Evaluation of starch based cryogels as potential biomaterials for controlled release of antibiotic drugs

L P BAGRI, J BAJPAI and A K BAJPAI*

Department of Chemistry, Government Autonomous Science College, Jabalpur 482 001, India

Abstract. In the present study starch has been blended with poly(vinyl alcohol) to design macroporous architectures following a repeated freeze-thaw method. These macroporous cryogels were loaded with an antibiotic drug, ciprofloxacin hydrochloride (Cfx), and evaluated for its *in vitro* delivery in a completely controlled manner thus exploring possibilities to use it as a biomaterial in burn or wound healing applications. The key advantage of the present system is that cryogels formed do not contain any chemical crosslinking agent which is often harmful to organic compounds. These Cfx loaded cryogels were characterized by infrared (FTIR) spectroscopy and scanning electron microscopy (SEM) techniques. The controlled release of Cfx drug from cryogels was investigated under varying experimental conditions such as percent loading of the antibiotic drug, chemical architecture of the cryogels and pH, temperature, and nature of the release media. The prepared cryogels show promise to provide a possible pathway for controlling delivery of antibiotic drug thus minimizing the known side effects and improving efficacy also.

Keywords. Cryogels; freeze–thaw; drug delivery; macroporous; ciprofloxacin hydrochloride.

1. Introduction

Hydrogels are insoluble, three dimensionally crosslinked polymer network structures composed of hydrophilic homo- or hetero-co-polymers, which have the ability to absorb significant amounts of water (Dimitrov 2003). From a biological viewpoint, this is an essential property to achieve an immunotolerant surface and matrix (i.e. with respect to protein adsorption or cell adhesion). Due to the significant water content, hydrogels also possess a degree of flexibility and texture very similar to natural tissue, which minimize potential irritation to surrounding membranes and tissues. The hydrophilic and hydrophobic balance of a gel carrier can be altered to provide tunable contributions which, in turn, influence the diffusive release of drug contained within the gel matrix (Szepes *et al* 2008; O'Brien *et al* 2009). Although hydrogels are made up of single polymers that have been extensively used in drug delivery studies, however, in many cases a single polymer alone cannot meet divergent demands in controlled drug release in terms of both properties and performance (Tong *et al* 2007).

Hydrogels are versatile materials and have potential applications as biomaterials in artificial muscle, drug delivery, contact lenses, dialysis membranes and tissue engineering. However, poor mechanical properties of these materials restrict their applications as structural biomaterials as in many cases the latter do need tolerable mechanical strength in addition to biophysical characteristics. Crosslinking by chemical agents (McGann *et al* 2009) or physical method (e.g. ionizing radiation, repeated freezing-thawing etc.) are common methods to produce a hydrogel matrix. The chemical crosslinking is rapid but may involve toxic chemicals whereas physical methods are quite safer and non-toxic but often slow in time scale. The freeze–thaw method is a simple and environment friendly technique to design highly elastic hydrogels (Hyon *et al* 1994; Peppas *et al* 1996).

It would be most desirable for a drug release system to match a patient's physiological need at proper time and/or proper site. This is why there is a great interest in the development of such controlled delivery systems (Wu *et al* 2001). Drug delivery technology can be brought to the next level by the fabrication of smart materials into single assembled device that is responsive to the individual patient's therapeutic requirements and able to deliver a certain amount of drug in response to biological state. Such smart therapeutics should possess one or more properties such as proper drug protection, local targeting, precisely controlled release, self-regulated therapeutic action, permeation enhancing, enzyme inhibiting, imaging and reporting. This is clearly a highly challenging task and it is difficult to add all of these functionalities in a single device (Juang *et al* 1996; Elvira *et al* 2002).

The controlled delivery of drugs has recently become an intensively developed field of pharmaceutical activity.

---

*Author for correspondence (akbmrl@yahoo.co.in)

The purpose behind controlling the drug delivery is to achieve more effective therapies, less side effects, prolonged efficacy, enhanced safety and reliability of drug therapy, regulated drug release rate and reduced frequency of drug administration to encourage patient compliance while eliminating the potential for both under and over dosing (Qiu and Park 2001).

Ciprofloxacin hydrochloride (Cfx), also called 1-cyclopropyle-6-fluoro-1-4-oxo-7-C-piprazinyl)-3quinoline carboxylic acid hydrochloride monohydrate, has been used as clinical medicine to kill green pus bacillus, large intestine bacillus, strain coccus, streptococcus and gold grape coccus that can result in some type of infections such as respiration, urethra, alimentary canal, etc (He *et al* 2004). Ciprofloxacin is a leader among the third generation fluoroquinolones with a broad spectrum of antibacterial activity and good penetration in most tissues (Chen *et al* 1994; Désévauxa *et al* 2002). Fluoroquinolones such Cfx are considered to be an appropriate choice for the prevention and treatment in infections in bone and soft tissues (Chouhan and Bajpai 2009).

Poly(vinyl) alcohol (PVA) based hydrogels have been used in a number of biomedical applications because of their inherent non-toxicity, non-carcinogenicity, good biocompatibility and desirable physical properties such as rubbery or elastic nature and high degree of swelling in aqueous solutions. PVA has also gained wide pharmaceutical applications as drug-delivery matrices (Korsmeyer and Peppas 1981; Basmadjian and Sefton 1983; Colombo *et al* 1985) or in the form of powders added to mixtures of other excipients for tablet formation (Thanoo *et al* 1993).

Starch is composed of two distinct fractions: amylase is the fraction containing about 4000 glucose units and amylopactin is the branched fraction containing about 100000 glucose units. Starch is naturally hydrolyzed by several amylolytic enzymes like α-amylase which is an endo enzyme specific to α-(1,4)–D-glucopyranosidic bonds located within polyglucose chains. The degradation products of starch amylolysis are mainly composed of oligosaccharaides, dextrins and maltose (Lia *et al* 1998).

An exhaustive work has been done in the field of controlled drug delivery of antibiotic drugs through polymeric matrices. The delivery of this drug by starch–PVA macroporous cryogels offers the possibility of maximizing its efficacy and safety and provides a suitable rate of delivery of the therapeutic dose, at the most appropriate site in the body. The drug loaded PVA–starch cryogels matrix may find application as antibiotic patches in post-surgical periods.

2. Experimental

2.1 *Preparation of polymer blends*

The freez–thaw method was adopted for preparation of blend hydrogels of starch and PVA as described elsewhere

(Bajpai and Saini 2005). In a typical experiment, definite amounts of starch and PVA were dissolved into distilled water in a Petri-dish and kept at −20°C for 24 h. The frozen gel was then thawed for 1 h at room temperature and again kept at −20°C for freezing. Such freezing–thawing cycles were repeated at least three times so that the entire mass was converted into a soft, spongy, white blend. The blend so prepared was purified by equilibrating it in distilled water for 72 h in order to leach out the unreacted chemicals as soon as possible. The swollen blend was cut into smaller discs and dried at room temperature for a week. The dried blend pieces were stored in airtight polyethylene bags.

2.2 *Characterization of blends*

2.2a *FTIR spectral analysis:* The infrared spectral analysis was performed on an FTIR spectrophotometer (FTIR-8400S, Shimadzu spectrophotometer) by recording the IR spectra of a dry thin film of the blend.

2.3 *ESEM studies*

The morphological features of the cryogels were investigated by recording their scanning electron micrographs (STEREO SCAN, 430, Leica, SEM, USA).

2.4 *Loading of drug*

An important aspect in the use of blends as drug vehicle was the effect of the drug loading level on the drug release rate. Higher drug loading may be achieved either by using highly concentrated drug solution or repeated soaking of blends in the drug solution and then drying them. Ideally, a successful drug delivery system should have a high drug loading capacity thereby reducing the quantity of matrix material for administration. Drug may be bound to the blends either (i) by polymerization in the presence of drug in most cases in the form of solution (incorporation method) or (ii) by absorbing/adsorbing the drug after the formation of blends by incubating them in the drug solution. In the present work, the former method was adopted as in the latter one, however, purification of the loaded device remains a problem (Bajpai and Choubey 2006).

For loading drugs onto the blends, known volume of drug (ciprofloxacin) was taken and diluted with appropriate amount of PBS solution and shaken vigorously for mixing of drug and PBS solution. Drug loaded devices were prepared by allowing 100 mg of blends to swell in freshly prepared drug solution (10 ml) until equilibrium swelling was reached. The percent of drug loaded onto blends was calculated by (1)

$$\% \text{Loading} = \frac{W_d - W_0}{W_0} \times 100, \qquad (1)$$

where W_d and W_0 are the dry weights of drug loaded and unloaded gels, respectively.

2.5 *In vitro release experiments*

In vitro release experiments were carried out by placing the ciprofloxacin loaded blends (0·1 g) in a test tube containing a definite volume (10 ml) of phosphate buffer saline (PBS) as the release medium (pH = 7·4) (1·2 mM KH_2PO_4, 1·15 mM Na_2HPO_4, 2·7 mM KCl, 1·38 mM NaCl). The amount of ciprofloxacin released from the cryogel blends was measured spectrophotometrically at 273 nm (Shimandzu 1700 Pharma Spec.) and the released amount of drug was determined from calibration plot (Knop 1996).

2.6 *Release kinetics*

The suspension of drug loaded blends was shaken for 1·5 h to study kinetics of the drug release process. In order to monitor the progress of the release process, 5 ml aliquots were withdrawn at desired time intervals and instantly replaced by the same volume of fresh release medium (PBS). In the aliquots withdrawn at different time periods, the amount of drug released was estimated spectrophotometrically.

The release of drug from polymeric blend systems is usually considered as a combination of Fickian (diffusion) and non-Fickian movements of drug molecules through polymer chains (Kosmidis *et al* 2003). In the present study, the kinetic data were analysed with the help of (2), which could be helpful in determining the mechanism of the release process

$$W_t / W_\infty = K t^n, \qquad (2)$$

where W_t and W_∞ are the amounts of the drug released at time, t and at infinity time (equilibrium amount of drug released), respectively and K is a rate constant. The constants, K and n, are characteristics of the drug–polymer system. Hassan and Peppas (2000) were the first to give an introduction to the use and limitations of these equations. For evaluating the diffusion constant of loaded drugs, (3) can be used:

$$\frac{W_t}{W_\infty} = 4 \left(\frac{D_t}{\pi L^2} \right)^{0.5}, \qquad (3)$$

where D is the diffusion constant of the drug and L the thickness of dry blends.

2.7 *Blood compatibility*

In order to evaluate the suitability of a polymer matrix to biomedical applications, particularly as artificial implant, the biomaterial must meet certain criteria prior to its actual realization. Thus, in order to judge the blood compatibility nature of the material, certain test procedures were established for determination of *in vitro* blood compatibility of the polymer material as discussed below.

2.7a *Protein adsorption*: The foremost event occurring at the interface of the blood–material contact was the adsorption of plasma proteins (bovine serum albumin, fibrinogen, etc) which was subsequently followed by the adhesion of leukocytes, macrophages or platelets and ultimately leading to fibrous encapsulation. Thus, the adsorption of proteins could be one of the determinants of biocompatible nature of the material. The adsorption of proteins was studied as below.

A known volume of protein solution of definite concentration was mildly shaken with the polymer blend for a definite time period and the remaining concentration of protein was monitored in the solution spectrophotometrically. The amount of the adsorbed protein was calculated according to (4)

$$\text{Adsorbed amount (mg m}^{-2}) = \frac{(C_0 - C_a)V}{A}, \qquad (4)$$

where C_0 and C_a being the concentrations of protein solution (mg per ml) before and after adsorption, respectively, V the volume of the protein solution and A the surface area of the adsorbent.

2.7b *Clot formation test*: The antithrombogenic potential of the blends surface was judged by the blood clot formation test as described elsewhere (Bajpai and Kankane 2007). In brief, the starch blends were equilibrated with saline water (0·9% w/v NaCl) for 72 h in a constant temperature bath and to these swollen blends, 0·5 ml of acid citrate dextrose (ACD) blood was added followed by the addition of 0·03 ml of $CaCl_2$ solution (4 M) to start the thrombus formation. The reaction was stopped by adding 4·0 ml of deionized water and the formed thrombus was separated by soaking in water for 10 min at room temperature and then fixed in a 36% formaldehyde solution (2·0 ml) for another 10 min. The fixed clot was placed in water for 10 min and weighted after drying.

2.7c *Haemolysis assay*: Haemolysis experiments were performed on the surfaces of the prepared blends as reported elsewhere (Bajpai and Bundela 2008). In a typical experiment, a dry blend film was equilibrated in normal saline water (0·9% NaCl solution) for 24 h at 37°C and human ACD blood (0·25 ml) was added onto the blend films. After 20 min, 20 ml of saline was added on the surface to stop haemolysis and the sample was incubated for 60 min at 37°C. Positive and negative controls were obtained by adding 0·025 ml of human ACD blood and saline solution, respectively to 2 ml of distilled

water. Incubated samples were centrifuged for 45 min, the supernatant was taken and its absorbance was recorded on a spectrophotometer at 545 nm. The percent haemolysis was calculated according to (5)

$$\% \text{ Haemolysis} = \frac{A \text{ test sample} - A(-) \text{ control}}{A(+) \text{ control} - A(-) \text{ control}}, \quad (5)$$

where A is the absorbance.

3. Results and discussion

3.1 *Mechanism of cryogels formation*

Mechanism of hydrogels preparation involves physical crosslinking due to crystallite formation. This method addresses toxicity issues because it does not require the presence of a crosslinking agent. Such physically cross-linked materials exhibit higher mechanical strength than PVA blends crosslinked by chemical or irradiative techniques because in the former case the mechanical load can be distributed along the crystallites of the three-dimensional structure (Gruber *et al* 1974). Although formation of an elastic blend by either having an aqueous solution of PVA at room temperature or successively freezing–thawing a moderately concentrated PVA solution is not a new concept, however, a molecular explanation for this phenomenon is not yet known. Three basic models including hydrogen bonding, polymer crystallites formation and liquid–liquid phase separation have been suggested to explain the mechanisms involved in blend formation.

In the present study, since a mixture of PVA and starch solution was taken, the above three models may be considered to operate simultaneously, thus yielding a highly elastic blend. The reason for this assumption is that starch is a multifunctional biopolymer and its presence in PVA solution will enhance the processes of hydrogen bond making, polymer crystallite formation and phase separation, respectively. The porous nature of blends produced by freezing–thawing method may be explained by the fact that whereas the freezing of a PVA–starch mixture results in the formation of ice crystal domains within the polymer matrix, the thawing process results in melting of the ice crystals, thus leaving wide pores in the blend. A repeated performance of the processes widens the pore sizes and thus enhances the porous nature of the blends. The formation of porous network due to freezing–thawing hypothetically is shown in figure 1.

3.2 *FTIR spectra*

FTIR spectra of the unloaded and Cfx loaded blends composed of PVA and starch are depicted in figures 2(a) and (b), respectively. The FTIR spectra clearly shows a broad band around 3583 cm^{-1} which is typical of bonded O–H stretching of alcoholic hydroxyls of starch and PVA (figure 2(a)). The spectra shows prominent peaks at 2924 cm^{-1} due to =CH stretching of methylene group, 1795 cm^{-1} due to overtone or combination bands, 1483 cm^{-1} due to sp^3-CH bending, and 1151 cm^{-1} due to C–O stretching of alcohol. The spectra (figure 2(b)) also mark the presence of drug (Cfx) as evident from the observed bands at 1163 cm^{-1} (C–N stretching) and 1003 cm^{-1} (C–F stretching). Similarly C–H out of plane bending vibration of aromatic ring also indicates the presence of Cfx in the cryogels.

3.3 *Scanning electron microscopy*

The morphological features of the proposed cryogel have been studied by SEM analysis. In order to demonstrate how the freeze–thaw method develops the formation of pores over the cryogels surfaces, a blend of PVA–starch was also prepared by simple solution cast method and the SEM images of both the types of blends were compared.
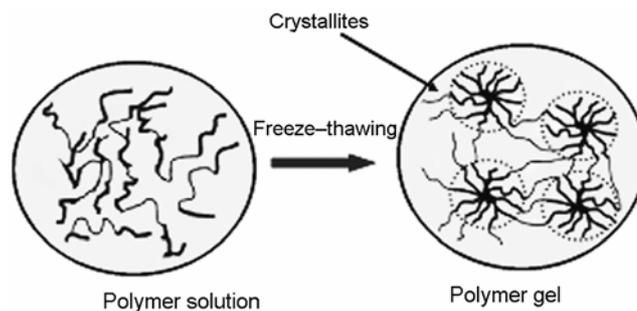

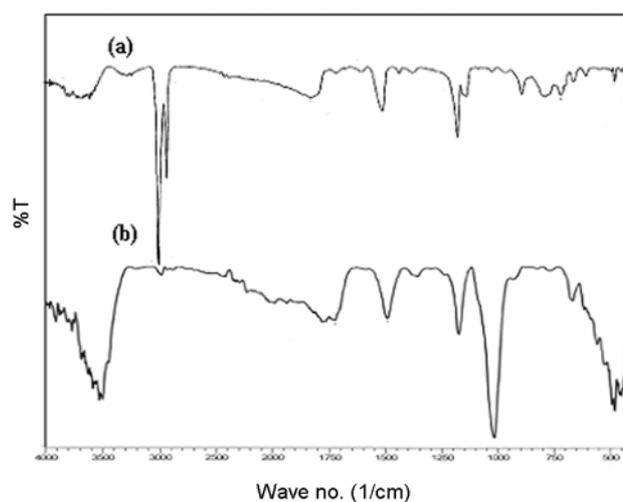**Figure 1.** A schematic presentation of mechanism of cryogel formation.

Figure 2. FTIR spectra of (a) Cfx unloaded blend of PVA and starch and (b) Cfx loaded blend of PVA and starch.
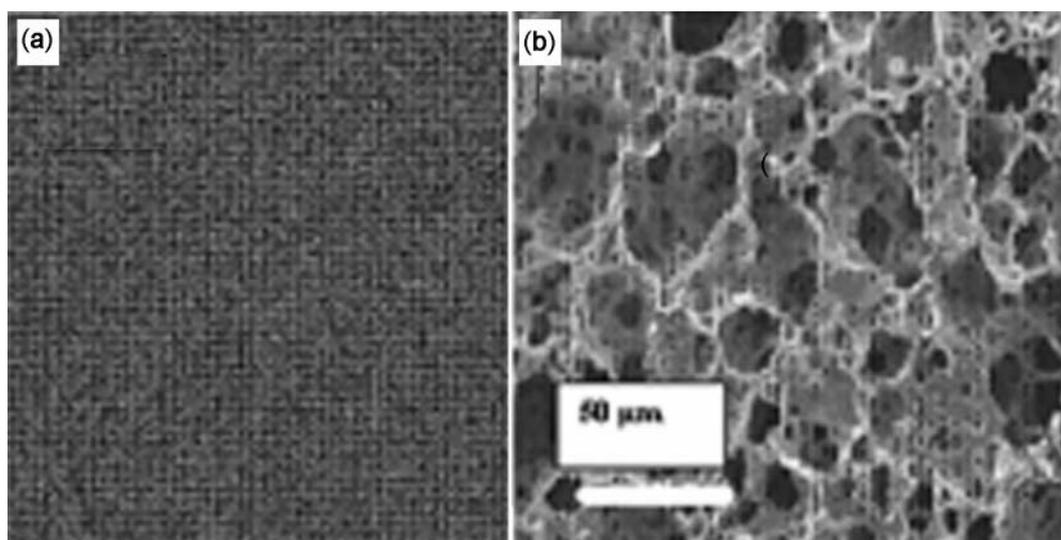
Figure 3. SEM images of blend of PVA and starch synthesized (**a**) simple solution casting method and (**b**) freezing–thawing method.
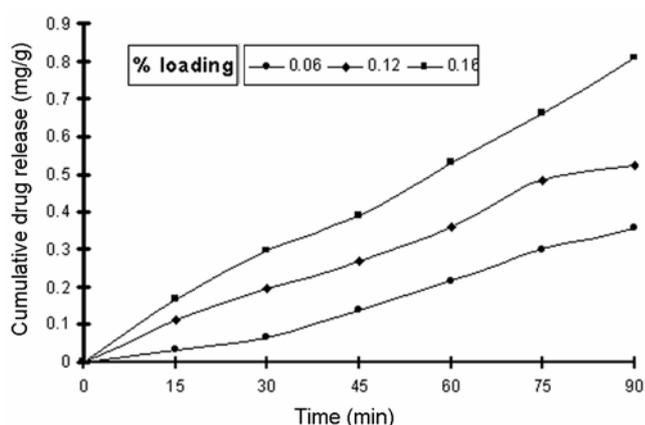

Figure 4. Effect of % loading on cumulative release of Cfx drug from blend composed of PVA and starch (40/60% w/w) (pH = 7·4, temp. = 37°C, FTC = 3).

The SEM images of simple and cryogels blends of PVA and starch are shown in figures 3(a) and (b), respectively. Figure 3(a) clearly shows that both the PVA and starch form a homogeneous blend having no porosity, unevenness, cracks or voids on the surfaces. On the other hand, figure 3(b) presents a porous morphology with pore sizes varying in the range 25–50 μm. In this way, a clear distinction between the two images provides an experimental evidence for the proposed formation of crystallites during the freezing–thawing method.

3.4 *Effect of % loading on Cfx release*

In the present work, blends of definite composition were loaded with different amounts of Cfx by allowing the blends to swell in the drug solution of concentration varying in the range 0·5–2·0 mg/ml. The loaded blends were allowed to release the entrapped drug into a definite volume of release medium. The release results are shown in figure 4, which reveal that initially on increasing drug concentration up to 2·0 mg/ml a larger release of the drug is obtained. The observed results may be attributed to the fact that in the blends with higher loading (up to 2·0 mg/ml) of drug concentration, the solvent front (PBS) advances faster into the polymer matrix and, therefore, results in a larger release of the drug. Similar type of results has also been reported by other workers (Roy *et al* 2009).

3.5 *Effect of wt.% of composition on drug (ciprofloxacin) releases*

As PVA and starch are the main constituents of the cryogel, their varying weight fraction in the hydrogels exerts a significant influence on the water sorption capacity, as well as other allied properties. Thus, the effect of PVA/starch on the drug release has been investigated by varying the wt. fraction of PVA in the range 20–71.4 (% w/w) or that of starch in the range 29–80 (% w/w), respectively. The results are summarized in figure 5, which clearly indicate that the cumulative drug release increase when the weight fraction of the PVA/starch increases from 20/80 to 42/58 while a decrease in the amount of released drug is noticed beyond 42/58% weight fraction of PVA. The observed results may be explained as below.

When the weight percent of PVA increases from 20–42, the tendency of crystallites formation in the blend also increased which results in generation of wide pores in the cryogel blend. These wide pores frequently allow water

molecules to enter the gel and consequently the Cfx drug molecules dissolve and release out from the blend. However, beyond a certain weight fraction (40/58) of PVA/starch the blend becomes highly crystalline in nature and, therefore, shows fewer tendencies to absorb water. Thus, due to reduction in swelling the extent of cumulative drug release also decreases.

Another possible reason for the observed decrease may be that increasing wt. fraction of PVA may also produce hydrogen bonding between the polymer chains and may result in highly crosslinked blend network which shows less tendency of water sorption and as a result the amount of released drug decreases.

3.6 *Effect of pH on drug (ciprofloxacin) releases*

For controlled drug delivery system, pH plays an important role. The utilization of pH change within the GI tract
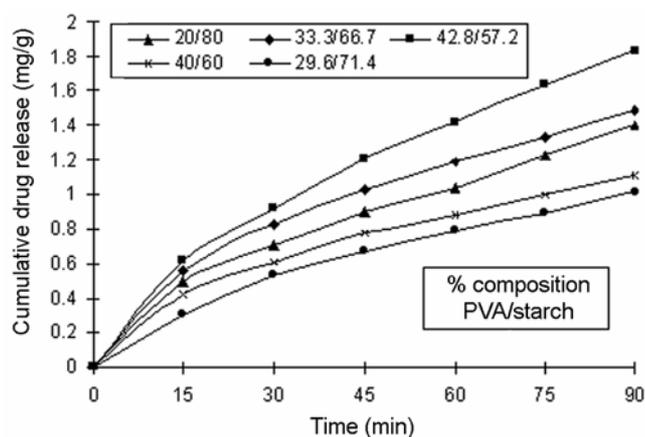

Figure 5. Effect of wt.% of composition on cumulative release of Cfx drug from blend composed of PVA and starch (pH = 7·4, temp. = 37°C, FTC = 3, %loading = 0·16%).
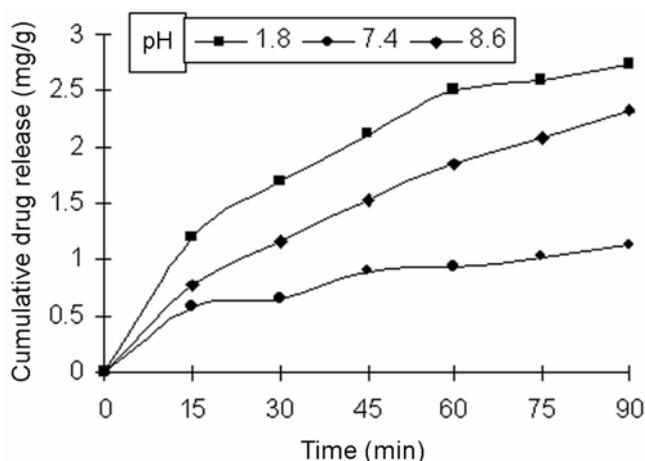

Figure 6. Effect of pH on cumulative release of Cfx drug from blend composed of PVA and starch (40/60% w/w) (temp. = 37°C, FTC = 3, %loading = 0·16%).

and exploitation of bacterial enzyme localized within the colon is one of current interest in controlled drug delivery. In the present study, the release dynamics of the drug has been observed under varying pH as found in the GI tract. The release results are depicted in figure 6 which indicates that with increasing pH of the medium the amount of released Cfx decreases in the range 1·8–7·4 while beyond 7·4, an increase is observed. It means that the release of Cfx is more at 1·8 (acidic) and 8·6 (basic) medium than 7·4 pH. The results can be explained by the fact that the Cfx (amphoteric in nature) undergoes partial hydrolysis, and consequently produce cationic charged centres (NH^{2+}) and these cationic charged centres increase the hydrophilicity of matrix as well as repulsion between cationic charged centres. As a result chains are easily relaxed and thus produced wide free volumes in the network.

Ciprofloxacin hydrochloride
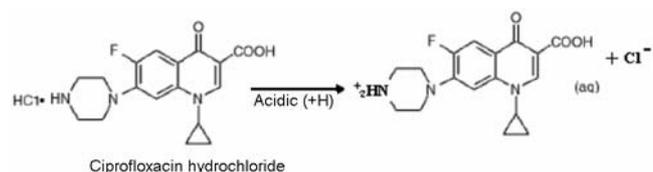
Another reason may be that the sustained release is caused by osmotic pressure difference $\pi_{in} - \pi_{out}$ at the inside and outside of the cryogel (Vilches *et al* 2002). The osmotic pressure (π) results from a net difference in concentration of mobile ions between the interior of the nanogel at external medium. Increasing ionic concentration difference obviously results in an enhanced swelling of the polymer which in turn will bring about an increase in the amount of released drug. The osmotic pressure is given as

$$\pi_{ion} = RT \sum (C_{ig} - C_{is}), \qquad (6)$$

where C_{ig} and C_{is} represent the mobile ion concentration in the cryogel and solution, respectively. When the pH is 1·8, ionic concentration within the cryogel becomes quite huge. At this pH due to the NH^{2+} ion of Cfx, there occurs an increase in differences in ionic concentration between the external and internal medium which results in an increase in the degree of water sorption and this obviously enhances the extent of Cfx release.

Similar types of results have also been obtained in alkali medium. The results can be explained by the fact that the COOH groups undergo a partial hydrolysis to produce anionic groups, COO^-. Now these anions produce repulsive forces and cause macromolecular chains to undergo a fast relaxation which facilitates the penetration of water into the loaded cryogel and subsequently the amount of the released Cfx increases.

3.7 *Effect of temperature on drug (ciprofloxacin) release*

Temperature sensitivity is one of the most important characteristics in drug delivery technology. It has a direct

influence on the release behaviour of a hydrogel as it affects both the segmental mobility of the hydrogel chains as well as the diffusion of penetrant molecules. In the present study, the effect of temperature on the drug release from the starch/PVA cryogel has been investigated by varying the temperature of the swelling medium in the range of 5–40°C. The results are depicted in figure 7, which show that with increasing temperature the released amount also increases.

The observed increase in the released amount of ciprofloxacin can be explained by the fact that with increasing temperature, the network chains undergo faster relaxation due to increased kinetic energy and thus facilitates water sorption process.

3.8 *Effect of number of FTC on drug (ciprofloxacin) releases*

The effect of number of repeated freeze–thaw cycles on the Cfx drug release from cryogels has been studied and
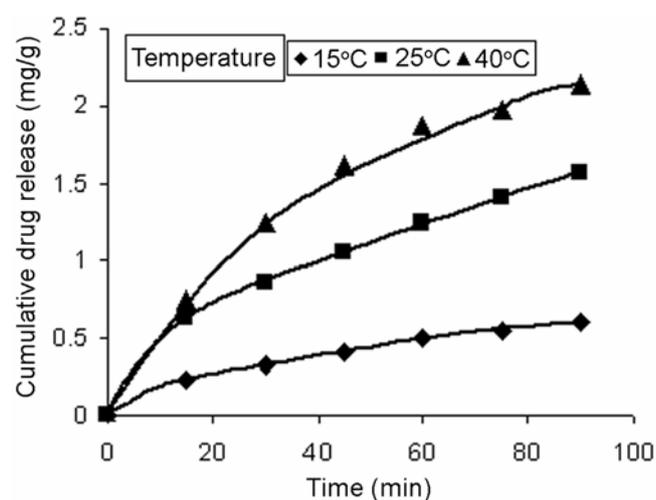
Figure 7. Effect of temperature on cumulative release of Cfx drug from blend composed of PVA and starch (40/60% w/w) (pH = 7·45, FTC = 3, %loading = 0·16%).
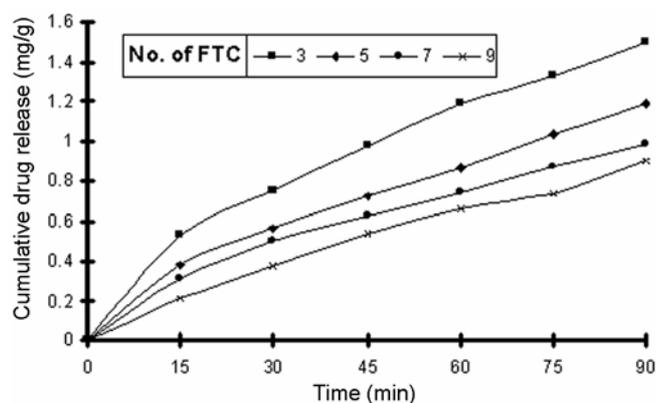
Figure 8. Effect of number of freeze–thaw cycles on cumulative release of Cfx drug from blend composed of PVA and starch (40/60% w/w) (pH = 7·4, temp. = 37°C, %loading = 0·16%).

shown in figure 8. The results clearly reveal that the extent of drug release decreases with increasing number of FTC. The observed results may be attributed to the fact that in the case of 3rd cycle, the cryogels does not have large crystalline regions and therefore, the chains of PVA and starch keep on relaxing with increasing releasing time. However, with increasing number of FTC, the gel acquires increasing crystallinity which restricts the mobility of PVA and starch chains and consequently results in a suppressed drug release (Bagri *et al* 2009).

Another reason for the observed decrease in the amount of drug release with increasing number of FTC is that the pore sizes of the cryogel decreases with increasing number of FTC. Thus, due to shrinkage of the pore sizes of the cryogel the number of incoming water molecules and outgoing drug molecules decreases which results in lower amount of drug release. The reduction in pore sizes with increasing number of FTC is evident from SEM images as shown in figure 9.

3.9 *Effect of physiological fluids on drug (ciprofloxacin) release*

The influence of solutes on the release kinetics of ciprofloxacin was examined by performing release experiments in the presence of solutes such as urea (5% w/v) and D-glucose (5% w/v) and in physiological fluids such as saline water (0·9% NaCl) and synthetic urine. The results are depicted in figure 10 which show that the presence of solutes suppresses the drug release. The reason for the lower release of ciprofloxacin in these fluids may be that the presence of salt ions in the release medium lowers the osmotic pressure of the gel and consequently decreases the extent of swelling of loaded blends (Dredán *et al* 1998).

3.10 *Evaluation of biocompatibility*

The selection of a material to be employed as a biomaterial for a specific end use must meet several criteria such as physicochemical properties, function desired, nature of the physiological environment, adverse effects in the case of failure, expected durability and consideration relating to cost and case of production. Whatever the type of materials, the biocompatibility is the foremost requirement for all biomaterials. In the present study, the assessment of biocompatibility has been made on the basis of three *in vitro* tests viz. BSA (bovine serum albumin) adsorption test, blood clot formation and haemolysis assay as discussed below.

3.10a *BSA adsorption*: In the present work, *in vitro* biocompatibility of prepared blends has been judged by monitoring the amount of protein (BSA) adsorbed by the blend. The results are shown in table 1, which indicates
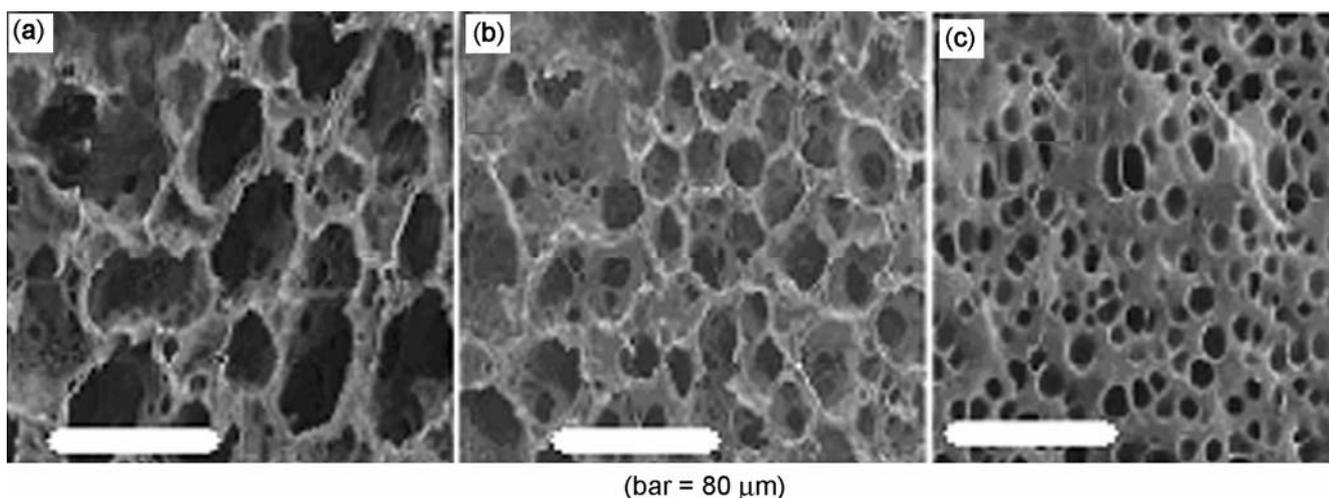
(bar = 80 μm)

Figure 9. SEM images depicting reduction in pore sizes of cryogel with increasing number of FTC: (**a**) 3, (**b**) 5 and (**c**) 7.

Table 1. Data showing biocompatibility parameters with varying compositions of cryogels.

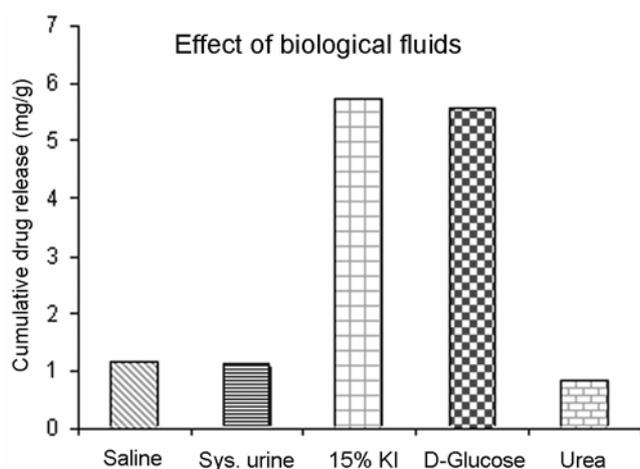| PVA/starch % (w/w)	Freeze–thaw cycles	% Haemolysis	BSA adsorbed (mg g^{-1})	Blood clot (mg)
20/80	3	18·19	0·0092	0·0008
33·3/66·7	3	16·24	0·0075	0·0006
42·8/57·2	3	13·28	0·0022	0·0003

Figure 10. Effect of biological fluids on cumulative release of Cfx drug from blend composed of PVA and starch (40/60 % w/w) (pH = 7·4, temp. = 37°C, FTC = 3, %loading = 0·16%).

that the amount of adsorbed BSA decreases with increaseing wt. fraction of PVA in the range 20–44 (% w/w). The observed findings may be explained on the basis of the fact that PVA is non-ionic, biocompatible and hydrophilic polymer and it does not provoke either any damage of blood cells or any change in the surface of plasma proteins which are the main factors for the biocompatibility.

The data summarized in the table also indicates that as the wt. fraction of starch increases in the range 56–80 (% w/w), the amount of adsorbed BSA increases which

suggests increasing thrombogenicity. The increase observed with increasing starch content may be explained by the fact that with increasing starch content, the wt. fraction of PVA decreases which results in a less biocompatible nature of the matrix. Moreover, increasing starch content may bring about heterogeneity in the blend matrix which may activate adsorption of the protein.

3.10b *Blood clot formation*: The blood clot experiments were performed on different compositions of the hydrogels and the amount of blood clot formed on the surfaces was recorded. The results are summarized in table 1, which indicate that a much less amount of blood clot is formed. This obviously implies an antithrombogenic nature of the hydrogel surfaces.

3.10c *Haemolysis test*: The prepared blends were also tested for hemolytic activity and the results obtained are shown in table 1. The results obtained clearly indicate that with increasing PVA content, the extent of haemolysis constantly decreases. The observed results are attributed to the reason that with increasing wt. fractions of PVA in the blends, the surface composition favourably changes which improves the blood compatible quality of the material.

3.11 *Analysis of kinetic sorption data*

It is a well established fact that the phenomenon of controlled drug release is basically ruled by the water sorption

Table 2. Data showing variation of diffusional (release) exponent '*n*' and diffusion constant (*D*) with varying compositions of cryogels.

PVA/starch % (w/w)	Freeze–thaw cycles	Temp. (°C)	$D \times 10^{-6}$ (cm^2 s^{-1})	*n*	Release mechanism
20/80	3	37	2·41	0·52	Anomalous
29·6/71·4	3	37	2·52	0·58	Anomalous
33·3/66·7	3	37	2·65	0·61	Anomalous
40/60	3	37	3·10	0·70	Anomalous
42·8/57·2	3	37	3·42	0·72	Anomalous

process that a loaded hydrogel undergoes when immersed into a suitable solvent. The swelling of the hydrogel could be due to the hydration of the polymer which results in a rapid decrease in its glass transition temperature to the temperature of the dissolution medium. Macroscopically, there is a relaxation response of the polymer chains due to stress induced by the presence of the dissolution solvent. This results in an increase in the radius of gyration and end-to-end distance of the polymer chains, causing a significant increase in the molecular volume of the hydrated polymer (Ranga Rao and Devi 1988).

It is clear from table 2 that for cryogel compositions prepared at varying concentration of PVA and starch, the value of *n* fluctuates between 0·5 and 1, thus, suggesting an anomalous type of release that is characterized by almost equal rates of solvent diffusion and chain relaxation.

4. Conclusions

Repeated freeze–thaw method of preparation of starch and PVA cryogels results in a spongy macroporous polymeric matrix which shows promise to function as swelling controlled drug release system. The structural characterization of prepared blends by FTIR spectral analysis confirms the presence of functional groups of starch, PVA and loaded drug (Cfx) in the blends. The morphology of the blends as studied by SEM analysis suggests the porous nature with pore size ranging from 10 μm. The addition of model drug, Cfx, to polymeric blends proved to be successful with a 28% drug entrapment. It is found that release profiles of Cfx are greatly influenced by varying the experimental parameters such as percent loading of Cfx, composition of the cryogel, pH and temperature of the medium.

With an increase in percentage loading of drug onto the hydrogels, the release of Cfx constantly increases. The released amount of drug constantly decreases with increasing number of freeze–thaw cycles in the cryogels.

An optimum drug release is obtained near pH 1·8 and 8·0 while lower release is noticed in physiological pH (7·4) range. A rise in temperature up to 40°C brings about an increase in the released Cfx while a decrease is noticed beyond 25°C. The physiological fluids suppress

the extent of drug release. The loaded drug shows retention of its chemical and bioactivity when released into PBS and other pH media as release mediums. With increasing concentrations of PVA and starch, the biocompatibility of cryogel increases.

References

Bagri L P, Bajpai J and Bajpai A K 2009 *J. Macromol. Sci. Pure & Appl. Chem.* **46** 1060

Bajpai A K and Saini R 2005 *Polym. Int.* **54** 1233

Bajpai A K and Choubey J 2006 *J. Appl. Polym. Sci.* **101** 2320

Bajpai A K and Kankane S 2007 *J. Appl. Polym. Sci.* **3** 1559

Bajpai A K and Bundela H 2008 *Xpress Polym. Letts* **2** 201

Basmadjian D M and Sefton V 1983 *J. Biomed. Mater. Res.* **17** 509

Chen D H, Leu J C and Huang T C 1994 *J. Chem. Technol. Biotechnol.* **61** 351

Chouhan R and Bajpai A K 2009 *J. Mater. Sci. Mater. Med.* **20** 1103

Colombo P, Caramella C, Conte U, Gazzaniga A and La Manna A 1985 *J. Controll. Rel.* **1** 283

Désévauxa C, Dubreuilc P and Lenaerts V 2002 *J. Controll. Rel.* **82** 83

Dimitrov M 2003 *Acta Pharm.* **53** 25

Dredán J, Zelko R, Antal I, Bihari E and Rácz I 1998 *Int. J. Pharmaceut.* 160

Elvira C, Mano J F, San Roman J and Reis R 2002 *Biomaterials* **23** 1955

Gruber E, Soehendra B and Schurz J 1974 *J. Polym. Sci. Symp.* **14** 44105

Hassan C M and Peppas N A 2000 *Adv. Polym. Sci.* **153** 37

He H, Cao X and Lee L 2004 *J. Controll. Rel.* **95** 391

Hyon S H, Cha W I, Ikada Y, Kita M, Ogura Y and Honda Y 1994 *J. Biomater. Sci. Polym. Ed.* **5** 397

Juang H, Bonner W S, Ogawa Y J, Vacanti P and Weir G C 1996 *Transplantation* **61** 1557

Knop K 1996 *Eur. J. Pharmaceut. Sci.* **4** 293

Korsmeyer R W and Peppas N A 1981 *J. Membr. Sci.* **9** 211

Kosmidis K, Argyrakis P and Macheras P 2003 *Int. J. Pharm.* **254** 183

Lia J K, Wang N and Wu X S 1998 *J. Controll. Rel.* **56** 117

McGann M J, Higginbotham C L, Geever L M and Nugent M J D 2009 *Int. J. Pharmaceut.* **372** 154

O'Brien S, Wang Y J, Vervaet C and Remon R J 2009 *Carbohyd. Polym.* **76** 557

Peppas N A, Ratner B D, Haffman A S, Schoen F J and Lemons J E 1996 *Biomaterials science: an introduction to materials in medicine* (Toronto: Academic) p. 62

Qiu Y and Park K 2001 *Adv. Drug Deliv. Rev.* **53** 321

Ranga Rao K V and Devi K 1988 *Int. J. Pharm.* **48** 1

Roy A, Bajpai J and Bajpai A K 2009 *Carbohyd. Polym.* **10** 1016

Szepes A, Makai Z, Blümer C, Mäder K, Peter Kasa Jr and Révész P S 2008 *Carbohyd. Polym.* **72** 571

Thanoo B C, Sunny M C and Jayakrishnan A 1993 *J. Pharm. Pharmacol.* **45** 16

Tong X, Zheng J, Lu Y C, Zhang Z F and Cheng H 2007 *Mater. Letts* **61** 1704

Vilches A P, Jimenez-Kairuz A, Alovero F, Olivera M E, Allemandi D A and Manzo R H 2002 *Int. J. Pharm.* **246** 17

Wu L U, Zhang Y T, Cai C S and Hu S H 2001 *New Chem. Mater.* 29