In vitro release dynamics of insulin from a loaded hydrophilic polymeric network

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A hydrophilic semi-interpenetrating polymer network of polyvinyl alcohol (PVA), poly(ethylene glycol) (PEG) and crosslinked polyacrylamide (PAM) chains has been synthesized and its potential for controlled release of macromolecular drugs has been assessed by taking insulin as a representative drug. The semi-IPN was characterized by IR studies 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 effect of chemical architecture of the IPN was investigated on the percent loading of insulin and its subsequent release from the loaded device. Other parameters such as the thickness of the gel, molecular weight of PEG and pH and temperature of the release medium were also studied for their possible impact on the release of insulin. The whole release data was analyzed by Ficks power law and the influence of various factors on the plausible mechanism of insulin release was investigated.

Introduction

Hydrogels are very versatile materials [1], widely used in several biomedical applications such as drug delivery systems [2], soft contact lenses [3] and as artificial implants [4]. Other practical applications include the use of hydrogels as flocculants for treatment of sludge [5], release of essential oils [6], release of agrochemicals [7], etc.

Hydrogels may be impregnated with biologically active agents, such as antibiotics, enzymes, contraceptives, drug antagonists, anticoagulants, anticancer, etc. and may serve as a system for controlled release of the agent absorbed over a prolonged time period at a specific body site [8, 9]. Controlled drug delivery systems are being used with increased frequency in treatment of many diseases [10, 11]. Cases where long-term delivery and minimal fluctuation of plasma concentration of the drug are needed, these systems are mainly used. Moreover, controlled drug delivery is aimed at providing not only sustained action but also constant, that is, ideally zero-order release rates in which the amount of drug released to the absorption site remains reasonably constant over prolonged periods of time [12].

For many years, controlled release systems have been capable of slowly releasing drugs of only low molecular weight (< 600). Large molecules such as proteins have not been considered feasible candidates, because polypeptides have been considered too large to slowly diffuse through most polymeric materials, even after swelling of the polymer [13, 14]. The development of protein-delivery systems is a real challenging problem, since the molecular size of the macro drug is a decisive factor in hindering the diffusion and release from hydrophilic network [15]. Another critical consideration in protein delivery from hydrogel system is that the encapsulated protein may denature [16] or aggregate as a result of exposure to moisture at 37 °C, causing the loss of biological activity and possible changes in immunogenicity [17]. Thus, the area of controlled delivery of macromolecular drugs possesses challenges and, therefore, deserve much more attention.

Thus, being motivated from the challenges coming across in the controlled delivery of macromolecular compounds, the objective of this study is to design a polymeric system; which could exhibit an effective delivery of a variety of proteins and peptides along with a zero-order release. In the present paper, therefore, we are reporting results on the controlled release of insulin from a swellable hydrophilic semi-interpenetrating network (IPN) of poly vinyl alcohol (PVA), polyethylene glycol (PEG) and polyacrylamide (PAM). The choice of constituent polymers PVA and PEG rests upon the fact that both are water soluble, biocompatible, non-toxic and non-immunogenic and find much use in biomaterials, biotechnology, and medicine [18–20]. The insulin on the other hand has been opted as a model protein drug, taking into account the widespread occurrence of diabetes together with the recognized shortcomings of conventional therapy. Controlled release of insulin is useful for the treatment of diabetes because the conventional

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insulin administration by injection causes a wide fluctuation in blood glucose level. Thus, for an ideal delivery of insulin, the delivery system should be controlled uniformly during the span of release time.

Experimental

Materials

Polyethylene glycol (PEG) (mol wt 400, 600 and 6000) were obtained from Wilson Laboratories, Bombay, India and used as received. Polyvinyl alcohol (hot processed, 98.6% acetalized, mol wt 30 000) was obtained from Burgoyne Burbidges and Co. India, and used without any pretreatment. Acrylamide (Research Lab, Poona, India) was crystallized twice from methanol (GR) and dried under vacuum over anhydrous silica for a week. N,N′-methylene bisacrylamide (MBA) (Central Drug House, Bombay, India) was employed as a crosslinking agent while potassium persulfate (Loba Chemie, India) as a polymerization initiator. Insulin (Knoll Pharmaceuticals, India, Activity 40 IU/ml) was purchased as 10 ml ampules.

Preparation of IPN

The IPNs of varying composition were prepared by the free radical polymerization method as described in our earlier communications [21]. In brief, into different petridishes (100 mm diameter, Corning) were added PVA (0.75–1.5 g), acrylamide (AM) (10.5–28 mM) PEG (0.22–1.32 g), MBA (0.006–0.038 mM), potassium persulfate (KPS) (0.003 mM) and water (1.1 M). The mixtures (20 ml) were homogenized and kept at 70 °C for 4 h so that the whole mass converted into thin white circular films. The films were cut into preweighed pieces of equal dimensions (1 × 1 cm) and equilibrated with bidistilled water for a week. The swollen hydrogel pieces were then dried at room temperature for 72 h and weighed again. This process was continued till the constant weights of IPNs were obtained. This clearly assured a complete removal of unreacted chemicals and monomers from the hydrogels.

Swelling kinetics and subsequent loading of insulin

The loading of an active agent is normally performed by two general methods. In one method, the hydrogel monomer is mixed with the active agent, an initiator, with or without a crosslinking agent and allowed to polymerize, trapping the agent within the matrix [22]. In the second approach, the hydrogel is allowed to swell in the bioactive solution till equilibrium and then dried to obtain the release device. The later method has some advantages over the first method as polymerization conditions may have deleterious effects on the drug properties and the difficulties in device purification after loading and polymerizations often remain.

In the present work, the second method was adopted for loading the IPN with insulin. In a typical experiment a preweighed dry piece of the hydrogel was allowed to swell in 5 ml insulin solution till equilibrium. The progress of swelling of the hydrogel was monitored by recording weights of swollen gels at different time intervals. The swelling process was characterized by the parameters given below:

$$\text{Swelling ratio} = \frac{W_s}{W_d} \tag{1}$$

The swelling IPNs after having attained a state of equilibrium were removed from insulin solutions and then dried at room temperature for 72 h. The following equation was used to calculate the amount of loaded insulin (mg/g),

$$\text{Loaded insulin} = \frac{W_d - W_o}{W_o} \tag{2}$$

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

Release experiments

The release of the encapsulated insulin were carried out by placing the dried and loaded IPNs (0.1 g) into a definite volume (1 ml) of double distilled water as the release medium. After definite time intervals, the release medium was withdrawn and assayed using Lowry's method [23] for protein estimation. An aliquot of 5 ml was taken out for final estimation by recording its absorbance at 750 nm (Systronics, Model No. 106, India). The amount of insulin released was determined with the help of calibration plot.

Kinetic analysis of release data

The potentiality of a drug delivery system is normally evaluated on the basis of first 50–60% release performance of the device as beyond this level therapeutically ineffective amounts of drug are present in the blood plasma. If a hydrogel film is equilibrated with a drug by soaking the hydrogel in an aqueous solution of the drug, the film can act as a vehicle for subsequent release of the drug when it is transferred to an aqueous sink. Release of solute from slab can be one dimensional if it takes place predominantly from the two main surfaces and according to Crank [24]

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty}\left\{\frac{8}{(2n+\ell)^2\pi^2}\right\} \cdot \exp\left\{\frac{-D(2n+\ell)^2\pi^2 t}{4\ell^2}\right\} \tag{3}$$

where M_∞ is the total drug content, M_t is the amount desorbed at time t, ℓ is the film thickness and n is an integer. This equation can be reduced to a simplified form as,

$$\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{\pi\ell^2}\right)^{0.5} \tag{4}$$

for $0 \leq M_t/M_\infty \leq 0.6$.

The following equation was used to study the release mechanism when the release and swelling data are analyzed

$$\frac{M_t}{M_\infty} = Kt^n \tag{5}$$

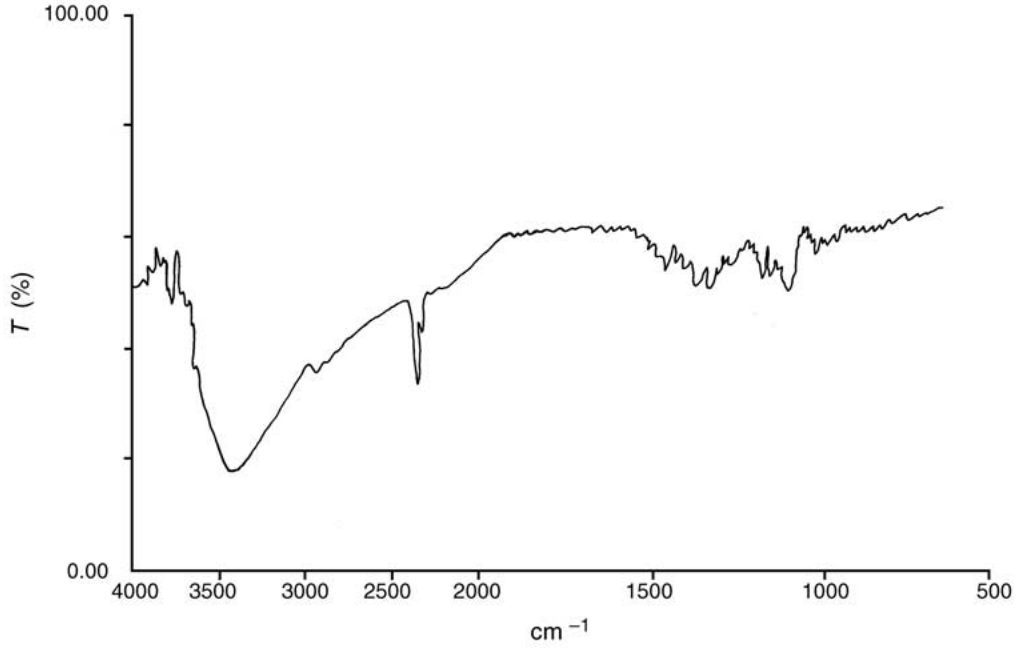

Figure 1 IR spectra of the IPN.

where M_t/M_∞ is the fractional release at time t and K is a rate constant. The exponent n is an indicator of mechanism of transport and has a value between 0.5 and 1. When $n = 0.5$ release is described as Fickian. When $n = 1$, the release is zero order, i.e. constant with time. In between these values, i.e. $0.5 < n < 1$, the closer is the release pattern to steady state release. When $M_t/M_\infty = 0.5$, t is the half life which is another extremely significant parameter in comparing systems. In the light of Equations 4 and 5, the released data will be analyzed.

Results and discussion

Characterization of network

Prior to discussing the results obtained, it is worth to characterize the structure of the prepared hydrogel.

IR spectral analysis

The spectra shows presence of hydroxyls of alcohol at 3650 cm^{-1} (O–H stretching), amide group at 3424 cm^{-1} (N–H stretching), 1596 cm^{-1} bending), and 1656 (C=O stretching). IR spectra also shows the presence of poly(ethylene glycol) (PEG) in the semi-IPN as evident from observed absorption bands at 1351 cm^{-1} (Fig. 1).

The spectra also shows the presence of carboxylates of amino acids at 1590 cm^{-1} and also weak absorption near 1400 cm^{-1}. These bands result from asymmetrical and symmetrical $C(\cdots\cdots O)_2$ stretching. Strong asymmetrical NH_3^+ bending occurs near 1560 cm^{-1} and N–H bending at 1609 cm^{-1}.

Network studies

A crosslinked polymer is often characterized by an important structural parameter M_C, the average molar mass between crosslinks, which is directly related to crosslink density. The magnitude of M_C affects the physical and mechanical properties of crosslinked polymers and its determination has wide range of practical significance. Equilibrium swelling is widely used to determine M_C. Early research by Flory and Rehner laid the foundation of the analysis of equilibrium swelling. According to the theory of Flory and Rehner, for a perfect network

$$M_C = -V_1 d_p \frac{(V_S^{1/2} - V_{S/2})}{\ln(1 - V_S) + V_S + \chi V_S^2} \quad (6)$$

where M_C is the number average molar mass of the chain between crosslinks. V_1 is the molar volume, d_p is the polymer density ($g\,ml^{-1}$), V_S is the volume fraction of polymer in the swollen gel, and χ is the Flory–Huggins interaction parameter between solvent and polymer [25].

The swelling ratio is equal to $1/V_S$. Here, the crosslink density q is defined as the mole fraction of crosslinked units.

$$q = M_O/M_C \quad (7)$$

where M_O is the molar mass of the repeating unit.

Some other authors defined a crosslink density, V_e, as the number of elastically effective chains, totally included in a perfect network, per unit volume, V_e is simply related to q since

$$V_e = d_A N_A / M_C \quad (8)$$

The value of V_1, d_p and χ were taken from related literature [26, 27].

The values of M_C, q and V_e of the networks have been calculated and summarized in Table I for varying compositions in the hydrogel.

Appearance of the IPN

In dry state the IPN was like a smooth thin film which upon swelling changed into semi-transparent enlarged mass as evident from the photograph shown in Fig. 2.

TABLE I Network parameters of the IPNs of different compositions

PEG (g)	PVA (g)	AM (mM)	MBA (mM)	KPS (mM)	M_C	$q \times 10^3$	$V_e \times 10^{-20}$
0.22	0.75	14	0.006	0.003	5536	12.8	10.4
0.55	0.75	14	0.006	0.003	14180	5.0	4.07
0.88	0.75	14	0.006	0.003	18510	3.83	3.12
1.32	0.75	14	0.006	0.003	21360	3.32	2.7
0.55	0.50	14	0.006	0.003	10152	6.9	5.69
0.55	0.75	14	0.006	0.003	14180	5.0	4.07
0.55	1.00	14	0.006	0.003	17036	4.16	3.3
0.55	1.50	14	0.006	0.003	20111	3.5	2.8
0.55	0.75	10	0.006	0.003	2477	28.6	23.3
0.55	0.75	21	0.006	0.003	14180	5.0	4.07
0.55	0.75	28	0.006	0.003	8173	8.6	7.0
0.55	0.75	14	0.012	0.003	6671	10.6	8.6
0.55	0.75	14	0.025	0.003	14180	5.0	4.07
0.55	0.75	14	0.038	0.003	7370	9.6	7.8
0.55	0.75	14	0.012	0.003	5575	12.7	10.3
0.55	0.75	14	0.038	0.003	3904	18.1	14.8

Dynamic release model

Based on the release mechanisms the controlled release delivery systems have been classified as bioerodible, biodegradable, swelling controlled and diffusion controlled. The present system falls into swelling controled delivery systems where a hydrogel is considered as an intimate mixture of macromolecular chains bonded to each other via chemical crosslinks or weak intermolecular forces. When a drug loaded dry hydrogel comes into contact with a thermodynamic compatible solvent, relaxation of polymeric chains takes place. This happens when the characteristic glass transition temperature of the polymer is decreased below the temperature of the experiment. The dissolved drug releases into the external release medium crossing the swollen polymeric layer formed around the matrix. Now, the following possibilities could arise,

(i) If the glass transition temperature of the polymer (T_g) is well below the experimental temperature, the polymer will be in the rubbery state and the polymer chains will have a greater mobility that allows an easier penetration of the solvent [28]. This clearly results in a Fickian diffusion (Case I) which is characterized by the solvent diffusioin rate; R_{diff}, slower than the polymer relaxation rate $R_{relax}(R_{diff} \ll R_{relax})$.

(ii) If the experimental temperature is below T_g, the polymer chains may not be sufficiently mobile to permit immediate penetration of the solvent in the polymer case. This gives rise to non-Fickian diffusion process which includes Case II diffusion and anomalous diffusion depending on the relative rates of diffusion and chain relaxation (for Case II, $R_{diff} \gg R_{relax}$ and for anomalous $R_{diff} \sim R_{relax}$).

Effect of composition on loading

The amount of the insulin loaded onto the 3D IPN matrix depends mainly on the potentiality of the IPN to imbibe the insulin solution when placed in its reservoir. In other words the loading of insulin will be regulated by the

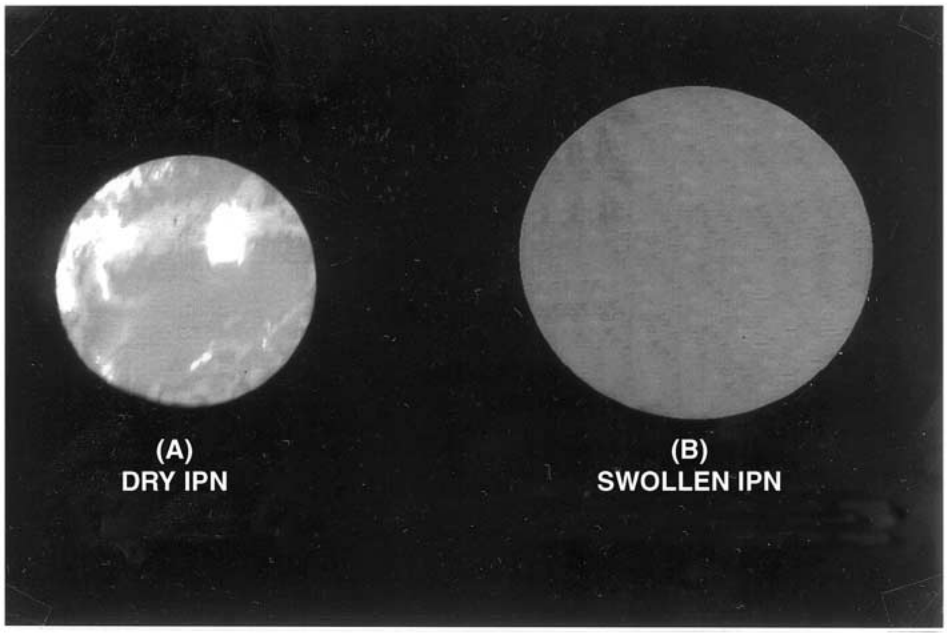

Figure 2 A photograph displaying dry and swollen IPNs.

TABLE II Data showing the variation of percent loading of insulin with varying composition of the IPNs

PEG (g)	PVA (g)	AM (mM)	MBA (mM)	KPS (mM)	Amount of insulin loaded (%)
0.22	0.75	14	0.006	0.003	18.2
0.55	0.75	14	0.006	0.003	20.5
0.88	0.75	14	0.006	0.003	24.0
1.32	0.75	14	0.006	0.003	28.2
0.55	0.50	14	0.006	0.003	24.6
0.55	0.75	14	0.006	0.003	20.5
0.55	1.0	14	0.006	0.003	16.4
0.55	1.5	14	0.006	0.003	15.2
0.55	0.75	10	0.006	0.003	25.0
0.55	0.75	14	0.006	0.003	20.5
0.55	0.75	21	0.006	0.003	18.7
0.55	0.75	28	0.006	0.003	16.2
0.55	0.75	14	0.006	0.003	20.5
0.55	0.75	14	0.012	0.003	19.2
0.55	0.75	14	0.025	0.003	17.5
0.55	0.75	14	0.038	0.003	14.0

extent of the swelling which, in turn, depends on the chemical architecture of the network.

The IPNs of different compositions were prepared by varying the amounts of PEG, PVA, AM, and MBA in the feed mixture and the insulin was loaded by equilibrating them in the insulin reservoir. The following discussion clearly reveals that the chemical architecture of the IPN has a pronounced effect on the percent loading of the insulin. The observed results are summarized in Table II and may be interpreted as below.

When the amount of PEG is increased in the feed mixture in the range 0.22–1.32 g, there is observed an increase in the percent loading. This may be explained by the fact that increasing molar concentration of PEG results in longer chain lengths of PEG, thereby increasing the mesh size of the free volumes available in between the macromolecular chains. This obviously allows insulin molecules to penetrate into the network of the hydrogel thereby resulting in a higher percent loading of the protein.

On varying the concentration of PVA in the feed mixture in the range 0.5–1.5 g, there is observed a decrease in the percent loading. The results may be explained by the fact that on increasing the concentration of PVA, the number of PVA chains in the hydrogel increases, which produces a more compact arrangement of macromolecular chains that contain small-size free volumes within the gel. Thus, larger insulin molecules are prevented from entering into the hydrogel, leading to a lower insulin content.

Similar type of results have been obtained when the amount of AM increases in the range 10.5–28 mM. This observed decrease may be explained by the fact that increasing number of AM chains in the hydrogel decreases the mesh size of the free volumes available in between the macromolecular chains, which hinder the penetration of the giant insulin molecules into the network structure of the hydrogel. This obviously results in a decline in the percent loading of the insulin.

When the crosslinker (MBA) was varied in the range 0.006–0.38 mM in the feed mixture of the gels, the loading drastically decreases. The observations are quite expected, as a greater number of crosslinks squeeze the free volumes available between the chains of the macromolecular network, thereby, forbidding the entrance of insulin molecules into the gel and consequently a lower loading of insulin is obtained.

Effect of percent loading on released insulin

An important aspect in the use of hydrogels as drug vehicle is the effect of drug loading level on the rate of drug release. The amount of drug delivered also depends on the extent of loading and its subsequent release into the release medium. Use of either, highly concentrated feed solutions or repeated soaking of gels in drug solution and drying them results in higher loading.

In the present study, a hydrogel of definite composition was loaded with different amounts of insulin in the concentration range (10–40% v/v). The release results are displayed in Fig. 3 which reveal that the released amount of insulin increases with increasing percentage loading in the range 14–35.2%. The results are quite expected and may be explained by the fact that larger the initial load, the faster the movement of the solvent front

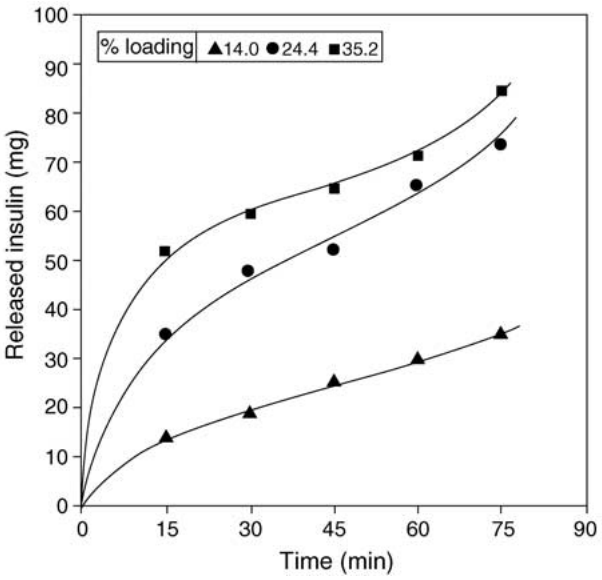

Figure 3 Effect of percentage loading of insulin on its release profiles for a given composition of the IPN [PEG] = 0.55 g, [AM] = 14 mM, [MBA] = 0.006 mM, [KPS] = 0.003 mM, pH = 7.4, temp. = 27 °C.

penetrating the surfaces of the loaded gel [29]. A higher loading of the hydrogel may also facilitate relaxation of macromolecular chains of the gel, and thus, a larger swelling of loaded hydrogel is expected in the release medium, which obviously results in a greater amount of released insulin.

The release profiles also present a typical feature that at higher loading of the IPNs, a major fraction of insulin is released just within 15 min. This can be described as ''burst effect''. The initial rapid release of insulin was due to the loading technique used in which the gel was soaked in a concentrated solution of insulin for 4–5 h. What actually happens is that during drying process of the gel, the insulin molecules migrate on the surface due to capillary action thus giving rise to the ''burst effect''.

Effect of hydrogel composition on released insulin

Effect of PEG

PEG is a hydrophilic polymer and a variation in its concentration with IPN enhances hydrophilicity of the network which ultimately affects the release profile of insulin. In the present work, the amount of PEG has been varied in the range 0.22–1.32 g and the corresponding release profiles are shown in Fig. 4 which clearly imply that the amount of released insulin increases with increasing PEG content of the IPN. The observed results are quite obvious as with increasing hydrophilicity of the network, water sorption by the loaded IPN increases which results in a greater release of entrapped insulin. Another explanation could be that with increasing PEG content in the IPNs the mesh sizes of the free volumes available in between the network chains widen, thus facilitating the release of large insulin molecules.

However, at 1.32 g PEG content, a fall in the released amount of insulin is noticed. This fall may be because of the reason that at higher concentration of PEG, number of hydrated PEG chains becomes so large in number that the release of big insulin molecules from within the network into the release medium becomes rather difficult and, therefore, the amount of released insulin decreases.

Effect of PVA

When the amount of PVA is varied in the feed mixture of the IPN in the range 0.50–1.5 g, the release profiles are greatly affected as shown in Fig. 5. The results clearly imply that the fractional release significantly increases with increasing PVA content (0.5–0.75 g) while at higher PVA concentration (> 0.75 g), it constantly decreases. The observed increasing fractional release is quite obvious and can be explained on the basis that with increasing concentration of PVA in the feed mixture, the number of PVA chains in the hydrogel increases, which produces a more hydrophilic arrangement of macromolecular chains. Thus, more and more water molecules enter into the loaded IPN and as a consequence, entrapped insulin molecules are released out. However, beyond 0.75 g of PVA content the dense macromolecular network restricts the expulsion of insulin molecules from the loaded gel and, therefore, the amount of released insulin decreases.

It is also clearly depicted in the figure that at lower concentration of PVA (0.5 g), a zero-order release profile is obtained, i.e. the fractional release remains almost constant with increasing time. On the other hand, a burst effect is noticed at higher concentration (0.75 g) of PVA. The observed zero order release at low PVA content of the IPN can be explained on the basis of the water sorption behavior of the gel. When the swelling zone travels across the gel at a constant rate, solvent uptake is proportional to time and zero-order kinetics are predicted if diffusate release follows the rate of swelling [30]. In several cases, the release has been related to the rate of solvent uptake [31].

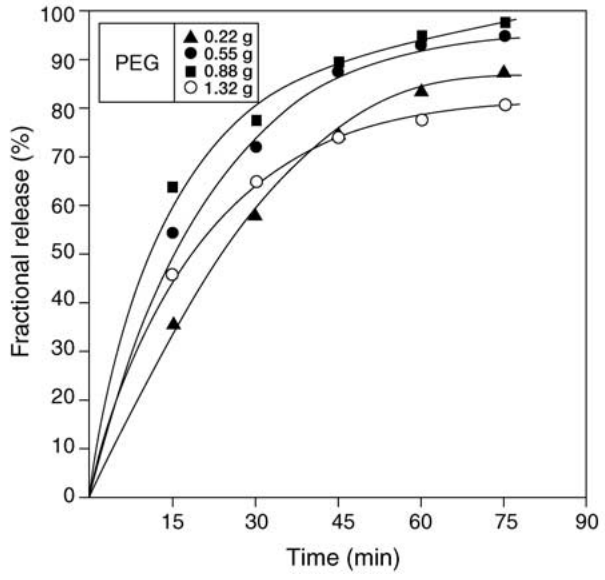

Figure 4 Effect of variation in PEG content of the IPNs on the fractional release insulin for a given composition of the IPN [PVA] = 0.75 g, [AM] = 14 mM, [MBA] = 0.006 mM, [KPS] = 0.003 mM, pH = 7.4, Temp = 27 °C, percentage loading = 20.5, thickness = 0.035 cm.

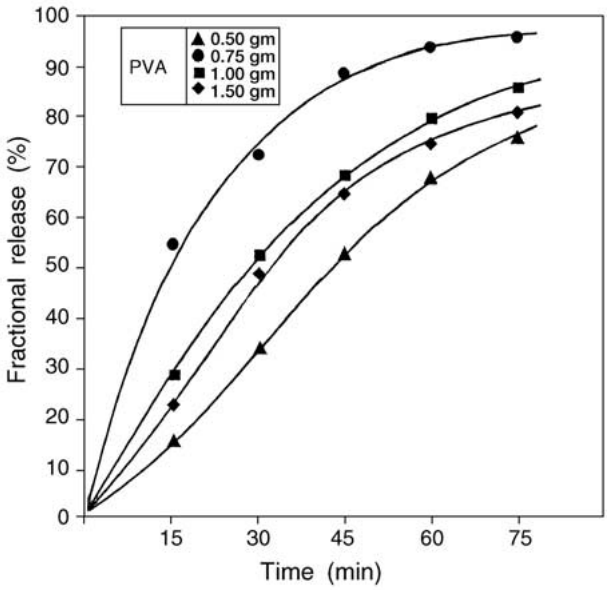

Figure 5 Variation in the fractional release of insulin with varying PVA content of the IPNs for a given composition of the IPN. [PEG] = 0.55 g, [AM] = 14 mM, [MBA] = 0.006 mM, [KPS] = 0.003 mM, pH = 7.4, Temp. = 27 °C, percentage loading = 20.5, thickness = 0.035 cm.

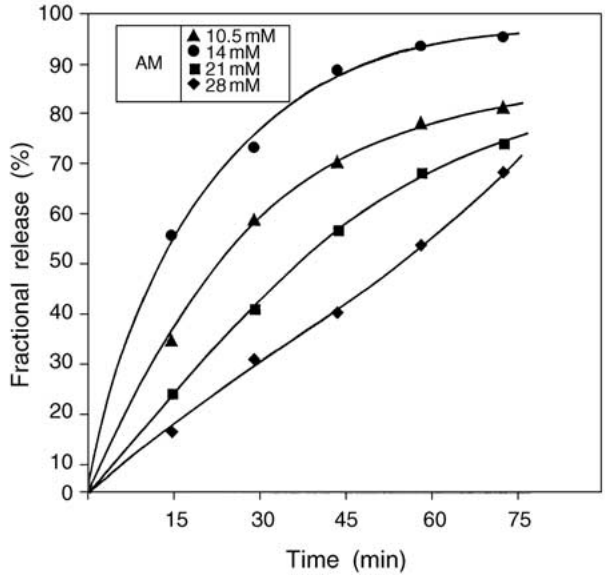

Figure 6 Effect of variation in AM content of the IPNs on the fractional release of insulin for a given composition of the IPN. [PEG] = 0.55 g, [PVA] = 0.75 g, [MBA] = 0.006 mM, [KPS] = 0.003 mM, pH = 7.4, Temp. = 27 °C, percentage loading = 20.5, thickness = 0.035 cm.

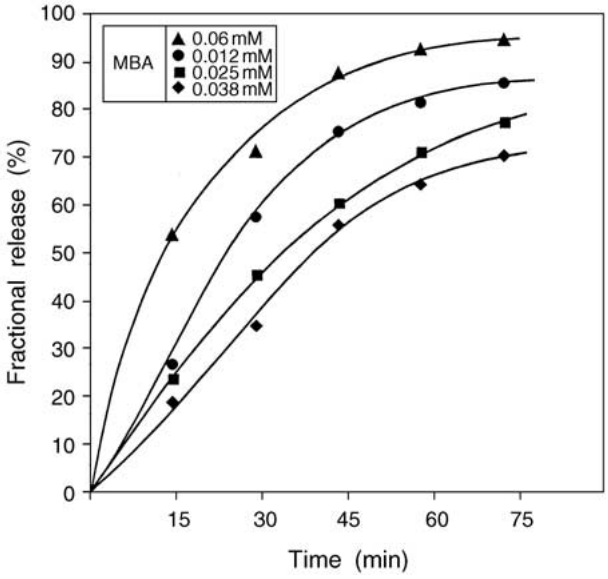

Figure 7 Effect of crosslinker (MBA) content of the IPNs on the fractional release of insulin for a given composition of the IPN [PEG] = 0.55 g, [PVA] = 0.75 g, [AM] = 14 mM, [KPS] = 0.003 mM, pH = 7.4, temp. = 27 °C, percentage loading = 20.5, thickness = 0.035 cm.

Effect of acrylamide

Acrylamide, a hydrophilic monomer, has been found to significantly affect the released amount of insulin. It was found that on increasing AM in the range 10.5–28 mM, the fractional release profiles of insulin are greatly influenced. The results are depicted in Fig. 6 which reveal that an initial increase in the concentration of AM (10.5–14 mM) in the feed mixture of the IPNs increases the hydrophilicity of the IPN which results in a greater degree of water sorption, and hence a greater amount of released insulin is obtained. However, on further increasing the concentration of AM in the range 21–28 mM, the amount of released insulin decreases because a further increase of AM content in feed mixture of IPNs enhances the crosslink density of the network which results in a lower degree of water sorption, which in turn leads to a lower release of entrapped insulin.

The release profiles also indicate a zero-order release behavior at highest concentration (28.1 mM) which may be attributed to the fact that at this concentration, crosslink density of the network will be greatest because of a great number of crosslinked polyacrylamide chains. Thus, with a compact macromolecular network, the loaded gel will swell to a lower extent and, therefore, the solvent zone travels across the IPN with nearly constant velocity. This will obviously result in a constant delivery of insulin into the release medium, thus giving rise to a zero-order release behavior.

Effect of crosslinker

One of the effective means of modifying the swelling characteristics of a hydrogel is by manipulating the amount of crosslinker in the feed mixture. This usually results in a change in the swelling behavior of the hydrogel in a complex way. In the present investigation, the amount of crosslinker is varied in the concentration range 0.006–0.038 mM in the feed mixture. The results are shown in Fig. 7 which clearly indicate that on increasing MBA in the studied range, the fractional release is appreciably reduced. The observed results are because of the reason that on increasing MBA content in the hydrogel, the number of crosslink points increases in the hydrogel, which increases the network density of the macromolecular network, thus reducing the mesh sizes of the free volumes available between the network chains. Thus, an increased crosslink density not only makes more difficult the passage of water molecules from the release medium into the loaded gel network but also hinders the diffusion of larger insulin molecules from within the gel into the release medium. This obviously decreases both the swelling of the IPN and fractional release of insulin. It is also clear from the release profile curves that at low concentration of MBA, a burst effect is observed while with increasing crosslinker in the IPN, the burst effect decreases and ultimately at higher concentration of MBA (0.038 mM), it completely disappears. The observed change in burst effect may be explained by the fact that at low MBA, the mesh size of the free volume available between the network chains is large, thus allowing the entrapped insulin molecule to migrate to the surface and release out quickly. On the other hand, at higher concentration of crosslinker due to a decrease in mesh size of the available free volume, the insulin molecules experience difficulty in releasing out of the gel, thus avoiding the burst effect.

Another remarkable feature visible in the release profile is that, a nearly zero-order kinetics is observed at highest MBA concentration. The possible reason for the observed zero-order behavior may be that at highly crosslinked state the hydrogel may not swell significantly and, therefore, the insulin molecules do not have to travel a larger distance within the gel to come out on the surface. This clearly reveals that diffusion constant of the releasing insulin molecules remain almost constant with releasing time.

Effect of pH

pH responsive macromolecular devices have been most frequently used to develop controled release formulations for oral administration which remains the most clinically acceptable way of drug-delivery. Oral administration of macromolecular drugs remains a significant challenge because peptides and proteins are susceptible to hydrolysis and digestion by the acid and enzymes in the gastrointestinal (GI) tract. Also, the bioavailability of orally delivered peptides and proteins is very low due to poor membrane permeability [32]. Thus, to improve therapeutic efficiency and to reduce or eliminate side effects of oral controlled drugs, it is reasonable to deliver drugs to specific regions of the GI tract.

pH sensitive polymers are used to prepare insulin releasing carriers via loading in aqueous solution. This system has additional advantage that only physical interaction between the polymer and protein is used to entrap drug in the matrix. No chemical modification of protein occurs which, otherwise, would result in a decrease in the bioactivity of the insulin.

Several methods of targeting the specific have been proposed. Two of these, i.e. utilization of pH changes within the GI tract [33] and exploitation of bacterial enzymes localized within the colon [34] are of current interest in controlled drug delivery system.

In the present investigation, the effect of change in pH of the release medium was investigated with respect to the released amount of insulin in the pH range 3.0–11.0. The results are depicted in Fig. 8 which indicate that whereas the released amount of insulin decreases in the acidic and alkaline range, it attains an optimum value at neutral pH 7.4. The observed results are in accordance with the swelling result. In the acidic and alkaline pH, the IPN's did not swell sufficiently as a result the protein loading decreased, hence under these conditions only the surface bound drug is mostly released. At pH 7.4, the IPN swelled and accordingly drug release was observed. Similar type of results have been reported elsewhere [35]

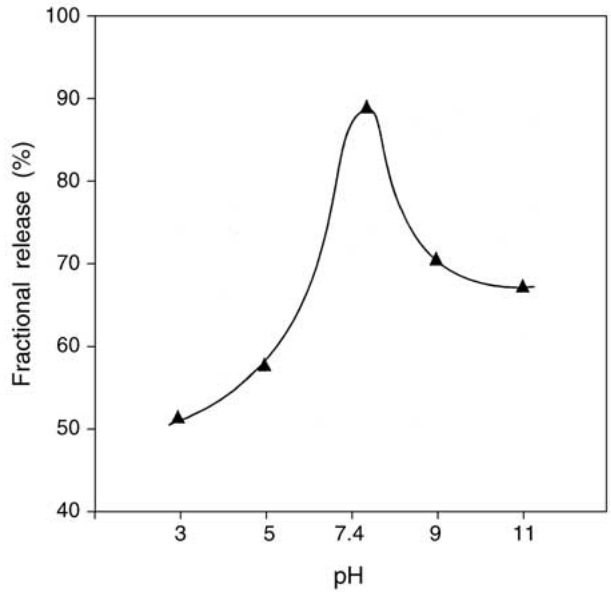

Figure 8 Effect of pH of the release medium on the fractional release of insulin for a given composition of the IPN. [PVA] = 0.75 g, [PEG] = 0.55 g, [AM] = 14 mM, [MBA] = 0.006 mM, [KPS] = 0.003 mM, temp. = 27 °C, percentage loading = 20.5, thickness = 0.035 cm.

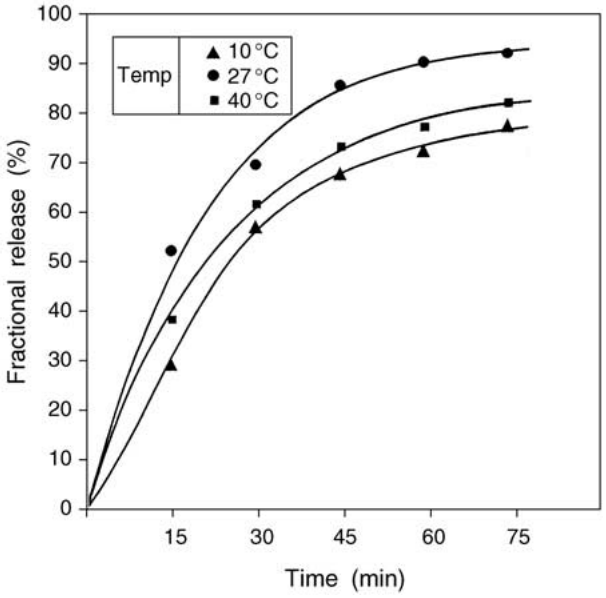

Figure 9 Effect of temperature on the fractional release of insulin for a given composition of the IPN. [PEG] = 0.55 g, [PVA] = 0.75 g, [AM] = 14 mM, [MBA] = 0.006 mM, [KPS] = 0.003 mM, pH = 7.4, percentage loading = 20.5, thickness = 0.035 cm.

Effect of temperature

The release behavior of the IPN is greatly concerned with the temperature of the release medium, as a rise in temperature affects the rate of diffusion of both the water and drug molecules into the gel as well as that of relaxation of network chains. In addition, a higher temperature may detach water molecules bound to network chains and thus cause a fall in the degree of swelling. In the present study, the temperature of release medium has been varied in the range 10 to 40 °C and its effect on the fractional release of insulin has been investigated. The results are depicted in Fig. 9 which indicate that with increasing temperature (10–27 °C), the fractional release of insulin increases while a decrease is observed beyond 27 °C. The observed increase in the fractional release may be attributed to the fact that with increasing temperature of the release medium, the mobility of macromolecular chains of the network also increases, thus permitting the entrance of greater number of water molecules into the semi-IPN. Thus, an increased water sorption results in a greater release of entrapped insulin molecules as evident from the Fig. 9.

However, there is a decrease in fractional release with increasing temperature because of the breaking of the hydrogen bonds between the water molecules and network chains, thus converting ''bound water'' to ''free water'' [36] which because of faster relaxation of polymer chains is forced out resulting in lower degree of swelling. Since the present release system is a swelling controled type, fractional release of insulin is also reduced.

Molecular weight effect

Many parameters like polymer hydrophilic/hydrophobic balance, crosslink density, device geometry, size and molecular weight of polymer are used to control the release rate of drug from polymeric systems. The present study deals with the effect of molecular weight on release

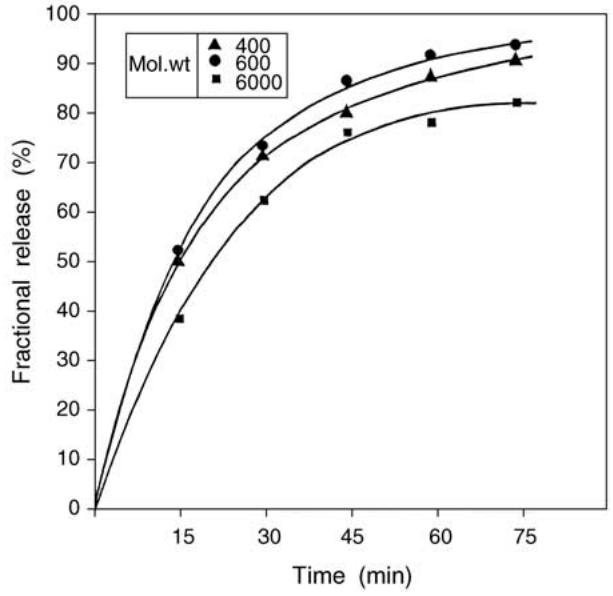

Figure 10 Effect of molecular weight of PEG on the fractional release of insulin for a given composition of the IPN. [PEG] = 0.55 g, [PVA] = 0.75 g, [AM] = 14 mM, [MBA] = 0.006 mM, [KPS] = 0.003 mM, pH = 7.4, temp. = 27 °C, percentage loading = 20.5, thickness = 0.035 cm.

profiles of insulin by using PEG of molecular weights 400, 600, and 6000, respectively. The results obtained are shown in Fig. 10 which indicate that the amount of the released insulin marginally increases with increasing molecular weights of PEG in the range 400–600. On further increasing the molecular weight (6000) the amount of insulin released shows a prominent decrease. The results can be explained as below.

The IPN in the present case is a crosslinked network of PEG, PVA, and PAM chains which are entangled into one another via physical forces. Secondly, PEG is a hydrophilic biocompatible polymer.

When the molecular weight of PEG is increased from 400 to 600, fractional release of the loaded insulin is found to increase. This increase in fractional release is due to the reason that on increasing the molecular weight of PEG from 400 to 600, the hydrophilicity of PEG increases which results in a greater loading of the drug into the IPN. Since the molecular weights of PEG are not too large, fewer crosslinks are present which easily enhances the mobility of polymer chains. This results in a rapid release of the polymer matrix, thus releasing the entrapped insulin with a faster rate. On further increasing the molecular weight of PEG (6000), the macromolecular mesh size or the free space available for diffusion of insulin and water molecues is reduced and this give rise to a slow releasing system. In such a system, the release mechanism generally involves movement of the drug through a complex porous path in the polymer matrix. If the polymer erodes, this will affect the pore structure and accelerate the release [37]. Release profiles also does not show any ''burst effect'' with high molecular weight polymer. This could be due to the reason that because of greater number of pseudo-crosslinks the swelling of the IPN is low in insulin solution which result in the absence of burst effect.

Another significant factor is that at higher molecular weight of PEG, the release profiles follows a fair zero-order release behavior. The reason for the observed behavior is that in a highly crosslinked matrix both the swelling and release processes shall be slower and will have constant swelling and release rates. On the other hand, in a less crosslinked IPN, of lower molecular weight PEG, because of greater swelling in release medium, the insulin molecules will have to travel a much longer path and, therefore, a constant amount of drug cannot be delivered in a definite time.

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 law 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 [38] 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 work the effect of the surface area of the gel on the release kinetics has been observed by taking insulin loaded IPNs of varying thickness in the range 0.035–0.184 cm. The results are depicted in Fig. 11 which reveal that the fractional release decreases with increasing thickness of the IPNs. The observed results are quite obvious as a thinner gel has a greater surface

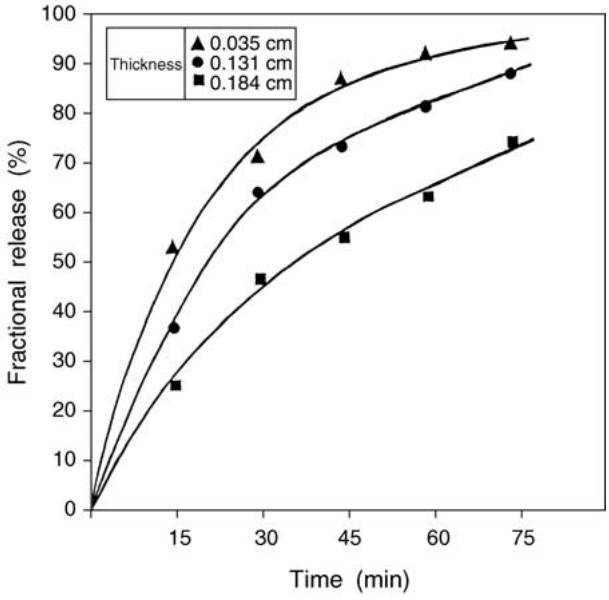

Figure 11 Influence of thickness of the loaded IPNs on the release profiles of insulin for a given composition of the IPN. [PEG] = 0.55 g, [PVA] = 0.75 g, [AM] = 14 mM, [MBA] = 0.006 mM, [KPS] = 0.003 mM, pH = 7.4, temp. = 27 °C, percentage loading = 20.5, thickness = 0.035 cm.

TABLE III Data showing the kinetic parameters of the release of insulin through the IPNs of different compoitions

Hydrogel composition					n	$D \times 10^5$ cm^2/min^{-1}	Release mechanism
PEG (g)	PVA (g)	AM (mM)	MBA (mM)	KPS (mM)			
0.22	0.75	14	0.006	0.003	0.76	3.4	Anomalous
0.55	0.75	14	0.006	0.003	0.76	6.6	Anomalous
0.88	0.75	14	0.006	0.003	0.61	3.4	Anomalous
1.32	0.75	14	0.006	0.003	0.71	4.9	Anomalous
0.55	0.50	14	0.006	0.003	1.05	2.75	Case II
0.55	0.75	14	0.006	0.003	0.76	6.6	Anomalous
0.55	1.0	14	0.006	0.003	0.96	2.75	Anomalous
0.55	1.5	14	0.006	0.003	1.00	3.4	Case II
0.55	0.75	10	0.006	0.003	0.66	3.4	Anomalous
0.55	0.75	14	0.006	0.003	0.76	6.6	Anomalous
0.55	0.75	21	0.006	0.003	0.80	2.6	Anomalous
0.55	0.75	28	0.006	0.003	1.12	1.5	Case II
0.55	0.75	14	0.006	0.003	0.76	6.6	Anomalous
0.55	0.75	14	0.012	0.003	0.95	4.1	Anomalous
0.55	0.75	14	0.025	0.003	0.90	2.2	Anomalous
0.55	0.75	14	0.038	0.003	1.00	2.2	Case II

area and, therefore, will show a larger release. Another reason could be that the thicker is the IPN, greater would be the force required to stretch it, as evident from the slower swelling of the thickest (0.184 cm) IPN. The degree of swelling of a gel is controled 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 [39].

Analysis of dynamic release data

The mechanistic aspects of the release process in swelling controled drug delivery systems may be well discussed in light of the Fick's Equation 5 in which the release exponent n varies in accordance with the release mechanism. The forthcoming paragraph describes the influence of chemical architecture of the hydrogel on the mode of insulin release.

When the concentration of PVA is increased in the feed mixture of the semi-IPN in the range 0.5–1.5 g, the release process follows a zero-order pattern except at the optimum release where the release process shows an anomalous value $(n = 0.76)$. The relaxation controlled nature of the release process at lower (0.5 g, PVA) and higher (1.0 and 1.5 g, PVA) concentration of PVA could be attributed to the fact that at lower and higher content of PVA, the IPN exhibits a lower degree of swelling because of lower hydrophilicity and high crosslink density of the IPN, respectively. Thus, because of a smaller swollen dimension of the IPN, the rate of chain relaxation process slows down and the release process follows Case II mechanism. Another interpretation of the observed zero order release process may be that when the gel is not adequately swollen, the releasing insulin molecules do not have to travel a longer path and, therefore, will diffuse out with almost constant velocity as evident from nearly the same values of diffusion constants.

On increasing the concentration of acrylamide, a hydrophilic monomer, in the feed mixture of the IPN in the range 10.5–28 mM, the value of the release exponent n increases in the anomalous range tending towards a zero-order release behavior. The observed results may be explained by the fact that with increasing AM content in the semi-IPN, the number of crosslinked PAM chains increases in the network which restrains the mobility of macromolecular chains in the hydrogel, thus rendering the release process to be relaxation controlled.

PEG was used as a hydrophilic polymer in the semi-IPN. However, a variation in the PEG content of the gel in the range 0.22–1.32 g is not found to cause a remarkable change in the release mechanism as evident from the release exponent values summarized in Table III. It is clear from the data that the release process follows anomalous behavior, i.e. a relaxation controlled process.

The effect of increasing crosslinker concentration on the mechanism of insulin release has been investigated by varying the MBA concentration in the range 0.006–0.038 mM. The release exponent data clearly implies that the release process tends to acquire a zero-order release behavior when the crosslinker content increases in the feed mixture of the semi-IPN. The obtained results may be attributed to the fact that with increasing degree of crosslinking in the gel, the network chains undergo a forbidden relaxation, thus imparting a relaxation controlled behavior to the release process. Moreover, because of a greater level of crosslinking in the semi-IPN, the degree of swelling is quite low and as a result the insulin molecules travel across the network and release out with same diffusion velocity. This obviously results in a zero-order release behavior (Table IV).

The release mechanism of insulin is also influenced by

TABLE IV pHs of various human-body fluids

Human-body fluids	pH
Saliva (in mouth)	6.7
Stomach (gastric juice)	1.0
Small intestine	
(i) Deu lenum (bile duct)	8.0–8.6
(ii) Pancrease (pancreatic duct)	7.5–8.0

TABLE V Data showing the kinetic parameters of the release of insulin through the IPNs of varying thickness and molecular weight

IPN composition					Thickness (cm)	Molecular weight	n	$D \times 10^5$ $cm^2\ min^{-1}$	Release mechanism
PEG (g)	PVA (g)	AM (mM)	MBA (mM)	KPS (mM)					
0.55	0.75	14	0.006	0.003	0.035	600	0.76	6.6	Anomalous
0.55	0.75	14	0.006	0.003	0.131	600	0.84	2.8	Anomalous
0.55	0.75	14	0.006	0.003	0.184	600	0.86	4.1	Anomalous
0.55	0.75	14	0.006	0.003	0.035	400	0.48	4.9	Fickian
0.55	0.75	14	0.006	0.003	0.035	600	0.76	6.6	Anomalous
0.55	0.75	14	0.006	0.003	0.035	6000	0.84	4.1	Anomalous

the thickness of the drug carrier as indicated by the data summarized in Table V. The data clearly implies that with increasing thickness of the semi-IPN, the release exponent n shifts from anomalous to zero-order value. The reason for the observed shift in release mechanism may be that as the thickness of the gel increases, its water sorption capacity also decreases. Thus, because of lower degree of water sorption, the encapsulated insulin molecules diffuse out with a constant velocity and as a consequence provide nearly zero-order release.

The molecular weight of the hydrophilic polymer (PEG) also affects the release mechanism of insulin. As shown in Table I, the release process is Fickian in nature when the PEG is of lowest molecular weight, i.e. 400, while at higher molecular weight (6000), the release tends to attain a zero-order (on Case II) value. The observed results could be attributed to the fact that when the mol wt of PEG is low, its chains are small and, therefore, are likely to undergo a faster relaxation. This obviously results in a diffusion controlled release process, i.e. Fickian type. On the other hand, with high mol wt PEG, the longer chains of PEG undergo a slow relaxation, thus rendering the release process to become more relaxation controlled. Moreover, because of high mol wt of PEG, the water sorption capacity of IPN also decreases and the releasing insulin molecules diffuse out with a constant rate. This obviously imparts a zero-order pattern to release mechanism.

Conclusions

The semi-IPN prepared by polymerizing acrylamide in the presence of PEG and PVA display a fair potentiality to release insulin from within their macromolecular matrices. The loading of insulin is found to be greatly influenced by the chemical composition of the gel. It is found that with increasing amounts of PEG, PVA, and AM in the IPNs, the percent loading increases. The fractional release of insulin also increases with increasing percent loading of the insulin.

The release profiles of insulin are greatly influenced by the chemical architecture of the IPNs. It is found that on increasing the amount of PEG in the feed mixture in the range 0.22–1.32 g, not much remarkable change is noticed in the release behavior.

When the amount of PVA increases in the IPN, the fractional release increases with increasing PVA content, although at lowest concentration the release remains unaffected showing a zero-order release profile. A ''burst effect'' is noticed at higher concentration.

In case of increase in AM content, the fractional release increases in the range 10.5–14 mM and shows a further decrease with increasing concentration. A zero-order release behavior is observed at highest concentration of AM whereas a ''burst effect'' is noticed at lower concentrations.

When MBA contents are increased in the IPNs, a decrease in fractional release is noticed and for their greatest concentration, a zero order delivery of insulin is noticed. Moreover, a ''burst effect'' is noticed at low concentrations of MBA.

The release profiles are greatly influenced by pH of the release medium. It is found that the fractional release attains an optimum value at neutral pH (7.4). a zero-order release behavior is obtained in the acidic range.

Other factors such as temperature of the release medium, molecular weights of PEG and thickness of IPNs are also found to suppress the fractional release of insulin at higher temperature(40 °C), molecular weight (6000) and thickness of the IPN. The release of insulin also follows a zero-order delivery.

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