Hemoglobin-Crosslinked Polyacrylamide Hydrogels: Swelling Behaviour Analysis

S.K. BAJPAI

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

Hydrogels have been prepared by polymerizing and crosslinking acrylamide in the alkaline solution of hemoglobin. The swelling behaviour of hemoglobin-crosslinked polyacrylamide hydrogels was analyzed in water and in citric acid-phospate buffer solutions at various pH. The effect of temperature on the swelling behaviour was also analyzed by varying the temperature from 25 to 60°C. Amount of crosslinking agent also affected the swelling capacity. Swelling parameters such as penetration velocity and diffusion coefficient were also evaluated. The gels were found to show reversible swelling and deswelling processes.

INTRODUCTION

Hydrogels are polymeric systems designed to contain a significant amount of water. They find a wide range of applications in the biomedical field including soft contact lenses,[1] drug delivery systems,[2,3] and burn dressings.[4] The medical and pharmaceutical applications of hydrogels have been extensively reviewed.[5]

Most of the research work on polymer gels is mainly focused on synthetic polymeric gels thus neglecting the considerable variety of networks in natural polymers such as naturally occurring polysaccharides and proteins. Since the biological responses to polymer surfaces are complex, each polymer system should meet certain requirements for biomedical applications. Biocompatibility of the material is critical, and for some applications biodegradability is desirable. The natural gels are ideal candidates for these biomedical applications. For example, they could be used to encapsulate and cultivate cells inside the gel, where the network will act as a semipermeable membrane allowing only growth factors to enter to aid the growth of the cells. The study of natural gels could be useful in the development of novel synthetic polymer networks that mimic natural gels,[6] and a knowledge of the response of natural gels to the changes in the environment could be invaluable verifying the trends observed in the experimental behaviour of synthetic polymeric gels.

Although some literature is available related with the studies of swelling behaviour of hydrogels with one of the constituents as natural polymer,[7–9] still there are possibilities of extensive research work in this field. In this connection, the present communication describes the analysis of swelling behaviour of hemoglobin-crosslinked polyacrylamide hydrogels. The equilibrium swelling has

been studied as a function of degree of crosslinking, pH and temperature of the external solution. Reversibility of the swelling process has also been tested.

EXPERIMENTAL

The raw materials used have been described in Table-1. Acrylamide was recrystallized in methanol before use and other materials were used as received. The doubly distilled water was used throughout the work.

TABLE-1
RAW MATERIALS EMPLOYED AND THEIR SOURCE

Case number	Name and description	Source (India)
1.	Acrylamide (Am)	Encore Chemicals
2.	N,N'-Methylene bis-acrylamide	Central Drug House
3.	Hemoglobin powder	Loba Chemie Industries
4.	Potassium persulphate (KPS)	Loba Chemie Industries
5.	Sodium metabisulfite (SMB)	S.D. Fine Chemicals

Synthesis of Crosslinked Hydrogels

A definite amount of hemoglobin powder was dissolved in NaOH solution and to this, acrylamide and N,N'-methylene bis-acrylamide (BIS) solutions were added and mixed thoroughly, followed by addition of calculated quantities of potassium persulfate (KPS) and sodium metabisulfite (SMB). The total mixture was stirred well quickly to avoid lumping, poured into 10×75 mm test tubes and set aside undisturbed. The resulting smooth, semi-transparent cylindrical hydrogels were removed from the test tube and then sliced into discs, washed with Tris-HCl buffer, pH 7.0, followed by acetone and water and dried in a dust-free glass chamber at room temperature. In all, two samples of hydrogels were prepared with different degree of crosslinking. The degree of crosslinking is represented as crosslinking ratio, which is defined as the ratio of the amount of the monomer to the amount of crosslinker.

According to this definition, an increase in the crosslinking ratio means a decrease in the degree of swelling.

Dynamic Swelling Studies

Hydrogels were swollen to equilibrium in water at physiological temperature 37°C. Equilibrium was attained in 24 h; the approach to equilibrium was monitored by measurement of mass of the swollen hydrogels. Once the equilibrium was attained, the swelling capacity was calculated using the formula

$$\text{Grams of water per gram of gel} = \frac{W_e - W_0}{W_0} \tag{1}$$

where W_e is the weight of the equilibrated hydrogel and W_0 is the initial weight of the dry hydrogel.

The penetration velocity (V) of buffer in each polymer was determined by weight gain method as reported elsewhere.[10] The penetration velocity was calculated from the slope of the initial portion of the penetrant uptake curve by using the equation

$$V = \frac{1}{2dA} \frac{dW_q}{dt} \qquad (2)$$

where 'V' denotes penetration velocity, $\frac{dW_q}{dt}$ denotes the slope of the weight gain versus time curve, and 'd' denotes the density of water at 37°C while 'A' denotes area of one face of the disc.

The mass uptake of the swelling solution 'M_t' as a function of time 't' was analyzed according to the equation,[11]

$$\frac{M_t}{M_\alpha} = Kt^n \qquad (3)$$

which could be used to find out the Fickian and non-Fickian absorption of water by hydrogel. 'M_α' is the mass uptake of the solvent at equilibrium, 'K' is a constant and 'n' is the exponent describing the Fickian or anomalous swelling mechanism.

On taking natural log of eq. (3),

$$\ln \frac{M_t}{M_\alpha} = \ln K + n \ln t \qquad (4)$$

The values of 'n' and 'K' were calculated from the slope and intercept of the plotes of $\ln (M_t/M_\alpha)$ against $\ln t$ respectively.

The diffusion coefficient 'D' of solvent was calculated using the following equation,[12]

$$D^n = \frac{K}{4} (\pi r^2)^n \qquad (5)$$

where 'r' is the radius of the gel disc.

To study the swelling behaviour of the hydrogels in medium of different pH, two pieces of preweighed hydrogels were placed in buffer solution of required pH and allowed to equilibrate. Mass measurements of the haydrogels were taken at different time intervals to monitor the attainment of equilibrium, and swelling capacities (water sorbed per g of gel) were determined. In each case the final water content was determined for two pieces of hydrogels. Good agreement was found in the degree of swelling for both pieces, each having the same history, the average of two determinations being used for calculations.

In order to study the reversibility of the swelling process, the hydrogel samples were allowed to equilibrate in water and then placed in 1 M NaCl solution, which caused the gel to deswell. The deswelling was then followed by weighing the gel at various time intervals. The reversibility of swelling and deswelling was determined using the same samples for consecutive swelling and deswelling experiments. The degree of swelling has been expressed as the swelling ratio, the ratio of final weight of the gel to initial weight of the gel.

RESULTS AND DISCUSSION

Effect of Crosslinking on Swelling: The effect of the concentration of BIS crosslinker on the equilibrium water absorption of hydrogels is depicted in Fig. 1. It is clear that as the amount of crosslinker in the reaction system increases the swelling capacity of the resulting hydrogel decreases which is a normally observed effect of increasing levels of crosslinker. The reason for the observed decrease in the swelling capacity is that water absorption is controlled by two, sometimes competing, factors: the chain stiffness or flexibility and the polarity of the system. When concentration of crosslinker increases, the flexibility of polymeric chains in the system decreases which results in the lower extent of chain relaxation process and hence swelling.
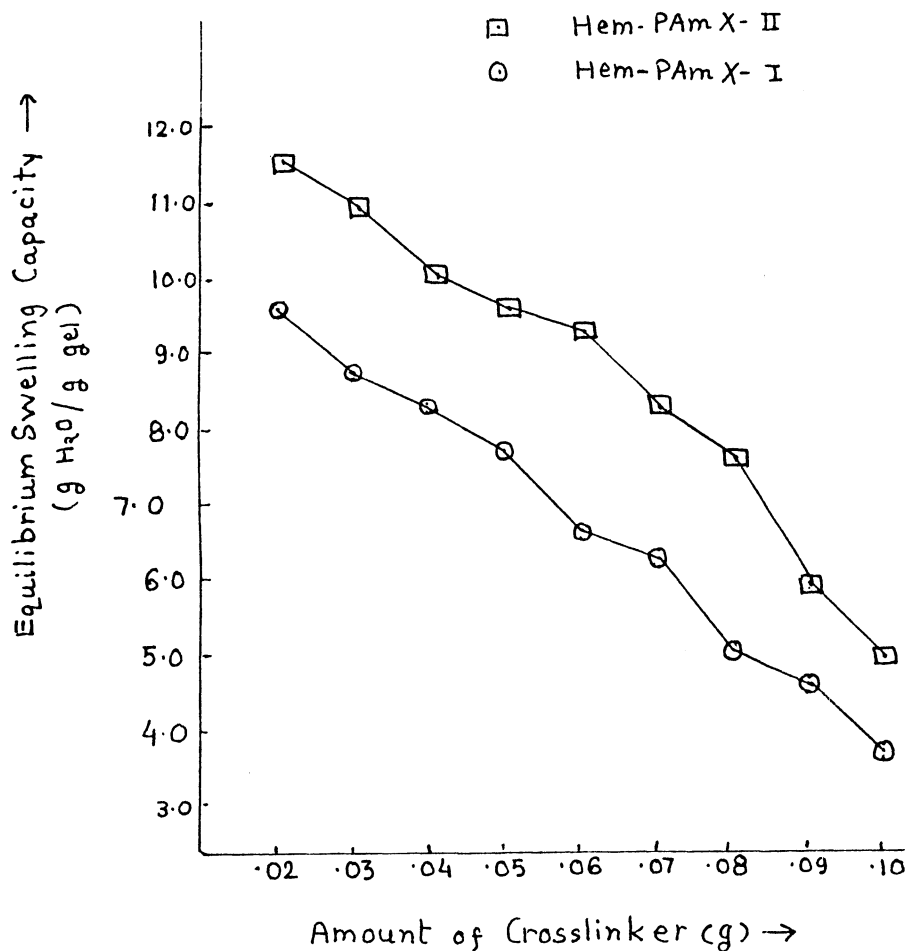

Fig. 1.   Effect of amount of crosslinking agent on the equilibrium swelling capacity of Hem-PAmX hydrogels; Temperature = 37°C; pH = 7.0.

Moreover, due to increase in the amount of crosslinker, the number of efficinet crosslinks per unit volume increases, therefore crosslink density and network chain density increases but by increasing the later parameter, there is less free
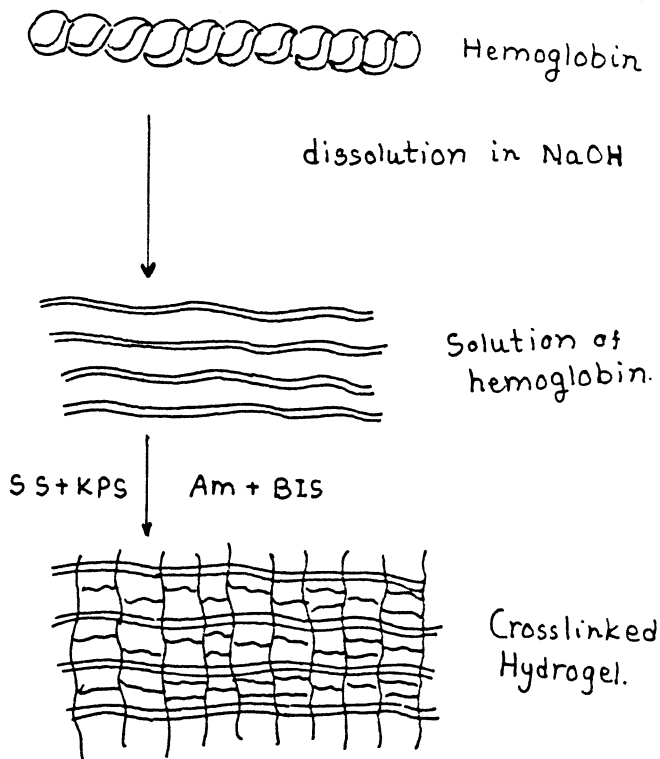
Scheme-I   Schematic diagram showing the formation of Hem-PAmX hydrogels.
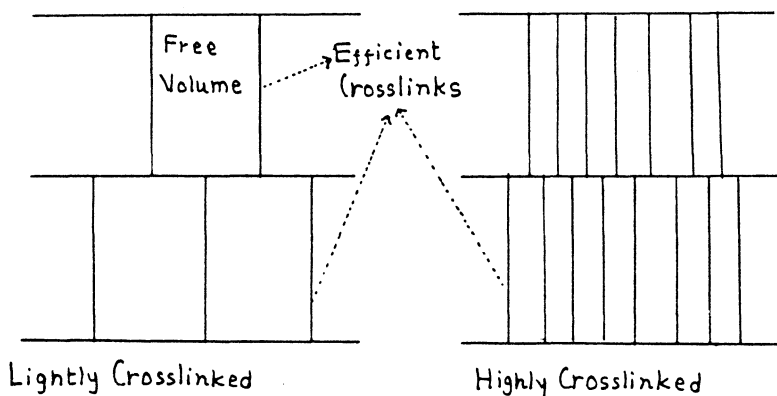

Fig. 2.   Effect of degree of crosslinking on the availability of free space
for accommodation of water inside the hydrogels.

volume or room to accommodate water and hence degree of swelling or swelling capacity decreases. The above reasoning is well depicted in Fig. 2. Here it is significant to mention that hemoglobin-noncrosslinked polyacrylamide hydrogels were also prepared but their disintegration started after 6 h when placed in water for swelling measurements.

Effect of pH: The swelling behaviour of hydrogels is greatly affected by variation in pH of the external solution as depicted in Fig. 3. The results so obtained are quite different from those obtained in the case of gelatin-polyacrylamide hydrogels,[9] where the equilibrium swelling was found to decrease with increase in pH of the external solution. It is clear from Fig. 3 that the equilibrium swelling capacity increases with rise in pH of the external solution, attains maximum value at pH 7.0, and then starts decreasing, thus attaining a constant value. It is also clear from the Figure that swelling is suppressed to a greater extent in the acidic range as compared to the alkaline. The observed experimental findings may be explained as below.
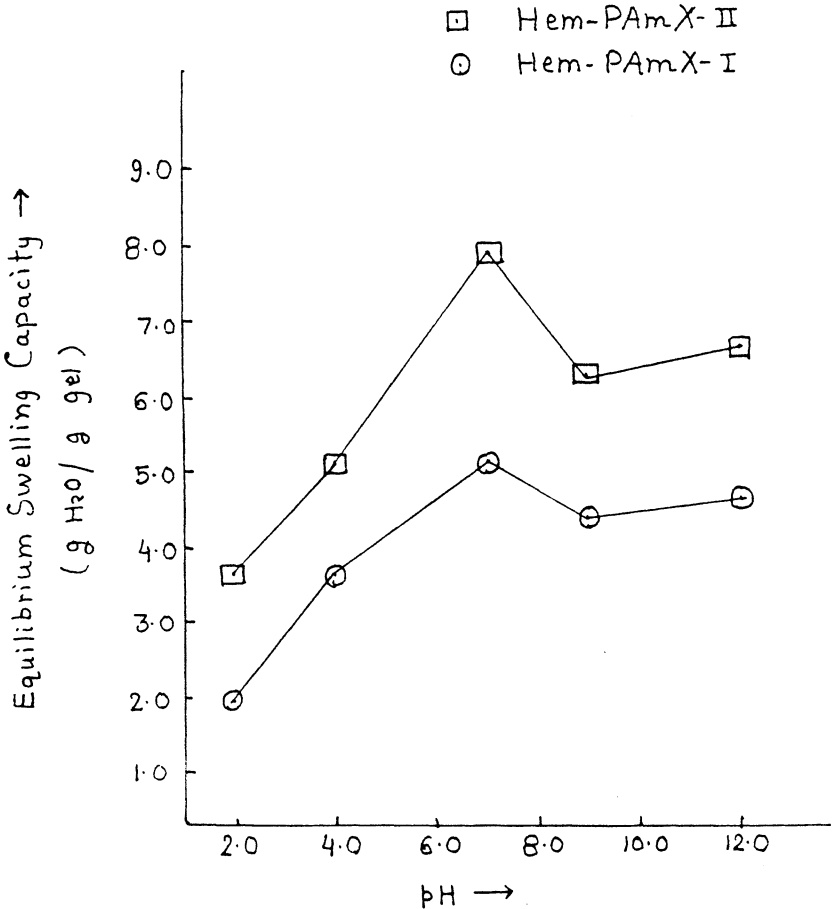

Fig. 3.   Effect of pH on the equilibrium swelling capacity; temperature = 37°C.

The swelling of a hydrogel in a solvent/solution is usually governed by two processes. First is migration of osmotically active ions between the gel phase and the external solution, in accordance with the Donnan-membrane equilibrium and the other is the relaxation of polymeric chains in the polymer matrix owing to the charge profile of polymer segments. When hydrogel is placed in deionised water or salt solution, the osmotically active ions migrate between the two phases thus creating a net osmotic pressure and ultimately causing swelling of the gel. Here it is the Donnan-membrane equilibrium which governs the process of swelling. However, when gel is placed in solutions of different pH, the charge profile of the polymeric chains is affected greatly and this results in operation of electrostatic and H-bonding interactions among charged groups thus resulting in decrease in chain relaxation or formation of complex structure. In other words, due to some electrostatic and H bonding interactions among various groups across the polymeric chains, flexibility of chains is reduced which ultimately causes less swelling of the hydrogel.

When pH of the solution is very low (*i. e.* pH = 2.0) the hydrogel shows almost minimum swelling. This may be attributed to the fact that in acid medium, the amide groups present in the polyacrylamide molecules undergo hydrolysis to form —COOH groups as shown in Scheme II. Therefore polymer matrix contains both acid and amide groups which ultimately results in the formation of a complex structure[13] through H-bondings as shown in the scheme II. Such a complex structure reduces the flexibility of polymer segments and hence solvent entrance
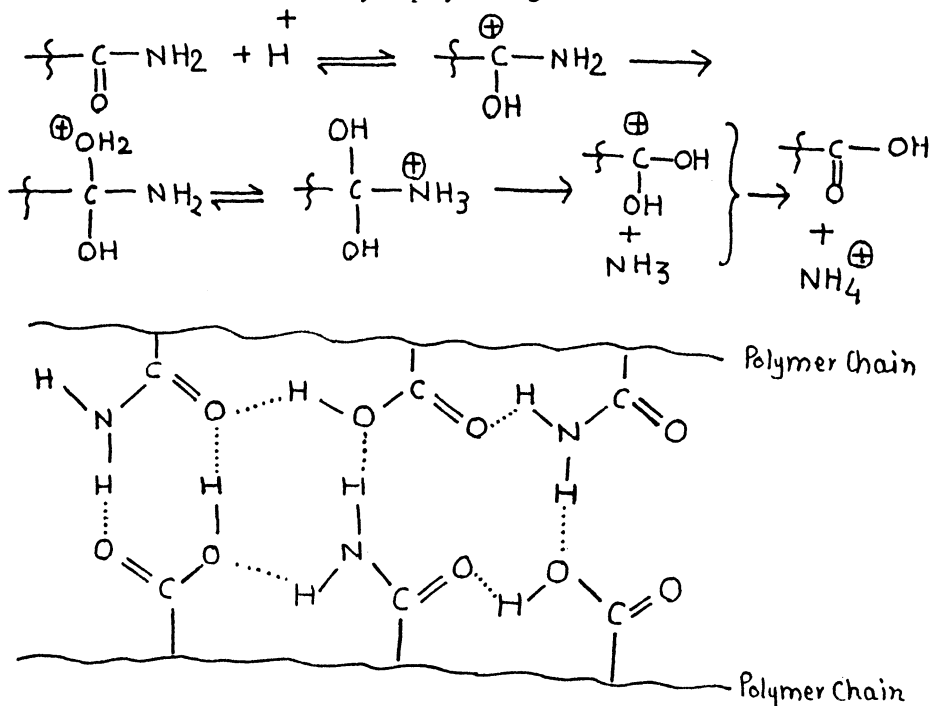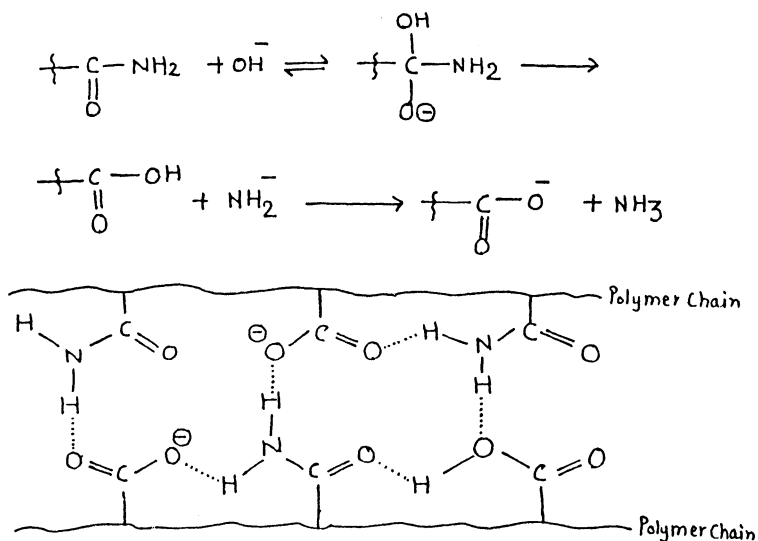

Scheme-II.   Scheme showing acid hydrolysis of amide groups in hydrogel and the formation of complex structure.

into the gel phase is discouraged. Here it is worth mentioning that as the experiments have been carried out at 37°C, the possibility of hydrolysis of amide groups cannot be ruled out which was further confirmed by getting a positive test for NH_4^+ ions in the external solution after the attainment of equilibrium.

When pH of the external solution is increased the extent of hydrolysis decreases which results in decrease in number of —COOH groups in the polymer matrix. Therefore H-bonding interactions become less operative in the polymer matrix which finally reduces the complexity of the structure or stiffness of the polymeric chains. This, in fact, results in more swelling of the gel.

When hydrogel is placed in doubly distilled water at pH 7.0, the swelling process is mainly governed by migration of osmotically active ions between the two phases in accordance with the Donnan-membrane equilibrium, and maximum swelling is observed. As hydrogels have been prepared by dissolving hemoglobin in NaOH solution, gel phase contains Na^+ ions which migrate towards the external solution and hence some OH^- ions migrate towards the outer solution so that electrical neutrality of the solution be maintained.

Scheme-III.   Scheme showing alkaline hydrolysis of amide groups and the formation of rather loose or less complex structure.

As the concentration of osmotically active ions outside the gel phase is very small, a net high osmotic pressure is developed between the two phases which results in extensive swelling of the hydrogel.This phenomenon is usually termed as gel hydrolysis. After attainment of equilibrium the pH of the external solution was found to be slightly alkaline (*i.e.*, pH = 7.4) which also confirms the proposed mechanism. Here it may be pointed out that absence of H-bonding interactions also helps the hydrogel to attain maximum swelling.

When hydrogel is placed in the external buffer solution of pH 9.0 the equilibrium swelling capacity is found to decrease which may be explained by

the fact that in alkaline medium, hydrolysis of amide groups takes place, thus generating —COOH groups. Hence polymer matrix contains both acid and amide groups which form complex structure. Now as the medium is alkaline, some of the —COOH groups dissociate to produce —COO⁻ ions. In this way the extent of H-bonding interactions is reduced and the complex structure produced is rather less rigid or stiff. Therefore decrease in the equilibrium swelling capacity does not occur to a greater extent as observed in acid medium.

TABLE-2

COMPOSITION OF HEM-POLYACRYLAMIDE HYDROGELS

S. No.	System	Composition (g)					
		Hem	Am	BIS	KPS	SMB	H_2O
1.	Hem-PAmX-I	4.00	4.00	0.08	0.06	0.06	35.00
2.	Hem-PAmX-II	3.00	5.00	0.05	0.07	0.07	35.00

When pH of the external solution is further increased (*i.e.*, pH = 12.0), most of the amide groups have been hydrolyzed to —COOH groups, thus forming the complex structure. However, at such a high pH an appreciable number of COOH groups ionise to produce —COO⁻ groups. Hence the gel structure is not so complex or stiff, but polymeric chains are rather relaxed due to mutual repulsion of —COO⁻ groups and hence a slight rise in swelling capacity is observed.

Table-3 describes various swelling parameters of hydrogel samples at different pH of the external solution. It is clear from the table that sample Hem-PAmX-II shows non-Fickian swelling behaviour at pH 7.0 (n > 0.50) while at other pH of the external solutions the two samples show Fickian behaviour.

TABLE-3

SWELLING PARAMETERS OF HEM-PAmX HYDROGELS AT DIFFERENT
pH OVER A PERIOD OF 24 h, TEMPERATURE = 37°C

pH	System	Equilibrium swelling capacity $g(H_2O)/(g\ gel)$	Penetration velocity $(V \times 10^5)$ cm/s	n	k	Diffusion coefficient $(D \times 10^6)$ cm²/s
2.0	Hem-PAmX-I	1.90	0.65	0.11	0.18	1.78
	Hem-PAmX-II	3.62	2.18	0.26	0.28	3.05
4.0	Hem-PAmX-I	3.61	2.17	0.29	0.27	3.04
	Hem-PAmX-II	5.18	2.18	0.41	0.40	4.26
7.0	Hem-PAmX-I	5.19	3.88	0.47	0.41	4.29
	Hem-PAmX-II	7.82	6.98	0.65	0.57	6.81
9.0	Hem-PAmX-I	4.30	3.15	0.39	0.38	4.01
	Hem-PAmX-II	6.23	4.87	0.48	0.47	5.10
12.0	Hem-PAmX-I	4.76	3.20	0.38	0.41	4.02
	Hem-PAmX-II	6.69	4.96	0.48	0.47	5.13

Effect of Temperature: Fig. 4 shows the effect of temperature on the swelling behaviour of the hydrogels. It is clear from the figure that equilibrium swelling capacity of hydrogels increases up to 55°C and then becomes almost constant. When the temperature of the hydrogel is raised up to 55°C, the velocity of the swelling front increases leading to a faster swelling as well as higher chain relaxation. Beyond 55°C, the equilibrium swelling capacity does not increase further due to possible hydrolysis of amide groups into carboxylic groups, which bind to the remaining amide groups through H-bonding, thus producing a complex structure. A further increase in the temperature does not cause any more increase in the equilibrium swelling capacity.
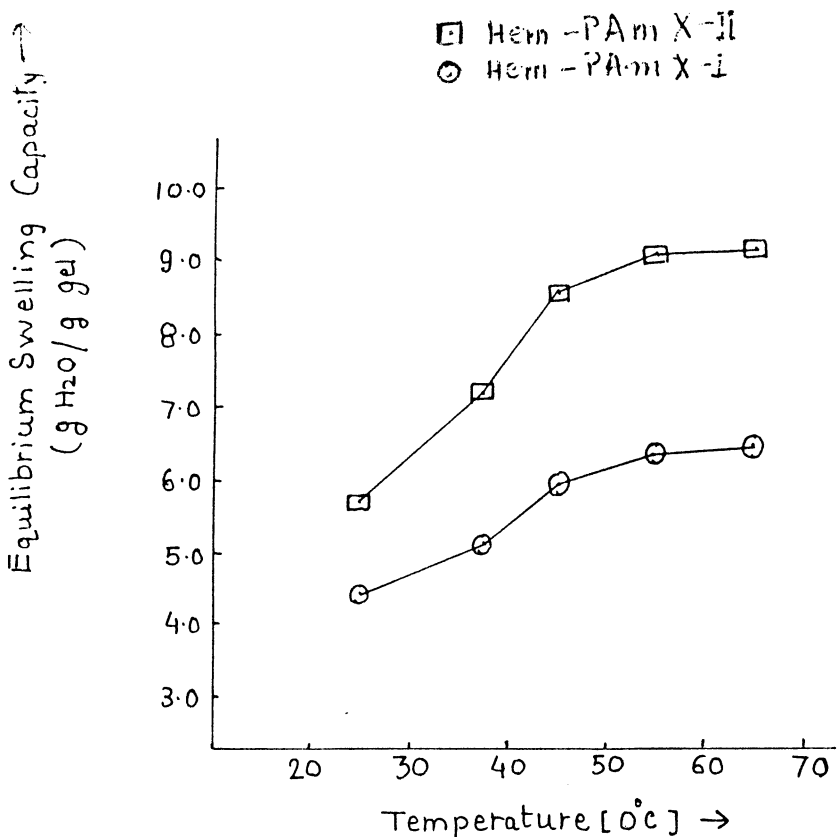

Fig. 4.   Temperature dependent swelling behaviour of hydrogels; pH = 7.0.

Reversibility of Hydrogel Swelling: The ability of hydrogel to undergo several cycles of swelling and deswelling is shown in Fig. 5. It can be seen from the Figure that after the first cycle the gel did not achieve its original swollen state and in all of the following cycles it swelled back to its previous swollen state. Since the virgin gel was used without any prior washing, some salt might

have been present which could have leached out upon deswelling, thus reducing
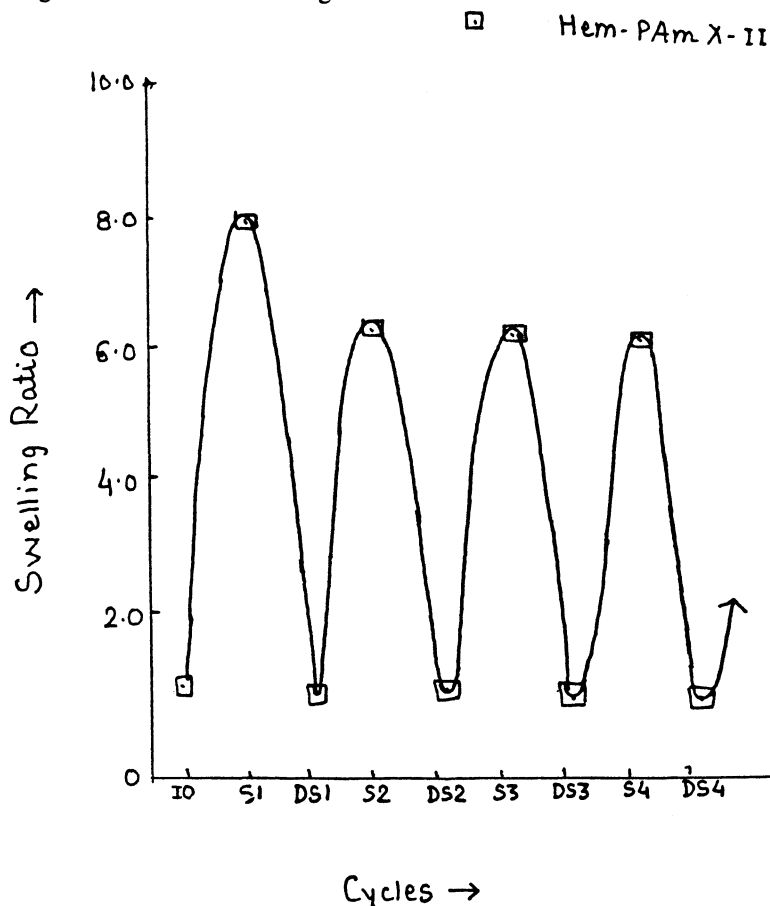the degree of successive swelling.

Fig. 5. Reversibility of Hem-PAmX-II hydrogels swelling. pH = 7.0; temperature = 37°C; IO
indicates initial condition; Si = swelling cycle equilibrium condition; and
DSi = deswelling cycle equilibrium condition.

When the freshly prepared gel is placed in the doubly distilled water at pH
7.0, the swelling occurs to maximum in accordance with the Donnan-membrane
equilibrium. Now when this swollen gel is placed in 1 M NaCl solution, the
concentration of osmotically active ions in the external solution becomes high
and hence this results into diffusion of water molecules from the gel phase into
the outer solution, thus causing the gel to deswell. Due to reversibility and rapidity
of swelling, the gel could be considered as a mechanochemical system in which
chemical ionisation energy could be transformed directly into mechanical
energy.[14]

Conclusions

The swelling behaviour of hemoglobin-crosslinked polyacrylamide hydrogels

has been found to be dependent on pH and temperature of the external solution. Moreover, the amount of crosslinking material also influences the swelling capacity. A gel with a low degree of crosslinking swells profoundly in water but its mechanical integrity is reduced with higher swelling. These observations are in consistent with the observed behaviour of other hydrophillic synthetic hydrogels. The Donnan-membrane equilibrium and chain relaxation of polymeric segments explain the pH dependent swelling behaviour statisfactorily. The pH dependent and reversible swelling behaviour could be very useful in the development of artificial muscle or physiologically sensitive drug delivery system and extractions of biocomponents from dilute solutions by a modified gel filtration technique.

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