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Release of ciprofloxacin from poloxamer-graft-hyaluronic acid hydrogels in vitro

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Abstract

Recently, in situ gel formation has extensively been studied to enhance ocular bioavailability and duration of the drug activity. In this study, we report grafting of poloxamer onto the hyaluronic acid for application of tissue engineering oriented ophthalmic drug delivery system. Graft copolymers were prepared by coupling mono amine-terminated poloxamer (MATP) with hyaluronic acid (HA) backbone using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and *N*-hydroxylsuccinimide (NHS) as coupling agents. The coupling of MATP with HA was clarified by ^{1}H NMR and FT-IR spectroscopy. The gelation temperature of graft copolymers was dependent on the content of HA and the concentration of poloxamer. From drug release studies in vitro, ciprofloxacin was sustainedly released from the poloxamer-g-hyaluronic acid hydrogel due to the in situ gel formation of the copolymer and viscous properties of HA.

Keywords: Poloxamer; Hyaluronic acid; Ophthalmic drug delivery; Mucoadhesive

1. Introduction

Eye drops as conventional ophthalmic delivery systems result in poor bioavailability and therapeutic response because of lacrimal secretion and nasolacrimal drainage in the eye (Patton and Robinson, 1976; Sieg and Robinson, 1977). Most of the drug is drained away from the pre-corneal area in few minutes. As a result, frequent instillation of concentrated solutions is needed to achieve the desired therapeutic effects (Chein et al., 1982). But, by the tear drainage the main part of the administered drug is transported via the naso-lacrimal duct to the GI-tract where it may be absorbed, sometimes causing side effects (Middleton et al., 1990). In order to increase the effectiveness of the drug a dosage form should be chosen, which increases the contact time of the drug in the eye. This may then increase the bioavailability, reduce systemic absorption and reduce the need for frequent administration leading to improved patient compliance.

Recently, in situ gel formation has extensively been studied to enhance ocular bioavailability and duration of the drug activity. Edsman et al. used poloxamer 407 as an in situ gel to increase ocular residence time (Edsman et al., 1998). Sechoy et al. reported that carteolol was slowly released in the rabbit eyes using in situ gel formation of alginic acid in the eye (Sechoy et al., 2000). It was also reported that thermosensitive

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poly(*N*-isopropylacryamide) hydrogel was potential in controlled release of antiglaucoma drugs (Hsiue et al., 2002). In situ gels are interesting since these are conveniently dropped as a solution into the conjunctival sac (Cohen and Lobel, 1997), where they undergo a transition into a gel with their favorable residence time. The sol–gel transition occurs as a result of a chemical/physical change induced by the physiological environment. Among them are poloxamer 407 (Miller and Donovan, 1982; Desai, 1998) and tetronics (Vadnere et al., 1984; Spancake et al., 1991), whose solution viscosity increases upon increasing the temperature to that of the eye, cellulose acetophthalate (CAP) latex (Gurny, 1981; Gurny et al., 1985) that coagulates when its native pH of 4.5 is raised by the tear fluid to pH 7.4 and GelriteTM (Rozier et al., 1989; Sanzgirl et al., 1993), a polysaccharide that gels in the presence of mono or divalent cations.

Poloxamer 407 (trade name, Pluronic F-127), a non-toxic poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) (PEO/PPO/PEO) triblock copolymer with a weight-average molecular weight of 12,600, contains 70% hydrophilic ethylene oxide units and 30% hydrophobic propylene oxide units. It forms a gel on warming to body temperature by undergoing a sol–gel transition. As a result of this reverse thermal gelation and extremely low toxicity, the administered solution containing drug turns into a gel and renders slow release characteristics to the drug delivery system in the pharmaceutical fields (Katakam et al., 1997; Edsman et al., 1998; Lin and Sung, 2000; Moghimi and Hunter, 2000).

Hyaluronic acid (HA) is a naturally occurring linear polysaccharide composed of alternating disaccharide units of *N*-acetyl-D-glucosamine and D-glucuronic acid joined by alternatiing β-(1–3) glucuronidic and β-(1–4) glucosaminidic bond. The HA serves a critical role as a signaling molecule in cell motility, cell differentiation, wound healing (Miyauchi et al., 1996), cancer metastasis (Entwistle et al., 1996), growth factor action, morphogenesis and embryonic development. Unmodified HA has many important applications in drug delivery and surgery. For example, HA was used as an adjuvant for ophthalmic drug delivery (Saettone et al., 1994), and was found to enhance the absorption of drugs and proteins via mucosal tissues. In addition, HA has important applications in the fields of viscosurgery and viscosupplementation (Balazs and Denlinger, 1989). The immunoneutrality of HA makes it an excellent building block for biomaterials to be employed for tissue engineering and drug delivery systems (Vercruysse and Prestwich, 1998).

In this study, we report grafting of poloxamer onto the HA for application of tissue engineering oriented ophthalmic drug delivery system. Graft copolymers were prepared by coupling mono amine-terminated poloxamer (MATP) with HA backbone using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and *N*-hydroxylsuccinimide (NHS) as coupling agents. The gelation temperature of graft copolymers was measured by vertical method. Ciprofloxacin release from the graft copolymer gel was performed in vitro. This poloxamer-graft-hyaluronic acid hydrogel will be expected to slow down drug elimination by lacrimal flow, both by undergoing in situ gel formation and by interacting with the mucus. Also, this hydrogel will be used to enhance wound healing of injured mucus layer of the eye.

2. Experimental

2.1. Materials

Sodium salts of HA from bovine trachea, EDC, diaminoethylene and poloxamer 407 were purchased from Aldrich Chemical Co. (Milwaukee, WI), NHS, 4-nitrophenyl chloroformate and ciprofloxacin were provided by Sigma (St. Louis, MO). All other chemicals were of extra pure reagent grade and were used as received.

2.2. Synthesis of MATP

MATP was prepared by two step reactions (Fig. 1). In the first step, 0.3968 mM of poloxamer was reacted with 0.496 mM of 4-nitrophenyl chloroformate dissolved in methylene chloride in the presence of triethylamine at room temperature for 4 h to yield a 4-nitrophenyl formate-derivatized intermediate. This intermediate was recovered by extraction, three times using petroleum ether. In the second step, 0.3398 mM of the intermediate was reacted with 1.0195 mM of diaminoethylene dissolved in methylene chloride at room temperature overnight. After reaction, the mixture was extracted three times with petroleum ether,

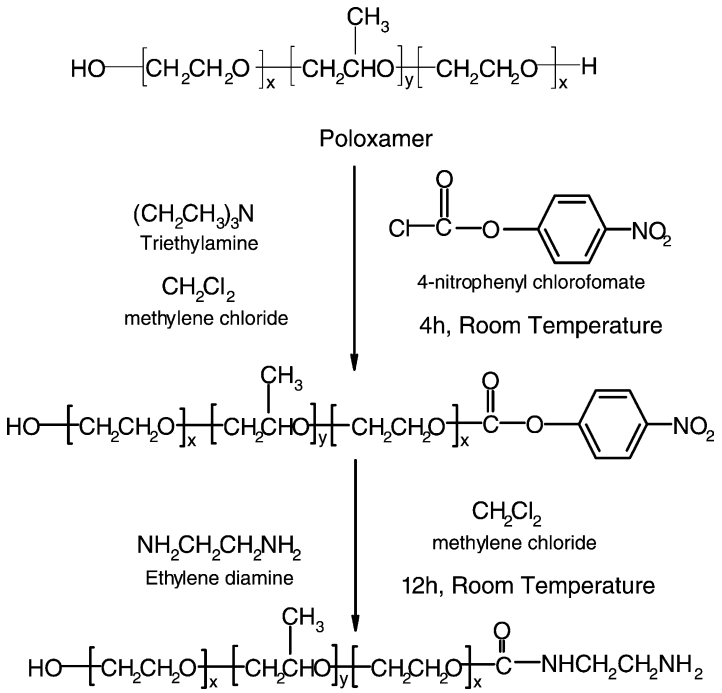

Fig. 1. Preparation of amine-terminated poloxamer.

then dialyzed against distilled water using a membrane with a molecular weight cut-off of 3500 for 3 days at room temperature, and then lyophilized to result in the product.

2.3. Grafting of poloxamer onto HA

Graft copolymer was prepared by coupling MATP with the HA backbone. The reaction was carried out at room temperature for 24 h by EDC/NHS methods. Specifically, reaction between the amine groups of poloxamer and carboxyl ones of HA in the presence of EDC resulted in amide bond formation (Fig. 2). The reaction was carried out at room temperature for 2 days. After reaction, the mixture was dialyzed against distilled water using a membrane with a molecular weight cut-off of 12,000–14,000 for 3 days at room temperature, and then lyophilized to result in the product.

2.4. ^1H NMR spectroscopy measurement

Proton nuclear magnetic resonance (^1H NMR) spectra were measured at 25 °C using AVANCE 600 spectrometer operating at 600 MHz. ^1H NMR spectra of graft copolymer were measured in D_2O to estimate the copolymer composition.

2.5. FT-IR spectroscopy measurement

The tablet of graft copolymer, poloxamer and HA, were obtained by KBr pellet. The infrared absorption (IR) spectra were recorded using a M series (MIDAC CORPERATION, USA) FT-IR spectrometer.

2.6. Measurement of gelation temperature

The gelation temperature of graft copolymer was measured by vertical method. One milliliter of poloxamer or graft copolymer was added into a 5 ml vial at the concentration of 18–20 wt.%, and then the gelation was checked by a visual inspection in an incubator. The gelation medium was simulated by the tear fluid which was prepared daily and equilibrated at 34 °C. The composition of artificial tear fluid used was sodium chloride, 0.670 g; sodium bicarbonate, 0.200 g; calcium chloride·$2H_2O$, 0.008 g and purified water, 100 g.

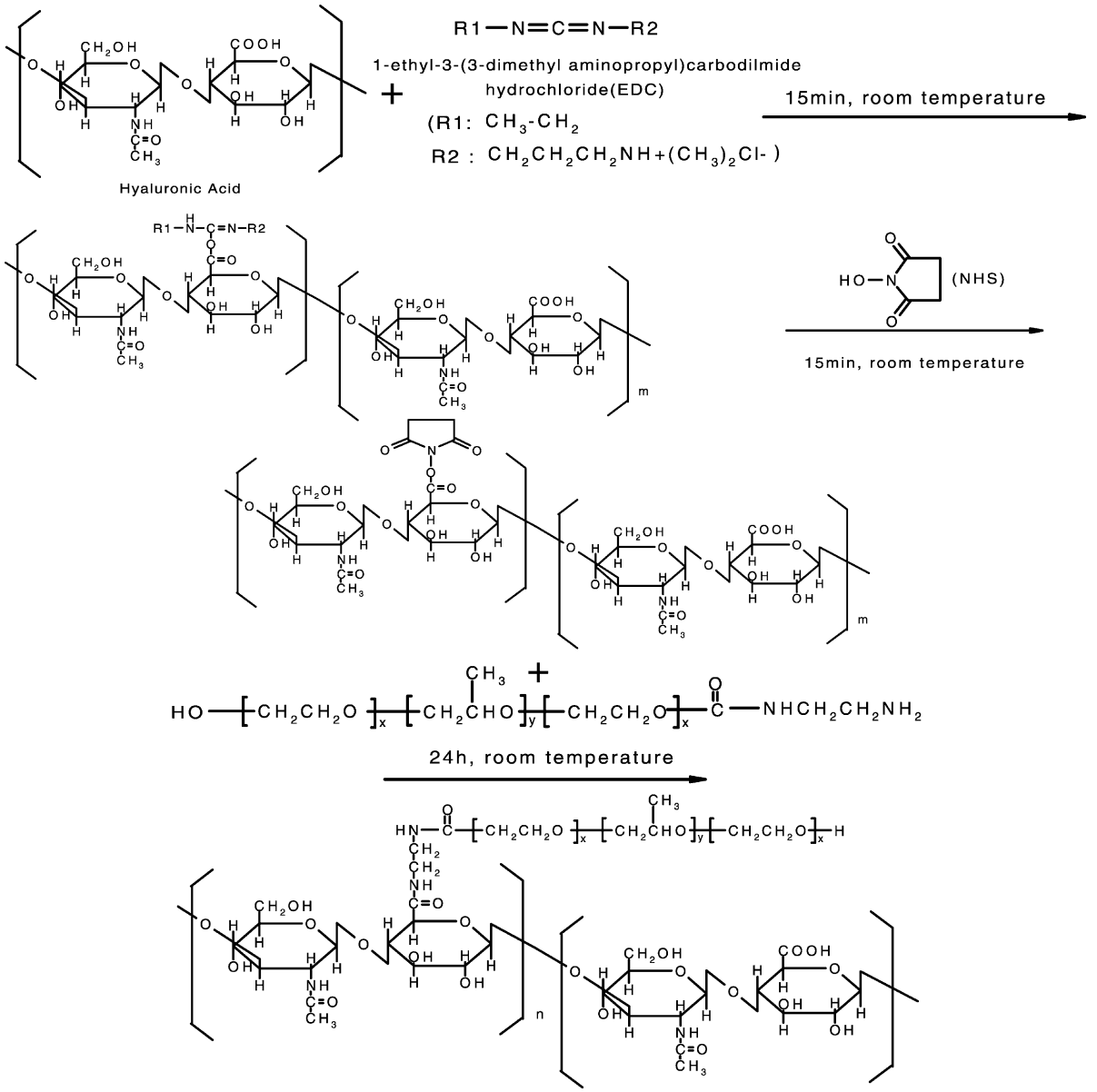

Fig. 2. Preparation of poloxamer grafted onto hyaluronic acid.

2.7. In vitro release studies

The in vitro drug release from graft copolymer was carried out by filling 1 ml of ciprofloxacin (0.35 wt.%)-loaded graft copolymer (20 wt.%) solution into 5 ml vials in triplicate. The above vials were placed for gelation at 34 °C in an incubator. The vials were then filled with 2 ml simulated artificial tear fluid. The temperature and stirring rate were maintained at 34 °C and 50 rpm, respectively. Aliquots (1 ml) were withdrawn from the release medium and replaced by an equal volume at each sampling time. The aliquot was diluted with the simulated tear fluid and the amount of drug released was determined by a

spectrophotometric assay at 287 nm using a UV-Vis spectrophotometer.

3. Results and discussion

3.1. Preparation of poloxamer grafted onto HA

Graft copolymer was prepared by coupling MATP with HA backbone at room temperature for 48 h by EDC/NHS methods previously reported (Chung et al., 2002). The synthesis schemes of MATP and poloxamer-graft-hyaluronic acid are illustrated in Figs. 1 and 2. The amide bonds were formed through the reaction between the amino groups of MATP and carboxyl ones of HA (Fig. 2).

3.2. ^1H NMR spectra of graft copolymer

The coupling of MATP with HA and graft copolymer composition were clarified by ^1H NMR spectroscopy. Fig. 3 shows ^1H NMR spectrum of graft copolymer. A typical signal of methyl groups (CH_3) of polypropylene oxide in poloxamer was detected at 1.2 ppm. An apparent *N*-acetylate proton peak ($NCOCH_3$) in HA was detected at 2.2 ppm. The other

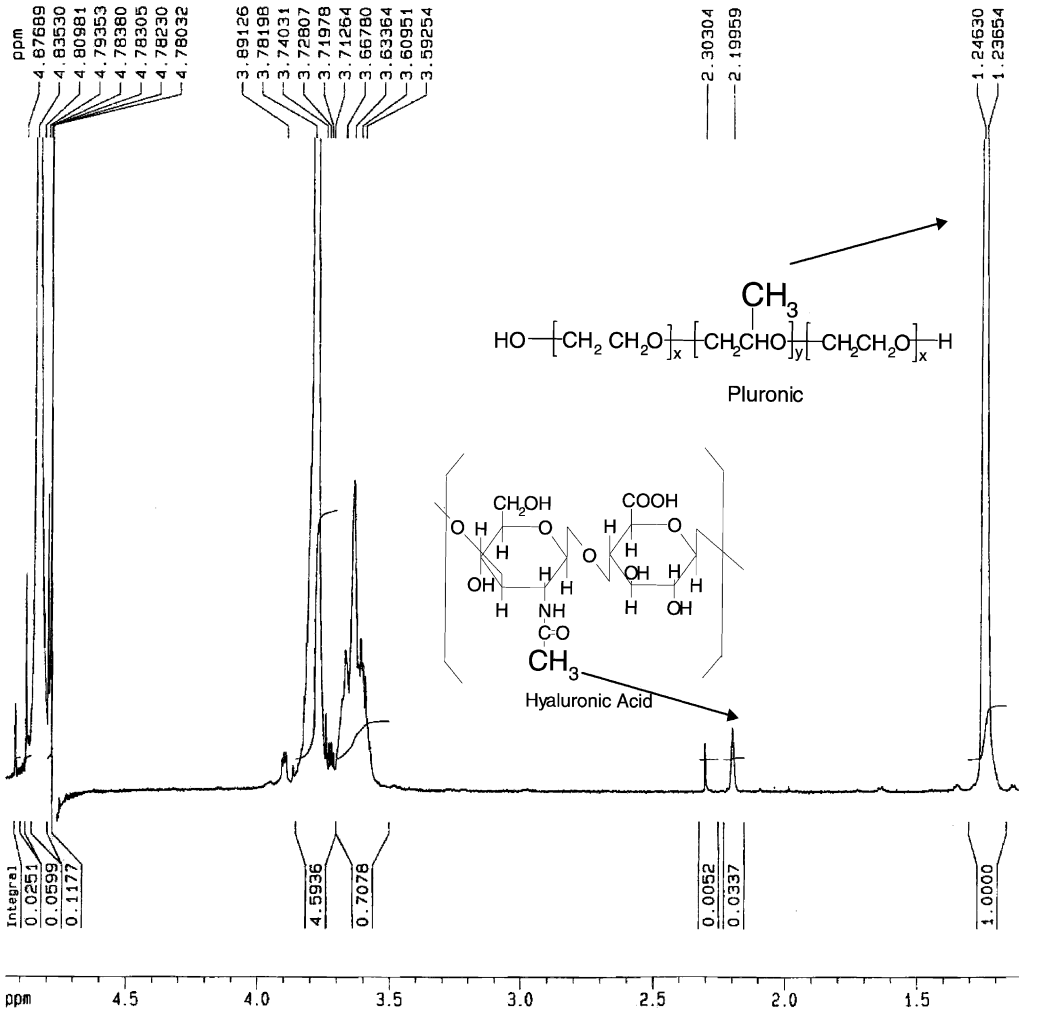

Fig. 3. ^1H NMR spectrum of poloxamer grafted onto hyaluronic acid.

proton peaks that ranged in 3.5–4.0 ppm were undistinguished by the ^{1}H NMR spectroscopy, because of the overlap of glucose unit peak of HA and methylene peak of poloxamer. Therefore, the compositon of graft copolymer was calculated by the integration of a methyl group peak of poloxamer and a *N*-acetylate proton one of HA. The content of HA in graft copolymer was 6.87 wt.% per poloxamer.

3.3. FT-IR spectroscopy

The amide bond formation resulted from the reaction between amine groups of the MATP and carboxyl ones of HA was clarified by FT-IR spectroscopy. Fig. 4 shows FT-IR spectra of HA and graft copolymer. HA exhibited the characteristic absorption at 1639 cm^{-1}, which is asymmetric carbonyl stretching vibration. The frequency of C=O absorption (1639 cm^{-1}) was lowered from the value formed for the parent carboxylic acid because of resonance. In the IR spectrum of graft copolymer, the carbonyl absorption band of carboxylate sodium salt in HA became weak and the new characteristic amide I band appeared at 1660 cm^{-1}. The result suggested that the amide bonds between carboxylic groups of HA and amine ones of MATP were formed.

3.4. Gelation temperature

The gelation temperature of graft copolymers is shown in Fig. 5. The change of gelation temperature was dependent on the content of HA and the concentration of poloxamer. The more the content of HA and ciprofloxacin, higher was the gelation temperature. This result suggested that hydrophilic properties of HA and ciprofloxacin interfered the hydrophobic interaction of poloxamer (PL). Also, sol–gel transition of graft copolymer did not occur if HA was over 15 wt.% in the copolymer owing to the increased hydrophilic property of HA.

3.5. In vitro release studies

The cumulative percent of ciprofloxacin released from the copolymer gel as a function of time is shown in Fig. 6. For the drug containing poloxamer as shown

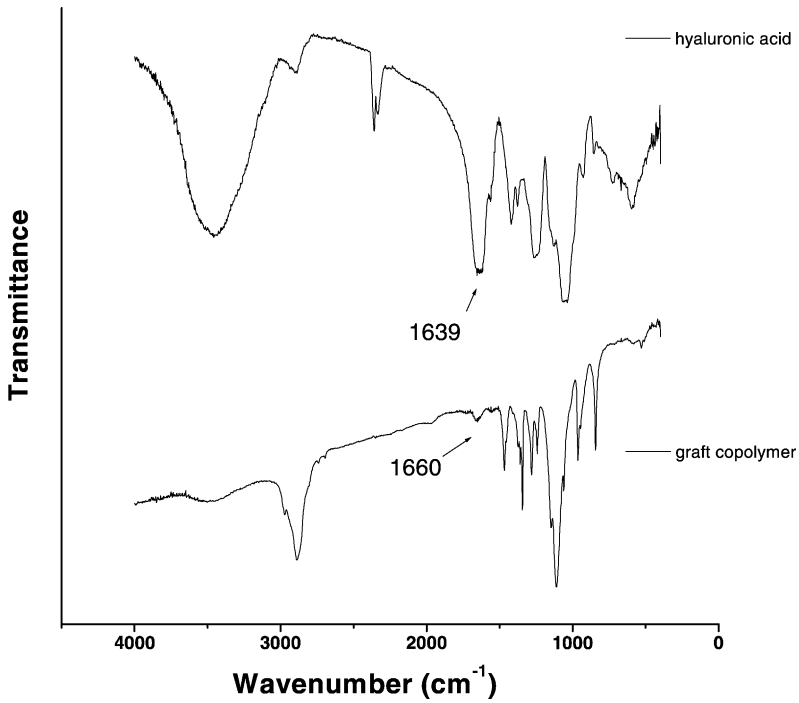

Fig. 4. FT-IR spectra of hyaluronic acid and poloxamer-g-hyaluronic acid.

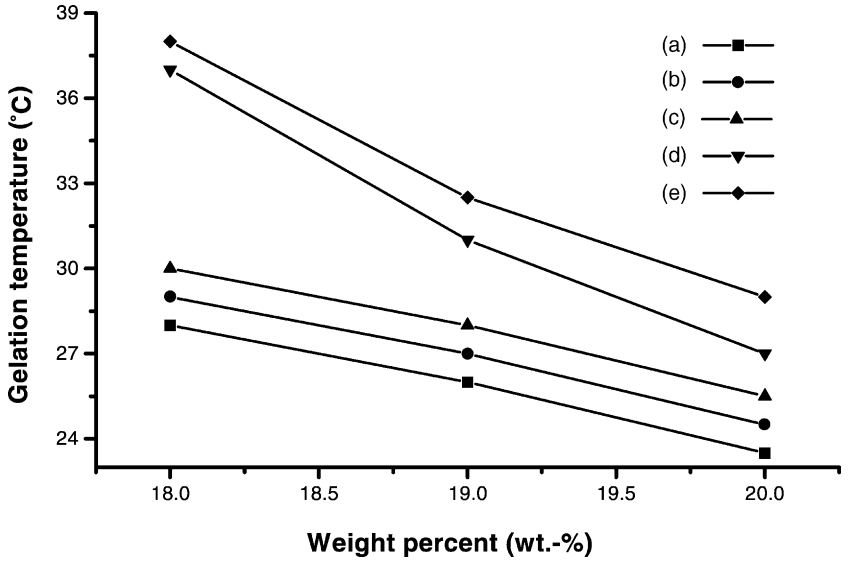

Fig. 5. The gelation temperature of (a) poloxamer, (b) graft copolymer (hyaluronic acid, 1.18 wt.%), (c) graft copolymer (hyaluronic acid, 6.87 wt.%), (d) graft copolymer (hyaluronic acid, 1.18 wt.% + ciproxacin, 0.88 wt.%) and (e) graft polymer (hyaluronic acid, 6.87 wt.% + ciprofloxacin, 0.88 wt.%).

in Fig. 6a, almost all ciprofloxacin was released after 3 h. On the other hand, for the ciprofloxacin-loaded graft copolymer (HA, 13.99 wt.%), the drug was released about 90% after 20 h as shown in Fig. 6d. These results suggested that ciprofloxacin was sustainedly released, as the content of HA in the copolymer increased due to the mucoadhesive property and high viscosity of HA. While in case the content of

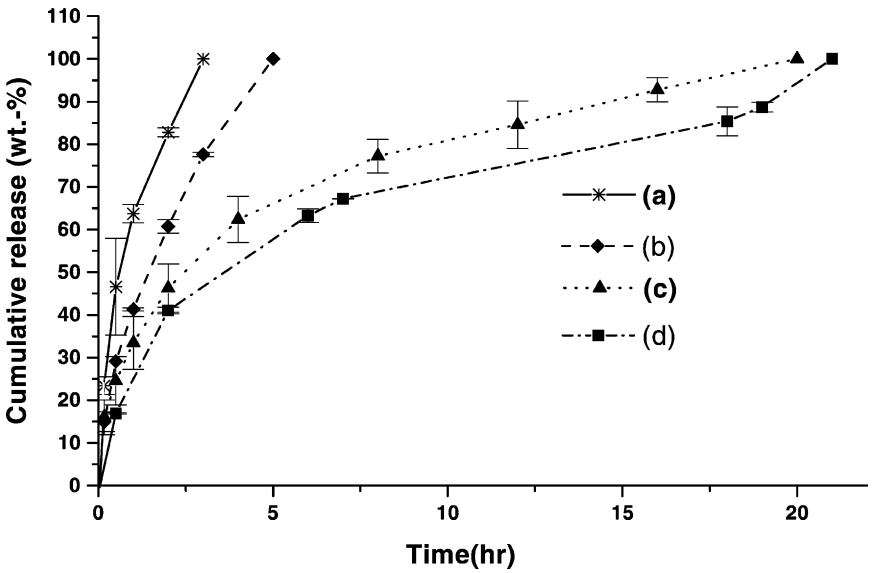

Fig. 6. Release of ciprofloxacin from (a) poloxamer, (b) graft copolymer (hyaluronic acid, 1.18 wt.%), (c) graft copolymer (hyaluronic acid, 6.87 wt.%) and (d) graft copolymer (hyaluronic acid, 13.99 wt.%) with 1.75 wt.% ciprofloxacin in vitro.

HA in graft copolymer increased in more than limited amount, a reversible sol–gel transition was not undergone. This result indicated that a relative increase of hydrophilicity of HA prevents the hydrophobic interaction of polypropylene oxide in poloxamer, leading to no gelation of graft copolymer. But the drug release from HA alone in vitro could not be performed due to the non-occurrence of sol–gel transition of HA itself.

4. Conclusions

Graft copolymers were prepared by coupling MATP with HA backbone by EDC/NHS method. The coupling of MATP with HA was clarified by ^{1}H NMR and FT-IR spectroscopy. The gelation temperature of graft copolymers was dependent on the content of HA and the concentration of poloxamer. A sustained ciprofloxacin release in vitro was dependent on the content of HA. The results of this study indicate that the bioadhesive, thermally gelling and tissue regeneration properties of these graft copolymers will be expected to be an excellent drug carrier for the prolonged delivery to surface of the eye.

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