguished and well resolved, the molar ratio of acrylic acid/Pluronic of the copolymer can be calculated through the following equation:

$$\text{Molar ratio of acrylic acid/Pluronic} = 3x/y$$

Here, x is the peak area at $\delta = 2.4$ ppm; y is the peak area summation at $\delta = 1.2$ ppm, and $\delta = 1.0$ ppm.

The GPC analysis results demonstrated that Pluronic-g-poly(acrylic acid) copolymer with narrow molecular weight distribution ($M_w/M_n < 1.2$) was obtained (see Table 1). The copolymers with acrylic acid/Pluronic molar ratios of 1.07, 1.79, and 3.12 were denoted as copolymer 1, copolymer 2, and copolymer 3, respectively.

Rheology Properties

Rheological experiments are frequently used for investigating the stress response of the gel subjected to a sinusoidally varying strain, providing information on the viscoelastic hydrodynamic properties.

The rheograms of various copolymers are shown in Figure 5A. The phase transition process of the copolymer solutions exhibited exponential increase of elasticity modulus with temperature. The copolymer solutions were free-flowing liquid below 25°C and converted to gels at relatively higher temperature. The complex viscosity of each copolymer increased with the increase in temperature. The complex viscosity at the same temperature increased with the increase of acrylic acid/Pluronic molar ratio (see Table 2).

The typical differential curves of the rheogram are shown in Figure 5B. The gelation temperature of copolymer aqueous solution decreased with the increase of the molar ratio of acrylic acid/Pluronic (the gelation temperature of 4.0% [w/v] aqueous solution of copolymer 1, copolymer 2, and copolymer 3 were 36.9, 35.1, and 32.6°C, respectively).

Figure 6 showed that the gelation temperatures of 4.0%, 6.0%, and 8.0% (w/v) copolymer 2 solution were 35.1, 35.6, and 35.6°C, respectively. The results showed that copolymer concentration had no obvious effect on the gelation temperature. The complex viscosity of each concentration increased with the increase of temperature. The complex viscosity at the same temperature increased with the increase of concentration (see Table 3).

In rheological terms, a gel is defined as a preparation that shows frequency-independent elasticity modulus G′ and

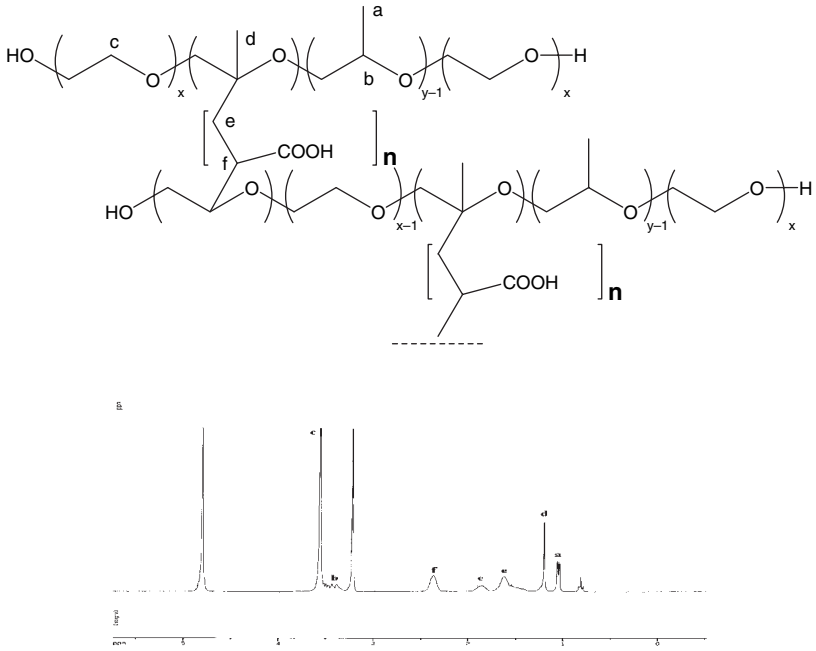


FIGURE 4. ^{1}H-NMR spectrum (300 Hz, CD_3OD) of Pluronic-g-PAA copolymer.

TABLE 1
The Molecular Weights, Compositions, and Polydispersity Indexes of the Copolymers

Copolymer	Molecular Weight M_w	Molecular Weight M_n	AA/Pluronic Molar Ratio[a]	Polydispersity Index
Copolymer 1	20,010	17,170	1.07/1	1.17
Copolymer 2	21,136	18,429	1.79/1	1.15
Copolymer 3	24,338	21,379	3.12/1	1.14

[a]Determined by ^{1}H-NMR.

TABLE 2
The Complex Viscosity and the Gelation Temperature of Various Copolymers

Copolymer	Complex Viscosity (Pa·s) 25°C	30°C	35°C	T_{gel} (°C)
Copolymer 1	0.09	7.92	36.3	36.9
Copolymer 2	0.11	14.6	63.0	35.1
Copolymer 3	0.53	40.8	103.0	32.6

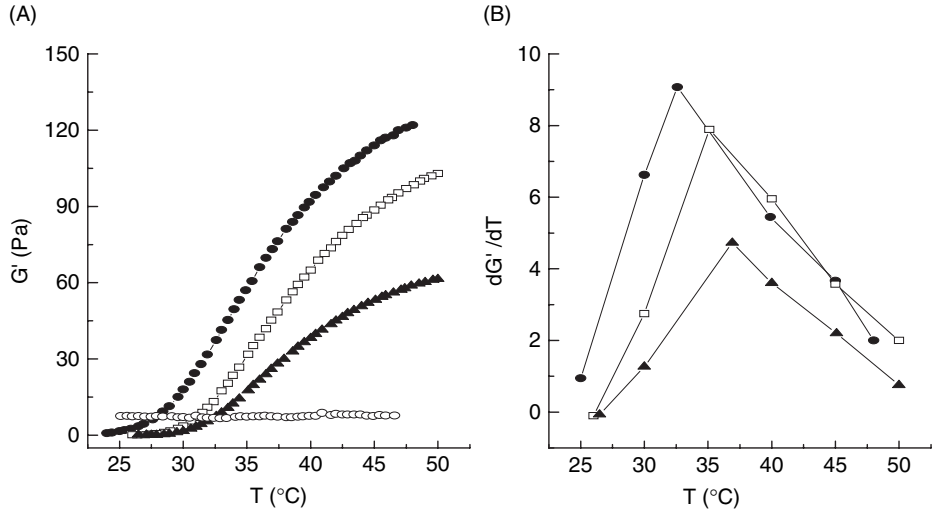


FIGURE 5. Temperature-dependent elasticity modulus (G') (A) and differential curve (B) of 4.0% (w/v) aqueous solutions of copolymer 3 (●), copolymer 2 (□), copolymer 1 (▲), and physical mixture (the molar ratio of PAA/Pluronic = 1) (○).

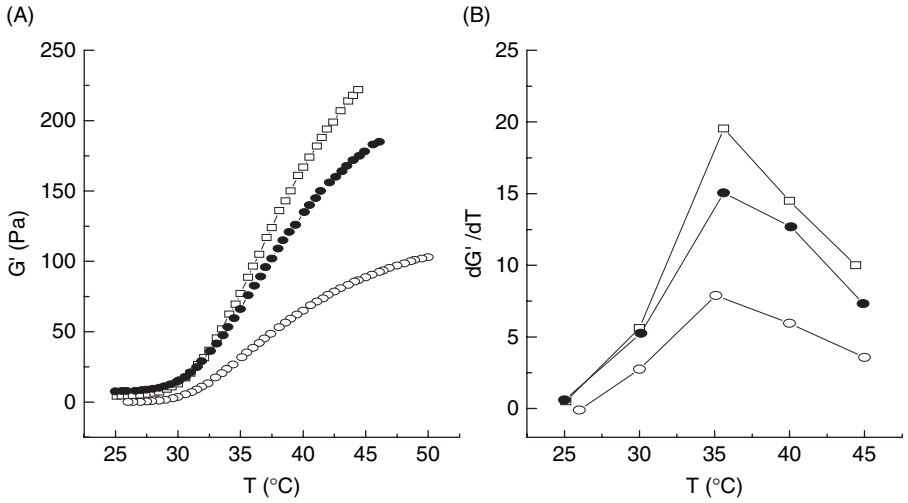


FIGURE 6. Temperature-dependent elasticity modulus (G') (A) and differential curve (B) of 8.0% (□), 6.0% (●), and 4.0% (○) (w/v) aqueous solutions of copolymer 2.

TABLE 3
The Complex Viscosity and the Gelation Temperature of Copolymer 2 at Various Concentration

Concentration (%)	Complex Viscosity (Pa·s) 25°C	30°C	35°C	T_{gel} (°C)
4	0.11	14.6	63.0	35.1
6	1.25	41.1	130.0	35.6
8	2.00	44.0	157.0	35.6

viscosity modulus G″, and low loss angle δ (tanδ = G″/G′) at all frequencies. This is in contrast to viscous polymer solutions, which show frequency-dependent G′ and G″ and the loss angle shifts when frequency increases (Edsman, Carlfors, Harju, 1996). At 25°C, both the copolymer solutions and the physical mixture solution behaved as free-flowing viscoelastic solutions (Figure 7A, B). At 35°C, the copolymer solutions exhibited typical rheological behavior for a gel (consistently low loss angles during a frequency sweep), whereas the physical mixture solution still behaved as free-flowing viscoelastic solutions (Figure 7C, D).

In Vitro Release Studies

The drug release from gel formulations was normally investigated with dissolution apparatus (Moore, Croy, Mallapragada, & Pandit, 2000). This method might interpret the release behavior of the drugs from the gels, but not be suitable for studying the release behavior and gel erosion of in situ gels for ophthalmic drug delivery. Because of the special configuration in the eyes, the ophthalmic gels will be continuously rinsed with tear fluid. In order to simulate lacrimal secretion and nasolacrimal drainage, and completely understand the release behavior of such an in situ gelling vehicle, the flow-through cells were used in our experiments.

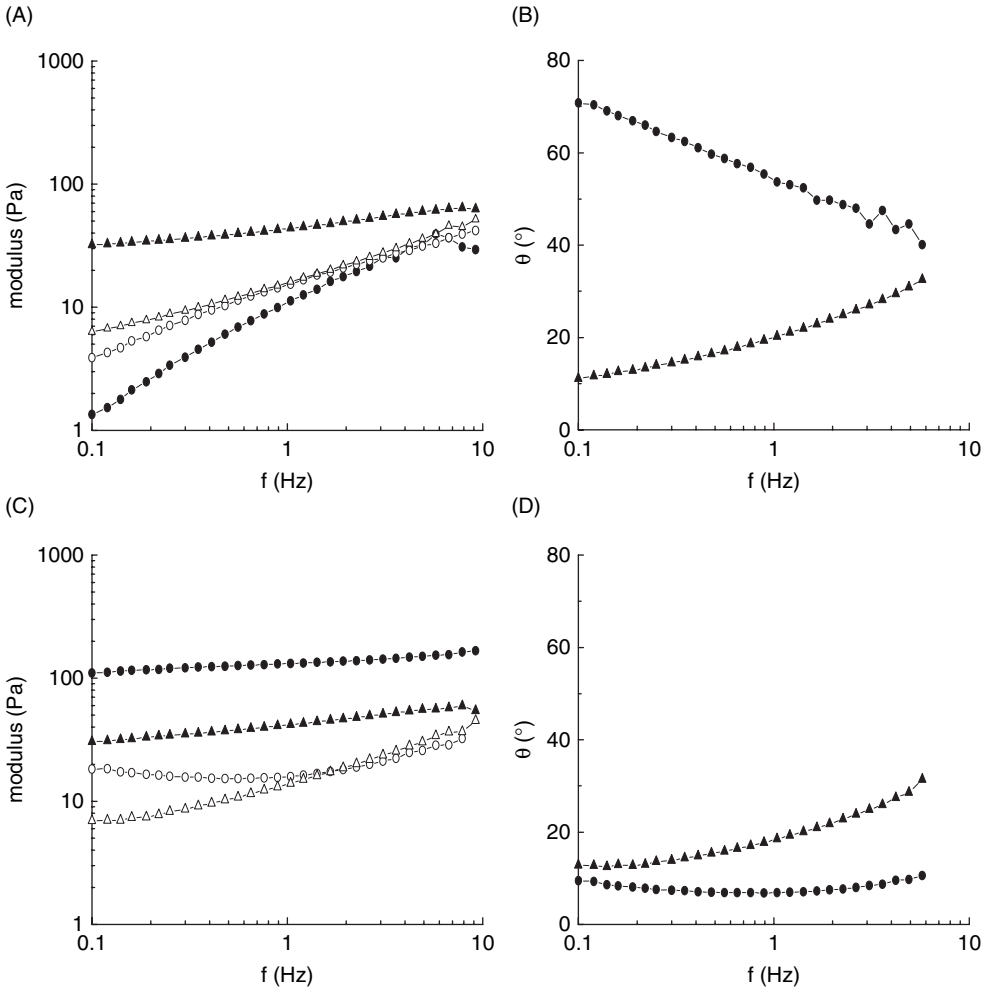


FIGURE 7. Rheological properties of aqueous solutions of copolymer (●) and physical mixture (▲) as a function of frequency at different temperature. (A) Modulus (elasticity modulus, G′, filled symbols; viscosity modulus, G″, unfilled symbols), 25°C. (B) Loss angle, 25°C. (C) Modulus (elasticity modulus, G′, filled symbols; viscosity modulus, G″, unfilled symbols), 35°C. (D) Loss angle, 35°C.

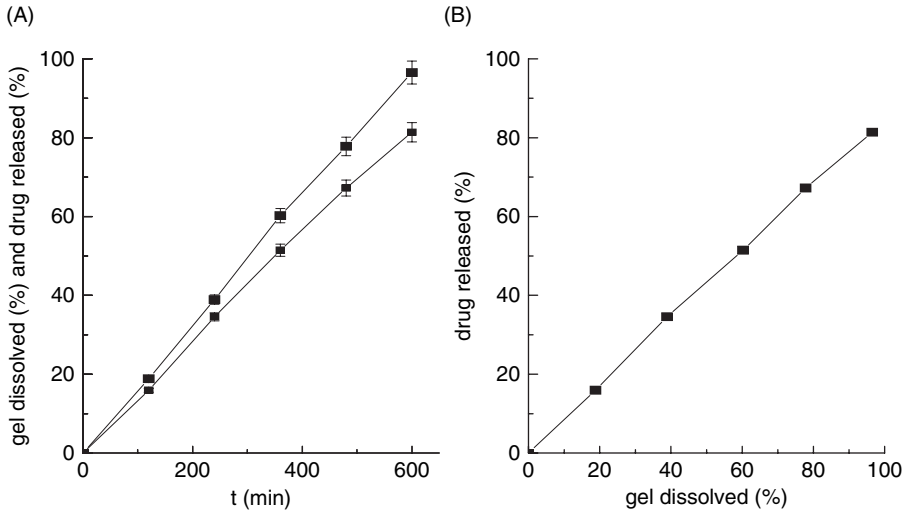


FIGURE 8. (A) Gel dissolution (■) and drug release (●) profiles of 0.2% VB12 in situ gels containing 4.0% (w/v) copolymer 3. (B) Plot of percent of drug released versus percent of gel dissolved.

Figure 8A is a typical plot showing the drug release and gel dissolution versus time. Both the drug release and gel dissolution profiles were linear until about 90% of the gel dissolved. After this point, the surface area and the dissolution rate of the gels changed due to the gel surface exposed to release medium became irregular. Figure 8B illustrated the correlation between drug release and gel dissolution. The results indicated gel dissolution controlled drug release property. Bhardwaj and Blanchard (1996) observed similar phenomenon in the release of melanotan-I from 25% F127 gels.

The zero-ordered release properties were observed under various experimental factors, such as drug concentration, copolymer concentration, the molar ratio of acrylic acid/Pluronic, and the flow rate of the release medium, as shown in Figure 9 and Table 4.

It was apparent that the drug release rates increased as flow rate increased. It was obvious that the drug release rates decreased as acrylic acid/Pluronic molar ratio increased. In addition, the data of GPC and the rheology also indicated that the weight-average molecular mass and the complex viscosity of the copolymers increased as acrylic acid/Pluronic molar ratio increased. The drug release behavior was correlated with the weight-average molecular mass and the complex viscosity of the copolymers. The results showed that the drug release rates decreased as copolymer solution concentration increased. Similar observations had been reported for Pluronic gel formulation (Bhardwaj & Blanchard, 1996). An explanation for this behavior was the increased number of micelles at higher copolymer solution concentrations, resulting in a more entangled system and more rigid gel. It was shown that drug concentration had no obvious effect on drug release. The release rates of VB12 from such copolymer gels were mainly dependent on the gel dissolution.

In Vivo Resident Experiments

For in vivo resident experiment, copolymer 3, which showed relatively low gelation temperature and higher gel strength, was applied. Figure 8 shows the level of VB12 in the conjunctival sacs of rabbits after instillation of 0.2% VB12 eye drops and 0.2% VB12 in situ gel containing 4.0% (w/v) copolymer 3. After administration of VB12 in situ gel formulation, drug concentration in the rabbit's conjunctival sac was significantly higher than that of conventional eye drops at all time points except for the first point, especially within 60 minutes. VB12 was diffused out quickly from eye drops during the first 20 minutes and almost vanished completely from the eye sac. Compared with eye drops, the diffusion of VB12 from in situ gel was slow, the drug resident time and total resident amount of in situ gel increased by 4.0- and 1.2-fold.

CONCLUSIONS

The rheological measurements showed the Pluronic-g-PAA copolymers can form temperature-responsive gels with suitable gelation temperature at relatively lower concentration compared with Pluronic F127, which had a gelation concentration of about 18% (w/v). The zero-ordered release properties were observed under various experimental factors, such as drug concentration, copolymer concentration, the molar ratio of acrylic acid/Pluronic, and the flow rate of the release medium. The rates of release drug from such copolymer gels were mainly dependent on the gel dissolution. In vivo experimental results, along with the rheological and in vitro drug

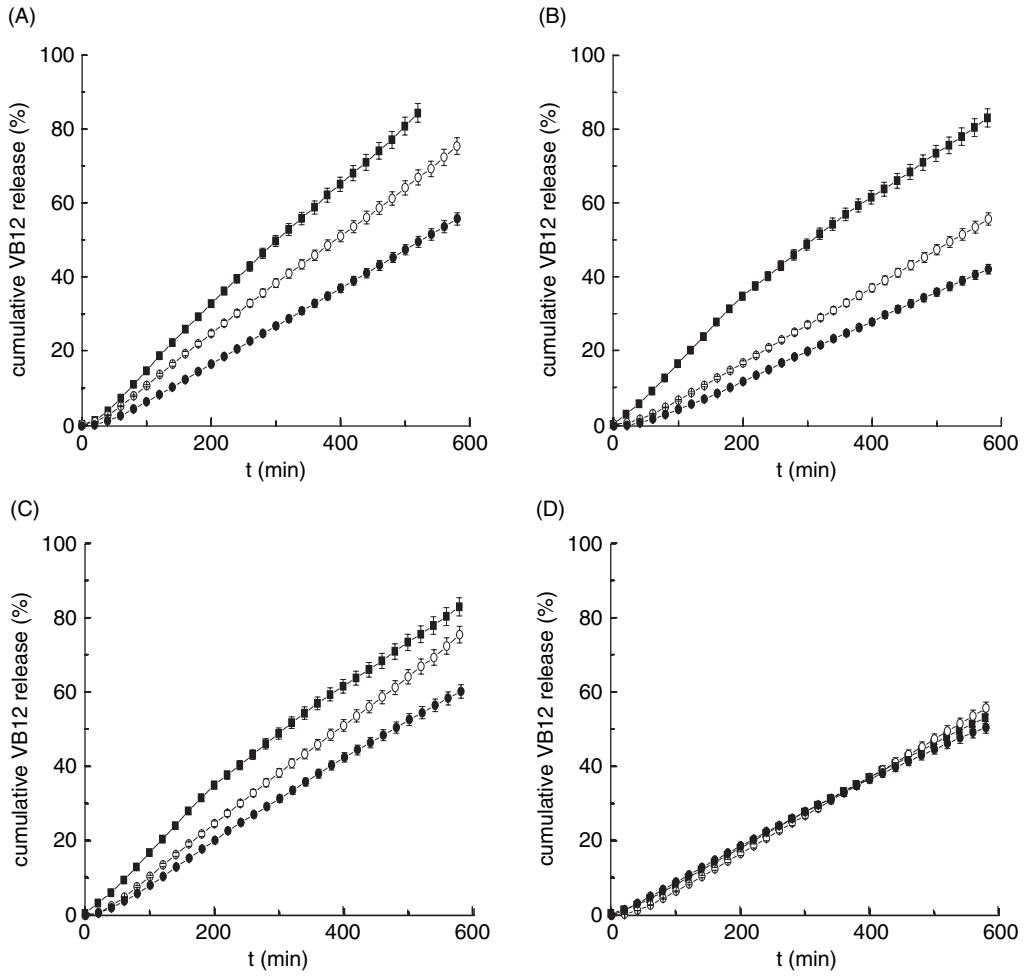


FIGURE 9. Effect of various parameters on drug release. (A) Flow rate of the release medium: 2.00 ml·min^{-1} (■), 1.50 ml·min^{-1} (○) and 1.00 ml·min^{-1} (●). (B) Acrylic acid/Pluronic molar ratio: 1.07 (■), 1.79 (○), and 3.12 (●). (C) Copolymer solution concentration: 4.0% (■), 6.0% (○), and 8.0% (●) (w/v). (D) Entrapped drug concentration: 2mg·ml^{-1} (■), 4mg·ml^{-1} (○) and 8mg·ml^{-1} (●).

TABLE 4
Kinetic Assessment of Release Data of VB12

Copolymer	Flow Rate (ml·min^{-1})	Copolymer C (%, w/v)	Drug C (mg·ml^{-1})	Equation	
				Slope	R^2
Copolymer 2	2.00	4.0	4	0.169	0.999
Copolymer 2	1.50	4.0	4	0.133	0.999
Copolymer 2	1.00	4.0	4	0.100	0.999
Copolymer 1	1.00	4.0	4	0.143	0.992
Copolymer 2	1.00	4.0	4	0.100	0.999
Copolymer 3	1.00	4.0	4	0.078	0.997
Copolymer 1	1.00	4.0	4	0.143	0.992
Copolymer 1	1.00	6.0	4	0.123	0.999
Copolymer 1	1.00	8.0	4	0.109	0.999
Copolymer 2	1.00	4.0	2	0.094	0.999
Copolymer 2	1.00	4.0	4	0.100	0.999
Copolymer 2	1.00	4.0	8	0.089	0.998

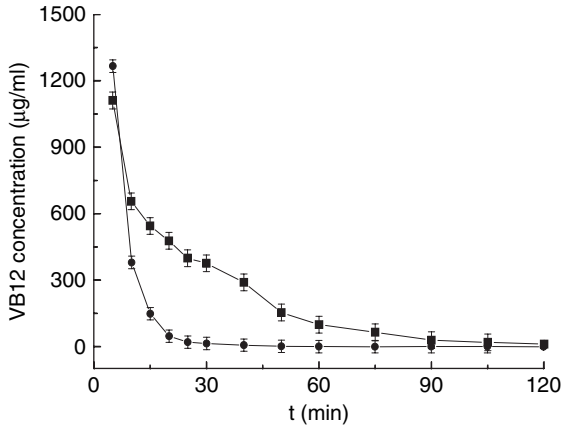


FIGURE 10. The level of VB12 in rabbit's conjunctival sac after instillation of 0.2% VB12 eye drop (●) and as in situ gel containing 4.0% (w/v) copolymer 3 (■).

release studies, demonstrated that in situ gels containing Pluronic-g-PAA copolymer may significantly prolong the precorneal resident time, and may further improve ocular drug bioavailability.

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