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Pluronic F127-*g*-poly(acrylic acid) copolymers as *in situ* gelling vehicle for ophthalmic drug delivery system

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Abstract

To prolong the precorneal resident time and improve ocular bioavailability of the drug, Pluronic F127-*g*-poly(acrylic acid) copolymers were studied as *in situ* gelling vehicle for ophthalmic drug delivery system. The rheological properties and *in vitro* drug release of Pluronic-*g*-PAA copolymer gels 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. But the drug concentration had no obvious effect on drug release. The release rates of the 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 in rabbit's conjunctiveal sac increased by 5.0 and 2.6 folds for *in situ* gel, compared with eye drops. The decreased loss angle at body temperature and prolonged precorneal resident time also indicated that the copolymer gels had bioadhesive properties. 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. Pluronic-*g*-PAA copolymer can be a promising *in situ* gelling vehicle for ophthalmic drug delivery system.

Keywords: Ophthalmic drug delivery system; Temperature-responsive *in situ* gel; Rheology; Pluronic F127-*g*-poly(acrylic acid) copolymers; Bioadhesive properties

1. Introduction

The conventional liquid ophthalmic formulation is eliminated from the precorneal area immediately upon instillation because of lacrimal secretion and nasolacrimal drainage (Makoid et al., 1976). As a result, frequent instillation of concentrated solutions is needed in order to achieve the desired therapeutic effects (Chein et al., 1982). Various ophthalmic vehicles, such as inserts, ointment, suspensions, and aqueous gels, have been developed in order to lengthen the resident time of instilled dose and enhance the ophthalmic bioavailability (Lee and Robinson, 1986). These ocular drug delivery systems, however, have not been used extensively because of some drawbacks, such as blurred vision from ointment or low patient compliance from inserts (Lee, 1990).

Several *in situ* gels forming systems have been developed to prolong the precorneal resident time of the drug and improve ocular bioavailability. *In situ* gels refer to polymer solutions which can be administrated as liquid, and undergo a phase transition to semisolid gel upon exposure to physiological environments. The gelation can be triggered by temperature change, such as Pluronic (Bochot et al., 1998; Desai and Blanchard, 1998) and ethyl(hydroxyethyl) cellulose (Lindell and Engstom, 1993), by pH change, such as cellulose acetate phthalate (Gurny et al., 1985) and carbopol (Srividya et al., 2001), or by the presence of cations, such as deacetylated gellan gum (Carfors et al., 1998) and alginate (Cohen et al., 1997).

Due to its unique thermo-reversible gelation properties, Pluronic F127 became one of the most extensively investigated temperature-responsive materials. Aqueous solution of Pluronic F127 at a concentration equal to or greater than 18% formed non-chemically crosslinked hydrogel upon warming to ambient temperature. The phase transition temperature strongly depended on Pluronic F127 concentration (Edsman et al., 1998). Such concentration of Pluronic F127 solution had a lower phase transition temperature (<25 °C) at which temperature Pluronic F127 solution would already become gels at room temperature

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and difficult to instill into the eye. Otherwise such concentration of Pluronic F127 solution made the cornea-damage (Vadnere et al., 1984). To develop a temperature-responsive gel with suitable phase transition temperature for ophthalmic drug delivery system, Wei et al. incorporated Pluronic F68 into Pluronic F127 solutions in order to modulate the phase transition temperature (Wei et al., 2002). The phase transition temperature of the Pluronics 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 poly(acrylic acid) (PAA) polymer, which showed pH-responsive properties (Davies et al., 1991). The mixture of 0.3% carbopol and 14% Pluronic F127 solutions had a suitable phase transition temperature (Lin and 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 in decreasing the concentration of Pluronic F127.

Recently, various temperature-responsive materials emerged, such as chitosan-β-glycerophosphate copolymers (Ruel-Gariepy et al., 2002), poloxamer-*g*-hyaluronic acid copolymers (Cho et al., 2003) and PLGA-PEG-PLGA copolymers (Qiao et al., 2005). Most of the applications of such materials were concerned with injection route for its convenient administration and favorable local residence period. Pluronic-*g*-poly(acrylic acid) copolymers were investigated as a novel temperature- and pH-responsive materials (Bromberg, 2001a,b). These copolymers had a unique graft-comb like structure whereby PAA were bonded to the polyether chains (primarily PPO segments with tertiary carbons) via C–C bonding. Because of the prominent ability of the polypropylene oxide (PPO) segments to aggregate in response to temperature increase, the Pluronic-*g*-PAA copolymer solutions exhibited reversible sol–gel transitions and presented in the form of viscoelastic gels at body temperature. When *in situ* gels formulation containing the Pluronic-*g*-PAA copolymer were 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. The Pluronic-*g*-PAA copolymers were investigated for nasal (Bromberg, 2001c), and oesophageal drug delivery (Bromberg and Ron, 1998). But to date, few efforts on the application of the 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, the Pluronic-*g*-PAA copolymers were studied as *in situ* gelling vehicle for ophthalmic drug delivery system. The rheological behaviors and *in vitro* drug release properties of such copolymer gels were evaluated. The resident properties of such *in situ* gels formulation containing gatifloxacin in rabbit's conjunctiveal sac were investigated.

2. Materials and methods

2.1. 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. Gatifloxacin (GTX) was purchased from Hubei Qianjiang Pharmaceutical Ltd. Co. (Hubei, China). All other chemicals were of reagent grade.

2.2. Copolymer synthesis and characterization

The 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,b). 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. Identical synthetic procedure (but without Pluronic F127) was used to synthesize poly(arylic acid) (PAA).

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 with a flow rate of 1 mL min^{-1}. ^1H nuclear magnetic resonance (^1H NMR) spectra of the copolymers were obtained in CD_3OD using a NMR instrument (ARX-300, Bruker, Switzerland).

2.3. Preparation of copolymer solutions

Aqueous solutions of the Pluronic-*g*-PAA copolymer (4.0, 6.0 and 8.0%, w/v) were prepared by dispersing the copolymer in deionized water with gentle stirring at 4 °C for 48 h. 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 GTX 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.

2.4. 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 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 and 10 Hz at 25 and 35 °C, respectively. Complex viscosity η^*, defined as complex modulus G^* divided by angular frequency (ω), were 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 Fig. 3).

2.5. In vitro release studies

In order to study the gel erosion and drug release behavior of the 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 et al., 1998), with only an acceptor compartment and a straight channel, was used for the release tests (see Scheme 1).

The *in vitro* release apparatus consisted of the upper part and the 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 collected graduated tube. The lower part was also a 50 mm long channel with rectangular cross-section of 5 mm × 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, two parts were fixed together. Then the gels were continuously rinsed with simulated tear fluid (STF) at a flow rate of 1 mL min^{-1} from the entrance, in order to simulate the eye blinking. The simulated tear fluid (1000 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 per 10 min. Then the solution was transferred to a 15 mL pyxis. The concentration of GTX 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 and carried out at 35 °C.

2.6. 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 2.5–3 kg, were positioned into restraining boxes. Fifty microliters *in situ* gels or commercially available eye drops (Anhui shuangke Pharmaceutical Ltd. Co., Anhui, China), both containing 0.2% GTX, were dosed by a microinjector. *In situ* gel was administered into the lower conjunctiveal sac, approximately midway between the inner and outer canthus at room temperature. 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 min. The filter paper was then put in a 1.5 mL centrifugal tube, diluted with 1.0 mL simulated tear fluid (STF), vortexed for 3 min and centrifuged at 4000 rpm for 10 min. Twenty microliters of supernatant was injected into the HPLC system to determine the concentration of GTX.

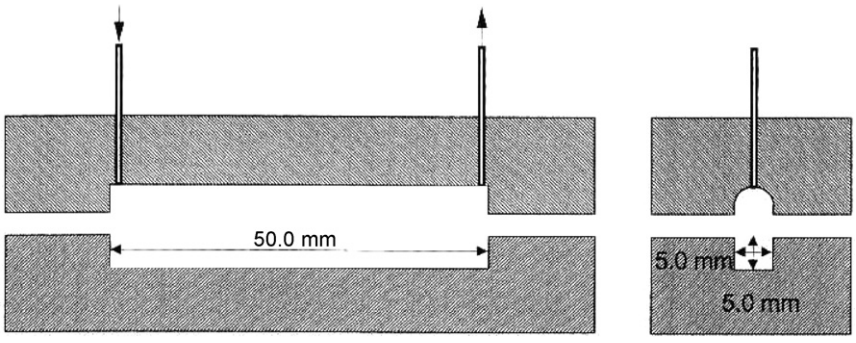
The mobile phase was a filtered and degassed mixture of acetonitrile and deionized water (24:76) and adjusted with phosphoric acid to a pH of 3.0. The liquid chromatograph was equipped with a 286 nm detector and a 4.6 mm × 200 mm column that contained packing C_{18}. The flow rate was 1 mL min^{-1}.

3. Results and discussion

3.1. Characterization of copolymers

The FTIR spectra of Pluronic F127, PAA and Pluronic-*g*-PAA copolymer are shown in Fig. 1. The spectrum of the Pluronic-*g*-PAA copolymer showed two characteristic absorption bands at about 1733 (stretch vibration of C=O in PAA segments) and 1105 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 (1100 cm^{-1}) and stretch vibration band of C=O (1715 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*-PAA copolymer is shown in Fig. 2. The characteristic chemical shifts at 2.4, 1.2, and 1.0 ppm are assigned to the methine hydrogen of the acrylic acid units, the



Scheme 1. Schematic drawing of the release cell: left, longitudinal section and right, cross-section (Tardi et al., 1998).

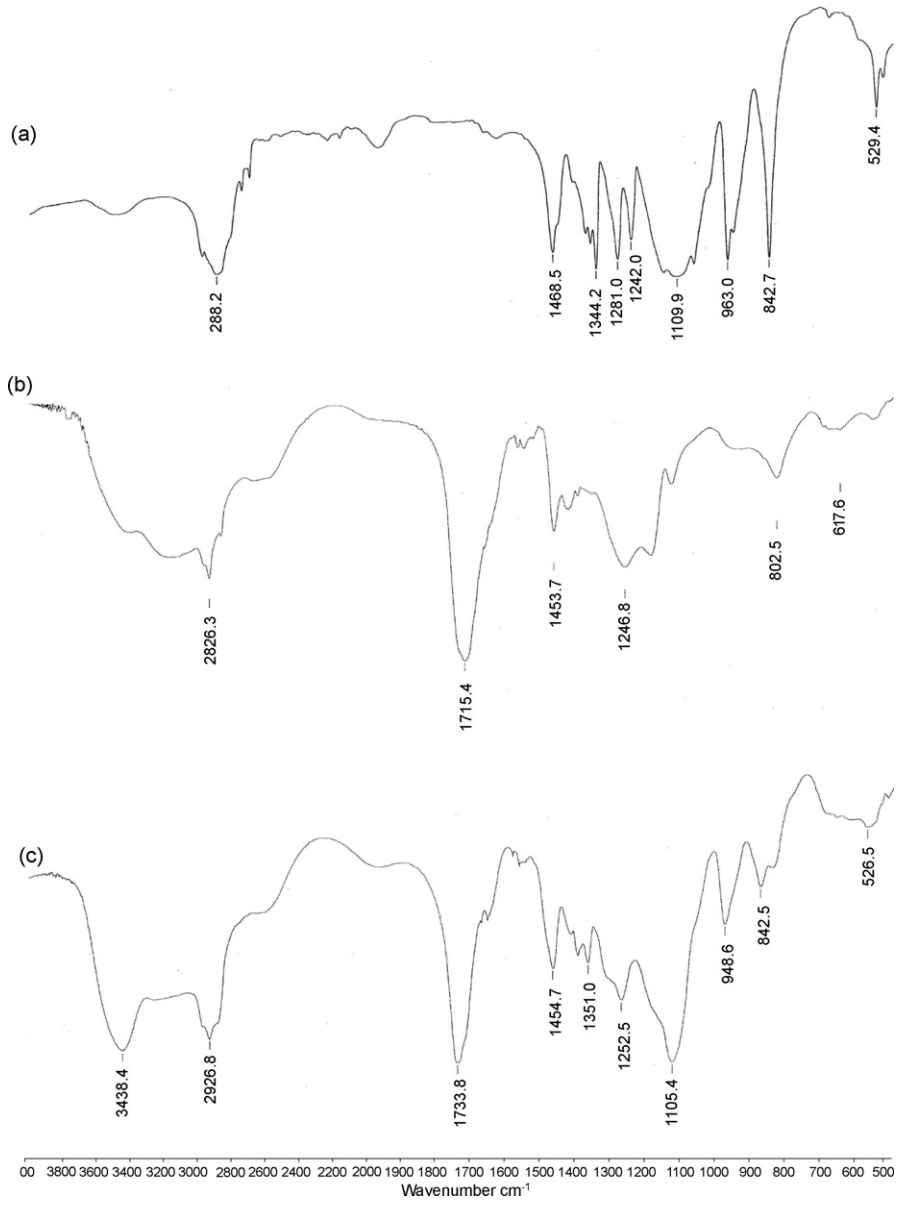


Fig. 1. FTIR spectra of Pluronic F127 (a), PAA (b) and Pluronic-*g*-PAA copolymer (c).

methyl hydrogen of Pluronic segments grafted on poly(acrylic acid), and the methyl hydrogen of free Pluronic unit, 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:

$$\frac{\text{acrylic acid}}{\text{pluronic}} = \frac{3x}{y}$$

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

The GPC analysis results demonstrated that Pluronic-*g*-PAA 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 copolymer1, copolymer2 and copolymer3, respectively.

3.2. Rheological properties

Rheological properties of the Pluronic-*g*-PAA copolymer solutions exhibited an exclusive temperature-responsive which was lacking for PAA hydrogels typically used in bioadhesive formulations. Pluronic F127 solution, PAA solutions, or their physical mixture did not show temperature-responsive gelation properties at the same concentration (4.0%, w/v). Fig. 3 illustrated the temperature-induced viscoelasticity of 4.0% (w/v) aqueous solution and differential curve of elasticity modulus of the Pluronic-*g*-PAA copolymer (copolymer2). The Pluronic-*g*-PAA aqueous systems aggregated at critical aggregation temperature (CAT) (Bromberg et al., 2004b). The transition from a viscous-dominated response at temperatures below CAT to an elastic-dominated response above CAT was indicative of the formation of the networks of aggregates. The Pluronic-*g*-PAA copolymers solution underwent a typical sol–gel transition

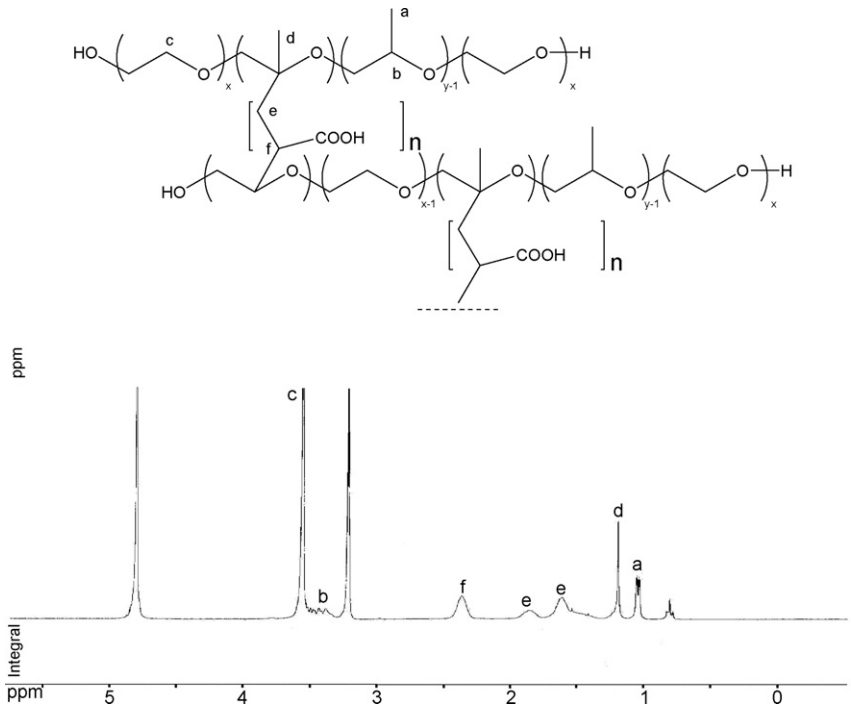


Fig. 2. ^{1}H NMR spectrum (300 Hz, CD_3OD) of Pluronic-*g*-PAA copolymer.

Table 1
The molecular weights, compositions and polydispersity indexes of the copolymers

Copolymer	Molecular weight		AA/Pluronic molar ratio[a]	Polydispersity index
	M_w	M_n		
Copolymer1	20,010	17,170	1.07/1	1.17
Copolymer2	21,136	18,429	1.79/1	1.15
Copolymer3	24,338	21,379	3.12/1	1.14

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

from a region with $G' < G''$ below CAT to a $G' > G''$ region above CAT. The transition point was characterized by a specific transition temperature $T = 33.4\,^{\circ}C$ measured at $G' = G''$. In our experiments, the gelation temperature (T_{gel}) was defined as the corresponding temperature at maximal value in the differential rheological curve. The gelation temperature of the Pluronic-*g*-PAA copolymer aqueous solution (copolymer2) was 35.1 °C.

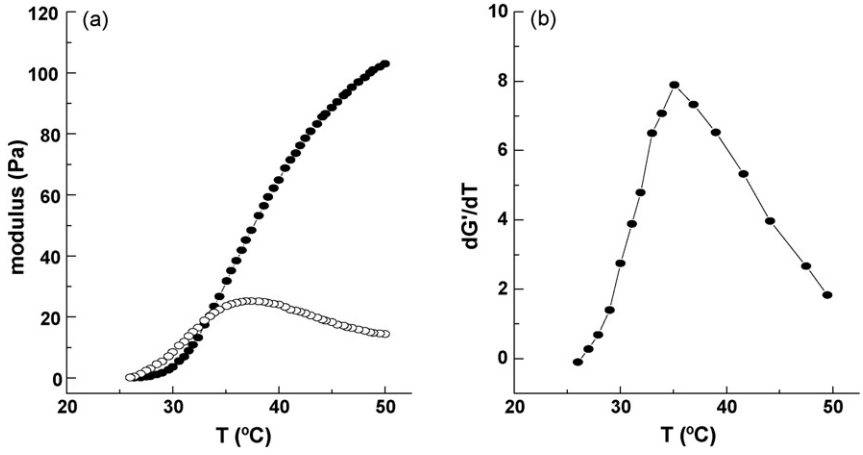


Fig. 3. (a) Temperature-dependent elasticity modulus (G', ●) and viscosity modulus (G'', ○) of 4.0% (w/v) aqueous solution of the Pluronic-*g*-PAA copolymer. (b) Differential curve of elasticity modulus of the Pluronic-*g*-PAA copolymer (copolymer2).

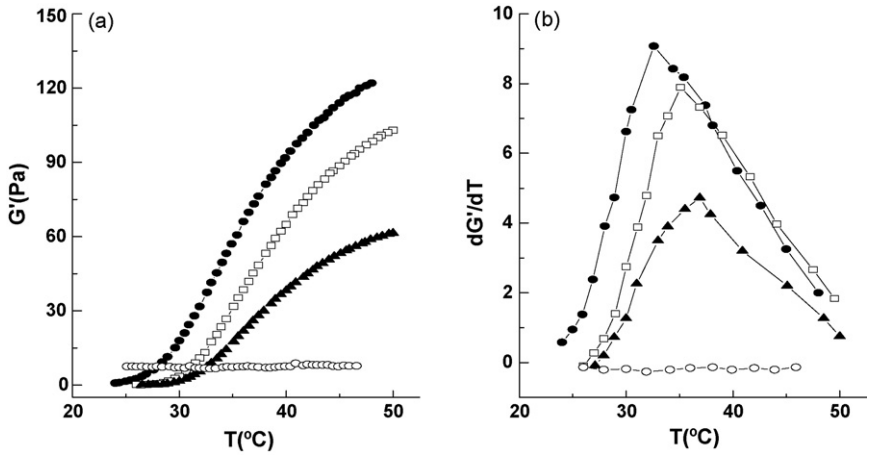


Fig. 4. Temperature-dependent elasticity modulus (G') (a) and differential curve (b) of 4.0% (w/v) aqueous solutions of copolymer3 (●), copolymer2 (□), copolymer1 (▲), and physical mixture (the molar ratio of PAA/Pluronic = 1) (◯).

Table 2
The complex viscosity and the gelation temperature of various copolymers

Copolymer	Complex viscosity (Pa s)			T_{gel} (°C)
	25 °C	30 °C	35 °C	
Copolymer1	0.09	7.9	36.3	36.9
Copolymer2	0.11	14.6	63.0	35.1
Copolymer3	0.53	40.8	103.0	32.6

Table 3
The complex viscosity and the gelation temperature of copolymer2 at various concentration

Concentration (%)	Complex viscosity (Pa s)			T_{gel} (°C)
	25 °C	30 °C	35 °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

Rheological experiments were 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 Fig. 4(a). The phase transition process of the copolymer solutions exhibited exponential increase of elasticity modulus with temperature. While 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 of 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 elasticity modulus are shown in Fig. 4(b). 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 copolymer1, copolymer2 and copolymer3 were 36.9, 35.1 and 32.6 °C, respectively).

Fig. 5 shows that the gelation temperature of 4.0, 6.0 and 8.0% (w/v) copolymer2 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).

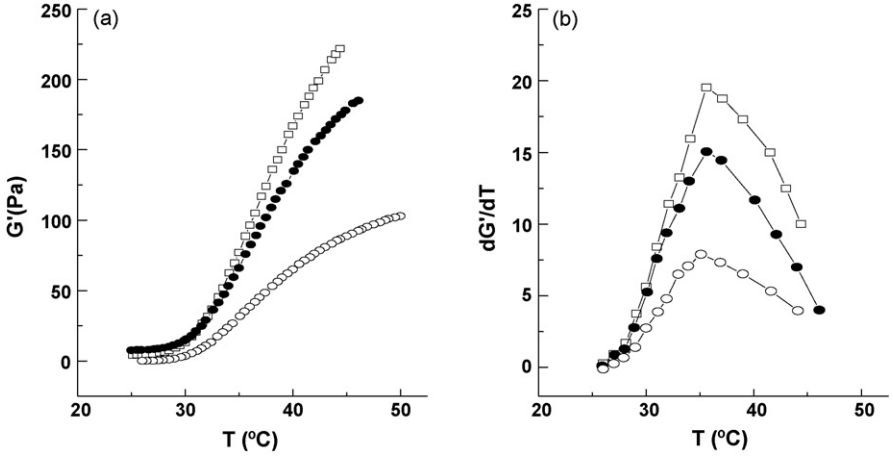


Fig. 5. Temperature-dependent elasticity modulus (G') (a) and differential curve (b) of 8.0% (□), 6.0% (●) and 4.0% (◯) (w/v) aqueous solutions of copolymer2.

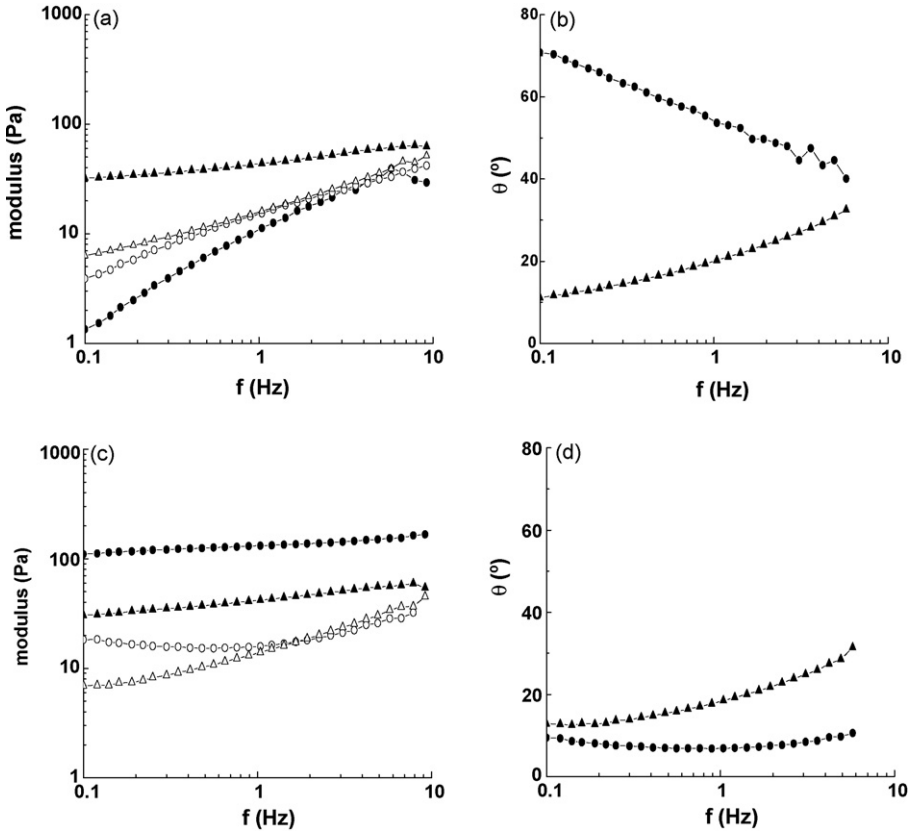


Fig. 6. Rheological properties of aqueous solutions of copolymer (●) and physical mixture (▲) as a function of frequency at different temperature. (a) Modulus (elasticity modulus, G', filled symbols; viscosity modulus, G'', unfilled symbols), 25 °C. (b) Loss angle, 25 °C. (c) Modulus (elasticity modulus, G', filled symbols; viscosity modulus, G'', unfilled symbols), 35 °C. (d) Loss angle, 35 °C.

In rheological terms, a gel is defined as a preparation which shows frequency independent elasticity modulus G' and viscosity modulus G'', and low loss angle δ ($\tan\delta = G''/G'$) 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 et al., 1996). At 25 °C, both the copolymer solutions and the physical mixture solution behaved as viscoelastic solutions (Fig. 6(a) and (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 viscoelastic solutions (Fig. 6(c) and (d)).

It should be noted that a linear correlation between the loss angle and the mucoadhesion work of various hydrogels had been reported previously, and was believed to be a link between the intrinsic properties of the gel and mucus-gel interface (Tamburic and Craig, 1995). It was observed that the energy stored in an elastic material ($\delta \rightarrow 0^\circ$) necessitates a higher work for the fracture to occur than in a Newtonian dampening, viscous liquid ($\delta \rightarrow 90^\circ$) (Bromberg et al., 2004a). In Fig. 6(b) and (d), the loss angle of the copolymer solutions decreased from 70° to 7° when temperature increased from 25 to 35 °C. The copolymer solutions may have potential bioadhesive properties at body temperature.

The rheological results showed that the copolymer solutions had both temperature-sensitivity and bioadhesive properties, which may be a promising *in situ* gelling vehicle for ophthalmic drug delivery system. Further studies were carried out on *in vitro* drug release/gel erosion and *in vivo* resident properties.

3.3. In vitro release studies

The drug release from gel formulations was normally investigated with dissolution apparatus (Moore et al., 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 *in situ* gelling vehicle, the flow-through cells were used in our experiments.

Fig. 7(a) 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. Fig. 7(b) illustrates the correlation between drug release and gel dissolution. The results indicated gel dissolution controlled drug release property. Bhardwaj et al. observed similar phenomenon in the release of melanotan-I from 25% F127 gels (Bhardwaj and Blanchard, 1996).

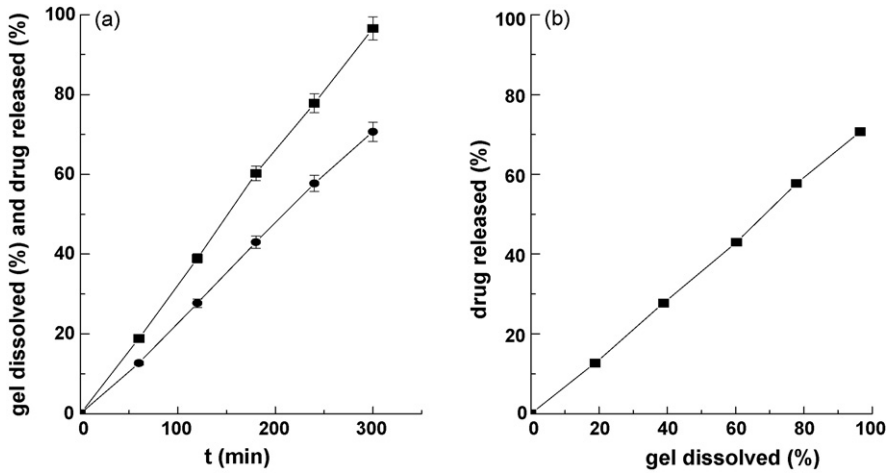


Fig. 7. (a) Gel dissolution (■) and drug release (●) profiles of 0.2% gatifloxacin *in situ* gels containing 4.0% (w/v) copolymer3. (b) Plot of percent of drug released vs. percent of gel dissolved.

The zero-ordered release properties were observed under various experimental factors, such as the flow rate of the release medium, the molar ratio of acrylic acid/Pluronic, copolymer concentration and drug concentration, as shown in Fig. 8 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

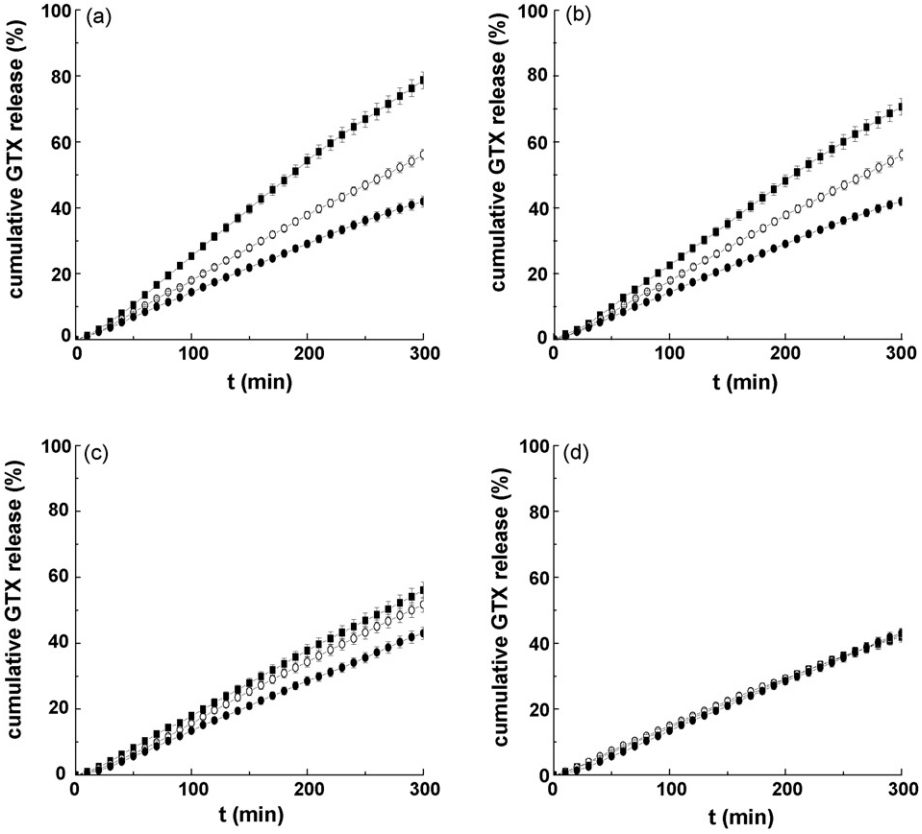


Fig. 8. Effect of various parameters on drug release. (a) Flow rate of the release medium: 2.00 mL min^{-1} (■), 1.00 mL min^{-1} (◯) and 0.50 mL min^{-1} (●). (b) Acrylic acid/Pluronic molar ratio: 1.07 (■), 1.79 (◯) and 3.12 (●). (c) Copolymer solution concentration: 4.0% (■), 6.0% (◯) and 8.0% (●) (w/v). (d) Entrapped drug concentration: 2.0 mg mL^{-1} (■), 1.0 mg mL^{-1} (◯) and 0.5 mg mL^{-1} (●).

Table 4
Kinetic assessment of release data of GTX

Copolymer	Flow rate (mL min^{-1})	Copolymer C (%, w/v)	Drug C (mg mL^{-1})	Equation	
				Slope	R^2
Copolymer2	2.00	4.0	2.0	0.274	0.999
Copolymer2	1.00	4.0	2.0	0.192	0.999
Copolymer2	0.50	4.0	2.0	0.144	0.999
Copolymer1	1.00	4.0	2.0	0.245	0.999
Copolymer2	1.00	4.0	2.0	0.192	0.999
Copolymer3	1.00	4.0	2.0	0.143	0.999
Copolymer2	1.00	4.0	2.0	0.192	0.999
Copolymer2	1.00	6.0	2.0	0.180	0.999
Copolymer2	1.00	8.0	2.0	0.148	0.999
Copolymer3	1.00	4.0	2.0	0.143	0.999
Copolymer3	1.00	4.0	1.0	0.143	0.999
Copolymer3	1.00	4.0	0.5	0.144	0.999

(Bhardwaj and 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 GTX from such copolymer gels were mainly dependent on the gel dissolution.

3.4. *In vivo* resident experiments

For *in vivo* resident experiment, copolymer3 with relatively low gelation temperature and higher gel strength was used. Fig. 9 shows the level of GTX in rabbit's conjunctiveal sac after instillation of 0.2% GTX eye drops and 0.2% GTX *in situ* gel containing 4.0% (w/v) copolymer3. After administration of GTX *in situ* gel formulation, drug concentration in the rabbit's conjunctiveal sac was significantly higher than that of conventional eye drops at all time points except for the first point, especially within 60 min. GTX was diffused out quickly from eye drops during the first 10 min and almost vanished completely from eye sac. Compared to eye drops, the diffusion of GTX from *in situ* gel was slow, the drug resident time and total resident amount of *in situ* gel increased by 5.0 and 2.6 folds.

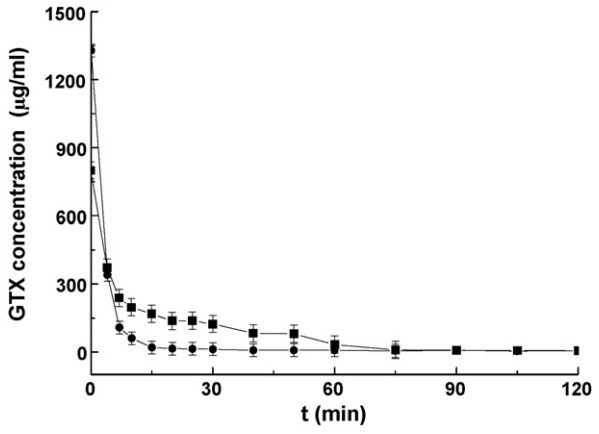


Fig. 9. The level of gatifloxacin in rabbit's conjunctiveal sac after instillation of 0.2% gatifloxacin eye drop (■) and as *in situ* gel containing 4.0% (w/v) copolymer3 (●).

4. Conclusions

The rheological measurements showed the Pluronic-*g*-PAA copolymers which had bioadhesive properties formed temperature-responsive gels with suitable gelation temperature at a relatively lower concentration (4.0%, w/v) compared to 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 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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