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Diffusion and binding of 5-fluorouracil in non-ionic hydrogels with interpolymer complexation

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ABSTRACT

Hydrogen-bonded interpolymer complexes can be used for development of novel dosage forms. In this study, two types of crosslinked hydrogels, copolymer networks of *N*-vinyl pyrrolidone and acrylamide (PVP-co-PAM) and interpenetrating polymer networks (IPN) composed of crosslinked PVP-co-PAM and poly(vinyl alcohol) (PVA), were synthesized at three different degrees of crosslinking. The side chain groups in such polymers can form non-ionic complexes through H-bonding, resulting in additional "crosslinks" in the hydrogels. Both kinds of hydrogels have significantly larger swelling sensitivities than the networks formed with ionizable side chains. In the IPNs, introduction of the PVA chains into the PVP-co-PAM networks raises the permeability, indicating more open pores. The permeability decreases with the increasing degree of crosslinking of the copolymer. For probing the drug binding in the hydrogels, Fourier transform infrared spectra (FTIR) difference spectroscopy indicated the presence of significant H-bonding interactions between 5-fluorouracil (5-FU) and the side chains of the polymers. Such interactions are larger in the PVP-co-PAM copolymers than in the IPN hydrogels, thereby causing an additional source of the slower release kinetics in the copolymer hydrogels as revealed by the Peppas model, albeit both types of the networks followed a non-Fickian transport mechanism.

1. Introduction

Considerable interest has been devoted to development of chemically crosslinked hydrogels as drug carriers (Tanaka et al., 1980; Vervoort et al., 1998; Qiu and Park, 2001; Lin and Metters, 2006). The controlled drug delivery devices based on chemically crosslinked hydrogels can assure a sustained release in targeted areas (Griffith, 2000) and offer good biocompatibility and improvement in patient compliance (Park, 1993; Peppas and Brazel, 1994).

Hydrogels can also be generated by forming polymer/polymer interactions without covalent crosslinking (Noble et al., 1999; Ozeki et al., 2005). For example, interpolymer complexes between poly(*N*-vinyl pyrrolidinone) (PVP) and poly(acrylic acid) (PAA) have been investigated in order to develop new mucoadhesive drug carriers by taking the advantages of interactions between the carboxyl groups of PAA and the carbonyl groups of PVP (Chun et al., 2002). Majority of the reported interpolymer complexes involve the use of an ionizable polymer as a component, e.g. polyacrylic acid, especially for pH responsive drug delivery (Berger et al., 2004; Khutoryanskiy, 2007; Ozeki et al., 2005; Park et al., 2008). The electrostatic polymer/polymer interactions take place between the ionizable groups of the polymers, and hence, the release of non-ionized drugs can be controlled by the swelling/eroding ratio, whereas the release of ionized drugs can be controlled by electrostatic polymer/drug interactions and drug diffusion is retarded as the ionic interactions augment within the network structure (de la Torrea et al., 2003).

In practice, interpolymer complexes can be formed by homogeneously mixing two polymers with non-ionic side chains and yet absorb larger quantities of water in aqueous solutions than those with ionizable side chains. Such non-electrostatic complex systems have not been well studied for drug carrier applications, partly because the forces purely based on hydrogen bonding (H-bonding) and van der Waals interactions become much weaker. However, their tendency to disassociate in biological fluid can be overcome by introducing so-called interpenetrating polymer network structures (IPN). IPNs are typically composed of two polymer networks, which are interlaced on a molecular scale. IPNs can attain a combination of properties of two component polymers, resulting in the amphiphilicity valuable for drugs with different hydrophilicities (Bae et al., 1991; Bae and Kim, 1993; Katono et al., 1991; Agnihotri and Aminabhavi, 2005, 2006; Kulkarni et al., 2001; Kurkuri and Aminabhavi, 2004). Interpenetration of two networks may also result in porous structures for transport of

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drug molecules with different sizes and better stability to different environment (such as variable pH and salt levels) than the individual homopolymer network. IPN hydrogels with networks of opposing or complementing properties (P(MAA-g-EG)) and hydrophobic IPNs (poly(butyl acrylate)) have been developed for oral chemotherapy delivery (Liechty et al., 2011). The non-ionic interpolymer complex augmented IPN hydrogels should serve as a versatile drug delivery system once the drug release kinetics and drug/polymer interactions are better understood.

At present, the effect of drug/polymer interactions on drug release kinetics has not been well investigated. Previous studies (am Ende et al., 1995; Peppas and Wright, 1998; Alvarez-Lorenzo and Concheiro, 2002) have focused on the presence of ionic interactions between the loaded drug and the polymer chains and their influence on the release rate from such systems. For example, am Ende et al. (1995) reported that that a cationic solute oxyprenolol strongly interacted with ionic hydrogels and showed increased hindrance in the drug transport from an anionic solute at high pH. Other studies (Yu and Grainger, 1995; Wu et al., 2005) suggest hydrophobic binding between the substituents on the drug molecules used, although the implication for drug release and hydrogel swelling was not apparent. Coughlan reported that direct interactions between vitamin B12 (or diltiazem) and poly(*N*-isopropylacrylamide) (PNIPA) hydrogels were insignificant, and only hydrophobic binding between PNIPA and benzoic acid was observed (Coughlan and Corrigan, 2006). Recently, we showed significant intermolecular interactions between vitamin B12 and crosslinked PAA and PAA–PVP copolymer hydrogels (Jin et al., 2009).

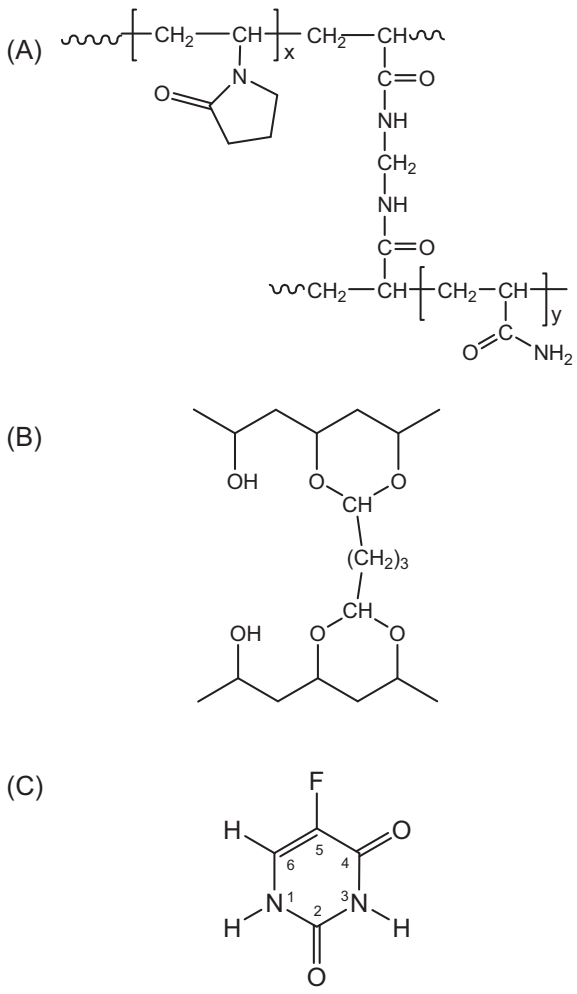
In this study, non-ionic copolymer based hydrogels chemically crosslinked are investigated for controlled release of 5-fluorouracil (5-FU). 5-FU has been used as a chemotherapy drug for decades. It is an active medicine against many cancers and effectively blocks the replication of DNA viruses (Presant et al., 1994; Schroeder et al., 1990). 5-FU is also used as antibacterial (Garrett et al., 1977) and antiviral drugs (Agudo et al., 2008; Kim et al., 1992; Pariente et al., 2001). For targeted delivery of 5-FU, a controlled release of 5-FU can effectively inhibit tumor growth and metastases (Jin et al., 2011; Wolinsky et al., 2012). Crosslinked copolymers of *N*-vinyl pyrrolidone (NVP) with acrylamide (AM) (PVP-co-PAM) and IPNs consisting of crosslinked polyvinyl alcohol (PVA) and PVP-co-PAM copolymer were synthesized and their interactions with 5-FU during diffusion were compared in the present study. The copolymer and IPN hydrogels can form intra- and inter-polymer complexes between their side chain groups. H-bonding interactions between 5-FU and the hydrogels are significantly present as probed by Fourier transform infrared spectra (FTIR) difference spectroscopy.

2. Materials and method

2.1. Materials and instrumentation

Analytical grade glutaraldehyde (25% aqueous solution), AM, potassium persulfate, 5-FU, *N*,*N*′-methylenebis(acrylamide) (MBAM), and PVA with a number averaged M_n = 22,100, weight averaged M_w = 70,000, polydispersity index PDI = 2.79 (as determined by gel permeation chromatography) and a degree of saponification > 99.8%, available from Sinopharm were used as received. *N*-vinyl pyrrolidone (NVP) monomer was obtained from Hangzhou Nanhang Co.

UV–visible absorption spectra were recorded on HP8453 spectrophotometer. FTIR were collected on Thermo Nicolet Nexus 310 infrared spectrometer.



Scheme 1. (A) Structure of vinyl pyrrolidone unit, acrylamide unit and crosslinking by MBAM in the PVP-co-PAM network; (B) structure of vinyl alcohol units crosslinked by glutaraldehyde in the PVA network; (C) atomic labeling in the 5-FU structure.

2.2. Preparation of crosslinked PVP-co-PAM and PVP-co-PAM/PVA IPN hydrogels

2.0 g of NVP (18 mmol), 1.0 g of AM (14 mmol) and 0.15 g of MBAM (1.0 mmol) were dissolved in 30 mL of water and mixed thoroughly with the initiator potassium persulfate (20 mg). The mixture was heated for 6 h at 70 °C to form copolymer gels (see Scheme 1A for the structure). After cooling, the gels were repeatedly soaked in distilled water for 4–5 h to remove residues of unreacted reagents, and dried in vacuum at 60 °C for 5 h to form crosslinked homogeneous membranes with constant weights and a nominal degree of crosslinking (XR) of 6.0%. The XR was defined as the ratio of moles of crosslinker MBAM per mole of total polymer repeating unit, in percentage. Similar procedures were used to obtain hydrogel membranes with the same fixed NVP:AM ratio, but with XR = 3.0% and 10.0%.

IPN hydrogels were prepared by crosslinking linear PVA polymers to form a network structure (see Scheme 1B for the structure) while AM and NVP monomers and related initiator and crosslinker were swollen into the network and polymerized and crosslinked among the crosslinked PVA to form an interpenetrated network. Thus, 2.0 g of NVP (18 mmol), 1.0 g of AM (14 mmol) and 0.15 g MBAM (1.0 mmol), were dissolved in 30 mL of water. 13 g of PVA aqueous solution (11% by weight, approximately containing 32.5 mmol of VA), 0.50 mL of glutaraldehyde (1.25 mmol), and

20 mg of potassium persulfate were added and mixed homogeneously. The mixture was heated for 6 h at 70 °C to form an IPN gel, which was cooled, repeatedly washed in water for 3–5 h, and dried in vacuum at 60 °C for 5 h to form uniform IPN membranes with constant weights. The nominal degree of crosslinking of the PVA chain is 7.7% while that of the vinyl pyrrolidone–acrylamide copolymer chain is 6.0%. Similar methods were used for preparation of IPN gels with the degree of crosslinking of the vinyl pyrrolidone–acrylamide copolymer chain XR = 3.0% and 10.0%, but that of the PVA chain fixed at 7.7%.

2.3. Characterization

The equilibrium swelling ratios ESR% were calculated after the dried gels were swollen completely with water and their weights (m_e) were measured to calculate the absorbed water weight by subtracting the dried gel weight (m_d):

$$\text{ESR}\ (\%) = \frac{m_e - m_d}{m_d} \times 100\% \quad (1)$$

Permeation test was performed by using a horizontal Valia–Chien cell assembly held on a magnetic stirrer. A hydrogel film was sandwiched between two circular windows, each on a half-cell positioned side-by-side. 300 mL of phosphate buffer (50 mM, pH 7.0) water were added to fill the receptor half-cell. The donor half-cell was filled with 300 mL of a 5-FU solution (0.5 mg/mL, in 50 mM phosphate buffer, pH 7.0). 10 mL of the permeated solution were withdrawn from the receptor cell at a time interval of every 1 h and 10 mL of distilled water was added immediately to compensate the receptor solution. The thickness of a hydrogel film is not important in the permeation test. Higuchi and co-workers have discussed the relationship between the permeability coefficient P and the drug concentration c at time t in the receptor cell (Corrigan et al., 1980). Only the hydrogel film area, liquid volume in the Valia–Chien cell, and drug concentrations are needed to calculate the permeability coefficients, as described in Eq (2) in the text below.

In order to determine the concentration of 5-FU, 10 mL of 5-FU solutions were prepared in phosphate buffer (50 mM, pH 7.0) solution at different concentrations (x, in 10^{-6} g/mL), and the absorbance values (y) at 268 nm were measured to obtain a standard calibration line $y = 0.0644x - 0.0201$, with a linear regression coefficient of $R^2 = 0.9986$.

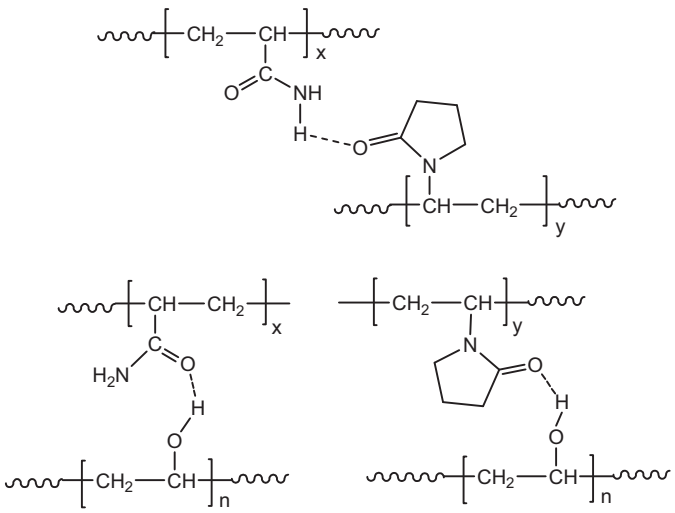
In the binding study, FTIR spectra were obtained from the vacuum dried hydrogel membranes before and after 5-FU had diffused in the above permeation tests. The FTIR spectrum of the 5-FU-free membrane was subtracted from that of the permeated membrane in order to cancel out the spectroscopic signals of the membrane material. The resulting difference spectrum shows the spectral features that are attributed to those of 5-FU in the membrane, as well as the features that are ascribed to the side chain groups of the polymers tightly bound to 5-FU, therefore giving the perturbed spectral bands of the polymers that cannot be subtracted out.

3. Results and discussion

3.1. Diffusion of 5-FU in crosslinked PVP-co-PAM and PVP-co-PAM/PVA IPN hydrogels

Hydrogels consisting of crosslinked copolymers of PVP and PAM were first synthesized by polymerization of *N*-vinyl pyrrolidone (NVP) with AM at a fixed molar ratio of 9:7 but at different degrees of crosslinking (XR = 3.0%, 6.0% and 10.0%) (see the structure in Scheme 1A). In comparison, these hydrogels were also transformed into IPN hydrogels by introducing crosslinked polyvinyl alcohol chains with a fixed degree of crosslinking XR = 7.7% (see the crosslinked PVA structure in Scheme 1B). The copolymers can form intrapolymer and interpolymer complexes via H-bonding between the amide and pyrrolidone side chain groups, as depicted in Scheme 2 (top), while part of the H-bonding structures can be replaced by new interpolymer hydrogen bonds between the hydroxyl side groups of the PVA chains and the amide or pyrrolidone groups of the copolymer chains in the IPN hydrogels, as shown in Scheme 2 (bottom). These H-bonding structures can be regarded as "crosslinkages" in addition to the covalent bond crosslinkages in the hydrogels. The copolymer and the copolymer/PVA IPN hydrogels are flexible membranes with a strong water absorption capability. The nominal crosslinking degree, XR, defined as the ratio of molar amount of crosslinker MBAM to that of polymer repeating unit, in percentage, varies only from 3.0% to 10.0% for the PVP-co-PAM and PVP-co-PAM/PVA IPN membranes. Table 1 shows that with increasing amount of the crosslinker used in the preparation, the ESR of the hydrogels decreases significantly by 40 to 50%, resulting in the decrease of the diffusional space between the crosslinkages. Noticeably, the ESR values of the copolymer and IPN hydrogels in this study are approximately 20 times larger than those of the PVP-co-PAA copolymers with ionizable acrylic acid (AA) side chains but approximately same levels of XR reported in our previous study (Jin et al., 2009). This can be interpreted by the strong electrostatic forces (Coulomb forces) in the hydrogels with ionic groups, and indicates that the hydrogels with solely H-bonding side chains may be more swellable and hydrophilic than those with ionizable side chains.

The release profiles of 5-FU in PVP-co-PAM and PVP-co-PAM/PVA IPN are compared in Figs. 1–3, respectively. The data describe the early release kinetics of the 5-FU solute. Table 2 lists linear regression results of 5-FU concentration (c, in μg/mL) in the



Scheme 2. Formation of interpolymer complexes: (top) between vinylpyrrolidone and acrylamide repeating units in the PVP-co-PAM; and (bottom) between PVP-co-PAM and PVA in the IPN.

Table 1
Equilibrium swelling ratio (ESR, in percentage), permeability coefficient (P), and release exponent (n) of PVP-co-PAM hydrogels and PVP-co-PAM/PVA IPN hydrogels with different nominal crosslinking ratios (XR, in percentage).

Hydrogel Sample	XR (%)	ESR (%)	P (cm/h)	n
PVP-co-PAM #1	3.0	420	0.0565	0.6349
PVP-co-PAM #2	6.0	301	0.0531	0.6148
PVP-co-PAM #3	10.0	207	0.0471	0.6096
PVP-co-PAM/PVA IPN #1	3.0/7.7	398	0.0737	0.6732
PVP-co-PAM/PVA IPN #2	6.0/7.7	268	0.0683	0.6328
PVP-co-PAM/PVA IPN #3	10.0/7.7	231	0.0589	0.6131

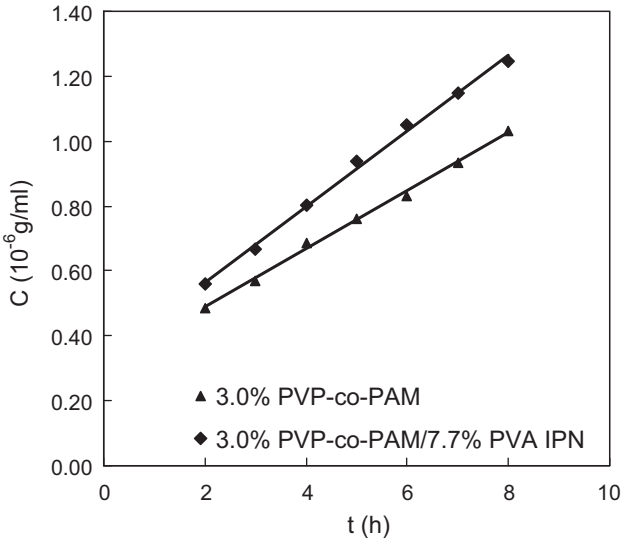


Fig. 1. In vitro release profiles of 5-FU in PVP-co-PAM copolymer hydrogel (solid triangle ▲, with XR = 3.0%) and in IPN hydrogel consisting of PVP-co-PAM and PVA (solid diamond ◆, with XR = 3.0% and 7.7% for PVP-co-PAM and PVA, respectively).

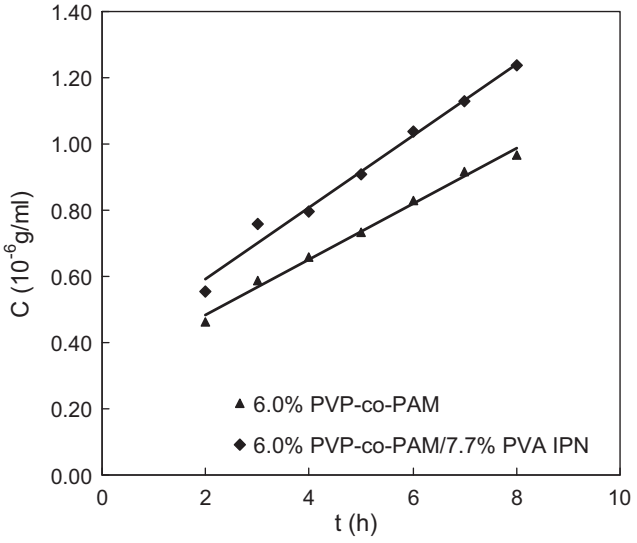


Fig. 2. In vitro release profiles of 5-FU in PVP-co-PAM copolymer hydrogel (solid triangle ▲, with XR = 6.0%) and in IPN hydrogel consisting of PVP-co-PAM and PVA (solid diamond ◆, with XR = 6.0% and 7.7% for PVP-co-PAM and PVA, respectively).

acceptor half-cell versus time (t, in h) for release in all copolymer hydrogels and copolymer/PVA IPN hydrogels prepared. The gradual decrease of the slopes of the linear relationships in Table 2 is consistent with the gradual increase of the crosslinking ratios. In each type of the hydrogels, the higher the nominal crosslinking ratio, the slower 5-FU is permeated. Furthermore, in Figs. 1–3, the

Table 2
Apparent correlation between the 5-FU concentration c in the acceptor half-cell and time t during the in vitro diffusion of 5-FU in PVP-co-PAM and in PVP-co-PAM/PVA hydrogels with different degrees of crosslinking.

Hydrogel sample (XR%)	Correlation (R^2: correlation coefficient)
PVP-co-PAM #1 (3%)	$c = 0.0895t + 0.3090$ $R^2 = 0.9966$
PVP-co-PAM #2 (6%)	$c = 0.0837t + 0.3179$ $R^2 = 0.9929$
PVP-co-PAM #3 (10%)	$c = 0.0744t + 0.2678$ $R^2 = 0.9987$
PVP-co-PAM/PVA IPN #1 (3%/7.7%)	$c = 0.1165t + 0.3336$ $R^2 = 0.9958$
PVP-co-PAM/PVA IPN #2 (6%/7.7%)	$c = 0.1082t + 0.3711$ $R^2 = 0.9836$
PVP-co-PAM/PVA IPN #3 (10%/7.7%)	$y = 0.0948t + 0.3612$ $R^2 = 0.9996$

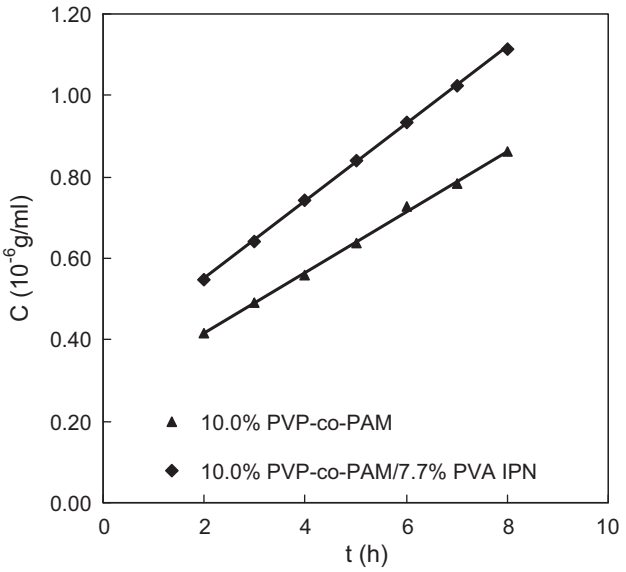


Fig. 3. In vitro release profiles of 5-FU in PVP-co-PAM copolymer hydrogel (solid triangle ▲, with XR = 10.0%) and in IPN hydrogel consisting of PVP-co-PAM and PVA (solid diamond ◆, with XR = 10.0% and 7.7% for PVP-co-PAM and PVA, respectively).

concentrations of 5-FU released from the IPN hydrogels are higher than from the copolymers, and the slope of the linear relationship listed in Table 2 for each IPN hydrogel is also higher than that of the corresponding copolymer, indicating permeation in the IPN hydrogels becomes easier than in the copolymers. In the IPN hydrogels, the molar ratio of the NVP:AM in the copolymer chain and its degree of crosslinking are the same as in the copolymer hydrogels. The hydroxyl groups in the PVA chains can form interpolymer H-bonding with the pyrrolidone and amide groups (Scheme 2 bottom), replacing some of the intrapolymer and interpolymer H-bonding between the pyrrolidone and amide groups (Scheme 2 top). Therefore the 5-FU release kinetics is controlled by the degree of the chemical crosslinking, the H-bonding interactions between the side chains, as well as the possible 5-FU/polymer interactions which will be addressed below.

Permeability coefficients (P) were calculated by using the following equation (Corrigan et al., 1980; Peppas and Wright, 1996, 1998):

$$-\ln\left(1 - \frac{2C_t}{C_0}\right) = \frac{2A}{V}Pt \quad (2)$$

where C_t is the concentration of 5-FU in the receptor half-cell at time t, C_0 is the initial concentration of 5-FU in the donor half-cell (0.5 mg/mL), V is the volume of each half-cell (300 mL), A is the effective area of the permeation window (1 cm^2), and P is the permeability coefficient of the membrane. A plot of $-(V/2A)\ln[1 - 2(C_t/C_0)]$ versus t gave the slope P and each plot for two types of the polymers are shown in Figs. 4–6, respectively. Table 1 also lists the permeability coefficients for all the hydrogel samples. The permeability coefficients P are reduced by approximately 16% to 20% when the nominal crosslinking ratio is increased from 3.0% to 10.0%, and the P values of the IPN are actually higher than those of the corresponding copolymer hydrogels at the same nominal crosslinking ratios. The introduction of the PVA chains brings about more open pores for 5-FU diffusion, which is likely due to the loss of some of the interpolymer H-bonding structures in the PVP-co-PAM chains by formation of new ones between the PVA side chains and the copolymer side chains.

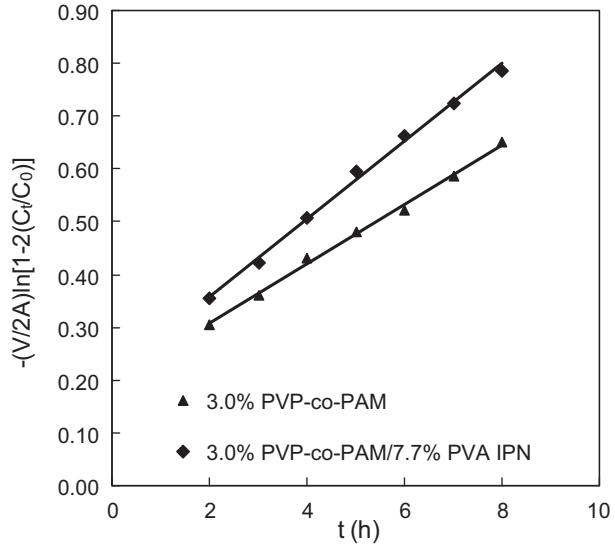


Fig. 4. Determination of the permeability coefficient through PVP-co-PAM copolymer hydrogel (solid triangle ▲, with XR = 3.0%) and through IPN hydrogel consisting of PVP-co-PAM and PVA (solid diamond ◆, with XR = 3.0% and 7.7% for PVP-co-PAM and PVA, respectively).

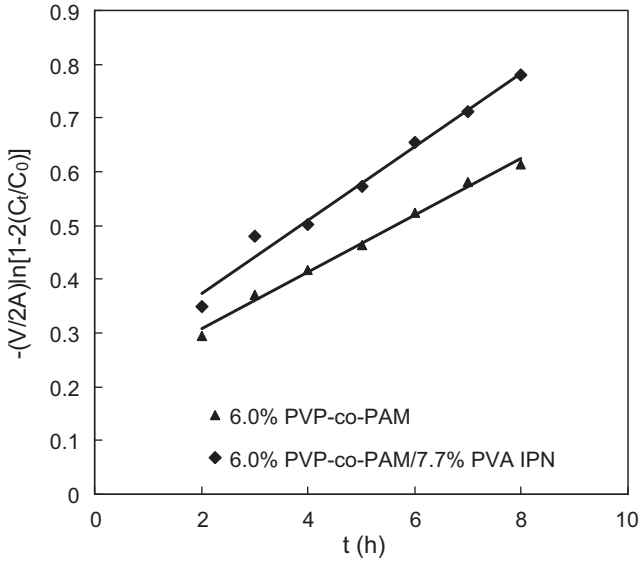


Fig. 5. Determination of the permeability coefficient through PVP-co-PAM copolymer hydrogel (solid triangle ▲, with XR = 6.0%) and through IPN hydrogel consisting of PVP-co-PAM and PVA (solid diamond ◆, with XR = 6.0% and 7.7% for PVP-co-PAM and PVA, respectively).

3.2. Cumulative release analyzed by different kinetic models

The simple empirical equation proposed by Peppas and coworkers for analyzing the solute release behavior from soluble polymer matrices can be employed for understanding the 5-FU release kinetics (Korsmeyer et al., 1983):

$$\ln M_t = n \ln t + C \tag{3}$$

where M_t is the cumulative fraction of drug released, C is the kinetic constant, t is release time and n is the diffusional exponent for drug release. The above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism (Ritger and Peppas, 1987). The log value of cumulative amount ($\ln M_t$) of 5-FU released at time t is plotted against $\ln t$. In general, if the diffusional exponent $n < 0.45$, the

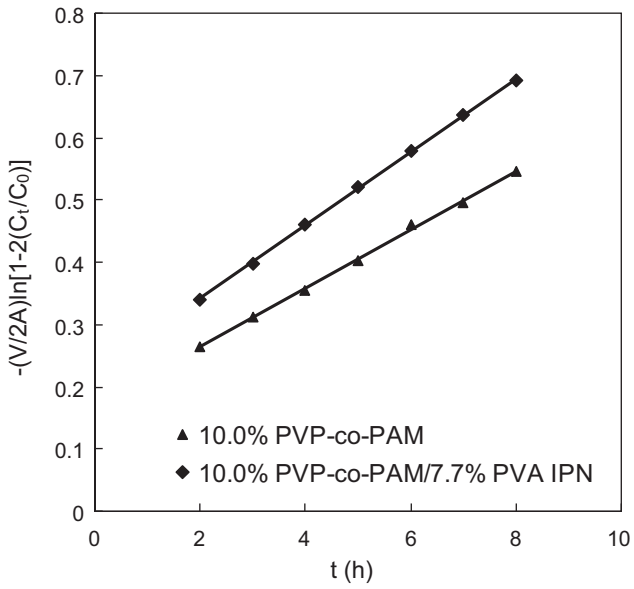


Fig. 6. Determination of the permeability coefficient through PVP-co-PAM copolymer hydrogel (solid triangle ▲, with XR = 10.0%) and through IPN hydrogel consisting of PVP-co-PAM and PVA (solid diamond ◆, with XR = 10.0% and 7.7% for PVP-co-PAM and PVA, respectively).

diffusion is Fickian; when $0.45 < n < 0.89$, the drug diffusion follows non-Fickian transport mechanism, corresponding to coupled effect of diffusion and polymer relaxation/erosion; $n > 0.89$, the diffusion is mainly aided by polymer relaxation/erosion. $n = 1$ indicates zero-order release mechanism (Case II transport mechanism). Values of $n > 1$ indicate Super Case II transport mechanism, implying swelling and relaxation of hydrophilic polymer chains help to transport.

The following equation is used to estimate the cumulative amount of 5-FU released

$$M_t = \frac{V\rho_n + \sum_{i-1}^{n-1} \rho_i V_i}{A} \tag{4}$$

where M_t is cumulative release amount (in grams), A is the effective permeation area (1 cm^2), V is the receptor half-cell volume (300 mL), ρ_n and ρ_i are the receptor half-cell's concentration at the nth sampling and at the ith sampling, respectively, V_i is the sampling volume (10 mL). We find that the cumulative release of 5-FU can be fitted by the above Peppas equation (3) very well for both types of the hydrogels (see Figs. 7–9). Summarized in Table 3 are the fitting results, with the linear regression coefficients R^2 in the range from 0.9863 to 0.9966. The release exponent values (n) of all the hydrogels vary from 0.6096 to 0.6732, indicating a non-Fickian transport mechanism, corresponding to coupled effect of diffusion and polymer relaxation. Furthermore, the release exponents from the copolymer hydrogels are slightly lower than those from the IPN hydrogels.

The diffusion of 5-FU was also examined by using Higuchi model,

$$M_t = kt^{1/2} + b. \tag{5}$$

As shown in Table 3, the fitting results obtained by using the Peppas equation (3) for all the hydrogels are found to be slightly better than those obtained by using the Higuchi model.

3.3. FTIR Study of binding of 5-FU in crosslinked PVP-co-PAM and PVP-co-PAM/PVA IPN hydrogels

Drug/polymer hydrogel interactions can be revealed by Fourier transform infrared spectroscopy. The raw data of FTIR spectra of

Table 3
Mathematic modeling and drug release kinetics of 5-FU from different hydrogels (R^2: correlation coefficient).

Hydrogel sample (XR%)	Peppas equation	Higuchi equation
PVP-co-PAM #1 (3.0%)	$\ln M_t = 0.6349 \ln t + 4.5543$ $R^2 = 0.9966$	$M_t = 151.93t^{1/2} - 73.16$ $R^2 = 0.9853$
PVP-co-PAM #2 (6.0%)	$\ln M_t = 0.6148 \ln t + 4.5764$ $R^2 = 0.9958$	$M_t = 144.06t^{1/2} - 58.89$ $R^2 = 0.9925$
PVP-co-PAM #3 (10.0%)	$\ln M_t = 0.6096 \ln t + 4.4461$ $R^2 = 0.9886$	$M_t = 126.70t^{1/2} - 53.97$ $R^2 = 0.9852$
PVP-co-PAM/PVA IPN #1 (3.0%/7.7%)	$\ln M_t = 0.6732 \ln t + 4.6983$ $R^2 = 0.9938$	$M_t = 195.47t^{1/2} - 108.99$ $R^2 = 0.9915$
PVP-co-PAM/PVA IPN #2 (6.0%/7.7%)	$\ln M_t = 0.6328 \ln t + 4.7699$ $R^2 = 0.9863$	$M_t = 184.44t^{1/2} - 83.17$ $R^2 = 0.9841$
PVP-co-PAM/PVA IPN #3 (10.0%/7.7%)	$\ln M_t = 0.6131 \ln t + 4.6898$ $R^2 = 0.9922$	$M_t = 162.61t^{1/2} - 69.36$ $R^2 = 0.9889$

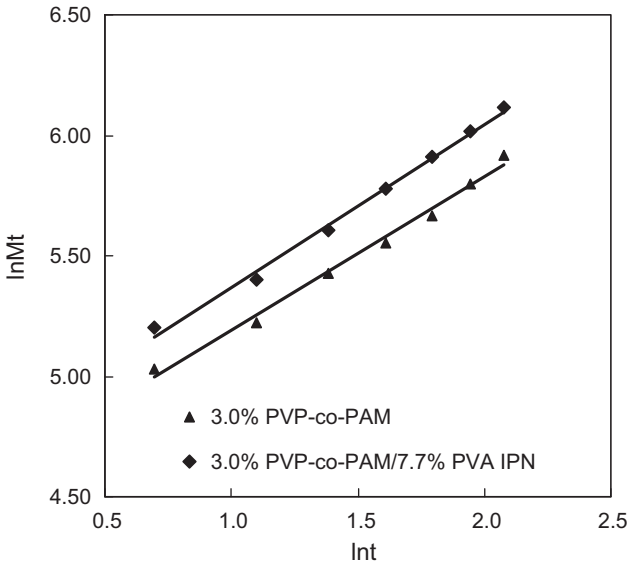


Fig. 7. Peppas equation analysis of diffusion of 5-FU through PVP-co-PAM copolymer hydrogel (solid triangle ▲, with XR = 3%) and through IPN hydrogel consisting of PVP-co-PAM and PVA networks (solid diamond ◆, with XR = 3% and 7.7% for PVP-co-PAM and PVA, respectively).

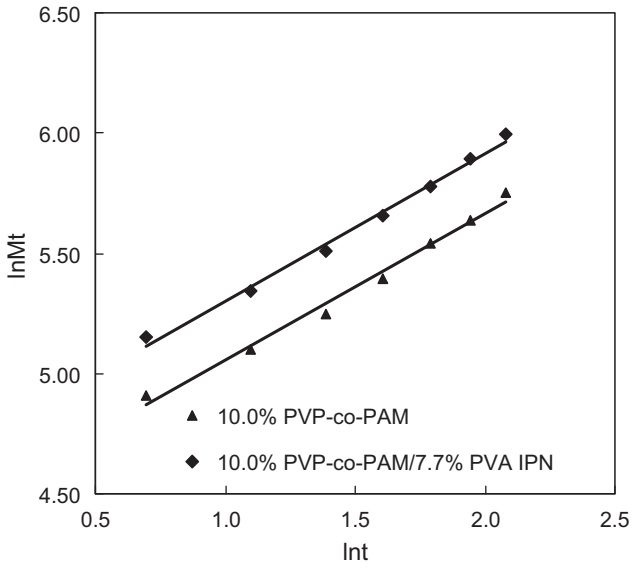


Fig. 9. Peppas equation analysis of diffusion of 5-FU through PVP-co-PAM copolymer hydrogel (solid triangle ▲, with XR = 10.0%) and through IPN hydrogel consisting of PVP-co-PAM and PVA (solid diamond ◆, with XR = 10.0% and 7.7% for PVP-co-PAM and PVA, respectively).

the hydrogels with drugs loaded are often dominated by the intense bands of the polymers, with a few minor bands showing the identity of the drug inside. The infrared spectroscopic features of the drug molecules are often overlapped with or buried under the broad polymer bands. In order to unravel the complexities, a special FTIR spectroscopy technique, FTIR difference spectroscopy, is employed to examine subtle changes in the 5-FU's uracil ring resulting from 5-FU/polymer interactions and surrounding groups of 5-FU from the side chains of the polymers. In this method, the FTIR spectra were obtained from the vacuum dried hydrogel membranes before and after 5-FU had diffused in the above permeation tests. The FTIR spectrum of the drug-free membrane could be subtracted out from that of the permeated membrane, and the intense spectroscopic signals of the hydrogel material unperturbed during drug binding were cancelled out. The resulting difference spectrum shows the spectral features of the vibrational modes that are attributed to those of 5-FU in the hydrogel, as well as the features that are ascribed to the side chain groups of the polymers tightly bound to 5-FU, giving the perturbed spectral bands of the polymers that cannot be subtracted out.

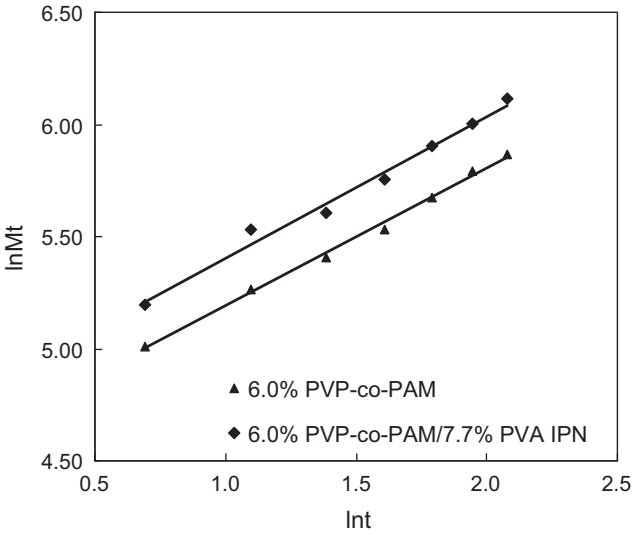


Fig. 8. Peppas equation analysis of diffusion of 5-FU through PVP-co-PAM copolymer hydrogel (solid triangle ▲, with XR = 6.0%) and through IPN hydrogel consisting of PVP-co-PAM and PVA (solid diamond ◆, with XR = 6.0% and 7.7% for PVP-co-PAM and PVA, respectively).

Vibrational analysis has been performed for 5-FU and spectral markers have been identified for 5-FU in the solid state (Dobrowolski et al., 2005; Rastogia and Palafox, 2011). A strong sharp band at 1245 cm^{-1} in the solid 5-FU spectrum (Fig. 10A) is attributed to fluorine-$C_{(5)}$ bond stretching mode (see Scheme 1C for labeling of the atoms in the 5-FU structure). Since the vibration of this bond is insensitive to H-bonding effect on the uracil ring (Rastogia and Palafox, 2011), the band can serve as an internal reference for the 5-FU-polymer interactions. It is hardly shifted and appears near 1246–1247 cm^{-1} in the spectra of 5-FU bound in the hydrogels (Fig. 10B and C).

The medium intensity band occurring at 1720 cm^{-1} in the solid crystalline state (Fig. 10A) is due to the $C_{(2)}$=O stretching mode in the uracil ring (Dobrowolski et al., 2005; Rastogia and Palafox, 2011). Upon binding in the hydrogels, the frequency of the $C_{(2)}$=O

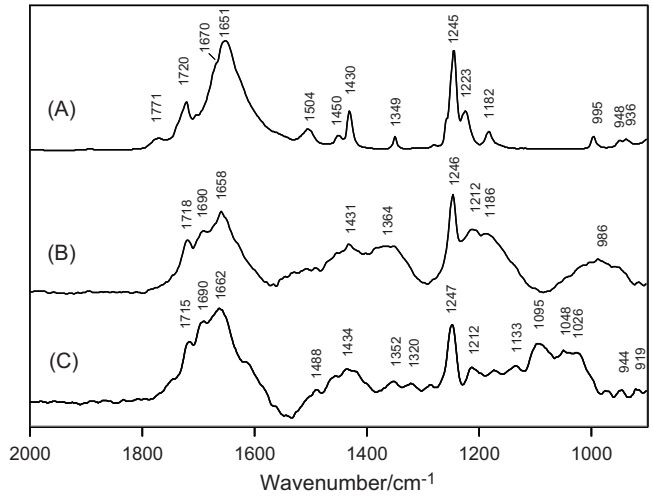


Fig. 10. FTIR spectra of 5-FU in the range 2000–900 cm^{-1}: (A) free 5-FU in solid state; (B) bound to PVP-co-PAM copolymer hydrogel with 6% of crosslinking; (C) bound to PVP-co-PAM/PVA IPN hydrogel with 6% of crosslinking of the copolymer.

stretching band is slightly downward shifted to 1718 cm^{-1} or 1715 cm^{-1} in Fig. 10B or C. The $C_{(4)}$=O stretching band which is shown only as a shoulder at 1670 cm^{-1} in the solid state in Fig. 10A is upward shifted by 20 cm^{-1} to 1690 cm^{-1} in the hydrogels. The $C_{(5)}$=$C_{(6)}$ stretching band appearing at 1651 cm^{-1} in the solid state 5-FU (Fig. 10A) is upward shifted by 7 cm^{-1} to 1658 cm^{-1} in the copolymer and upward shifted by 11 cm^{-1} to 1662 cm^{-1} in the IPN (Fig. 10B and C). The upward shift of the $C_{(5)}$=$C_{(6)}$ bands is due to the conjugation of the $C_{(5)}$=$C_{(6)}$ and $C_{(4)}$=O groups in the uracil ring. Since the $C_{(5)}$=$C_{(6)}$ and $C_{(4)}$=O stretching bands monitor the interaction of the $C_{(4)}$=O group in 5-FU with the polymer matrix, the frequency shifts indicate that the H-bonding interactions between the 5-FU's $C_{(4)}$=O groups and the polymer side chains in the hydrogels (most likely from the amide NH_2 groups in the copolymer and the —OH groups of the PVA chains) become weaker than those in the 5-FU neat solid. It should be noted that in non-hydrogen bonded environment (isolated in argon gas), the $C_{(2)}$=O, $C_{(4)}$=O and $C_{(5)}$=$C_{(6)}$ stretching bands of 5-FU actually appear at 1780.0, 1746.5, 1686.5 cm^{-1}, respectively (Dobrowolski et al., 2005; Rastogia and Palafox, 2011). This means the intermolecular H-bonding forces exerted by the side chains of the IPN lead to downward shift of the $C_{(2)}$=O, $C_{(4)}$=O and $C_{(5)}$=$C_{(6)}$ stretching bands of 5-FU by approximately 65, 56, and 24 cm^{-1}, respectively, when referenced to the non-hydrogen bonded state. The downward shifts of the C=O stretching bands are known to be related to the enthalpy of the H-bonding formation (or the so called H-bonding strength) (Thijs and Zeeger-Huyskens, 1984; Tonge et al., 1996). Since the downshift of the ring $C_{(5)}$=$C_{(6)}$ frequency is greater in the copolymer than in the IPN, it seems that the H-bonding forces applied by the copolymer is greater than those by the IPN. This provides a new source of the more retarded diffusion in the copolymer hydrogels than in the IPN hydrogels. The low frequency position of the $C_{(5)}$=$C_{(6)}$ band in the solid state of 5-FU at 1651 cm^{-1} actually implies even stronger H-bonding environment in the solid crystalline state than dispersed in the polymer hydrogels.

Additional spectral changes in Fig. 10 provide further evidence for the 5-FU/polymer interactions. The profiles of the bands near 1504 and 1430 cm^{-1} related to the $N_{(1)}$—H bending and those of the bands near 1349 and 1182 cm^{-1} associated with the $N_{(3)}$—H bending in 5-FU are broadened in the copolymer hydrogel, indicating direct interactions between the $N_{(1)}$—H and $N_{(3)}$—H groups in 5-FU and the polymer side chains. A new broad band appearing near 1095 cm^{-1} in Fig. 10C indicates the involvement of the PVA's —OH groups that surrounds the bound 5-FU in the IPN, since this band is due to the bending mode of the —OH groups from the PVA chains which has changed their interactions upon binding of 5-FU. A certain amount of phosphate ions from the buffer solutions are bound to the IPN, leading to a broad phosphate P=O band near 1026 cm^{-1} in Fig. 10C.

Therefore, significant binding of 5-FU to the polymers has mainly resulted in H-bonding interactions occurring near the uracil ring's carbonyl, $N_{(1)}$—H and $N_{(3)}$—H groups.

4. Conclusions

In this study, we have shown that non-ionic polymers with interpolymer complexes based on H-bonding can be used for controlling the release of a non-ionic drug 5-FU. The release kinetics in the PVP-co-PAM copolymer and PVP-co-PAM/PVA IPN hydrogels are controlled by the degree of chemical crosslinking introduced, the H-bonding between the polymer side chains, as well as the drug/polymer interactions. The permeability coefficient and swelling ratio of the hydrogels decrease with the increasing degree of crosslinking. At the same degree of crosslinking for the PVP-co-PAM copolymer chains, the IPN gels can have larger permeability coefficients than the copolymer gels. This can be caused by the reorganization of the H-bonding patterns between the side chains in the IPN gels. FTIR difference spectroscopy reveals that H-bonding interactions have occurred on the $C_{(4)}$=O and $C_{(2)}$=O, as well as the $N_{(1)}$—H and $N_{(3)}$—H groups of the uracil ring, therefore providing an additional source of more retarded diffusion through the copolymer hydrogels than through IPN hydrogels. The diffusion mechanism of 5-FU is a non-Fickian transport.

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References

Agnihotri, S.A., Aminabhavi, T.M., 2005. Development of novel interpenetrating network gellan gum-poly(vinyl alcohol) hydrogel microspheres for the controlled release of carvedilol. Drug Dev. Ind. Pharm. 31, 491–503.
Agnihotri, S.A., Aminabhavi, T.M., 2006. Novel interpenetrating network chitosan-poly(ethylene oxide-g-acrylamide) hydrogel microspheres for the controlled release of capecitabine. Int. J. Pharm. 324, 103–115.
Alvarez-Lorenzo, C., Concheiro, A., 2002. Reversible adsorption by a pH and temperature sensitive acrylic hydrogel. J. Control. Release 80, 247–257.
am Ende, M.T., Hariharan, D., Peppas, N.A., 1995. Factors influencing drug and protein transport and release from ionic hydrogels. React. Polym. 25, 127–137.
Agudo, R., Arias, A., Pariente, N., Perales, C., Escarmis, C., Jorge, A., Marina, A., Domingo, E., 2008. Molecular characterization of a dual inhibitory and mutagenic activity of 5-fluorouridine triphosphate on viral RNA synthesis. Implications for lethal mutagenesis. J. Mol. Biol. 382, 652–666.
Bae, Y.H., Okano, T., Ebert, C., Heiber, S., Dave, S., Kim, S.W., 1991. Heterogeneous interpenetrating polymer networks for drug delivery. J. Control. Release 16, 189–196.
Bae, Y.H., Kim, S.W., 1993. Hydrogel delivery systems based on polymer blends, block co-polymers or interpenetrating networks. Adv. Drug Deliv. Rev. 11, 109–135.
Berger, J., Reist, M., Mayera, J.M., Felt, O., Peppas, N.A., Gurny, R., 2004. Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. Eur. J. Pharm. Biopharm. 57, 19–34.
Chun, M.-K., Cho, C.-S., Choi, H.-K., 2002. Mucoadhesive drug carrier based on interpolymer complex of poly(vinyl pyrrolidinone) and poly(acrylic acid) prepared by template polymerization. J. Control. Release 81, 327–334.
Corrigan, O.I., Farvar, M.A., Higuchi, W.I., 1980. Drug membrane transport enhancement using high energy drug polyvinylpyrrolidone (PVP) co-precipitates. Int. J. Pharm. 5, 229–238.
Coughlan, D.C., Corrigan, O.I., 2006. Drug–polymer interactions and their effect on thermoresponsive poly(N-isopropylacrylamide) drug delivery systems. Int. J. Pharm. 313, 163–174.

de la Torrea, P.M., Enobakharea, Y., Torradob, G., Torrado, S., 2003. Release of amoxicillin from polyionic complexes of chitosan and poly(acrylic acid). Study of polymer/polymer and polymer/drug interactions within the network structure. Biomaterials 24, 1499–1506.

Dobrowolski, J.C., Rode, J.E., Kolos, R., Jamroz, M.H., Bajdor, K., Mazurek, A.P., 2005. Ar-matrix IR spectra of 5-halouracils interpreted by means of DFT calculations. J. Phys. Chem. A 109, 2167–2182.

Garrett, E.R., Hurst, G.H., Green Jr., J.R., 1977. Kinetics and mechanisms of drug action of microorganisms XXIII: microbial kinetic assay for fluorouracil in biological fluids and its application to human pharmacokinetics. J. Pharm. Sci. 66, 1422–1429.

Griffith, L.G., 2000. Polymeric Biomaterials. Acta Mater. 48, 263–267.

Jin, L., Lu, P., You, H., Chen, Q., Dong, J., 2009. Vitamin B12 diffusion and binding in crosslinked poly(acrylic acid)s and poly(acrylic acid-co-N-vinyl pyrrolidone)s. Int. J. Pharm. 371, 82–88.

Jin, Y., Ren, X., Wang, W., Ke, L., Ning, E., Du, L., Bradshaw, J., 2011. A 5-fluorouracil-loaded pH-responsive dendrimer nanocarrier for tumor targeting. Int. J. Pharm. 420, 378–384.

Katono, H., Maruyama, A., Sanui, K., Ogata, N., Okano, T., Sakurai, Y., 1991. Thermo-responsive swelling and drug release switching of interpenetrating polymer networks composed of poly(acrylamide-co-butyl methacrylate) and poly(acrylic acid). J. Control. Release 16, 215–228.

Khutoryanskiy, V.V., 2007. Hydrogen-bonded interpolymer complexes as materials for pharmaceutical applications. Int. J. Pharm. 334, 15–26.

Kim, H.O., Ahn, S.K., Alves, A.J., Beach, J.W., Jeong, L.S., Choi, B.G., Van Roey, P., Schinazi, R.F., Chu, C.K., 1992. Asymmetric synthesis of 1,3-dioxolane-pyrimidine nucleosides and their anti-HIV activity. J. Med. Chem. 35, 1987–1995.

Korsmeyer, R.W., Gurny, R., Docler, E., Buri, P., Peppas, N.A., 1983. Mechanism of solute release from porous hydrophilic polymers. Int. J. Pharm. 15, 25–35.

Kulkarni, A.R., Soppimath, K.S., Aminabhavi, T.M., Rudzinski, W.E., 2001. In vitro release kinetics of cefadroxil-loaded sodium alginate interpenetrating network beads. Eur. J. Pharm. Biopharm. 51, 127–133.

Kurkuri, M.D., Aminabhavi, T.M., 2004. Poly(vinyl alcohol) and poly(acrylic acid) sequential interpenetrating network pH sensitive microspheres for the delivery of diclofenac sodium to the intestine. J. Control. Release 96, 9–20.

Liechty, W.B., Caldorera-Moore, M., Phillips, M.A., Schoener, C., Peppas, N.A., 2011. Advanced molecular design of biopolymers for transmucosal and intracellular delivery of chemotherapeutic agents and biological therapeutics. J. Control. Release 155, 119–127.

Lin, C.C., Metters, A.T., 2006. Hydrogels in controlled release formulations: network design and mathematical modeling. Adv. Drug Deliv. Rev. 58, 1379–1408.

Noble, L., Gray, A.I., Sadiq, L., Uchegbu, I.F., 1999. A non-covalently crosslinked chitosan based hydrogel. Int. J. Pharm. 192, 173–182.

Ozeki, T., Yuasa, H., Okada, H., 2005. Controlled release of drug via methylcellulose–carboxyvinylpolymer interpolymer complex solid dispersion. AAPS PharmSciTech 6, E231–E236.

Pariente, N., Sierra, S., Lowenstein, P.R., Domingo, E., 2001. Efficient virus extinction by combinations of a mutagen and antiviral inhibitors. J. Virol. 75, 9723–9730.

Park, K., 1993. Biodegradable Hydrogels for Drug Delivery. Technomic Publishing, Lancaster, PA.

Park, S.-H., Chun, M.-K., Choi, H.-K., 2008. Preparation of an extended-release matrix tablet using chitosan/carbopol interpolymer complex. Int. J. Pharm. 347, 39–44.

Peppas, N.A., Brazel, C.S., 1994. Temperature- and pH-sensitive hydrogels for controlled release of heparin and streptokinase. In: Mikos, A.G., Murphy, R.M., Bernstein, H., Peppas, N.A. (Eds.), Biomaterials for Drug and Cell Delivery. Materials Research Society, Pittsburgh, pp. 211–216.

Peppas, N.A., Wright, S.L., 1996. Solute diffusion in poly(vinyl alcohol)/poly(acrylic acid) interpenetrating networks. Macromolecules 29, 8798–8804.

Peppas, N.A., Wright, S.L., 1998. Drug diffusion and binding in ionizable interpenetrating networks from poly(vinyl alcohol) and poly(acrylic acid). Eur. J. Pharm. Biopharm. 46, 15–29.

Presant, C.A., Wolf, W., Waluch, V., Wiseman, C., Kennedy, P., Blayney, D., Brechner, R.R., 1994. Association of intratumoral pharmacokinetics of fluorouracil with clinical response. Lancet 343, 1184–1187.

Qiu, Y., Park, K., 2001. Environment-sensitive hydrogels for drug delivery. Adv. Drug Deliv. Rev. 53, 321–339.

Rastogia, V.K., Palafox, M.A., 2011. Vibrational spectra, tautomerism and thermodynamics of anticarcinogenic drug: 5-fluorouracil. Spectrochim. Acta Part A 79, 970–977.

Ritger, P.L., Peppas, N.A., 1987. Simple equation for solute release. Part 1. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or disks. J. Control. Release 5, 37–42.

Schroeder, S.A., Krupp, M.A., Tierney Jr., L.M., McPhee, S.J. (Eds.), 1990. Current Medical Diagnosis and Treatment, Appleton and Lange. Prentice-Hall, London.

Tanaka, T., Fillmore, D., Sun, S., Nishio, I., Swislow, G., Shah, A., 1980. Phase transition in ionic gels. Phys. Rev. Lett. 45, 1636–1639.

Thijs, R., Zeeger-Huyskens, T., 1984. Infrared and Raman studies of hydrogen bonded complexes involving acetone, acetophenone and benzophenone-I. Thermodynamic constants and frequency shifts of the ν_{OH} and $\nu_{C=O}$ stretching vibrations. Spectrochim. Acta Part A 40, 307–313.

Tonge, P.J., Fausto, R., Carey, P.R., 1996. FTIR studies of hydrogen bonding between alpha,beta-unsaturated esters and alchols. J. Mol. Struct. 379, 135–142.

Vervoort, L., Mooter, V.G., Augustijns, P., Kinget, R., 1998. Insulin hydrogels. I. Dynamic and equilibrium swelling properties. Int. J. Pharm. 172, 127–135.

Wolinsky, J.B., Colson, Y.L., Grinstaff, M.W., 2012. Local drug delivery strategies for cancer treatment: gels, nanoparticles, polymeric films, rods, and wafers. J. Control. Release 159, 14–26.

Wu, J.Y., Liu, S.Q., Heng, P.W.S., Yang, Y.Y., 2005. Evaluating protein release from, and their interactions with, thermosensitive poly(N-isopropylacrylamide) hydrogels. J. Control. Release 102, 361–372.

Yu, H., Grainger, D.W., 1995. Modified release of hydrophilic, hydrophobic and peptide agents from ionised amphiphilic gel networks. J. Control. Release 34, 117–127.