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Sustained release of 5-FU from Poloxamer gels interpenetrated by crosslinking chitosan network

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ABSTRACT

This study investigates *in vitro* the drug delivery characteristics of new thermo-sensitive gels, P-CS/GA gels, in which a chitosan (CS) network is crosslinked with various concentrations of glutaraldehyde (GA) that interpenetrates Poloxamer (P) gels. The results indicate that the swelling ratios of all P-CS/GA gels are markedly superior to those of non-swelling P and P-CS gels. For example, P-CS/GA (0.1 wt.%) gels have swelling ratios of 13.2 ± 1.0, which are maintained for approximately 18 h in water at 37 °C. *In vitro* releases of 5-FU from P-CS/GA (0.1 wt.%) gels had significantly lower initial burst release ($P < 0.01$) and lasted much longer than those from gels without a CS network. For example, the duration of release of 5-FU was in a significantly sustained manner for up to 52 h, which was about 10 times or longer than the period of delivery using P or P-CS gels. The release of drugs from gels with an interpenetrating CS network could be modeled by Fickian diffusion; the characteristic constant '*k*' of drug–gel systems decreased as increasing GA concentrations in the P-CS/GA gels, and increasing the viscosities of the P, P-CS and P-CS/GA solutions.

1. Introduction

Poloxamer block copolymers comprise various poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) blocks arranged in a tri-block polymer structure, PEO–PPO–PEO. Since Poloxamers comprise various numbers of hydrophilic PEO and relatively hydrophobic PPO copolymers, various segments of copolymers in aqueous solution interact and aggregate to form micelles (Dumortier et al., 2006; Ruel-Gariepy and Leroux, 2006). At a sufficiently high concentration and temperature (higher than LCST, lower critical solution temperature), the Poloxamer micelles pack in an order that results in a transition of sol to a gel state called thermo-sensitive gel (Juhasz et al., 1989; Liu and Chu, 2000; Dumortier et al., 2006; Ruel-Gariepy and Leroux, 2006). Numerous researchers have examined the thermo-sensitivity of Poloxamer (P) gels with an interest in their potential use in various pharmaceutical applications (Liu and Chu, 2000; Amiji et al., 2002; Roques et al., 2007; Caffaggi et al., 2008). For example, injecting Paclitaxel loaded Poloxamer 407 (20%) into a tumor positively inhibits tumor growth (Amiji et al., 2002). Poloxamer 407 has also been employed as a carrier for the intra-pericardial administering of plasmid DNA in gene therapy (Roques et al., 2007). However, P gels such as Poloxamer 188 and 407 generally sustain the release of drugs for only a short period (such as <5 h) in an aqueous environment (Barichello et al., 1999; Jeong et al., 2002; Ricci et al., 2005), since P gels dissociate rapidly in (such an OR an aqueous) environment. Various chemical modifications of Poloxamer (Cho et al., 2003; Sosnik and Cohn, 2004; Chung et al., 2005), such as amine-termination or grafting of hyaluronic acid or chitosan to mono-carboxyl Poloxamers, can reduce the critical gelation concentration and the dissolution rate in aqueous solution (Cho et al., 2003; Chung et al., 2005). The development of a modified P gel which may reduce the dissolution rate but improve the sustained release of drugs is of interest.

Chitosan (CS), an amino polysaccharide (poly-1,4-D-glucosamine) has been extensively applied in drug delivery and tissue engineering (Lanza et al., 2000; Kumar et al., 2004; Chung et al., 2008). The cationic property of chitosan has been utilized to deliver hydrophilic drugs such as tissue plasminogen activator and insulin to substrates that carry negative charges, such as the fibrin network and mucosa surfaces (Lanza et al., 2000; Chung et al., 2008). Furthermore, CS has abundant reactive amine groups that can be crosslinked by various agents, such as glutaraldehyde, to generate a network to promote the sustained release of drugs (Wang et al., 2008).

5-Fluorouracil (5-FU) is an anti-cancer drug, which can effectively inhibit the synthesis of DNA and is extensively employed in

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solid tumor therapy such as against breast, colorectal and brain cancer. Since it is metabolized rapidly in the body, the maintenance of a high serum concentration to improve its therapeutic activity requires it to be continuously administered by intravenous injection or infusion. However, 5-FU in plasma above a certain concentration has a severely toxic effect (Yoneda et al., 1999; Johnson et al., 1999), which is frequently observed in cancer patients that undergo this treatment. Therefore, 5-FU delivery by other routes with controlled release characteristics, such as the irradiation of solutions of N-acryloylglycine (NAGly) mixed with chitosan or gelatin microspheres (El-Sherbiny et al., 2005; Sastre et al., 2007), has been investigated. Recently, a 2-hydroxy-ethylmethacrylate (HEMA) and acrylic acid based 5-FU-imprinted hydrogel system is developed and its delivery of 5-FU *in vitro* is characterized (Singh and Chauhan, 2008). However, the release of the drug from 5-FU-imprinted hydrogel can be sustained for only 5 h (Singh and Chauhan, 2008). 5-FU was therefore selected to evaluate the release characteristics of new developed gels.

In this investigation, P gels with an interpenetrating CS network that was crosslinked by GA, P-CS/GA gels, were developed with a view to overcoming the fast dissolution of P gels, improving swelling properties, and sustaining the release of 5-FU for longer.

2. Materials and methods

2.1. Preparing drug-loaded P, P-CS and P-CS/GA solutions/gels with various formulas

5-Fluorouracil (5-FU) with a molecular weight of 130.08 Da was purchased from Sigma Company (Sigma Company, St. Louis, MO, USA). In this study, sufficient amounts of F127 and F68 (BASF laboratory, Wyandoote, USA) were dissolved in distilled water to prepare a Poloxamer (P) solution of F127 (18 wt.%) and F68 (15 wt.%) at 4 °C. To prepare a P solution that contains CS (1.1 wt.% CS), F127 (18 g) and F68 (15 g) were dissolved in 1 wt.% acetic acid solution that contained 1.75 wt.% of CS (1.1 wt.% CS in final, assigned as solution A). In preparing 5-FU (0.3 wt.% in final solution) loaded P-CS solution, sufficient 5-FU was dissolved in solution A, which was diluted with D.I. water. To prepare various concentrations of glutaraldehyde (GA) (0.05, 0.075 and 0.1 wt.% final concentrations) in 5-FU-loaded P-CS/GA solutions, 0.25 ml of solutions of various concentrations of GA was added to 4.75 ml of the drug-loaded P-CS solutions, and mixed thoroughly to initiate crosslinking reactions. The aforementioned solutions were prepared at 4 °C.

2.2. Characteristics of P-CS and P-CS/GA solutions

2.2.1. Measuring viscosity of solutions

To measure the viscosities of the P-CS and P-CS/GA solutions, 1 ml of each solution was added to a cone/plate viscometer (Rheometer RS-100, HAAKE Mess-Technik, Karlsrhue, Germany), with a cone angle of 1°. The viscosities of the solutions were continuously measured at 30 °C at a shear rate of 75 s^{-1} using a computer-controlled program, and data were automatically recorded from 60 to 900 s. The effects of various shear rates on the viscosities of P-CS or P-CS/GA solutions were examined by making aforementioned measurements but with the shear rates varied from 30 to 300 s^{-1}.

2.2.2. Swelling ratios and dissolution of gels

The swelling ratios of the gels were determined in a manner similar to that employed in our earlier works, but with some modifications (Liu et al., 2004). To determine the swelling ratios of P-CS gel, 1 g of the gel in a stainless steel-mesh holder was immersed in a vial that contained 30 ml of dissolution medium (such as water), and shaken at 80 rpm at 37 °C. The weights of the gels were periodically measured after the water on the surfaces of the gels had been gently wiped away. After each measurement had been made, the gel was returned to the vial with a fresh dissolution medium to make a new measurement. The swelling ratio of a gel was defined as the ratio of the increase in the weight of the hydrated gel at each measurement time to the original weight of the gel (Liu et al., 2004). To study the swelling ratios of the P-CS/GA gels with various concentrations of GA, the P-CS solutions at 4 °C were first crosslinked with GA at a particular crosslinking duration, as described below, and then the temperature was increased to LCST (34.5 °C) to form the gel, which was then placed in the aforementioned holder to conduct the swelling experiments following the procedure that was used for other gels.

When the crosslinking times of P-CS/GA solutions were too long, the solutions would transform into thermo-irreversible P-CS/GA gels at LCST, in a manner that was nevertheless influenced by GA concentrations in the solutions. To evaluate the duration of the thermo-reversibility of P-CS/GA gels, a series of tests such like a glass capillary method (Dumortier et al., 2006) were performed that involved heating the solutions to gels and then cooling the gels to solutions repeatedly with a cycle time of 5 min. The results indicated that P-CS/GA solutions/or gels with 0.05%, 0.075% and 0.1% GA lost their thermo-reversibility at approximately 40, 30 and 20 min, respectively. The aforementioned a particular crosslinking duration for P-CS/GA solutions with 0.05%, 0.075% and 0.1% of GA were therefore set to 30, 20 and 10 min, respectively.

2.3. sangeeta In vitro release and diffusion mechanism of 5-FU from P, P-CS and P-CS/GA gels

The procedures for investigating the release of 5-FU (0.3 wt.%) from gels were similar to those employed in the authors' earlier works, but with a few modifications (Liu et al., 2004). To investigate the release of 5-FU (0.3 wt.%) from P-CS/GA gels, the gels were placed inside an osmosis membrane with a cut-off molecular weight of 300 kDa (Spectrum medical industries Inc., USA); suspended in vials that contained 30 ml of distilled water as the dissolution medium, and shaken at 60 rpm at 25 °C. 0.5 ml of dissolution medium was periodically drawn out to determine the 5-FU concentration using a UV/VIS spectrometer (Jasco-530, Kobe, Japan) at a wavelength of 266 nm (El-Sherbiny et al., 2005; Tasdelen et al., 2005). The dissolution medium was replaced with the same volume of fresh medium.

Although numerous simple mathematical models describe the release of drugs from swellable polymeric systems, including gels (Peppas et al., 1980; Ritger and Peppas, 1987), they do not fully predict all associated experimental observations. Generalized empirical equations such as the power law model have been extensively employed to evaluate drug release from swellable systems (Ritger and Peppas, 1987). In this work, the empirical power law equation, Eq. (1), is applied to analyze the release of 5-FU from various formulations of gels:

$$M_t/M_\infty = kt^n \quad (1)$$

where M_t/M_∞ is the fractional release of the drug at time t; 'k' is the characteristic constant of the drug–gel systems; and 'n' is the diffusion exponent characteristic of release mechanism. The equation applies until 60% of the drug has been released and effectively describes the release of the drug from discs of gels and other swellable materials, as well as that from non-swellable matrices. For evaluating the release mechanism of 5-FU from each gel, the k values were calculated according to the conditions limited by the equation.

All calculations were made using Sigmastat statistical software (Jandel Science Corp., San Rafael, CA, USA). In a Student t-test and

ANOVA, a confidence level of at least 95%, corresponded to statistical significance ($P < 0.05$). Data are presented as mean ± standard deviation, having been measured at least in triplicate.

3. Results

3.1. Effect of GA concentration on thermo-sensitivity of P-CS/GA gels

The LCST of thermo-sensitive P gels depends on the ratio of P407 (or F127) to P188 (or F68) in the gel (Dumortier et al., 2006). In this investigation, the P solution comprises F127/F68 (18 wt.%/15 wt.%), yielding an LCST value of 34.5 °C. The LCST value of the P-CS gels was only slightly affected by the low concentrations of CS, such as 0.3–2.0 wt.% (data not shown). In contrast, adding GA (0.05–0.10 wt.%) to P-CS solutions (such as with 1.0 wt.% CS) would finally transform the P-CS/GA solutions to a non-thermo-sensitive gels at LCST after a period of time. Additionally, adding high concentrations of GA to P-CS solutions shortened the period for which the thermo-sensitivity of the P-CS/GA gels could be maintained. Based on our tests, the thermo-sensitive characteristics of P-CS/GA solutions/gels with 0.05 and 0.10 wt.% GA were maintained for approximately 40 and 20 min, respectively, at 25 °C.

Fig. 1a schematically depicts the P/CS solutions. The crosslinking reactions between CS and GA in P-CS/GA solutions are assumed to form a CS crosslinked network (Fig. 1b). CS polymers or CS solutions at a high concentration emit broad fluorescence when they are excited by light with a particular wavelength. However, the emission of fluorescence by P-CS gels could not be observed using a laser confocal microscope (data not shown). Interestingly, P-CS/GA gels emit green fluorescence, and fluorescent images of entangled CS polymers or CS network-like configurations formed within the gels were captured as viewing angles of 0° and 90° (Fig. 1c and d). Moreover, the thickness of the entangled region of CS polymers or the CS network-like configurations formed in the gels is approximately 18.68 μm. The formation of an interpenetrating CS network in P-CS/GA gels (Fig. 1) might change their flow and drug release properties.

3.2. Effects of interpenetrating CS network on viscosities of P-CS/GA solutions

The viscosities of the P and P-CS solutions were about constants at various shear rates (data not shown), suggesting that their rheological behaviors are Newtonian fluids (Dumortier et al., 2006) at a tested temperature. Moreover, the viscosity of the P-CS solution increases with temperature in a manner that is similar to the rheological behavior of the P solution (data not shown) (Dumortier et al., 2006). For example, the viscosities of the P-CS solution increased from 145.0 through 350.0–448.1 mPa as temperature of the solution increased from 25 through 32–34.5 °C (at LCST of the P-CS gel). Fig. 2 presents the viscosities of P-CS and P-CS/GA solutions at 30 °C with various concentrations of GA measured from 60 to 900 s. Interestingly, at 30 °C, the viscosity of the P-CS solution was approximately three times that of the P solution, possibly because the entangled CS polymers (Fig. 1c) would hinder the flow of micelles in the P-CS solution. The viscosities of the P-CS/GA solutions increased with time, and the rates of increase of viscosities were positively correlated with the amounts of GA in the solutions (Fig. 2). For instance, the viscosities of the P-CS/GA solutions of 0.05 and 0.1 wt.% GA were 408.4 and 425.5 mPa, respectively, at 60 s, increasing to 463.4 and 575.4 mPa at 600 s (Fig. 2). According to the principles of chemical reaction kinetics, either prolonging the reaction times between GA and CS polymers or increasing the con-

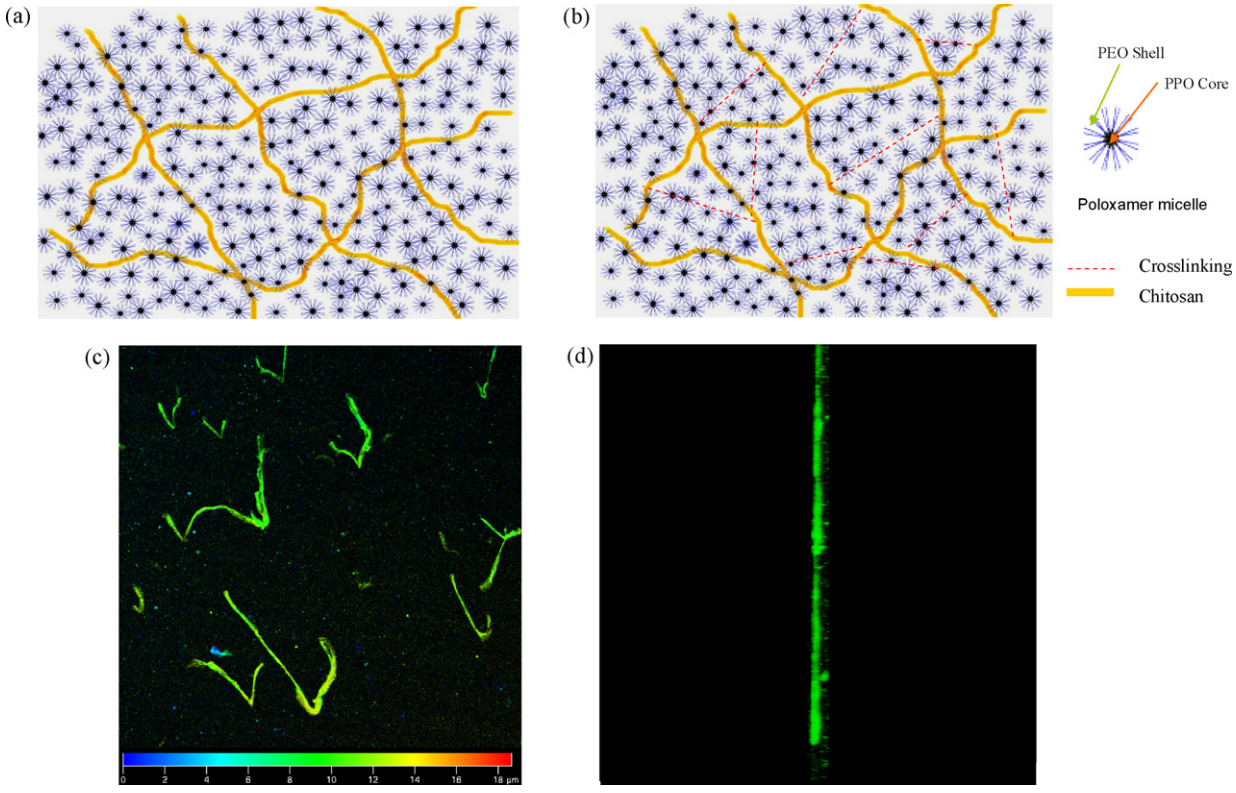

Fig. 1. (a and b) Hypothetical schematic diagrams of P-CS and P-CS/GA gels, respectively. (b) CS polymers are assumed to be crosslinked by GA to form a CS network. (c and d) Fluorescent photographs of P-CS/GA gels captured using a laser confocal microscope (Zeiss LSM 510) at viewing angles of 0° and 90°, respectively. The entanglements of CS polymers or CS network-like configurations (green color) with a thickness of 18.68 μm are observed in the gels (20×). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

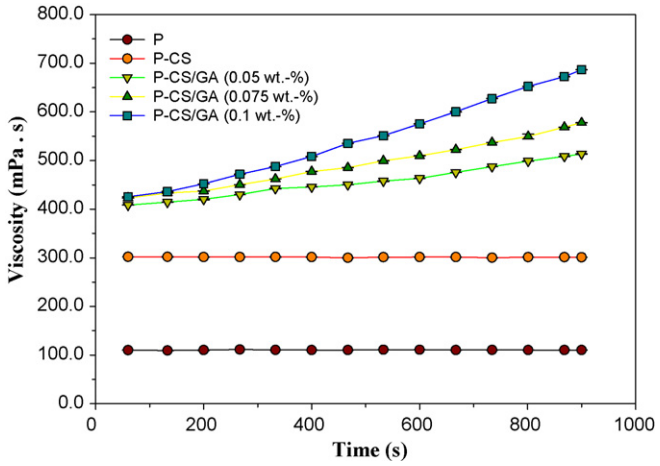

Fig. 2. Viscosity of P, P-CS and P-CS/GA solutions with various GA concentrations (0.05, 0.075 and 0.1 wt.%, respectively) as function of time. Viscosity of each solution was measured at a shear rate of 75 s^{-1} and 30 °C. Viscosity of P-CS/GA solution increased with GA concentration or the duration of measurements. In contrast, the viscosity of P or P-CS solution remained constant during measurements.

centrations of the cross-linker and also a reactant, GA, respectively, would increase the denseness of CS network in P-CS/GA solutions. Increasing viscosities of P-CS/GA solutions with increasing reaction times between GA and CS polymers, and GA concentrations (Fig. 2), respectively, would be due to increasing denseness of interpenetrating CS network in P-CS/GA gels (Fig. 1b) that hindered shear flow of the solutions.

3.3. Effects of CS network on swelling ratios of P-CS/GA gels

In aqueous state, the dissolution of P gel (or packed micelles) is well known to proceed rapidly (Lin and Sung, 2000; Jeong et al., 2002) since the inflow of water causes the rapidly dissolves the gels. For example, 25 wt.% of Poloxamer 407 gel was completely dissolved in a release medium *in vitro* for 4 h (Lin and Sung, 2000). Without further processing, the swelling ratios of P and P-CS gels in this work were very low (data not shown), as reported elsewhere (Lin and Sung, 2000; Jeong et al., 2002). Interestingly, the P-CS/GA gels absorbed much water than the P-CS gels, and so had higher swelling ratios (Fig. 3). The maximum swelling ratios of the P-CS/GA gels with 0.05, 0.075 and 0.1 wt.% GA were 7.1 ± 0.7, 12.5 ± 0.3 and 13.2 ± 1.0 (in wt.%, $n = 3$) times those of the P-CS gels, respectively, although the time to reach the maximum ratios varied with the amount of GA in the gels (Fig. 3). For instance, P-CS/GA gels with 0.05, 0.075 and 0.1 wt.% GA took 2, 8 and 24 h, respectively, to reach the maximum swelling ratio (Fig. 3). Notably, P-CS/GA gels with 0.075 and 0.1 wt.% GA maintained their maximum swelling state for around 6 and 18 h, respectively, until they were significantly dissociated, meaning that the weight had decreased by at least 5% from its maximum in the medium. However, the gel with only 0.05 wt.% GA could not maintain the maximum swelling state for any time (Fig. 3). Interestingly, the periods for which P-CS/GA gels can resist dissolution were also strongly related to the amount of GA that they contained. For example, the times required for dissolution of the gels with 0.075 and 0.1 wt.% of GA to points where their maximum swelling weight was 50% dissolved were approximately 22 and 66 h after they reached the maximum weights, respectively.

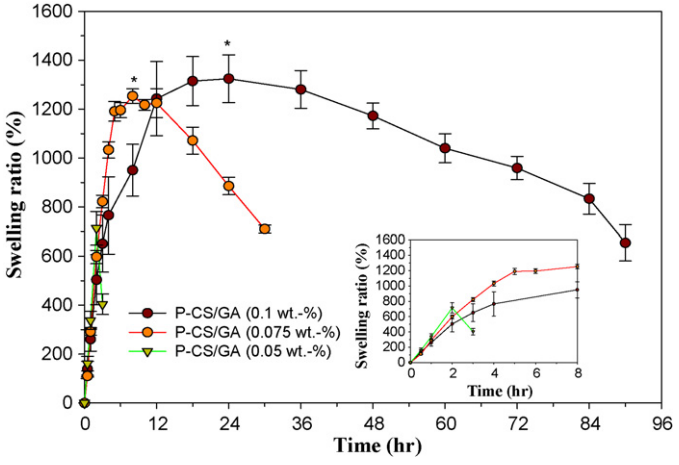

Fig. 3. Swelling ratios of P-CS/GA gels with GA at concentrations of 0.05, 0.075 and 0.1 wt.%, as functions of time. The times required to reach the maximum swelling ratios of these gels were approximately 2, 8 and 24 h, respectively, following hydration in water. The measurements were terminated when the gels were dissociated to 50% of their maximum weight.

3.4. Release of 5-FU from P-CS or P-CS/GA gels

Fig. 4 plots the *in vitro* cumulative releases of 5-FU from P, P-CS and P-CS/GA gels with various concentrations of GA. When 5-FU solution was present only in membranes, the burst release of a large amount of 5-FU was observed at 0.5 h, ending within 1.0 h (Fig. 4). The burst releases of 5-FU trapped in P and P-CS gels were a little lower than those of 5-FU solution, and the releases were sustained for 3.0 and 5.0 h, respectively. Interestingly, the duration of release of 5-FU from P or P-CS gel was similar to that from thermo-responsive hydrogel such as poly[N-acryloyglycine-chitosan] hydrogels (El-Sherbiny et al., 2005). Notably, the burst releases of 5-FU from P-CS/GA gels were significantly ($P < 0.01$, $n = 3$) lower than those from P-CS gels (from 59.0 ± 0.8% to 20.6 ± 0.2% at 1.0 h for gels with 0.1 wt.% GA) and the duration of those releases of 5-FU was extended significantly to 52 h, independently of the GA concentrations in the gels (Fig. 4). However, the release profiles and the total amounts of 5-FU released were somewhat affected by the GA concentrations in the P-CS/GA gels. For instance, 91.4% of the 5-FU was released from the gel with 0.05 wt.% GA while 84.7% of that was released from the gels with 0.075 and 0.10 wt.% GA. The durations of release of 5-FU from P-CS/GA gels were approximately 10 or more times of that from P or P-CS gels (Fig. 4).

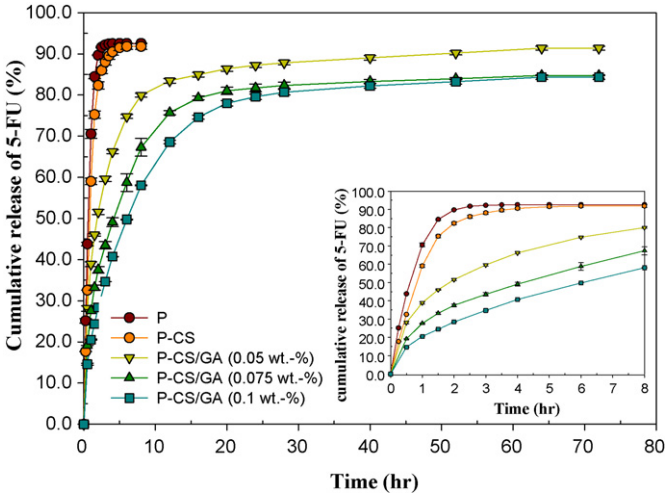

Fig. 4. *In vitro* cumulative release of 5-FU from gels of P, P-CS and P-CS/GA with various concentrations of GA. Burst releases of 5-FU from P and P-CS gels were significantly lower ($P < 0.01$, $n = 3$) in P-CS/GA gels with 0.075 and 0.1 wt.% GA. The duration of 5-FU releases in P-CS/GA gels were markedly sustained to 52 h, which is approximately 10 the period in P or P-CS gel.

Table 1
Gel characteristic constant '*k*' for release of 5-FU from drug-loaded gels with various formula are presented according to the plot of $M_t/M_\infty = kt^{0.5}$. The values are expressed in $k \times 10^2$.

Drug	Gel				
	P	P-CS	P-CS/GA (0.05 wt.%)	P-CS/GA (0.075 wt.%)	P-CS/GA (0.1 wt.%)
5-FU	12.7	11.7	4.30	3.40	3.10

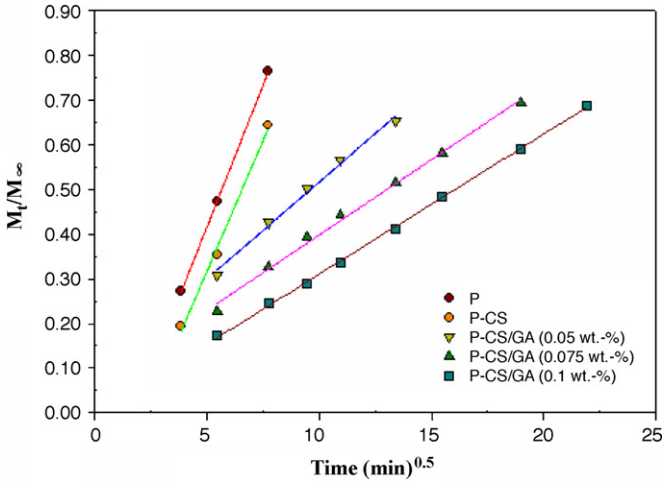

Fig. 5. Plots of M_t/M_∞ versus t^n; $n = 0.5$, the best-fitted value of n with a coefficient of correlation, R^2, of over 0.99, for evaluating the fractional release of 5-FU from the drug-loaded P, P-CS and P-CS/GA gels, the latter with various concentrations of GA.

3.5. Diffusion mechanisms of 5-FU from gels

According to the best fitting for the release of 5-FU presented in Fig. 4, '*n*' value for Eq. (1) (stated in Section 2.3) is 0.5. Fig. 5 plots M_t/M_∞ against $t^{0.5}$ for the fractional release of 5-FU from drug-loaded P, P-CS and P-CS/GA gels with various concentrations of GA. Moreover, Table 1 presents the '*k*' value for each gel. Moreover, to examine the possible correlation between the '*k*' values of gels and viscosities, η, of P, P-CS and P-CS/GA solutions in Fig. 2, the regression line of '*k*' values versus η (the η values at 900 s of Fig. 2) were calculated. Interestingly, the regression line was 'k' = $15.7–2.0 \times 10^{-2} \times \eta$, with a correlation coefficient, R, about 0.96, indicating that the release characteristics of the gel decreased with increasing the viscosity of the correspondent solution.

4. Discussion

Systems that provide the sustained release of drugs are receiving much attention from the pharmaceutical industry because they provide several advantages over conventional drug delivery systems. These systems may prolong drug action, reduce side effects and reduce the frequency with which drugs need to be administered. Numerous pharmaceutical researchers are interested in various potential applications of drug delivery using thermo-sensitive P gels (Liu and Chu, 2000; Amiji et al., 2002; Roques et al., 2007; Caffaggi et al., 2008). However, P gels such as Poloxamer 188 and 407 generally sustain the release of drugs for a short period in an aqueous environment (Barichello et al., 1999; Jeong et al., 2002; Ricci et al., 2005) because Poloxamer gels dissociate rapidly in an aqueous environment. The maximum duration of drug release is typically limited by the influx of water into the P gels, which dissolves the gel. The objective of the newly developed P-CS/GA gels is to reduce the rate of dissolution and prolong the 5-FU release period.

4.1. Fluorescent images of interpenetrating CS networks in P-CS-GA gels

Reactions between the amine groups of CS and the aldehyde groups of GA are well known to occur in P-CS/GA solutions (Wang et al., 2008), forming an assumed CS-crosslinking network (Fig. 1b). To the best of the authors' knowledge, no microscopic image of the assumed interpenetrating CS networks in gels has been presented. This work provided fluorescent images of the entanglement of CS polymers at different viewing angles (0° and 90°). They had a thickness of approximately 18.68 μm. They partly depicted the entanglement of CS polymers or the CS network-like configurations that formed within P-CS/GA gels (Fig. 1c and d). In contrast, no fluorescence was observed from P-CS gels without GA crosslinking reactions under a laser confocal microscope (data not shown). The fluorescent images partially revealed the presence of a CS network in the P-CS/GA gels that strongly influenced the characteristics of the release of 5-FU from the gels (Figs. 4 and 5). This crosslinked network may become denser as the crosslinking time increases. As the transition from a solution to the gel state occurs at LCST, the crosslinked CS network interpenetrates the P-CS/GA gels, preventing the aggregates of P micelles or P gels from being broken down to form free P micelles when the temperature is below LCST, finally losing the thermo-sensitivity of the gels. To avoid any possible difference between the thermo-sensitive and non-thermo-sensitive P-CS/GA solutions, thermo-sensitivity was maintained while the viscosity was being measured.

4.2. Interpenetrating CS network affects viscosity and swelling ratios of P-CS/GA solutions and gels

The increase in the viscosity of the P-CS/GA solution may have been caused by the formation of the CS network within it, which hindered the flow of the solution (Fig. 2). Furthermore, a higher concentration of GA in P-CS/GA solutions was associated with the formation of a denser CS network, possibly because of higher reaction rates, and therefore, a markedly increasing resistance to the flow of the solutions, which is responsible for a considerably high viscosity of them (Fig. 2).

Grafting carbohydrates, such as hyaluronic acid or chitosan onto amine-terminated or mono-carboxyl Poloxamers, can reduce the critical gelation concentration and the dissolution rates of the gels in aqueous environments (Cho et al., 2003; Chung et al., 2005). Covalently crosslinking ethoxysilane-capped PEO blocks of Poloxamers also prevents the rapid dissolution of gels in water (Sosnik and Cohn, 2004). In this work, without the aforementioned sophisticated chemical modifications, the dissolution times of P-CS/GA gels in aqueous environments were significantly longer than P gels (Fig. 3). The high swelling ratios of GA-containing gels may be associated with the CS network that interpenetrates the gels, and binds the aggregated P micelles/gels in compartments during hydration, thereby preventing the rapid dissolution of the gels.

4.3. Sustained release of 5-FU from P-CS/GA gels

The durations of the release of 5-FU in P-CS/GA gels (Fig. 4) exceeded those in chitosan-based or other thermo-sensitive hydrogels under similar release conditions in an *in vitro* investigation

(El-Sherbiny et al., 2005; Tas-delen et al., 2005; Lin et al., 2007). The highly sustained releases of 5-FU in P-CS/GA gels were due to reducing the diffusion rates of 5-FU from the core of the aggregated P micelles/gels (Fig. 5) that were trapped within the compartments of CS networks (Fig. 1b). However, details of the mechanisms by which the release of 5-FU from P-CS/GA gels were sustained must be investigated in the future.

Results concerning diffusion mechanism reveal that the releases of 5-FU from gels of various formulae follow the Fickian diffusion process. For the releases of 5-FU from P-CS/GA gels, k values declined as the GA concentrations in gels increased (Fig. 5). In addition, Fig. 2 presented that increasing GA concentrations in P-CS/GA solutions resulted in increasing their viscosities which might be resulted from increasing the denseness of interpenetrating CS networks within the solutions and gels. According to the Einstein equation, molecular diffusion coefficient is function of the viscosity of solution/gel. 'k' values of P-CS/GA gels declined as the viscosities of the solutions increased which were demonstrated in a linear regression line of 'k' versus viscosities of the solutions, η (presented in the end of Section 3.5). Therefore, the viscosities of the P, P-CS and P-CS/GA solutions with various concentrations of GA play an important role in affecting the release characteristics of 5-FU from the gels.

5. Conclusion

New thermo-sensitive P-CS/GA gels were designed, and the effects of the GA crosslinking CS network, which interpenetrated the gels, on their swelling ratios and drug release characteristics were examined. P-CS/GA gels with 0.1 wt.% of GA had a swelling ratio of up to 13.2 ± 1.0, which was maintained for approximately 18 h. The *in vitro* release of 5-FU in P-CS/GA gels with 0.1 wt.% of GA significantly reduced the burst release of the drug ($P < 0.01$) from the gels, and greatly sustained the release of 5-FU ($P < 0.01$) for up to 52 h, which was about 10 times of the duration of 5-FU release in P gels. The mechanisms of release of the drug for various formulations of the gels were modeled by Fickian diffusion, and the characteristic constant 'k' of the drug–gel systems declined as increasing the GA concentrations within the P-CS/GA gels, and increasing the viscosities of the P, P-CS and P-CS/GA solutions.

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