In Situ Gel Forming Systems of Poloxamer 407 and Hydroxypropyl Cellulose or Hydroxypropyl Methyl Cellulose Mixtures for Controlled Delivery of Vancomycin

Azita H. H. Talasaz,[1] Ali A. Ghahremankhani,[1] Shadi H. Moghadam,[1] Mazda R. Malekshahi,[1] Fatemeh Atyabi,[1] Rassoul Dinarvand[1,2]

[1]*Novel Drug Delivery Systems Lab, Faculty of Pharmacy, Medical Sciences/University of Tehran, Tehran, Iran*
[2]*Medical Nanotechnology Research Center, Medical Sciences/University of Tehran, Tehran, Iran*

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ABSTRACT: Poloxamers are a family of triblock copolymers consisting of two hydrophilic blocks of polyoxyethylene separated by a hydrophobic block of polyoxypropylene, which form micelles at low concentrations and form clear thermally reversible gels at high concentrations. The objective of this study was to develop an *in situ* gel forming drug delivery system for vancomycin using the minimum possible ratio of poloxamer 407 (P407). Decreasing the concentration of poloxamer could reduce the risk of hypertriglyceridemia induction. Different additives were added to the poloxamer formulations. It was observed that among different additives, hydroxypropyl methyl cellulose (HPMC) and hydroxypropyl cellulose (HPC) can decrease poloxamer concentration required to form *in situ* gelation from 18 to 10%. The dynamic viscoelastic properties of the samples were determined. Both the storage modulus and the loss modulus of the samples increased abruptly as the temperature passed a certain point. The gelling temperature was in the order of P407 : HPC (10 : 10 w/w) < P407 : HPMC (10 : 10 w/w) < P407 : HPMC (15 : 5 w/w) < P407 : HPC (15 : 5 w/w). Drug release rate could be controlled by changing the type and ratio of additives as well as the amount of drug loaded. It can be concluded that combining P407 and cellulose derivatives could be a promising strategy for preparation of thermally reversible *in situ* gel forming delivery systems with low poloxamer concentration. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 2369–2374, 2008

Key words: poloxamer 407; *in situ* gel forming systems; HPC; HPMC; vancomycin; block copolymers; rheology; gelation; gels; stimuli-sensitive polymers

INTRODUCTION

Poloxamers are nonionic, polyoxyethylene-polyoxypropylene-polyoxyethylene (PEO-PPO-PEO) triblock copolymers, which form micelles at low concentrations and clear thermally reversible gels at high concentrations. These polymers exist as a mobile viscous liquid at low temperatures but form a rigid semisolid gel at high temperatures. Thus, it is possible to use these polymers to design a formulation, which is liquid at room temperature, but forms gel once injected, thus providing a depot of drug at the injection site. Use of poloxamer gels to develop such a product would not only provide a sustained release formulation, but also enhance stability.[1] An additional advantage of this gel compared with an implant is that the gel can be injected as a solution, thus avoiding surgical techniques. They also may be prepared generally with fewer manufacturing steps than other injectable controlled drug delivery systems such as microparticles and implants and require mainly terminal filter sterilization.[2] The injectable gels usually consist of a solvent to dissolve the polymeric content and/or the therapeutic agent and they form an ''implant-like'' upon injection.

Pluronic F127 or poloxamer 407 (P407), with a nominal molecular weight of 12500 and a PEO/PPO weight ratio of 2 : 1, has been widely used in drug delivery systems, since reported to be the least toxic of the commercially available poloxamers.[2] It has been used for topical, transdermal, ophthalmic, and implantable applications.[3–7] In addition, P407 exhibits non specific antiadhesive properties that can prevent the adherence of proteins and bacteria, and thereby hinder infections.[8–10]

Aqueous solutions of P407 above a concentrations of about 20% (w/w) are liquid when refrigerated, but gel upon warming.[11] However, application of concentrated polymer solutions (>16%) in drug delivery may be disadvantageous as it changes the osmolality of the formulation, kinetics of gelation, and causes discomfort in ophthalmic applications

Correspondence to: R. Dinarvand (dinarvand@tums.ac.ir).

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due to vision blurring and crusting.[12] It has been shown that such concentration cause notable toxicity and increased cholesterol and triglycerides levels in plasma after intraperitoneal injection in rats.[13] This phenomenon may be due to the stimulation of HMG-COA reductase activity in the liver by poloxamer vehicle.[14] Thus, elevated levels of cholesterol and triglycerides resulting from the chronic administrations of poloxamer containing drug formulations to patients may potentially hinder therapeutic outcome. Therefore, formulations with poloxamer less than 16% by using injectable biodegradable polymers are of high value for long period drug delivery.[15]

P407 has been studied in a series of papers for controlled delivery of low-and high-molecular-weight bioactive agents such as melanotan,[16] lidocaine,[17] ibuprofen,[18] pilocarpin,[19] and interleukin-2.[20]

Vancomycin is a glycopeptide antibiotic used in the prophylaxis and treatment of infections caused by Gram-positive bacteria. Its utilization has increased markedly in the last few years, as a result of the increasing numbers of infections of bone and prosthetic devices by methicillin-resistant staphylococci.[15] However, its restricted clinical efficacy in poorly vascularized body sites during parenteral use, and the risks of toxicity associated with high serum concentrations, warrants the search for new carrier systems for local vancomycin release.

The objective of this study was to develop an *in situ* gel forming drug delivery system for vancomycin using the minimum possible ratio of P407. Preparation of an *in situ* gel forming system using low concentration of P407 is very important for avoiding the potential risk of hypertriglyceridemia connected to the high concentration of P407. The impact of different additives hydroxy propyl methyl cellulose (HPMC) and hydroxy propyl cellulose (HPC) on the physicochemical properties of the *in situ* gelling system was also investigated.

MATERIALS AND METHODS

Materials

P407 was purchased from SynoPharm, Germany. Vancomycin HCl (USP grade) was kindly donated by Jaber Pharmaceutical, Iran. Hydroxy propyl cellulose (HPC) was from Aldrich, Germany. Hydroxy propyl methyl cellulose (HPMC, 6cps) was provided by Merck, Germany. Sucrose, poly ethylene glycol 400 (PEG400), polyvinyl alcohol (PVA) MW 72000, NaCl, NaH_2PO_4, Na_2CO_3, sorbitol and isopropyl myristate were of Merck, Germany. Deionized water purified through a Millipore water purification system was used. The other chemicals were of analytical grade and used as received.

P407 gel preparation

Gels were prepared on a weight/weight basis by the cold method described by Schmolka.[11] Appropriate amounts of P407 and vancomycin were weighed out and added to 4 mL of deionized water. In some formulations, one of the following additives was added to the poloxamer (17%w/w) and vancomycin mixture: sucrose (5, 10, or 15% w/w), PEG 400 (2, 5, or 10%), PVA 72000 (0.5%), NaCl (1, 2.5%), Na_2CO_3 (1, 2.5%), NaH_2PO_4 (1, 2.5%), HPMC (1 to 10%), HPC (1 to 10%), sorbitol (5 to 25%), isopropyl myristate (5 or 10%) or TMC (up to 5%). The mixture was placed in a refrigerator until a clear, homogenous liquid was formed. Then, the formulations were put in the water bath and gradually heated from 10 up to 50°C. The formulations were checked whether they had been gelled or not by turning the vials upside down, so that the effectiveness of the additives in reducing the poloxamer concentration was determined.

If the additive was effective, efforts were made to minimize the poloxamer concentration as low as possible with a viscosity that could be injected via a syringe. In these formulations, poloxamer preserved its ability to form a gel upon warming up.

Rheological studies

The rheological analysis was performed using a stress and strain controlled rheometer model Paar Physica MCR300. Flow measurement were performed with a single gap cylinder CC27 geometry (outer radius 14.46 mm, max shear rate 1300 1/s, max shear stress 2800 Pa, gap 1.13 mm, sample volume 19.35 mL). The temperature range was between 10 and 40°C. At the minimum temperature the formulations were liquid, whereas upon warming they gelled. The storage modulus G', the loss modulus G'', and the complex viscosity [η^*] were recorded. The temperature at which G' suddenly varied corresponded to the temperature of the sol-gel transition.

Release studies

The formulations of P407 : HPMC (15 : 5 w/w), P407 : HPC (15 : 5 w/w), P407 : HPMC (10 : 10 w/w), P407 : HPC (10 : 10 w/w) were investigated for *in vitro* release kinetic studies. Different amounts of vancomycin (20, 50, 100, or 200 mg) were incorporated into these formulations. The cold P407 solution was introduced into the dialysis tube (cellulose membrane, cutoff 12,000, Merck, Germany), which was then placed in the dissolution apparatus I (CALEVA dissolution tester 10 + ST, Switzerland). Distilled water (900 mL) was used as the solution medium. The temperature was set at 37°C, and the rotating speed at 25 rpm. Sampling was performed

at 1, 2, 4, 8, 24, 30, 48, 54, 72, 78, 96, 144, 150, 168, 174, 192,198, and 216 h after starting the dissolution test. Amount of vancomycin released was determined spectrophotometrically at the wavelength of 282 nm. All experiments were carried out in triplicate.

RESULTS AND DISCUSSION

Rheological analysis

Aqueous solutions of P407 at concentrations of higher than 20% w/w become gel when temperature is increased above a certain point. At 25°C the solution behaves as a liquid, which is transformed into a semisolid transparent gel at body temperature (37°C). To develop an *in situ* gel forming delivery system for vancomycin using minimum concentration of P407, different additives were mixed with lower concentrations of P407 (17% w/w). The gelation property of these mixtures was then investigated. Sucrose (5, 10, or 15% w/v), PEG 400 (2, 5, or 10%), PVA 72000 (0.5%), NaCl (1, 2.5%), Na_2CO_3 (1, 2.5%), NaH_2PO_4 (1, 2.5%), sorbitol (up to 25%), isopropyl myristate (5, 10%), TMC (up to 5%), HPMC (up to 10%) or HPC (up to 10%) were evaluated. Results showed that among all additives, only two polymers from cellulose family (i.e., HPC and HPMC) could produce thermally reversible gels when added to aqueous solutions of P407 at concentrations <17% w/v.

Packaging of micelles and micelle entanglements have been suggested as the mechanisms of the gelation of aqueous solutions of p407. With increasing temperature, micellization becomes more important, and then at a definite point micelles come into contact and no longer move. Micelle entanglements do not allow micelles to separate easily from each other and that forms the rigidity of the gel. Proximity of micelles and micelle entanglements cause the sharp increase in viscosity close to the gel point. This mechanism also corresponds with the minimum concentration required for gelation, since the micelles must occupy a high volume fraction of the solution to come into contact and entangle.[21]

At low concentrations (1–10 wt %), some cellulose derivatives aqueous solutions such as hydroxy propyl cellulose are liquid at low temperatures, but gel upon heating.[22] Gelation of these cellulose derivatives is primarily caused by the hydrophobic interaction between molecules containing methoxy substitution. At low temperatures, the macromolecules are hydrated, and there is little polymer-polymer interaction other than simple entanglement. As the temperature is raised, the polymers gradually lose their water of hydration, which is reflected by a decline in relative viscosity. Eventually, when sufficient but not complete dehydration of the polymer occurs, polymer-polymer association takes place, and the system approaches an infinite network structure, as reflected experimentally by a sharp rise in relative viscosity.[23,24] Figure 1 shows the chemical structure of these polymers. The storage modulus (G′) and loss modulus (G″) of both P407 : HPC and P407 : HPMC formulations were measured by dynamic mechanical analysis (Fig. 2). The changes in rheological parameters can be divided into three phases. During the first phase, before the gelation point, G′ was very low as expected for liquids. The second phase corresponded to the gelation phase. From this point onwards, both G′ and G″ values increased abruptly, as the liquid becomes gel. The last phase consisted of stabilization, with G′ values tending to stabilize, while *G″* values fell after having reached a maximum.[15]

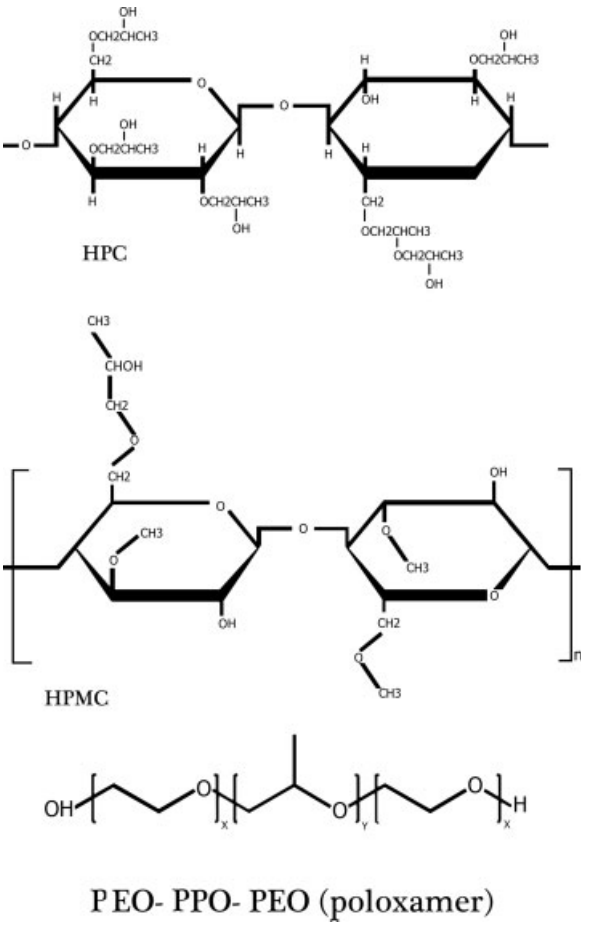

Figure 1 Chemical structure of poloxamer, HPC and HPMC.

The thermosensitive property of gels was evaluated by sol/gel transition temperature. The sol/gel transition temperatures correspond to the temperature characterized by a drastic change of the rheological behavior of the system. The storage modulus is a measure of the energy stored and recovered per

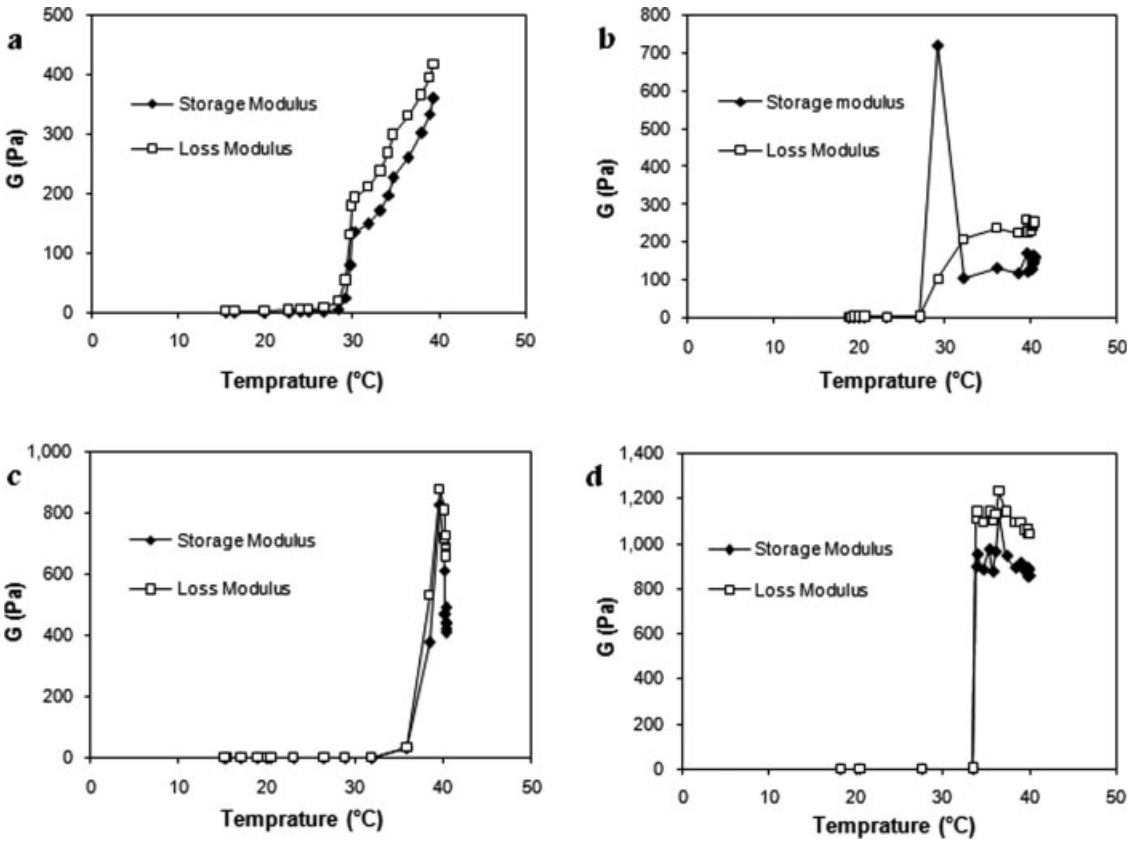

Figure 2 Temperature-dependent changes of the rheological properties of poloxamer gels: (a) P407 : HPC (15 : 5 w/w), (b) P407 : HPC (10 : 10 w/w), (c) P407 : HPMC (15 : 5 w/w), and (d) P407 : HPMC (10 : 10 w/w).

cycle of deformation and reflects the solid-like component of elastic behavior.[25] The storage modulus is thus low at solution stage but increases drastically at the gelation temperature.[26] The gelation temperature were 33.9, 29.8, 36.6, 29.1 for P407 : HPC (15 : 5 w/w), P407 : HPC (10 : 10 w/w), P407 : HPMC (15 : 5 w/w), and P407 : HPMC (10 : 10 w/w), respectively.

All the formulations exhibited a gelation temperature below body temperature, but presence of HPMC resulted in higher gelation temperature compared with HPC.

Figure 3 shows the complex viscosity change for formulations made of P407 18%, P407 : HPMC (15% : 5%), P407 : HPMC (10% : 10%), P407 : HPC (15% : 5%), and P407 : HPC (10% : 10%) as a function of temperature. As can be seen, the viscosity of each formulation increased abruptly at temperatures above 27°C. This suggests that these formulations have the potential of application as *in situ* gel forming systems for drug delivery purposes.

Drug release

Figure 4 shows vancomycin release from different *in situ* gel forming systems containing 100 mg drug. Gel composed of P407 (20%) released 80% of its drug content during the first 24 h. Replacing 5% or 10% of P407 in formulation with HPC decreased this burst release to 72 and 61%, respectively. HPMC had more significant effect on retarding vancomycin release rate. In case of gels comprising of P407 : HPMC (10 : 10 w/w) $\sim$ 50% of the loaded drug was released during the first day and the release continued by a slower rate until 216 h. This is contrary to the finding of Veyries et al.[15] They reported slower *in vitro* vancomycin release from P407 gels. They

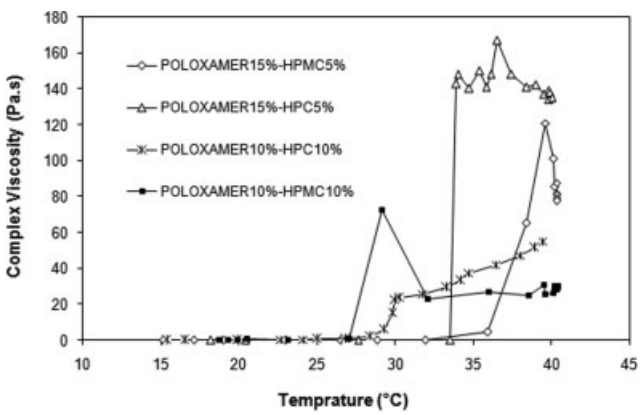

Figure 3 Complex viscosity of different mixtures of poloxamer with HPC or HPMC as a function of temperature.

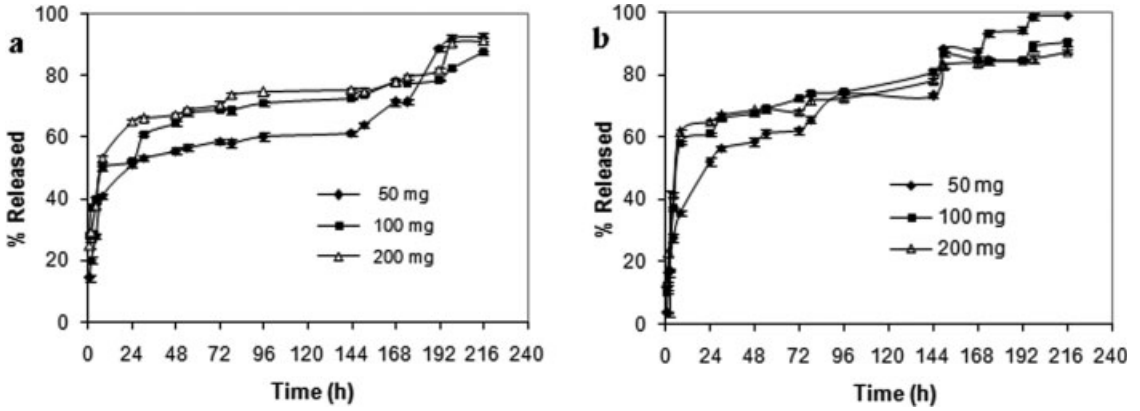

Figure 4 Effect of the type and percentage of cellulose derivatives on vancomycin release from *in situ* forming gels: (a) P407 : HPC (10 : 10 w/w) and (b) P407 : HPMC (10 : 10 w/w).

found that *in vitro* diffusion of vancomycin through the P407 gel was slowed by setting the pH at 7.4. This step may have allowed for the initial loading dose of vancomycin to be above the solubility limit in the gel and led to the formation of small particles of antibiotic dispersed throughout the matrix. Thus, saturation of the gel appeared to favor prolonged release of vancomycin. Another explanation for the slowing of vancomycin diffusion at pH 7.4 may be the involvement of a limiting factor, i.e., the dissolution rate of the antibiotic particles that form when the gel is saturated.[15] In contrast in our study the release media was not saturated with vancomycin as reported by Veyries et al.[15]

Figure 5 represents the effect of drug loading on vancomycin release from P407 : HPC and P407 : HPMC gels. In both cases, formulation containing 50 mg drug had slower release rate than those with

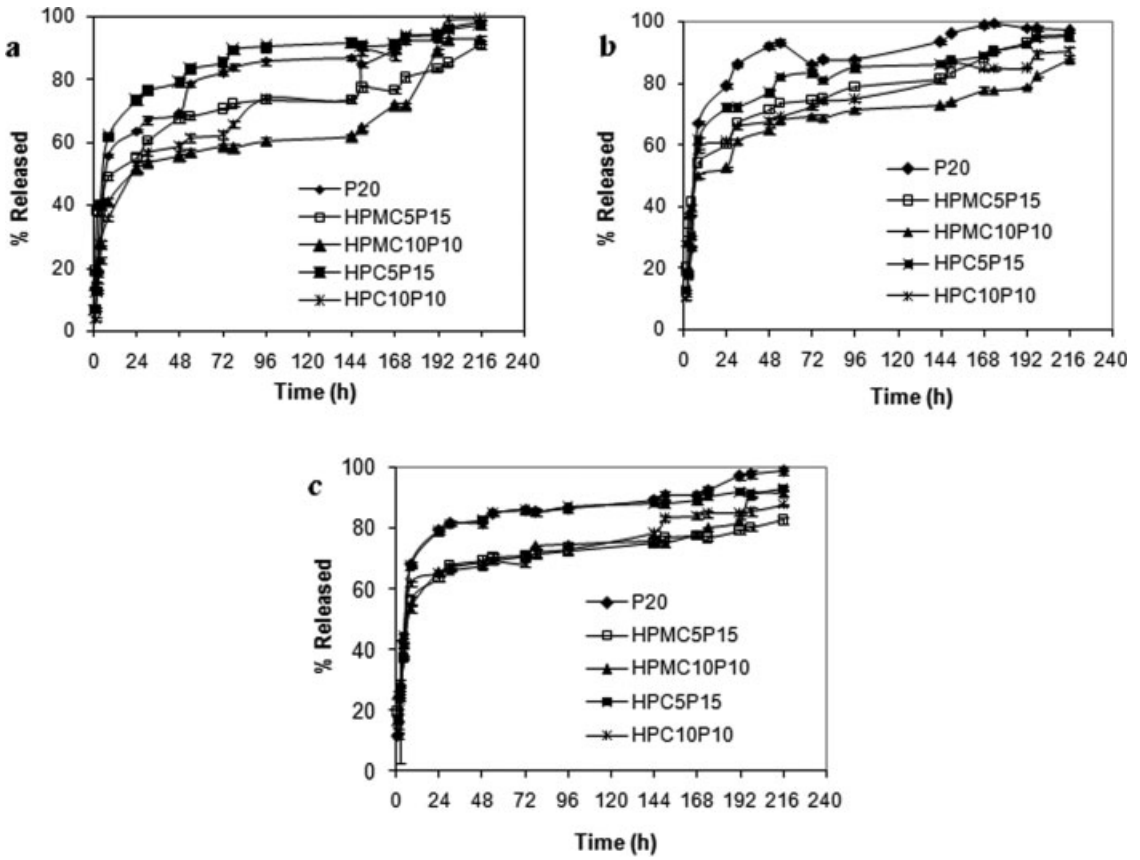

Figure 5 Effect of the amount of drug loading on vancomycin release from *in situ* forming gels: (a) 50 mg drug loading, (b) 100 mg drug loading, and (c) 200 mg drug loading.

100 or 200 mg drug. This may be due to the impact of higher drug diffusion when higher drug amount is loaded. In this study vancomycin was used as a model drug to prove the ability of P407 and cellulose derivative thermosensitive gel formulations to prolong drug release. This release profile could optimize the therapeutic efficacy. The high initial local concentration is desirable for inhibiting early bacterial adhesion to prosthetic devices, at a time when the risk of infection by exogenous bacteria is highest after surgery. The prolonged lower concentrations would protect the prosthetic device against endogenous infection. Moreover, antibiotic activity should be reinforced by the non specific antiadhesive effect of P407 that can limit protein and bacterial adhesion.[8,10] As the gels remain at the injection site, the duration of active vancomycin levels is determined by the dilution rate and diffusion through extra-micellar aqueous channels, and by the biological degradation of the gel matrix itself.[27]

CONCLUSIONS

It was shown that using cellulose derivatives such as HPMC and HPC can significantly decrease the necessary poloxamer concentration required for the preparation of *in situ* gel forming system suitable for the delivery of high molecular weight drugs such as vancomycin.

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