Release dynamics of tetracycline from a loaded semi-interpenetrating polymeric material of polyvinyl alcohol and poly(acrylamide-co-styrene)

A. K. BAJPAI*, J. BAJPAI, SANDEEP SHUKLA
Bose Memorial Research Laboratory, Department of Chemistry, Government Autonomous Science College, Jabalpur (M.P.) – 482001, India
E-mail: akbmrl@yahoo.co.in

A semi-interpenetrating polymer network (IPN) of polyvinyl alcohol (PVA) and poly(acrylamide-co-styrene) (PAMS) was prepared and its potential for controlled release of tetracycline was assessed. The IPN was characterized by IR spectral analysis and network parameters such as the average molecular weight between crosslinks (M_c), crosslink density (q) and number of elastically effective chains (V_e) were evaluated. The influence of various experimental conditions such as different percent loadings, composition of the IPNs, thickness of the loaded device, pH and nature of the release medium were investigated on the release profiles of the drug. Various kinetic constants such as the diffusional exponent (n), diffusion constant (D) and penetration velocity (v) were evaluated for different release processes and based on the dynamic release data, an analysis of transport mechanisms of tetracycline was made using Fick's equations.

1. Introduction

Hydrogels are three dimensional physically or chemically crosslinked polymeric materials that can absorb large amounts of water without dissolving and losing their shapes. The water reservoir in these polymeric networks develops certain critical biophysical properties such as soft and rubbery texture, living-tissue-like resemblance, physiological stability towards biofluids, low interfacial tension, permeability to biomolecules, blood-compatibility, etc. These biophysical characteristics enable hydrogels as a potential candidate for a number of biomedical applications including controlled release systems [1, 2]. Hydrogels are of special interest in controlled release applications because of their soft tissue biocompatibility, the ease with which the drugs are dispersed in the matrix and the high degree of control achieved by selecting the physical and chemical properties of the polymer network. Hydrogels are indeed a novel class of materials and find diversified applications in pharmaceutical science [3].

The recent past has witnessed a tremendous research activity in the technology of controlled delivery of bioactive compounds which often includes targeting biologically active molecules to specific sites and extending their therapeutic lifetime once inside the body. Polymeric drug carrier systems have several advantages in optimizing patient treatment regimes. In particular, swelling controlled release systems are capable of delivering drugs at constant rates over an extended period of time. In these systems, the rate of drug delivery is controlled by the balance between drug diffusion across a concentration gradient, the polymer relaxation occurring as the crosslinked polymer imbibes water, and the osmotic pressure occurring during the swelling process [4]. This osmotic pressure is related to the high drug concentration inside the network. Thus, swelling controlled release systems offer a fair degree of superiority over the conventional release systems.

Tetracycline is one of the most abundantly tested and used antibiotics in the treatment of periodontal diseases. Clinical studies using tetracyclines hydrochloride (TC) have shown that it has an effective spectrum of activity against many of anaerobic microbes associated with various periodontal diseases involving both adult and juvenile periodontitis patients [5]. TC has several inherent properties which enhance its potential use in the treatment of periodontal diseases. There are the substantiality of TC to definite and cementum surfaces, its ability to etch and/or remove the root surface smear layer and cause surface demineralization (chemical conditioning of the root surface), to delay pellicle and plaque formation, and to exhibit anti-collagenase activity [6]. Tetracyclines contained in hydrogels are also significant in tissue engineering where hydrogels are impregnated with respective cells of the tissue aimed.

As the systematic use of antibiotics may cause several side effects (sensitivity, resistant strains, superinfections), the local administration of antibiotics has received considerable attention. Sendil *et al.* [7] investigated controlled release of TC from biodegradable poly(3-

* Author to whom all correspondence should be addressed.

TABLE I Different effect of free drug and the polymer entrapped drug on the whole body

Free drug	Polymer entrapped drug
No specific accumulation	Specific accumulation
Fast elimination	Slow elimination
High side toxicity	Low side toxicity
Narrow therapeutic index	Broad therapeutic index
Sometimes poor solubility	Soluble
Immunosuppression	Low immunosuppression

hydroxybutyrate-co-3-hydroxy valerate) (PHBV) of various valerate contents. They observed that the encapsulation efficiency of the drug was influenced by the emulsifiers used in the continuous medium and the chemistry of the drug. The authors were unable to obtain a zero-release behavior.

Apart from tetracycline, other antibiotics such as amoxicillin and metronidazole, which are quite effective in treating *Helicobacter pylori*, were also undertaken for controlled release study, as indicated in a study by Risbud *et al*. [8] who designed drug-loaded pH-sensitive polymeric vehicles of chitosan and polyvinyl pyrrolidone by the freeze-dried method. The authors noted that freeze-dried membranes could serve as potent candidates for antibiotic delivery in an acidic environment.

Bittner and coworkers [9] prepared biodegradable poly(DL-lactide-co-glycolide) microspheres loaded with tetracycline HCl by a spray drying technique and studied the influence of γ-irradiation on radical formation and polymer degradation. They confirmed the presence of free radicals in TC-loaded microspheres while no free radicals could be detected in unloaded microsphere.

Thus, looking to the pharmacological importance of TC release studies, we, in the present investigation, are reporting results on the controlled release of tetracycline entrapped in a semi-interpenetrating polymer network (IPN) of polyvinyl alcohol (PVA) and poly(acrylamide-co-styrene). The entrapped drugs are of great concern in pharmaceutical science as they provide a number of benefits over free drugs in solution as shown in Table I. The choice for taking PVA as a preformed polymer in the IPN is due to the reason that it is used as a basic material for a variety of biomedical applications including contact lenses material [10], skin replacement [11], artificial cartilage replacement [12], etc. because of its inherent nontoxicity, noncarcinogenicity, good biocompatibility and desirable physical properties such as elastic nature, high degree of swelling in aqueous solutions and good film forming property. The poly(vinyl alcohol) IPNs have also gained wide pharmaceutical applications as drug delivery matrices [13], or in the form of powders added to mixture of other excipients for tablet formation [14]. In order to strengthen the TC loaded IPNs a copolymer of hydrophilic acrylamide and hydrophobic styrene has been employed in the present study which not only provides mechanical strength to the device but also regulates its swelling behavior which is a key parameter in controlling the release pattern of the loaded drug. Moreover, the use of hydrophobic segments in IPN synthesis has increasingly become popular because of the new trends particularly in the field of contact lenses which involves hydrophobic microdomains in the optical material for improved permeability of oxygen without loss of transparency and other optical properties.

2. Experimental

2.1. Materials

Polyvinyl alcohol (PVA) (hot processed M.Wt. – 40,000, degree of hydrolysis – 98.6%) was obtained from Burgoyne Burbidges & Co., Bombay, India and used without further purification. Acrylamide (AM) (Research Lab, Poona, India) was crystallized twice from methanol (G.R.) and dried under vacuum over anhydrous silica for a week. Styrene (Research Lab, Poona, India) was purified by washing sequentially with 10% NaOH, 2N H_2SO_4, and finally with double distilled water. The monomer so purified was distilled under vacuum conditions. N,N′-methylene bisacrylamide (MBA) (Central Drug House, Bombay, India) employed as a crosslinking agent, potassium persulfate (Loba Chemie, India) as a polymerization initiator were used as received. Tetracycline hydrochloride (TC) I.P. was gifted from Jagsonpal Pharmaceuticals Ltd., India. All other chemicals used were of analytical grade and bidistilled water was used throughout the experiments.

2.2. Buffers

The following buffers were used in maintaining pH of the release medium while studying pH effect :

1. 0.05 M HCl + 0.09 M KCl for pH 2.07.
2. 0.01 M acetic acid + 0.01 M sodium acetate for pH 4.70, and
3. 0.01 M KH_2PO_4 + 0.01 M Na_2HPO_4 for pH 6.85.

2.3. Physiological fluids

In order to study the release behavior of tetracycline under *in vivo* experimental conditions, the release experiments were also carried out in simulated physiological fluids whose compositions are described below:

1. Saline water—0.9% (w/v) NaCl solution
2. Synthetic urine—2.0 g NaCl (0.8% w/v), 250 mg $MgSO_4$ (0.10% w/v), 5.0 g urea (2% w/v) and 150 mg $CaCl_2$ (0.06% w/v).

2.4. Preparation of IPN

The IPN's were prepared by the free radical polymerization method as described in our earlier communications [15]. In brief, into a petridish (2 inch diameter, Corning) were added polyvinyl alcohol (PVA) 1.0 g, acrylamide (AM) 10.5 mM, styrene (ST) 8.6 mM, N, N′ – methylene bis acrylamide (MBA) 0.12 mM, potassium persulfate (KP) 0.073 mM and water 1.3 M. The reaction mixture was homogenized by manual mixing, deariated by purging N_2 gas for 1 h, and kept at 80 °C for 3 h. The IPNs so formed were dried at 60 °C for 5 h. They were further purified by equilibrating them in bidistilled water for 48 h. The equilibrated swollen IPNs were dried at room temperature for one week and cut into slabs of

identical sizes (0.75 cm × 0.80 cm × 0.051 cm) and weights (0.040 g).

2.5. IR spectral analysis

The IPNs synthesized were characterized by IR spectral analysis (Perkin Elmer, Paragon 1000 FTIR).

2.6. Swelling measurements

For performing swelling experiments, a general gravimetric procedure was adopted. In brief a preweighed piece of the gel (Xerogel) was immersed into bidistilled water at fixed pH and temperature (27 °C) and allowed to swell till equilibrium. The progress of the swelling of IPN was monitored by recording weights of the swollen gel at different time intervals. The swelling process was characterized by the parameter as given below:

$$\text{swelling ratio (SR)} = W_s/W_d \qquad (1)$$

where W_s and W_d are the weights of swollen and dried gels respectively.

2.7. Loading of tetracycline

The loading of a drug onto a carrier is normally performed by two general methods. In one method, the hydrogel monomer is mixed with the drug, an initiator, with or without a crosslinking agent and allowed to polymerize, trapping, the drug within the matrix [16]. In the second approach the gel is allowed to swell in the drug solution till equilibrium and then dried to obtain the release device. The latter method has some advantages over the first method as polymerization conditions may have deleterious effects on drug properties and difficulties in device purification after loading the polymerization often remain. Moreover, such a loading technique allows drugs to be physically entrapped into the polymer matrix which is a quite advantageous method of drug entrapment. The superiority of the technique lies in the fact that even hydrophobic and non-functional drugs could be loaded into the releasing device.

In the present study the latter method of loading was followed which involved swelling of preweighed pieces of IPN into the TC solution of known concentration and then taking out and drying them at room temperature for 48 h. The following equation was used to calculate the percent loading,

$$\text{Percent loading} = \frac{W_d - W_o}{W_o} \times 100 \qquad (2)$$

where W_d and W_o are the weights (in mg) of tetracycline loaded and dry IPNs, respectively.

2.8. Release experiments

The dried and loaded slabs of IPNs were placed at a definite pH into a fixed volume (10 ml) of bidistilled water as a release medium and mildly shaken in a thermostated shaker at 27 °C. The purpose of shaking was that the release of TC could take place through both the opposite faces of the loaded slabs. The IPNs were repeatedly removed and transferred into fresh water at predetermined time intervals. The amount of released tetracycline was assayed spectrophotometrically [17] as briefly described below :

Into a 5 ml of drug solution (0.2 to 1.0 mg/ml) were added 10 ml of $FeCl_3$ (0.2%) prepared in 1:100 HCl. The absorbance of the solution was read after 15 min at 490 nm (Systronics, India, Model No. 106). The absorbance was quite stable and the Lambert-Beer's law was perfectly obeyed in the specified drug concentration range. It is worth mentioning here that although the method is originally applicable to oxytetracycline, however, we obtained excellent results with tetracycline also. The release kinetics of TC was studied for 6 h.

In order to have mechanistic insights into the drug transport processes the kinetic data of the swelling process was fitted into the following power law equation,

$$\frac{W_t}{W_\infty} = kt^n \qquad (3)$$

where k is a constant incorporating structural and geometric characteristics of the device, n is diffusional exponent and W_t and W_∞ are the amounts of drug released at time t and equilibrium time, respectively. For $n > 0.5$, non-Fickian diffusion is observed, while $n = 0.5$ represents a Fickian diffusion mechanism. The value of $n = 1$ provides Case II transport mechanism in which drug releasing from hydrogel of slab geometry will be of zero order. It is notable here that Equation 3 is based on the assumption that release occurs as soon as the matrix is placed in contact with the fluid.

For calculating diffusion constant of the drug, the following Fickin diffusion equation can be used,

$$\frac{W_t}{W_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} \exp\left\{\frac{-D(2n+1)^2\pi^2 t}{\ell^2}\right\}$$

The above equation is further simplified to the following early-time approximation,

$$\frac{W_t}{W_\infty} = 4\left(\frac{Dt}{\pi\ell^2}\right)^{0.5} \qquad (4)$$

where D is the diffusion constant of the drug ($cm^2\ S^{-1}$) and ℓ is thickness of the dry and drug-loaded IPN slab. Equation 4 clearly implies that from the early-time slope of the plot drawn between W_t/W_∞ and $(t)^{0.5}$, the value of D can easily be calculated for any fractional release vs $(\text{time})^{0.5}$ curve.

2.9. Penetration velocity measurements

The penetration velocity for each IPN composition was determined by the weight gain method as described by Peppas and Franson [18]. The penetration velocity was calculated from the slope of the initial portion of the penetrant uptake curve from the equation

$$v = \left(dW_g/dt\right) \cdot \left(\frac{1}{\rho}\right)\left(\frac{1}{2A}\right) \qquad (5)$$

where v denotes the penetration velocity, dW_g/dt denotes the slope of weight gain vs time curve, and ρ denotes the density of water. A denotes the area of one face of the

TABLE II Structural parameters of the semi-IPNs of PVA and poly(AM-CO-ST) with varying crosslink densities and at fixed [AM] = 1.0 g, [ST] = 8.6 mM, [AM] = 10.5 mM, [KP] = 0.12 mM

Amount of MBA (mM)	Crosslink density $\times 10^3$ (q)	Average mol. wt between crosslinks (M_c)	Elastically effective chains (v_e) $\times 10^{19}$
0.12	1.43	61967	1.16
0.19	9.7	9175	7.87
0.25	10.6	8406	8.59
0.38	36.5	2444	24.90

slab and the factor 2 accounts for the fact that penetration takes place through both the faces. The penetration velocities calculated for different IPN compositions are listed in Table III.

It is worth mentioning here that for calculating the penetration velocity only early-time linear portion of the graph was considered and the latter portion was ignored. The reason is that the swelling (and release) data are significant up to 60% (i.e. $W_t/W_\infty \leq 0.60$) only.

3. Results and discussion

3.1. IR analysis of IPN

The IR spectra of the end-polymer is depicted in Fig. 1. The spectra clearly marks the presence of hydroxyls of alcohol at 3605 cm^{-1} (O–H stretching), methylene (CH_2) twisting and wagging vibrations at 1392, 1371, 1359 and 1143 cm^{-1}, amide groups at 3563 cm^{-1} (N–H stretching) and NH_2 bending vibrations at 1660, 1651 and 1633 cm^{-1} respectively. In addition to the above mentioned peaks, the spectra also contains absorption bands in the range 1667–1429 cm^{-1} indicative of C=C skeletal in plane vibration due to phenyl ring.

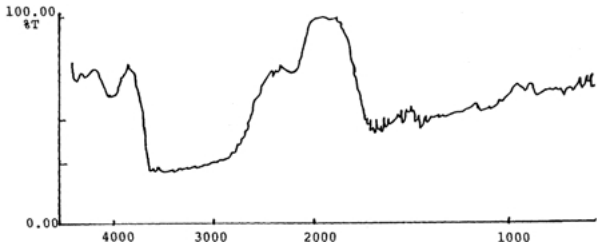

Figure 1 IR spectra of the semi-IPN.

The spectra analysis suggests for a grafted type of network structure in which crosslinked AM and ST chains are grafted onto the backbone of PVA via hydroxyls of the preformed polymer.

On the basis of the spectral analysis, a scheme of reactions may be proposed for the formation of semi-IPN as depicted in Fig. 2.

3.2. Network structure

Both theoretically [19] and experimentally [20] it has been demonstrated that polymer composition has profound effects on release profiles. For instance, the presence of polymer network could retard solute diffusion, through three primary variables: the molecular weight between crosslinks, the equilibrium volume swelling ratio and the solute radius.

In the case of swelling-controlled release systems and hydrogels used for drug delivery, it is important not only that swelling increases progressively with time, but also that the mesh size of the device is able to molecularly accommodate the drug. Thus, the mesh size of a network is an important parameter for prediction of hydrogel (or IPN) permeability. The mesh size is directly controlled by the molecular weight between crosslinks (M_c) which is also a significant parameter in characterizing the crosslinked polymer network. The magnitude of M_c greatly affects the physical and mechanical properties of crosslinked network, its determination is of practical significance. Equilibrium swelling is widely used to determine M_c. Early research by Flory and Rehner laid the foundation for analysis of equilibrium swelling.

TABLE III Data showing the variation of penetration velocity (v) for swelling of IPN and diffusion constant (D) for release of TC with varying composition of the IPNs

PVA (g)	ST (mM)	AM (mM)	MBA (mM)	Thickness (cm)	$v \times 10^5$ (cm/s)	$D \times 10^6$ (cm^2/s)
0.5	8.6	10.5	0.12	0.051	1.85	2.06
1.0	8.6	10.5	0.12	0.051	1.60	2.56
1.5	8.6	10.5	0.12	0.051	1.03	2.21
1.0	4.3	10.5	0.12	0.051	2.08	2.34
1.0	8.6	10.5	0.12	0.051	1.60	2.56
1.0	13.0	10.5	0.12	0.051	1.20	2.34
1.0	8.6	7.0	0.12	0.051	1.15	1.79
1.0	8.6	10.5	0.12	0.051	1.60	2.56
1.0	8.6	17.6	0.12	0.051	1.90	2.84
1.0	8.6	10.5	0.06	0.051	2.08	2.02
1.0	8.6	10.5	0.12	0.051	1.60	2.56
1.0	8.6	10.5	0.25	0.051	0.80	3.04
1.0	8.6	10.5	0.12	0.017	0.55	5.15
1.0	8.6	10.5	0.12	0.030	1.25	3.60
1.0	8.6	10.5	0.12	0.051	1.60	2.56

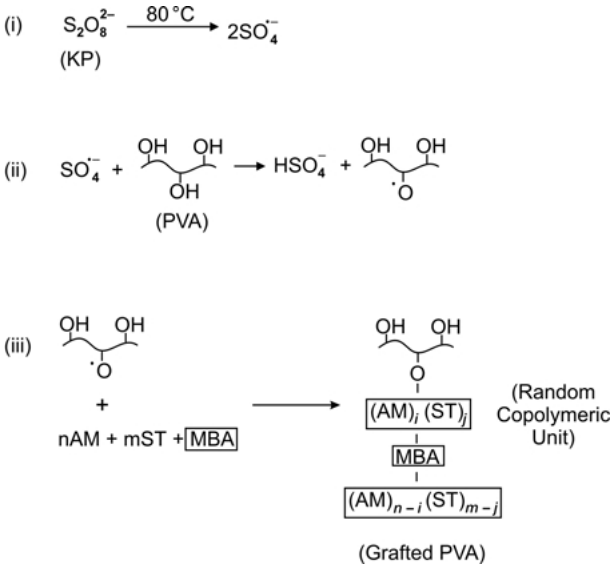

Figure 2 A proposed scheme of reactions for the synthesis of semi-IPN.

According to the theory of Flory and Rehner, for a network

$$M_c = -V_1 d_p \frac{V_s^{1/3 - V_s/2}}{\ln(1 - V_s) + V_s + XV_s^2} \tag{6}$$

where V_1 is the molar volume of water (mL mol^{-1}), d_p is the polymer density (g mL^{-1}), V_s is the volume fraction of polymer in the swollen IPN, X is the Flory-Huggins interaction parameter between solvent and polymer.

The swelling ratio (Q) is equal to $1/V_s$. Here, the crosslink density, q, is defined as the mol fraction of crosslinked units [21].

$$q = \frac{M_o}{M_c} \tag{7}$$

where M_o is the molar mass of the repeating unit.

Other authors define a crosslink density, v_e, as the number of elastically effective chains, totally included in a network, per unit volume, v_e is simply related to q since

$$v_e = \frac{d_p N_A}{M_c} \tag{8}$$

where N_A is Avogadro number.

Since the IPN in the present study contains a copolymeric structure, the molar mass of the polymer repeat unit, M_o, can be calculated by the following equation

$$M_o = \frac{n_{AM} \cdot M_{AM} + n_{ST} \cdot M_{ST}}{n_{AM} + n_{ST}} \tag{9}$$

where n_{AM} and n_{ST} are the mol. number of *AM* and *ST* (mol) and M_{AM} and M_{ST} are the molar mass of *AM* and *ST* (g mol^{-1}) respectively.

The density of the polymer d_p was determined by pyknometry and found to be 1.2 g cm^{-3}. Other parameters such as V_1 and X were noted from the literature [22, 23]. Using Equations 6, 7 and 8 the volume of M_c, q and v_e have been calculated for the IPN's containing different amounts of crosslinkers (MBA). The values summarized in Table II indicate that the average molar mass between crosslinks decreases with increasing crosslinker (MBA) content in the networks.

3.3. Mechanism of drug release

Hydrogels consist of macromolecular chains crosslinked to each other to create a tangled mesh structure, providing a matrix for the entrapment of drugs. When such loaded matrices come in contact with a thermodynamically compatible solvent, relaxation of polymeric chains take place. This happens when the characteristic glassy rubbery transition temperature of the polymer is decreased below the temperature of the experiment. Swelling is the macroscopic evidence of this transition. The dissolved drug diffuses into the external receiving medium, crossing the swollen polymeric layer formed around the matrix. Depending on the rate of the swelling process, the associated drug release may be Fickian or non-Fickian, including special Case II transport [24].

In the present study, the IPN could be imagined to be made up of PVA and crosslinked PAM-ST chains. Into the free volumes available between the IPN chains are accommodated the TC molecules in the loaded gel. When the IPN contacts the release medium, the penetrant water molecules invade the IPN surface and, thus, a moving front is observed that clearly separates the unsolvated glassy polymer region ahead of the front from the swollen and rubbery IPN phase behind it [25]. Just ahead of the front, the presence of solvent plasticizes the polymer and causes it to undergo a glass to rubber transition [26]. Now, the following possibilities arise:

1. If the glass transition temperature of the polymer (Tg) is well below the experimental temperature, the polymer will be in the rubbery state and polymer chains will have a higher mobility that allows an easier penetration of the solvent into the loaded IPN and subsequently release of the drug molecules into the release medium [27]. This clearly results in a Fickian diffusion (Case I) which is characterized by a solvent (or drug) diffusion rate, R_{diff}, slower than the polymer relaxation rate, R_{delax} ($R_{diff} \ll R_{\text{relax}}$).
2. If the experimental temperature is below Tg, the polymeric chains of IPN are not sufficiently mobile to permit immediate penetration of the solvent in the polymer core. This gives rise to a non-Fickian diffusion process which includes Case II and anomalous diffusions respectively depending on the relative rates of diffusion and chain relaxation (for Case II, $R_{\text{diff}} \gg R_{\text{relax}}$, and for anomalous, $R_{\text{diff}} \sim R_{\text{relax}}$).

3.4. Effect of drug loading on released TC

Analysis of drug release behavior requires an understanding of the factors influencing the drug loading into each sample, as the drug concentration is an important factor in the observed release rates. Water was chosen as a dissolution medium for drug entrapment into the IPNs. For increasing the percent loading of the drug into the IPNs the dried IPNs were equilibrated with TC solution of concentration range 5.0 to 15.0 mg/ml. This resulted in a loading of the drug in the range 43.3% to 66.6%. The loaded gels were allowed to release the entrapped TC

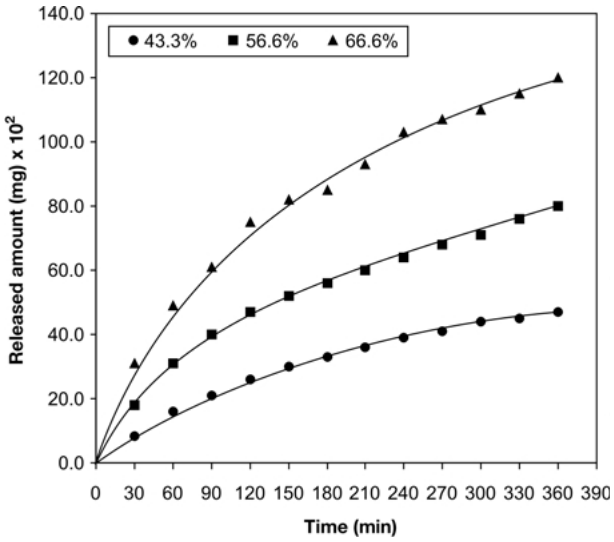

Figure 3 Effect of percent loading of TC on its release through a semi-IPN of [PVA] = 1.0 g, [ST] = 8.6 mM, [AM] = 10.5 mM, [MBA] = 0.12 mM, [KP] = 0.073 mM, pH = 3.0, temp. = 27 ± 0.2 °C, thickness = 0.051 cm.

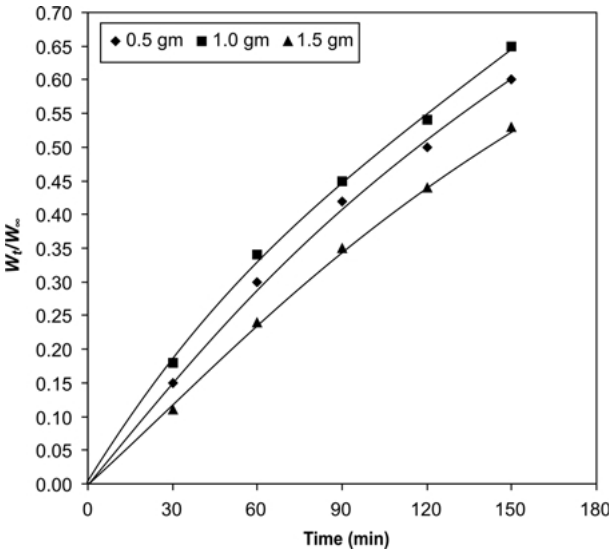

Figure 4 Effect of PVA content of the semi-IPN on the fractional release of TC at fixed composition of the IPN, [ST] = 8.6 mM, [AM] = 10.5 mM, [MBA] = 0.12 mM, [KP] = 0.073 mM, % loading = 43.3, pH = 3.0, temp. = 27 ± 0.2 °C, thickness = 0.051 cm.

into a definite volume of the release medium. The results are depicted in Fig. 3 which clearly indicate that the amount of released TC gradually increases with increasing percent loading. The observed increase in the release rate may be attributed to the fact that a larger loading of drug on the IPN facilitates a faster movement of the solvent front that penetrates the surface of loaded IPN [28].

Another reason for the observed higher release rate at higher loading could be that as TC is a charged molecule, a larger loading of TC in the IPN will originate repulsive forces within the network, thus resulting in a faster relaxation of macromolecular chains of the IPN. This obviously brings about a faster release rate of TC. It is notable here that a maximum of 85% of entrapped TC was found to be released.

3.5. Effect of IPN compositions on TC release

Swellable hydrophilic polymers have been used for the purpose of prolonged drug delivery and drug targeting [3]. Delivery systems based on relaxing networks are capable of slow release of an imbedded drug, with release controlled by the rate of swelling and relaxation of the polymer chains [29]. As the swelling pattern of a hydrogel is primarily dependent on the composition of the network, the release profiles of drug are directly and substantially regulated by the chemical architecture of the loaded device. In the present study also the release of TC is influenced by varying composition of the IPNs as discussed below.

3.5.1. Effect of PVA

It is an established fact [30] that the drug : polymer ratio is one of the important factors that significantly influences the release of a drug from the polymer matrices. In the present study the effect of PVA content of the IPN was investigated on the release amount of TC by varying the amount of PVA in the feed mixture in the range 0.5 to 1.5 g. The results are depicted in Fig.4 which indicate that the fractional release of TC increases upto 1.0 g of the PVA while beyond it a fall in the released amount as well as the release rate is noted. The reason for the observed initial increase is quite apparent as PVA is a hydrophilic polymer with distinct water associating properties and its increasing proportion in the IPN composition will result in a greater swelling of the network which as a consequence, will deliver increasing fraction of the drug into the release medium. However, beyond a certain concentration of PVA (1.0 g) the network density becomes so high that incoming water molecules as well as outgoing TC molecules are prevented by the network chains and, therefore, the fractional release rate slows down. The penetration velocities are not much different for PVA content of 0.5 and 1.0 g, however, at 1.5 g of PVA, penetration velocity appreciably decreases as shown in Table III.

3.5.2. Effect of styrene

An effective route to bring about the desired modification in the sorption property of a polymer is by introduction of a hydrophobic monomer into the hydrophilic system. This normally results in a change in the maximum hydration degree, and diffusion of the swelling agent into the matrix as well as the organization of water molecules depending on the chemical composition and the distribution of the hydrophobic monomeric units along the macromolecular chains [31]. For instance, the water gain property of a polymer of 2-hydroxyethyl methacrylate was affected by means of the introduction of a hydrophobic monomer such as furfurylacrylate [32]. Similarly, inclusion of a hydrophobic crosslinker into the polymer matrix has also been attempted for altering physical properties of the polymer [33].

The effect of increasing concentration of styrene on the release behavior of TC has been examined by varying its concentration in the range 4.3 to 13.0 mM in the feed

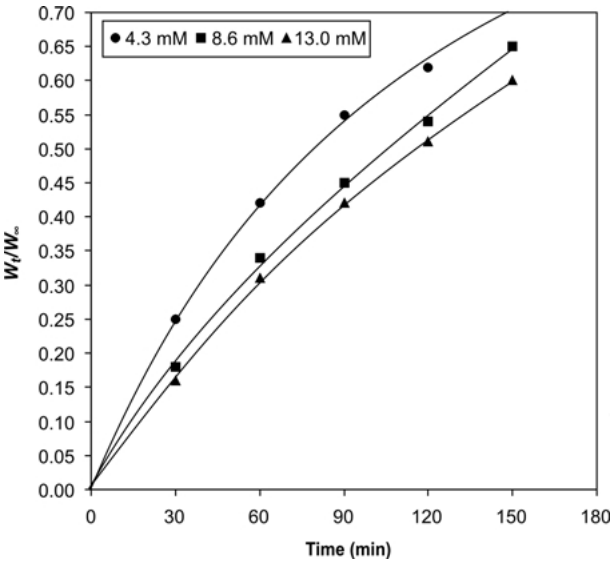

Figure 5 Effect of ST content of the semi-IPN on the fractional release of TC at fixed composition of the IPN, [PVA] = 1.0 g, [AM] = 10.5 mM, [MBA] = 0.12 mM, [KP] = 0.073 mM, % loading = 43.3, pH = 3.0, temp. = 27 ± 0.2 °C, thickness = 0.051 cm.

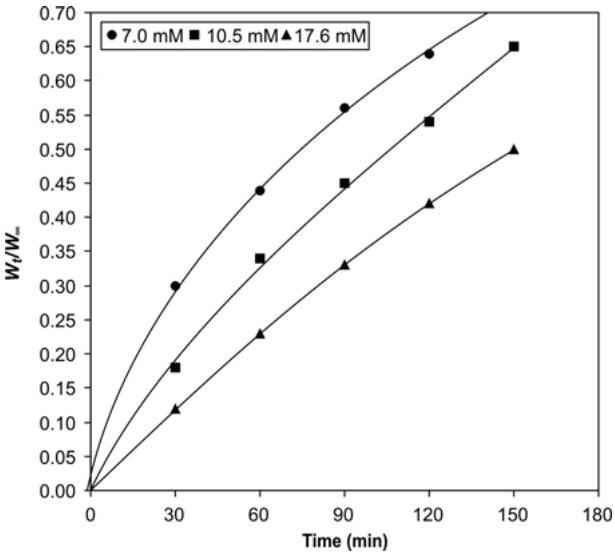

Figure 6 Effect of AM content of the semi-IPN on the fractional release of TC at fixed composition of the IPN, [PVA] = 1.0 g, [ST] = 8.6 mM, [MBA] = 0.12 mM, [KP] = 0.073 mM, % loading = 43.3, pH = 3.0, temp. = 27 ± 0.2 °C, thickness = 0.051 cm.

mixture of the IPNs. The results are displayed in Fig. 5 which indicate that with increasing proportion of styrene in the IPN the fractional release of TC constantly decreases. The observed decrease in fractional release may be attributed to the reason that increasing hydrophobicity in the IPN results in a lower water sorption which leads to a slow release of the entrapped drug. The observed lower water sorption is further confirmed by the decreasing values of penetration velocities as summarized in Table III.

3.5.3. Effect of acrylamide

The hydrophilic comonomer AM has been identified to have exerted a direct influence on the release profiles of the drug. In the present investigation the effect of AM variation on the release profile of TC has been studied by varying the monomer in the range 7.0 to 17.6 mM in the feed mixtures of the IPNs. The results are shown in Fig. 6 which indicate that with increasing AM content the fractional release constantly decreases. The observed findings may be explained by the fact that increasing crosslinked PAM chains in the gel network brings about a compactness of the macromolecular chains of the IPN and this restrains the penetrations of water molecules into the gel matrix and subsequent release of TC molecules into the release medium. A decreasing value of penetration velocity with increasing AM content in the IPN further confirms lower degree of swelling of the network.

Another notable observation from release curves is that on increasing the concentration of AM in the feed mixture the release mechanism shifts more towards zero order release which is a highly desirable goal in controlled drug-delivery technology. A number of approaches have been explored in the past to develop matrix systems which would deliver the active ingredient at a constant rate. These include: (a) the use of the rate controlling barriers, (b) modification of the geometry of the device, (c) establishing a nonuniform concentration distribution profile of the active ingredient across the matrix, and (d) swelling controlled delivery systems based on glassy hydrogels. It has also been suggested that the release of the active ingredient from the polymer would follow zero order kinetics as a result of time/position dependent diffusivities of the active ingredient.

In the present study also the observed tendency of release process to shift towards zero-order kinetics at higher AM content may be explained by the similar logic of time/position dependent diffusivities of the entrapped TC. It is clear from Table II that at higher AM content the diffusion constant of the drug has increased which is further supported by the observed increase in the penetration velocity of the moving front (water). It is also worth mentioning here that at higher AM content the IPN has low degree of water sorption which reveals that the drug molecules will not have to travel a longer path to come to the surface of the device and, therefore, an acceleration in the diffusivity of the TC molecules is expected. The phenomenon of time/position dependent diffusivity has been well cited in the literature.

3.5.4. Effect of crosslinker

The influence of increasing crosslinking of the IPN on the release behavior was investigated by employing different amounts of crosslinking agent (MBA) while in the IPNs preparation. When MBA was used in the concentration range 0.06 to 0.25 mM in the feed mixture of the IPNs a decrease in fractional release was noticed as shown in Fig. 7. The observed decrease in release rate may be attributed to the fact that increasing crosslinker in the IPN results in greater crosslink density of the network which, in turn, brings about a fall in the average molecular weight of the copolymer between the crosslinks as evident from the data presented in Table II. Thus, the decreasing molecular weight between crosslinks reduces the mesh sizes of the free volumes in the network and this obviously forbids both the entrance of water

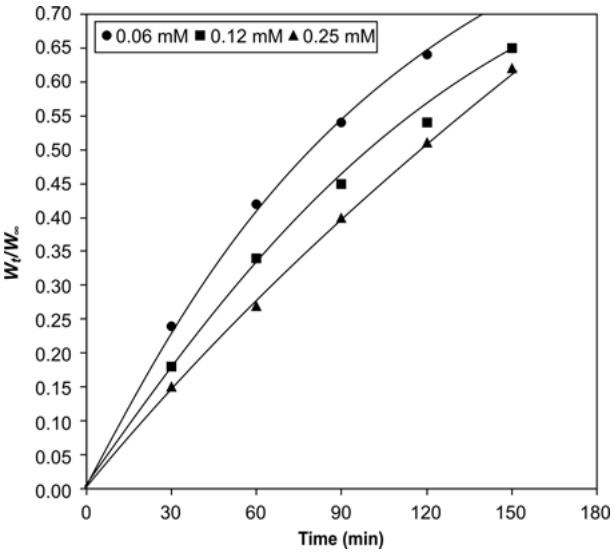

Figure 7 Effect of MBA content of the semi-IPN on the fractional release of TC at fixed composition of the IPN, [PVA] = 1.0 g, [ST] = 8.6 mM, [AM] = 10.5 mM, [KP] = 0.073 mM, % loading = 43.3, pH = 3.0, temp. = 27 ± 0.2 °C, thickness = 0.051 cm.

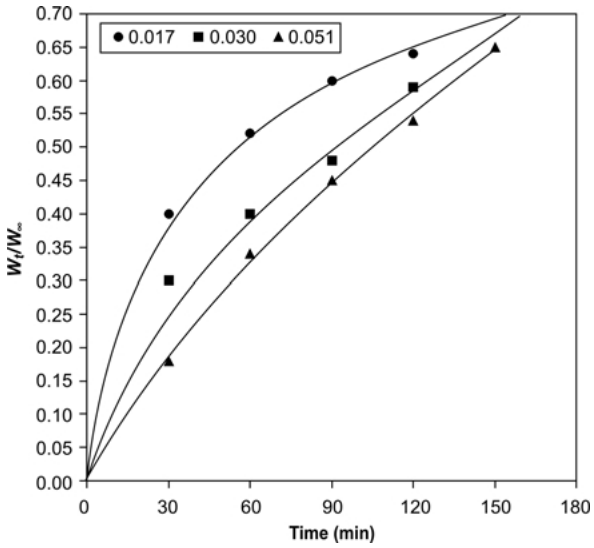

Figure 8 Variation of fractional release of TC with thickness of the semi-IPN of fixed composition, [PVA] = 1.0 g, [ST] = 8.6 mM, [AM] = 10.5 mM, [MBA] = 0.12 mM, [KP] = 0.073 mM, % loading = 43.3, pH = 3.0, temp. = 27 ± 0.2 °C.

molecules into the loaded matrix as well as the release of the drug into the outer medium.

Some authors [34] have reported an increase in the glass transition temperature of the polymer with increasing concentration of the crosslinker. This also results in a restrained mobility of the network chains and, therefore, slows down the swelling and the release processes.

As can be seen in Table III, the penetration velocities of the solvent have also decreased with increasing MBA concentration of the MBA, and hence this also supports the idea of slow penetration of water molecules into the IPN matrix.

Another typical feature visible in the released profiles of TC is the increasing zero order release tendency of the device with increasing MBA concentration in the IPN. This can again be explained by the fact that a greater crosslinked network swells to a lesser extent and, therefore, the releasing TC molecules have not to travel a longer path within the swollen network. Obviously, the TC molecules will diffuse out with greater diffusivity and this is further confirmed by the increasing values of diffusion constants as shown in Table III.

3.6. Thickness effect

Release of a water-soluble drug dispersed in a xerogel occurs only after water penetrates the network to swell the polymer and dissolve the drug followed by diffusion along aqueous pathways to the surface of the device. From Fick's laws of diffusion, flux (of drug) is directly proportional to surface area for fixed values of other dependent variables. Although the diffusion coefficient, D, depends on the water content [35] and has a fixed value for fully hydrated systems it will not be constant for systems which are swelling while they are releasing active additive. Until the hydrogel is fully swollen there will be a decreasing gradient of diffusivity from the surface to the center. Provided the supply of drug from the interior by diffusion, or dissolution of dispersant in the surface layer can maintain a constant level of concentration of dissolved drug, flux would be expected to increase along with expansion of the surface in accordance with Fick's 1st law.

In the present study, the influence of the surface area of the gel on the release kinetics has been observed by taking loaded hydrogels of varying thickness in the range 0.017 to 0.051 cm. The results are shown in Fig. 8 which indicate that the fractional release increases with decreasing thickness of the loaded hydrogel. The observed results are quite obvious as a thinner gel has a greater surface area and, therefore, will show a larger release. It has also been noted that thinner gel attains equilibrium release at earlier times than the thicker gel does (not shown). Another reason could be that the thicker is the gel greater would be the force required to stretch it, as evident from the slower swelling of the thickest (0.051 cm) gel. The degree of swelling of a hydrogel is controlled by a combination of free energies of mixing between water and the hydrophilic polymer chains and by the elastic response of the rubbery network to the expansion due to water uptake. Similar type of results have also been reported by other workers [36].

The penetration velocities calculated are summarized in Table III.

3.7. pH effect

The development of drug delivery systems capable of selective release of drugs in the colon have received much attention [37]. The major therapeutic applications which can be found for oral-colon-specific delivery are the treatment of local disorders in the colon and the delivery of peptide and protein drugs; colon is considered as less hostile environment (i.e. suitable as an absorption site for the drug because of less diversity and intensity of digestive enzymes) than the stomach and the small intestine [38]. The colon has long retention time and his highly responsive to desorption enhancers [39].

To improve therapeutic efficiency and reduce or

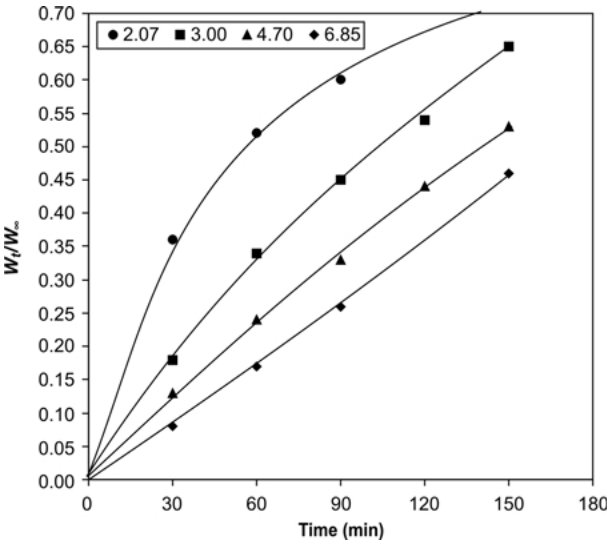

Figure 9 Effect of pH of the release medium on fractional release of TC through the semi-IPN of fixed composition, [PVA] = 1.0 g, [ST] = 8.6 mM, [AM] = 10.5 mM, [MBA] = 0.12 mM, [KP] = 0.073 mM, % loading = 43.3, temp. = 27 ± 0.2 °C, thickness = 0.051 cm.

eliminate side effects of oral administered drugs, it is reasonable to deliver drugs to specific regions of the gastrointestinal (GI) tract. Various compounds have been targeted to the colon in the form of prodrugs [40]. However, a more universal drug delivery system, which is not drug specific, is desirable. Several methods of targeting the specific regions have been used or proposed. Two of these, i.e. utilization of pH changes within the GI tract [41] and exploitation of bacterial enzymes localized within the colon [42] are of current interest in controlled drug delivery systems.

In the present investigation the effect of pH on the release pattern of tetracycline has been studied by varying the pH of the release medium in the range 2.07 to 6.85. The results are depicted in Fig. 9 which indicate that the amount and rate of fractional release of tetracycline decrease with increasing pH of the medium. The release-profile curves also imply that as the pH of the release medium increases the release kinetics shifts towards more and more zero-order type and particularly at pH 6.85 a zero-order release is achieved upto 65% of the released tetracycline. The results can be explained as below : Tetracycline produces three acidity constants (Table IV) in aqueous solutions of their salts as its molecule has three protonation sites as shown in Fig. 10. Thus, in the acidic range at lowest pH, i.e. 2.07, the amide groups become protonated and cause repulsion among the entrapped drug molecules within the gel network. This obviously results in a widening of the mesh sizes of the gel which facilitate release of the tetracycline molecules into the release medium. Another reason may be that the amide groups of the polyacrylamide segments may also be protonated at low pHs and produce repulsion among the protonated segments within the gel network.

TABLE IV Actidity constants for tetracycline

Antibiotic	pKa_1	pKa_2	pKa_3
Tetracycline	3.3	7.7	9.5

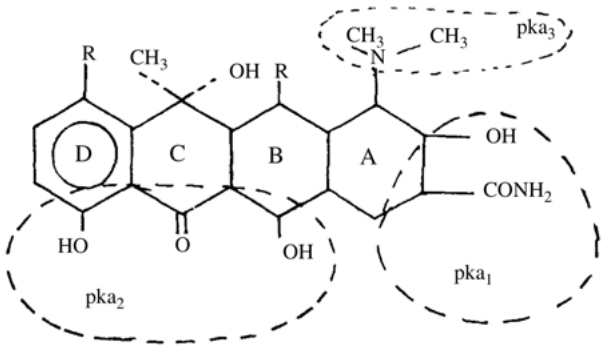

Figure 10 Structure of TC molecule.

It is also implied by Fig. 10 that with increasing pH, the release mechanism shifts towards zero order which can be interpreted as below:

The swelling of a polymer matrix normally results in a rapid decrease in its glass transition temperature (Tg) to the temperature of the dissolution medium. Microscopically, there is a relaxation response of the polymer chains due to stresses introduced by the presence of the dissolution solvent. This results in an increase in the radius of gyration and end-to-end distances of the polymer chains, causing a significant increase in the molecular volume of the hydrated polymer [43]. This reduces the free volume due to the presence of the micropores, which may manifest itself as a shift in the drug release mechanism. Thus, the observed zero order release may be attributed to a reduction in regions of low microviscosity and the closing of micropores in the swollen gel.

Another possible explanation could be based on the time/position dependent diffusivity of the active ingredient as explained earlier. With increasing pH of the release medium the tendency of PAM chains of the IPN to become protonated by producing $-CONH_3^+$ groups along the PAM segments of the network chains decreases which causes a lower degree of water sorption due to a decrease in repulsive forces between the network chains. Thus, because of low swelling of the network, the drug molecules have not to travel longer path for being released into the outer medium and this will result in an enhanced diffusion of TC molecules.

3.8. Effect of medium

The effect of nature of the medium on the release kinetics of TC has been investigated by performing the release experiments in physiological fluids such as saline water and artificial urine. The results are depicted in Fig. 11 which reveal that the fractional release rate is significantly suppressed in physiological fluids in comparison to that in distilled water. The possible reason for lower release rate of TC in these fluids may be due to the presence of salt ions in the release medium which lower the rate of penetration of water molecules into the loaded gel, thus resulting in a fall in the released amount of TC.

It can also be seen in Fig. 11 that in physiological fluids, the mode of the release process tends to acquire zero-order nature which may be explained by the fact that the presence of salt ions lowers the swelling of the

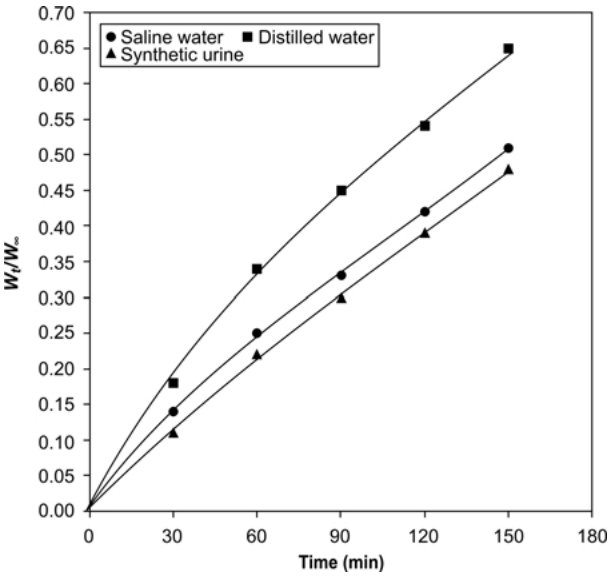

Figure 11 Effect of the nature of the release medium on the fractional release of TC through the semi-IPN of fixed composition, [PVA] = 1.0 g, [ST] = 8.6 mM, [AM] = 10.5 mM, [MBA] = 0.12 mM, [KP] = 0.073 mM, % loading = 43.3, pH = 3.0, temp. = 27 ± 0.2 °C.

loaded IPNs so that diffusion of drug molecules into the release medium is not significantly reduced, thus maintaining the condition for a zero-order release.

3.9. Analysis of release data

Release of a water soluble drug entrapped in a hydrogel occurs only after water penetrates the network to swell the polymer and dissolve the drug followed by diffusion along aqueous pathways to the surface of the device. The release profile of a drug is closely related to the swelling characteristics of the hydrogel which, in turn, is a key function of the chemical architecture of the gel. As mentioned previously, the analysis of release data provides valuable information about the transport mechanism of the drug within the hydrated gel and, thus, the following discussion highlights the correlation of release data and transport mechanism. The value of n and D calculated according to Equations 3 and 4 are summarized in Tables III and V.

When the concentration of PVA increases in the gel in the range 0.5 to 1.5 g the release exponent decreases from Super Case II to anomalous value as shown in Table V. The results observed can be explained by the fact that at lower PVA content in the gel the free volumes in between the network chains will have large mesh sizes and, therefore, the water sorption and subsequently the drug release would be faster displaying Super Case II transport. However, upon increasing PVA content in the gel the diffusional exponent decreases to an anomalous value indicating a relaxation controlled nature of release process. This appears justified also as greater PVA content in the gel will result in dense network which permits slow relaxation of macromolecular chains.

When the amount of styrene increases in the feed mixture of the gel in the range 4.3 to 13.0 mM, the diffusional exponent is found to increase in the anomalous region indicating that the release process becomes increasingly relaxation controlled. This can be explained by the fact that larger number of hydrophobic polystyrene chains, because of their bulky sites, experience restriction in relaxation and as a result the release process becomes relaxation controlled.

In case of addition of hydrophilic monomer (acrylamide) to the feed mixture of the gel in the concentration range 7.0 to 17.6 mM, the diffusional exponent n is found to increase from a nearly Fickian value of 0.47 to anomalous value of 0.82, thus implying a shift of release mechanism from diffusion controlled to relaxation controlled. The observed shift in release mechanism could be attributed to the fact that with increasing crosslinked polyacrylamide chains in the gel network, the relaxation process becomes less favorable thus making release of the tetracycline as relaxation controlled.

The crosslinker also has a pronounced effect on the release mechanism. When the concentration of crosslinker (MBA) increases in the range 0.06 to 0.25 mM the diffusional exponent decreases from a Super Case II value to anomalous value, thus implying that the release process acquires increasing chain relaxation controled nature. However, at the lowest MBA content the observed Super Case II value of the diffusional exponent suggests a faster water sorption process which is found experimentally verified also.

The thickness of the loaded gel also influences the transport mechanism of releasing tetracycline. It is

TABLE V Data showing the variation of diffusional (release) exponent n with varying composition of the IPNs

PVA (g)	ST (mM)	AM (mM)	MBA (mM)	Thickness (cm)	n	Swelling mechanism
0.5	8.6	10.5	0.12	0.051	1.25	Super Case II
1.0	8.6	10.5	0.12	0.051	0.76	Anomalous
1.5	8.6	10.5	0.12	0.051	0.70	Anomalous
1.0	4.3	10.5	0.12	0.051	0.70	Anomalous
1.0	8.6	10.5	0.12	0.051	0.76	Anomalous
1.0	13.0	10.5	0.12	0.051	0.80	Anomalous
1.0	8.6	7.0	0.12	0.051	0.47	Fickian
1.0	8.6	10.5	0.12	0.051	0.76	Anomalous
1.0	8.6	17.6	0.12	0.051	0.82	Anomalous
1.0	8.6	10.5	0.06	0.051	1.27	Super Case II
1.0	8.6	10.5	0.12	0.051	0.76	Anomalous
1.0	8.6	10.5	0.25	0.051	0.66	Anomalous
1.0	8.6	10.5	0.12	0.017	0.48	Fickian
1.0	8.6	10.5	0.12	0.030	0.62	Anomalous
1.0	8.6	10.5	0.12	0.051	0.76	Anomalous

noticed that with increasing thickness of the gel the diffusional exponent increases from Fickian to non-Fickian (anomalous) value indicating for a shift of transport mechanims from diffusion controled to relaxation controlled process.

The numerical values of the diffusion constants (D) have also been calculated and summarized in Table III.

4. Conclusions

The copolymerization of acrylamide (AM) and styrene (ST) in the presence of a crosslinker and a preformed polymer such as poly(vinyl alcohol) (PVA) results in the formation of a semi-interpenetrating polymer network (IPN) which shows potential to act as a vehicle for the controlled delivery of tetracycline (TC). It is observed that the amount of released TC increases with % loading of the drug onto the IPNs. The fractional release of TC is greatly influenced by the chemical architecture of the gel. In the case of variation in the PVA content of the IPN the fractional release initially increases while beyond an optimum PVA content a decrease in the release rate is noticed. When the ST and AM contents are varied in the fixed mixture of the IPNs, the fractional release also drops. It is also noticed that at higher AM content of the IPN the release process approaches zero order kinetics. A similar trend is also observed with increasing crosslinker (MBA) content of the IPN. The rate of fractional release is also found to decrease with decreasing thickness of the IPNs and increasing pH of the release medium. It is also found that when release experiments are performed in physiological fluids such as saline water and artificial urine, the fractional release not only decreases but also acquires zero-order kinetics. The release exponent (n), diffusion constant (D) and penetration velocity (v) also vary with varying IPN composition and experimental conditions.

References

1. N. PEPPAS, in ''Hydrogels in Medicine and Pharmacy'', edited by N. Peppas (CRC, Boca Raton, FL, 1986).
2. H. ICHIKAWA and Y. FUKUMORI, *J. STP Pharma. Sciences* **7**(6) (1997) 529.
3. M. DUMITRIU, in ''Polymeric Drug Carriers'', edited by S. Dumitriu (Polymeric Biomaterials, Marcel Dekker, New York, 1994).
4. P. COLOMBO, *Adv. Drug Deliv. Rev.* **11** (1993) 37.
5. R. M. PALMER, T. L. P. WATTS and R. F. WILSON, *J. Clin. Periodont.* **23** (1996) 670.
6. E. UNSAL and T. F. AKKAYA, *ibid.* **21** (1994) 351.
7. D. SENDIL, I. GURSEL, D. L. WISE and V. HASIRCI, *J. Control Release* **59** (1999) 207.
8. M. V. RISBUD, A. A. HARDIKAR, S. V. BHAT and R. R. BHONDE, *ibid.* **68** (2000) 23.
9. B. BITTNER, K. MADER, C. KROLL, H.-H. BROCHERT and T. KISSEL, *ibid.* **59** (1999) 23.
10. S. H. HYON, W. I. CHEA, Y. IKADA, M. KITU, Y. OYURA and Y. HONDA, *J. Biomater. Sci. Polym. Ed.* **5** (1994) 397.
11. J. H. TUANG, W. S. BANNER, Y. J. OGAWA, P. VACANTI and G. C. WEIR, *Transplantation* **61** (1996) 1557.
12. D. H. CHEN, J. C. LEU and T. C. HUANG, *J. Chem. Technol. Biotechnol.* **61** (1994) 351.
13. B. C. THANOO, M. C. SUNNY and A. JAYAKRISHANA, *J. Pharm. Pharmacol.* **45** (1993) 16.
14. J. T. CORSTENSEN, J. P. MARTY, F. PUISIEUX and H. FESSI, *J. Pharm. Sci.* **70** (1981) 222.
15. A. K. BAJPAI and M. SHRIVASTAVA, *J. Macromol. Sci. Pure Appl. Chem.* **A37**(9) (2000) 1069.
16. S. Z. SONG, S. H. KIM, J. R. CARDINAL and S. W. KIM, *J. Pharm. Sci.* **70** (1981) 216.
17. F. D. SNELL and C. T. SNELL, in ''Colorimetric Methods of Analysis'', Vol. IV AAA (Van Nostrand Reinhold Co., 1971).
18. N. A. PEPPAS and N. M. FRANSON, *J. Polym. Sci. Polym. Phys. Ed.* **21** (1983) 983.
19. R. W. KROSMEYER and N. A. PEPPAS, *J. Membr. Sci.* **9** (1981) 211.
20. C. I. REINHART and N. A. PEPPAS, *ibid.* **18** (1984) 227.
21. Z. Y. DING, J. J. AKINOIS and R. SALOYEV, *J. Polym. Sci. Polym. Phys.* **20** (1991) 1035.
22. J. ROSIAK, K. BURCZAK, T. CZOKYNOKA and W. PEKALA, *Radiat. Phys. Chem.* **22** (1983) 917.
23. H. H. HOOPER, J. P. BAKER, H. W. BLANCH and J. M. PRAUSNITZ, *Macromolecules* **23** (1990) 1096.
24. N. A. PEPPAS, *Pharm. Acta Helv.* **60** (1985) 110.
25. P. COLOMBO, A. GAZZANIGA, U. CONTE, M. E. SANGALLI and A. L. MANNA, *Proc. Int. Symp. Control Release Bioact. Mater.* **14** (1987) 83.
26. G. R. DAVISON III and N. A. PEPPAS, *J. Control Release* **3** (1986) 243.
27. R. A. GRINSTED, L. CLARK and J. L. KOENIG, *Macromolecules* **25** (1992) 1235.
28. S. W. KIM, Y. H. BAE and T. OKANO, *Pharmaceut. Res.* **9** (1992) 283.
29. N. A. PEPPAS and A. R. KHARE, *Adv. Drug Deliv. Rev.* **11** (1993) 1.
30. CO, XU and H. SUNADA, *Chem. Pharm. Bull.* **43** (1995) 483.
31. B. VOZQUEZ, J. S. ROMAN, C. PENICHE and M. E. COHEN, *Macromolecules* **8440** (1997) 30.
32. C. PENICHE, D. ZALDIVAR, A. GALLARDO and J. SAN ROMAN, *J. Appl. Polym. Sci.* **54** (1994) 959.
33. C. PENICHE, M. E. COHEN, B. VAZQUEZ and SAN ROMAN, *J. Polymer* **38** (1977) 5977.
34. B. RAMRAJ and G. RADHAKRISHNAN, *Polymer* **35**(10) (1994) 2167.
35. M. E. MCNEILL and N. B. GRAHAM, *J. Biomater. Sci. Polym. Edn.* **4**(3) (1993) 305.
36. M. E. MCNEILL and N. B. GRAHAM, *ibid.* **5**(1/2) (1993) 111.
37. D. R. FRIEND (ed.) ''Oral Colon-Specific Drug Delivery'' (Boca Raton FL, CRC Press, 1992).
38. K. IKESUE, P. KOPECKOVA and J. KOPECEK, *J. Int. J. Pharm.* **95** (1993) 171.
39. A. RUBINSTEIN, *Crit. Rev. Ther. Drug Carrier Syst.* **12** (1995) 101.
40. M. ASHFORD, J. T. FELL, D. ALTWOOD, H. L. SHAYAMA and F. J. WOODHEAD, *J. Pharm. Pharmacol.* **44** (1992) 1049.
41. H. S. CHANG, H. PARK, P. KELLY and J. R. ROBINSON, *J. Pharm. Sci.* **74** (1985) 399.
42. C. M. LANCASTER and M. A. WHEATLY, *Polym. Prep.* **30** (1989) 480.
43. K. V. RANGA-RAO and K. P. DEVI, *Int. J. Pharm.* **48** (1988) 1.
44. S. S. SHAH, M. G. KULKARNI and R. A. MASHELKAR, *J. Appl. Polym. Sci.* **41** (1990), 2437.

Received 12 September 2001
and accepted 13 June 2002