In Vitro Release Modulation of Hemoglobin from a Ternary Polymeric Delivery Vehicle

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ABSTRACT: The release of hemoglobin from hydrogel matrices of polyethylene glycol (PEG), polyvinyl alcohol (PVA), and crosslinked polyacrylamide (PAM) was investigated in phosphate-buffered saline (PBS, pH 7.4) as a release medium. The hemoglobin was loaded onto hyrogels by swelling them in hemoglobin solution and the effect of composition of the hydrogel on the amount of hemoglobin loaded was observed. The effects of experimental parameters such as varying concentrations of PEG, PVA, PAM, and crosslinking agent in the feed mixture, pH of the release medium, and the presence of salts were investigated on the release dynamics of the hemoglobin. The release results were analyzed on the basis of the kinetic data, and the kinetic parameters such as the diffusional exponent (n) and diffusion constant (D) were evaluated. From the kinetic parameters data an attempt was made to explore the nature of the mechanism of the hemoglobin release process. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 85: 104–113, 2002

Key words: hydrogel; hemoglobin; swelling-controlled release; dynamics

INTRODUCTION

Three-dimensional macromolecular matrices capable of imbibing a large amount (>20%) of water into their internal molecular structure are termed *hydrogels* or, more fascinatingly, *smart polymers.*[1] The presence of a water reservoir in these polymeric networks becomes the origin of a number of unusual properties such as soft and rubbery texture, living-tissue–like resemblance, physiological stability toward biofluids, low interfacial tension, permeability to biomolecules, biocompatibility, and so forth.[2] These biophysical characteristics of hydrogels enable them to be employed as a potential biomaterial in a wide number of biomedical applications such as, for example, artificial implants,[3] burn dressings,[4] dialysis membranes,[5] contact lenses,[6] and drug-delivery vehicles.[7–11]

In recent years, a considerable research effort has been invested in techniques to control release and delivery of peptides and proteins.[12–15] Controlled release of macromolecular drugs is not as easy as that of the low molecular weight drugs. These types of drugs have high molecular weights; therefore, conventional controlled release techniques, which are based on Fickian diffusion through polymeric matrix systems, are not always effective for desirable drug delivery. In addition, these drugs are chemically unstable in general. The loading of peptides and proteins into, and their controlled release from, polymeric devices without altering bioactivity still remain unsolved problems. Moreover, in the case of proteins, the role of solute molecular size is much more dramatic in hindering the diffusion and release from hydrophilic polymers.[16]

Another critical consideration in protein delivery from hydrogel systems is the potential for protein denaturation in the device. For diffusion-controlled delivery systems, where water is the

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main transporting medium, the protein solution stability governs the type of device. Extended releasing times can be achieved with reservoir systems composed of a protein solution enveloped by a hydrogel membrane.[17]

Another technologically important application of such protein loading and release studies lies in the concept of aqueous two-phase extraction (ATPE) method, which has potential value as a novel protein separation technique and is among the most widely studied techniques for protein isolation.[18]

In the present study, therefore, we report results on the controlled release of hemoglobin through a polymeric hydrogel of polyethylene glycol (PEG), polyvinyl alcohol (PVA), and polyacrylamide (PAM). The reason for selecting PEG and PVA as polymeric components of the hydrogel lies in the fact that, whereas PEG is a highly biocompatible, nontoxic, nonimmunogenic, and water-soluble polymer, PVA, on the other hand, is a hydrophilic polymer with a great film-forming property. Moreover, the polyacrylamide in the crosslinked state has been extensively employed in hydrogel preparations for different purposes.[19] Because of its biological potentiality hemoglobin was chosen as a model macromolecular drug for the release study.

EXPERIMENTAL

Materials

Polyethylene glycol (PEG; MW 600) was obtained from Wilson Laboratories (Bombay, India) and used as received. Polyvinyl alcohol (hot processed MW 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 1 week. *N,N′*-Methylene bisacrylamide (MBA; Central Drug House, Bombay, India) was employed as a crosslinking agent and potassium persulfate (Loba Chemie, India) was used as a polymerization initiator. Hemoglobin was supplied by Loba Chemie in a powder form and its solution was prepared in 0.1*N* NaOH. All other chemicals used were of analytical reagent grade and bidistilled water was used throughout the experiments.

Preparation of Hydrogels

The hydrogels of varying compositions were prepared by the free-radical polymerization method, as described in our earlier communications.[20] In brief, into different petri dishes (4-in. diameter; Corning) were added PVA (2.5 to 7.5% w/v), acrylamide (AM) (3.75 to 10.0% w/v), PEG (1.0 to 6.0% w/v), MBA (0.05 to 0.30% w/v), and potassium persulfate (KPS) (0.04% w/v). The mixtures (20 mL) were homogenized and kept at 80°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 1 week. The swollen hydrogel pieces were then dried at room temperature for 72 h and weighed again. This process was continued until the constant weights of hydrogels were obtained. This clearly ensured the complete removal of unreacted chemicals from the hydrogels.

Swelling Experiments

For performing swelling experiments, the general gravimetric procedure was adopted. In a typical experiment, a preweighed (0.1 g) and preloaded (272.7 mg/g gel) piece of hydrogel was immersed into a PBS solution (release medium) and allowed to swell to equilibrium. The progress of the swelling of the hydrogel was monitored by recording weights of the swollen gels at different time intervals. The swelling process was characterized by the parameter given as

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

where W_s and W_d are the swollen and dry weights of the loaded hydrogels, respectively.

Loading of Hemoglobin

The loading of a drug onto hydrogels 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.[21] In the second approach, the hydrogel is allowed to swell in the drug solution to equilibrium and then dried to obtain the release device. The latter method has some advantages over the first method, given that 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 hydrogel with hemoglobin. In a typical experiment a preweighed dry piece of the hydrogel was allowed to swell in a hemoglobin solution (4% w/v) for 24 h and then taken out and dried at room temperature for 72 h. The following equation was used to calculate the amount of loaded hemoglobin (mg/g gel):

$$\text{Loaded hemoglobin} = \frac{(W_d - W_0)}{W_0'} \tag{2}$$

where W_d and W_0 are the weights (in mg) of hemoglobin-loaded and dry gels, respectively, and W_0' is the weight of the dry gel (in g).

Release Experiments

The dried and loaded hydrogels (0.1 g) were placed into a definite volume (10 mL) of PBS (pH 7.4, 2.7 m*M* KCl, 1.2 m*M* KH_2PO_4, 138 m*M* NaCl, 1.15 m*M* Na_2HPO_4) as a release medium and 5 mL of release medium was taken out at different time intervals. The aliquot taken out was estimated for the released hemoglobin by recording its absorbance at 420 nm (Model No. 106; Systronics, India) and then returned into the release medium. The amount of hemoglobin was determined with the help of a calibration plot.

All experiments were performed in replicate numbers and a fair reproducibility was obtained.

Kinetic Analysis of Release Data

The potentiality of a drug delivery system is normally evaluated on the basis of the first 50–60% release performance of the device because, 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 (xerogel) 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 a slab can be regarded as one dimensional if it takes place predominantly from the two main surfaces and, according to Crank,[22]

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

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 that is still 99% accurate:

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

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

A more realistic view can be constructed about the release mechanism when the release and swelling data are analyzed in light of the following equation:

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

where M_t/M_∞ is the fractional release at time t and k is a rate constant. The exponent n is an important indicator of the mechanism of transport and in general has a value between 0.5 and 1.[23] When $n = 0.5$, the release is taken to be 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 release is described as anomalous. The closer n is to 1, the closer is the release pattern to steady-state release. When $M_t/M_\infty = 0.5$, the half-life t is another extremely significant parameter in comparing systems. In light of eqs. (4) and (5) the release data will be analyzed.

RESULTS AND DISCUSSION

Effect of Gel Composition on Protein Loading

The amounts of loaded hemoglobin (mg/g gel) onto gels of varying compositions are presented in Table I. The data clearly indicate that the composition of the hydrogel has a pronounced effect on the loading of the hemoglobin. The results are explained in the following discussion.

When the amount of PEG increases in the feed mixture in the range 1.0 to 6.0% v/v, a decrease is observed in the loaded hemoglobin. This observed decrease may be explained by the fact that increasing the number of PEG 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 hemoglobin molecules into the network structure of the

Table I Data Showing the Kinetic Parameters of Release of Hemoglobin from Hydrogels of Varying Compositions and Loading

Hydrogel Composition						
PEG (% v/v)	PVA (% w/v)	AM (% w/v)	Loaded Hemoglobin[a] (mg/g gel)	n[a]	Diffusion Constant D[a] (10^7 cm^2 s^{-1})	Release Mechanism
1.0	3.75	5.0	318.2 ± 5.26	0.80 ± 0.06	3.7 ± 0.38	Anomalous
2.5	3.75	5.0	272.7 ± 1.62	1.10 ± 0.24	3.6 ± 0.46	Case II
4.0	3.75	5.0	227.2 ± 2.34	1.20 ± 0.09	1.4 ± 0.16	Super case II
6.0	3.75	5.0	181.8 ± 1.82	1.42 ± 0.12	0.9 ± 0.08	Super case II
2.5	2.5	5.0	583.3 ± 3.46	1.1 ± 0.24	4.7 ± 0.12	Case II
2.5	5.0	5.0	192 ± 1.44	0.64 ± 0.018	1.4 ± 0.76	Anomalous
2.5	7.5	5.0	120.4 ± 1.20	0.52 ± 0.012	0.5 ± 0.08	Anomalous
2.5	3.75	3.75	272.7 ± 2.86	0.59 ± 0.022	1.4 ± 0.84	Anomalous
2.5	3.75	7.5	308.4 ± 3.62	0.41 ± 0.024	0.9 ± 0.09	Fickian
2.5	3.75	10.0	352 ± 5.22	0.38 ± 0.016	0.8 ± 0.02	Fickian

[a] Values represent the mean ± SD of at least three determinations.

hydrogel. This obviously results in a decline in the loading of the hemoglobin onto the hydrogels.

Similar type of findings have been observed when the hydrophilic polymer PVA increases in the feed mixture in the range 2.5 to 7.5% w/v. The observed decrease in the loaded hemoglobin may be attributed to the increasing number of PVA chains in the hydrogel, which produces a more compact arrangement of macromolecular chains that contain small-size free volumes within the gel. Thus, hemoglobin molecules are prevented from entering into the hydrogel, leading to a protein-loaded gel with low hemoglobin content.

An opposite trend is noticed when the hydrophilic acrylamide increases in the feed mixture in the range 3.75 to 10.0% w/v. The results clearly indicate that the loaded hemoglobin increases in the above-mentioned range of acrylamide. The results can be explained by the fact that, when the gel with increasing acrylamide content is allowed to swell in an alkaline solution of hemoglobin, the amide groups undergo an increasing extent of hydrolysis, producing $-COO^-$ groups along the network chains. Thus, a greater number of carboxylate-bearing chains causes a higher order of repulsion among the chains, thus widening the mesh sizes in between the network chains. This obviously favors a greater loading of hemoglobin molecules onto the hydrogels.

When the crosslinker (MBA) was varied in the range 0.05 to 0.3% w/v in the feed mixture of the gels, the loading drastically declines (data not shown). The observations are quite expected, given that a greater number of crosslinks squeeze the free volumes available between the chains of the macromolecular network and, thus, the loading of the hemoglobin decreases.

Effect of Protein Loading on Released Hemoglobin

An important aspect in the use of hydrogels as drug vehicle is the effect of drug loading level on the rate of drug release. For this purpose a hydrogel of definite composition was loaded with different amounts of hemoglobin and the loaded gels were released in PBS. The results obtained, as shown in Figure 1, reveal that the amount of released hemoglobin increases with increasing hemoglobin loading. The results are quite expected, given that the larger the initial load, the faster the movement of the solvent front penetrating the surface of the loaded gel.[24] 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 hemoglobin.

Effect of Hydrogel Composition on Released Hemoglobin

Because the hydrogel in the present work belongs to a swelling-controlled release system, the release profiles are primarily affected by the swelling characteristics of the network that, in turn, are influenced by the chemical architecture of the hydrogel. To investigate the effect of composition of the hydrogel on the release kinetics of the he-

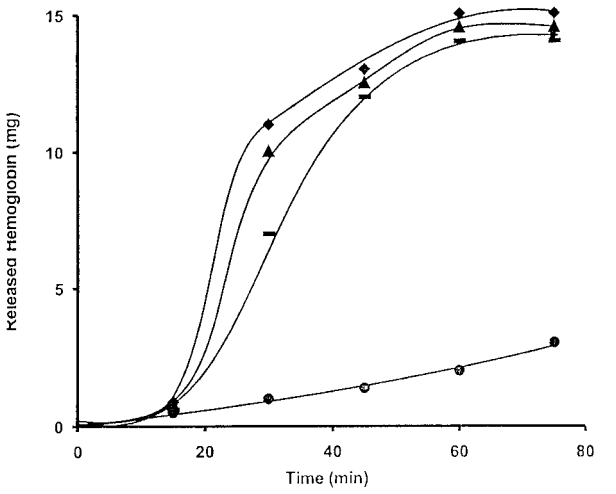


Figure 1 Effect of amount of loaded hemoglobin on the release profile of the loaded hydrogels. [PEG] = 2.5% v/v, [PVA] = 3.75% w/v, [AM] = 5.0% w/v, [MBA] = 0.05% w/v.

moglobin, it is essential to undertake almost identically loaded hydrogels, so that the effect of loading on the release rate could be eliminated. In the present study, therefore, the amounts of hemoglobin loaded onto hydrogels were maintained nearly constant in subsequent studies, as discussed next.

Effect of PEG

The effect of PEG on the released amounts of hemoglobin was investigated in the concentration range of 1.0 to 6.0% v/v of PEG in the feed mixture. The results, depicted in Figure 2, indicate that the released amount increases in the concentration range 1.0 to 4.0% v/v of PEG, whereas it decreases beyond 4.0% v/v.

The results can be explained by the fact that with increasing PEG content in the hyrogel, the hydrophilicity of the gel also increases and, consequently, the water molecules penetrate the gel, causing relaxation of macromolecular chains. This obviously results in a diffusion of entrapped hemoglobin molecules to the release medium following either a Fickian or a non-Fickian transport mechanism. This clearly explains the increased amounts of released hemoglobin with increasing PEG.

However, beyond 4.0% v/v of PEG a decline in the released hemoglobin is observed that is attributed to the fact that, beyond 4.0% v/v of PEG, the network density increases to an extent that does not allow the penetration of either the water molecules from the release medium to the gel or the hemoglobin molecules from the gel into the release medium. Thus, the released amount of hemoglobin decreases.

Effect of PVA

The release behavior of a drug vehicle is fundamentally dependent on its water-sorption characteristics that, in turn, are regulated by the chemical architecture of the gel, including the hydrophilicity of the gel, network density of macromolecular chains, mesh size of the free volumes, and so forth. Polyvinyl alcohol, a hydrophilic polymer of great potential uses, has been well documented in the literature for its notable biomedical applications.[25]

In the present study the influence of PVA on the release pattern of the hemoglobin was investigated by varying its composition in the feed mixture of the gel in the range 2.5 to 7.5% w/v. The release data, displayed in Figure 3, indicate that with increasing PVA in the gel the amount of released hemoglobin decreases. The results can be explained by the fact that when the swelling pattern of the hydrogels is observed it is found

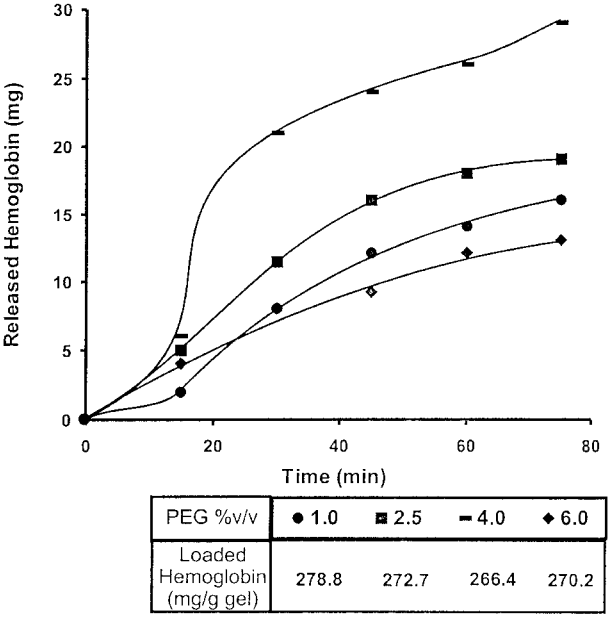
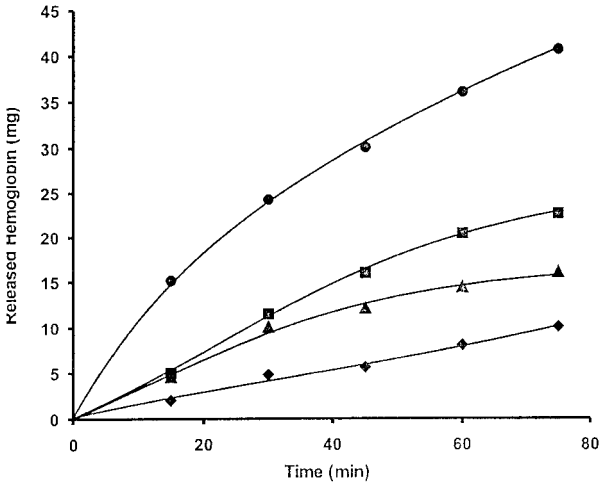


Figure 2 Variation in the amounts of released hemoglobin with varying PEG content of the hydrogel. [PVA] = 3.75% w/v, [AM] = 5.0% w/v, [MBA] = 0.05% w/v.



PVA % w/v	● 2.5	■ 3.75	▲ 5	◆ 7.5
Loaded Hemoglobin (mg/g gel)	260.8	272.7	264.2	276.6

Figure 3 Effect of PVA content of the hydrogel on the amounts of released hemoglobin. [PEG] = 2.5% v/v, [AM] = 5.0% w/v, [MBA] = 0.05% w/v, hemoglobin loading = mg/g gel.

that the swelling ratio also decreases with increasing PVA content of the gel. The swelling results are quite obvious, given that on increasing the proportion of PVA in the gel, although hydrophilicity also increases, at the same time the crosslink density of the network increases sufficiently so as to reduce the free volumes between the chains and thus decrease the swelling of the hydrogel. A decreased swelling of the hydrogel is also implied for lower amounts of released hemoglobin. Similar type of results have also been reported elsewhere.[26]

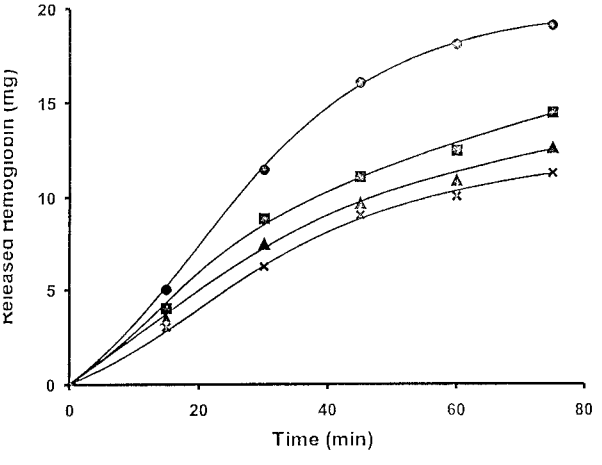
Effect of Acrylamide

Acrylamide, a hydrophilic monomer, has been found to significantly affect the released amount of hemoglobin. It was found that on increasing AM in the range 3.75 to 10.0% w/v, the amount of released hemoglobin decreases (data not shown). The observed decrease can be attributed to the fact that with an increasing number of AM chains in the hydrogel, the density of the network increases, which results in slow relaxation of polymer chains. This obviously retards the diffusion of entrapped hemoglobin molecules into the release medium and thus gives rise to lower amounts of released hemoglobin.

The observed results are further supported by the findings of swelling experiments in which a decrease in the swelling ratio is observed with increasing acrylamide content in the hydrogel.

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 (MBA) in the feed mixture was varied in the range 0.05 to 0.30% w/v, keeping other compositions constant (i.e., [PEG] = 2.5% v/v, [PVA] = 3.75% w/v, and [AM] = 5.0% w/v), and its effect was observed on the amount of released hemoglobin. The results, as shown in Figure 4, reveal that the amount of released hemoglobin decreases with increasing MBA content in the hydrogel. The results can be explained by the fact that with increasing MBA in the feed mixture, 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



MBA % w/v	● 0.05	■ 0.1	▲ 0.2	× 0.3
Loaded Hemoglobin (mg/g gel)	280.6	272.7	264.6	278.8

Figure 4 Effect of crosslinker content of the hydrogel on the release profiles of the hemoglobin. [PEG] = 2.5% v/v, [PVA] = 3.75% w/v, [AM] = 5.0% w/v.

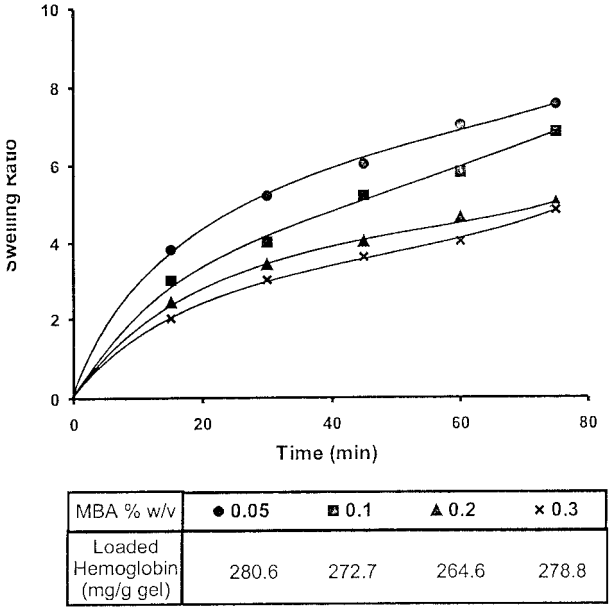


Figure 5 Variation in the swelling ratio of the hydrogel with varying amounts of crosslinker content of the hydrogel. [PEG] = 2.5% v/v, [PVA] = 3.75% w/v, [AM] = 5.0% w/v.

loaded gel network but also hinders the diffusion of larger hemoglobin molecules from within the gel to the release medium. This obviously decreases the amount of released hemoglobin. Similar results have also been reported by other investigators.[27]

The release results are further supported by the hemoglobin loading and swelling observations. As mentioned earlier, the loading of hemoglobin decreases with increasing crosslinker in the gel. Furthermore, the loaded gels exhibit decreased swelling with increasing MBA content in the gel, as shown in Figure 5. These observations can be attributed to the fact that increased crosslinking in the gel not only restrains the diffusion of water molecules into the gel but also results in a slower relaxation of network chains, and both of these factors imply a lower degree of swelling. Some investigators have explained the lower swelling of a highly crosslinked gel by the decrease in the glass-transition temperature of the polymer, which will restrict the mobility of macromolecular chains and result in a lower swelling.[28]

Effect of pH

The hydrogels responsive to external conditions such as pH, ionic strength, and temperature of aqueous systems are of great practical significance.[29] New controlled drug-delivery systems in response to changes in pH of the release medium are being explored,[30,31] to improve therapeutic efficiency and reduce or eliminate side effects of oral controlled drugs. It is also being attempted to deliver drugs to specific regions of the gastrointestinal (GI) tract by making use of pH changes within the GI tract.

In the present investigation the effect of change in pH of the release medium was investigated with respect to the released amount of hemoglobin in the pH range 4.0 to 11.0. The results, as depicted in Figure 6, reveal that both the release rate and released amount of hemoglobin increase with increasing pH of the release medium. The results can be explained by the fact that with increasing pH of the release medium, the amide groups ($-CONH_2$) of polyacrylamide undergo a partial hydrolysis to carboxylic groups (–COOH), which upon ionization produce anionically charged chains. Now, these charged chains cause repulsive forces to operate among themselves and thus the macromolecular chains undergo a fast relaxation, which facilitates the penetration of water molecules into the loaded gel and subsequently enhances the amounts of released hemoglobin. Similar results have also been published elsewhere.[32]

Effect of Salts on Released Hemoglobin

It is a well recognized fact that a balance between the osmotic pressure and the polymer elasticity

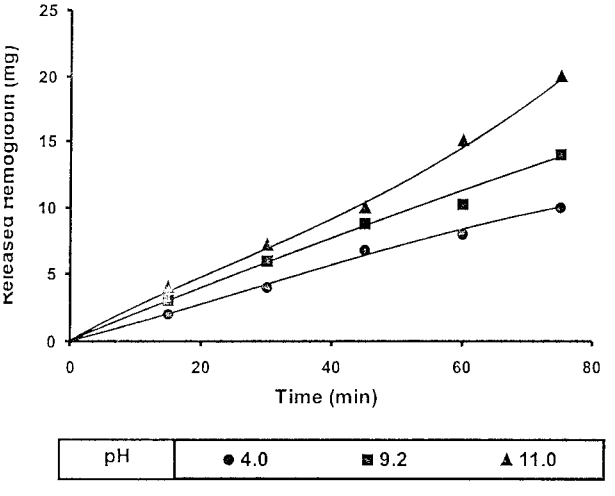


Figure 6 Effect of pH of the release medium on the amounts of released hemoglobin. [PEG] = 2.5% v/v, [PVA] = 3.75% w/v, [AM] = 5.0% w/v, [MBA] = 0.05% w/v, hemoglobin loading = 272.7 mg/g gel.

sets the physical dimensions of the swelling hydrogels.[33] Normally, the osmotic pressure results from a net difference in concentrations of mobile ion between the interior of the gel and the exterior solution. According to Donnan equilibrium theory, when a gel contacts a liquid, then the solvent chemical potential μ in both the gel and solution phases must be equal at equilibrium, as shown in the following equation:

$$\Delta\mu_i^g = \Delta\mu_i^s \tag{6}$$

where the superscripts g and s denote the gel and solution phases, respectively. In terms of the osmotic pressure π, the above equation can be written as

$$\pi = \frac{-(\Delta\mu_i^g - \Delta\mu_i^s)}{V} = 0 \tag{7}$$

where V is the molar volume of the solvent. Osmotic pressure π of the gel determines whether the gel will expand or shrink. Now, neglecting ion–ion, ion–solvent and, ion–polymer interaction, we can write

$$\pi_{\text{ion}} = RT \sum_i (C_i^g - C_i^s) \tag{8}$$

where C_i is the mobile ion concentration of species i. Equation (7) clearly implies that the larger the difference of ionic concentration between the gel and solution, the greater the swelling and, obviously, the greater the hemoglobin release.

In the present work the influence of the presence of NaCl in the release medium was investigated with respect to the released amount of hemoglobin by adding salt (NaCl) to the release medium in the concentration range 0.01 to 0.1M. The results, as shown in Figure 7, indicate that the amount of released hemoglobin decreases with increasing concentration of electrolyte in the release medium. The results are justified by eq. (7), given that increasing the salt concentration in the release medium decreases the difference of ionic concentration between the gel and release medium and this directly affects the swelling of the loaded hydrogel in the release medium. Obviously, a lower degree of swelling of the hydrogel results in a lower release rate of hemoglobin.

Analysis of Kinetic Release Data

It is a well-established fact that the release process basically results from the swelling of the loaded hydrogel in the release medium. In the present study the release data were treated with eqs. (4) and (5) and the evaluated kinetic constants, such as the diffusion constant D and the diffusional exponent n, are summarized in Table I. Now the summarized data could be well analyzed to give some information about the mechanism of the release process, as explained next.

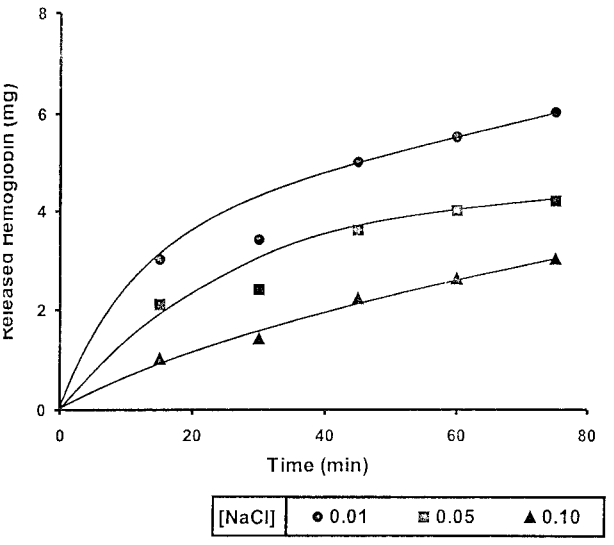


Figure 7 Effect of salt (NaCl) on the release profile of the hemoglobin. [PEG] = 2.5% v/v, [PVA] = 3.75% w/v, [AM] = 5.0% w/v, [MBA] = 0.05% w/v, hemoglobin loading = 272.7 mg/g gel.

When the amount of PEG increases in the concentration range of 1.0 to 6.0% v/v in the feed mixture of the hydrogel, the diffusional exponent n is found to increase in the non-Fickian range from 0.8 to 1.42, thus attaining a super Case II transport value of 1.42. The results suggest that with increasing PEG the release process becomes increasingly relaxation controlled. The observed results also appear justified, given that a greater PEG content in the gel may restrain the segmental mobility of polymer chains and, as a consequence, the diffusion of hemoglobin molecules becomes relaxation controlled. This explanation is further supported by the fact that less-mobile PEG chains slow down the diffusion of hemoglobin molecules from the interior of the gel into the release medium, as evident in Table I from decreasing values of the diffusion constants of hemoglobin molecules.

The effect of varying amounts of PVA on the release mechanism of hemoglobin was investigated by varying PVA in the concentration range of 2.0 to 6.0% w/v in the feed mixture of the

hydrogel. The results, summarized in Table I, reveal that with increasing PVA the diffusional exponent n decreases from 1.10 to 0.52, thus shifting the hemoglobin transport mechanism from a super Case II value of 1.10 to anomalous values of 0.52. In other words, the release mechanism tends to become relaxation controlled. The results are quite obvious because increasing PVA content enhances the compactness of the network and, therefore, slows down the relaxation of macromolecular chains. This clearly results in a relaxation-controlled release of hemoglobin. A decreasing value of diffusion constants also supports the idea of slow relaxation of polymeric chains of the hydrogel.

The data summarized in Table I also indicate that with increasing acrylamide content in the hydrogel in the range 3.75 to 10.0% w/v, the diffusional exponent constantly decreases from a super Case II value of 1.09 to a Fickian value of 0.38, thus shifting the release mechanism from relaxation controlled to diffusion controlled. The observed results can be explained by the fact that with increasing acrylamide content in the hydrogel, the number of crosslinked polyacrylamide chains increases that, because of increased molecular weight of the polymer molecules, widens the size of the free volumes available between the network chains. This, in turn, results in a rapid relaxation of polyacrylamide chains, which makes the hemoglobin release a diffusion-controlled process. The decreasing values of diffusion constants imply that increasing numbers of polyacrylamide chains hinder the diffusion of large hemoglobin molecules from within the loaded hydrogel into the release medium.

CONCLUSIONS

The hydrogel composed of polyethylene glycol, polyvinyl alcohol, and crosslinked polyacrylamide displays a fair potentiality to release the hemoglobin entrapped within the macromolecular matrix. An increase in hemoglobin loading is observed with increasing contents of acrylamide and crosslinker (MBA), whereas an increase in the proportions of hydrophilic polymers PEG and PVA in the feed mixture decreases the percentage loading of hemoglobin. The hydrogel loaded with hemoglobin exhibits the following release results:

1. The released amount of hemoglobin increases with increasing PEG in the range 1.0 to 4.0% v/v, whereas beyond 4.0% v/v, a fall in the released amount is observed. The release mechanism also shifts from anomalous to super Case II transport.
2. With increasing PVA content (2.5 to 7.5% w/v) in the feed mixture of the hydrogel, the released hemoglobin decreases continuously. The hydrogel also shows a shift in the release mechanism from Case II to Fickian transport with increasing proportion of PVA.
3. When the concentration of acrylamide increases in the hydrogel in the range 3.75 to 10% w/v, a decrease in the released amount is observed. The kinetic parameters reveal that with increasing monomer the release mechanism shifts from Case II to Fickian transport.

The release of hemoglobin also decreases with increasing crosslinker (MBA) in the gel. The presence of NaCl in the release medium lowers the release rate, whereas increasing the pH enhances the amount of released hemoglobin.

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