Preparation and Characterization of Macroporous Poly(2-hydroxyethyl methacrylate)-Based Biomaterials: Water Sorption Property and *In Vitro* Blood Compatibility

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ABSTRACT: A semi-interpenetrating polymer network (IPN) comprising gelatin and poly(2-hydroxyethyl methacrylate-*co*-acrylamide) was prepared and characterized by FTIR, environmental scanning electron microscopy (ESEM) and differential scanning calorimetry (DSC) techniques. The water sorption potential of the prepared semi-IPNs was investigated for varying chemical architecture of the IPN, and experimental conditions such as pH, temperature and ionic strength of the swelling media. The semi-IPNs were also judged for *in vitro* blood compatibility by performing blood clot, percent hemolysis, protein adsorption and platelet adhesion tests. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 1559–1571, 2007

Key words: hydrogel; interpenetrating polymer network; swelling; blood compatibility

INTRODUCTION

Polymers remain the most versatile class of biomaterials, being extensively applied in medicine and biotechnology, as well as in the food and cosmetic industries. Applications include surgical devices, implants and supporting materials such as artificial organs, prosthesis and sutures, drug delivery systems with different routes of administration and design, carriers of immobilized enzymes and cells, biosensors, components of diagnostic assays, bioadhesives, ocular devices, and materials for orthopaedic applications.[1]

Polymers to be used as biomaterials can be designed in such a way that appropriate chemical, physical, interfacial, and biomimetic characteristics can be tailored to the polymer material and consequently may be used for various specific applications. Compared with other type of biomaterials, such as metals and ceramics, polymers offer the advantage that they can be prepared in different compositions with a wide variety of structures, shapes, and properties. Current research and development is focused on tissue engineering, for which such materials are considered to have a particularly significant potential.

Polymers used as biomaterials can be naturally occurring, synthetic, or a combination of both.[2] Naturally derived polymers are abundant and usually biodegradable. Their principal advantage lies in the development of reproducible production methods because their structural complexity often renders modification and purification difficult. On the other hand, synthetic polymers are available in a wide variety of compositions with readily adjustable properties as per the end use. Processing, copolymerization, and blending provide simultaneous means of optimizing a polymer's mechanical characteristics and its diffusive and biological properties.[3] The primary difficulty is the general lack of biocompatibility of majority of synthetic materials. Synthetic polymers are therefore often blended with natural polymers to achieve a uniform matrix with improved and enhanced favorable properties.

Among various kinds of materials being used in biomedical application, hydrogels find a unique place in the biomedical and pharmaceutical community.[4] Hydrogels are water swollen, three dimensional structures composed of hydrophilic homopolymers or copolymers.[5] They are rendered insoluble due to the presence of chemical (covalent or ionic) or physical crosslinks. The latter can be entanglements, crystallites, or hydrogen bonded structures.[6] The crosslinks provide the network structure and physical integrity. Over the last 35 years, hydrogels have been extremely useful in biomedical and pharmaceutical applications mainly due to their high water content and rubbery nature, which is similar to natural tissue, as well as their biocompatibility.[7] Whereas all these unusual properties qualify them to be used as im-

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plantable material in surgical practices, their property to provide desirable protection of drugs, peptides and especially proteins from the potentially harsh environment in the vicinity of the release site make them a suitable carrier in drug delivery technology.[8]

Depending on the intended medical application, all biomaterials are evaluated in terms of biocompatibility,[9] defined as the acceptance (or rejection) of an artificial material by the surrounding tissues and by the body as a whole.[10] The term biocompatibility encompasses many different properties of the materials, including toxicity, tissue compatibility, and blood compatibility. What actually happens is that as soon as a material is placed within the body, it is covered in blood due to the incision made in the surrounding tissue. The blood–material interaction starts a series of biological reactions (host response)[11] that in the best case leads to successful integration of the implant but in the worst case lead to a significant encapsulation.

Polymeric biomaterials can also be produced by copolymerization of conventional monomers to achieve nearly monodisperse polymers. It is possible to produce polymers containing specific hydrophilic or hydrophobic entities, biodegradable repeating units, or multifunctional structures that can become points for three dimensional expansions of networks.[12]

Thus, being inspired by the biomedical utility of macromolecular structures, we, in the present article, report preparation and characterization of semi-interpenetrating polymer networks (IPNs) of gelatin and poly(2-hydroxyethylmethacrylate-*co*-acrylamide).

The selection of 2-hydroxyethyl methacrylate (HEMA) as one of the components of the IPN lies in its certain unusual but desirable properties such as high water content, nontoxicity, and favorable tissue compatibility. The presence of a hydroxyl and carboxy group on each repeat unit makes this polymer compatible with water, whereas the hydrophobic methyl groups and backbone impart hydrolytic stability to the polymer and support the mechanical strength of the matrix.[13]

The selection of gelatin as another component of the prepared gel rests upon two reasons. First, gelatin, a connective tissue protein, is well known for its nontoxic, nonirritant and biodegradable properties and good living body compatibility and, therefore, has been widely used in food, pharmacy, and cosmetic applications.[14] Second, the formation of specific intermolecular interactions through hydrogen-bond formation between two or more polymers is responsible for its mixing behavior and therefore, the properties of gelatin are justified from this point of view also. Thus, the incorporation of gelatin in the HEMA-based gel is expected to enhanced blood compatibility of the semi-IPN gel. As far as polyacrylamide is concerned, its inert and hydrophilic nature and subsequent biomedical applications are well recognized.[15]

EXPERIMENTAL

Materials

Acrylamide (AM) used as a monomer (Research Lab, Pune, India) was crystallized twice in methanol (GR) and dried in vacuum over anhydrous silica for a week. Gelatin (Merck, India), type A (isoelectric point 7.6) was used as supplied. HEMA (2-hydroxyethyl methacrylate) was obtained from Sigma Aldrich (USA) and freed from the inhibitor by the prescribed method. The crosslinker used in polymerization was ethyleneglycol dimethacrylate (EGDMA) obtained from Merck (Germany) and used as received. Potassium persulphate and metabisulphite were of Loba Chemie (India) and used without pretreatment. Ethylene glycol (EG) (Merck, India) was used as a cosolvent. All other chemicals used were of analytical grade and bidistilled water was used throughout the experiments.

Preparation of semi-IPN

IPN hydrogels were prepared by conventional redox polymerization method. A typical procedure for the copolymerization can be described as follows: Gelatin 0.5 g was dissolved in 5 mL of distilled water at 60°C and to this solution are added calculated amounts of AM, EG, HEMA, EGDMA (as a crosslinker), and the redox initiator comprising potassium metabisulphite (KMBS) and potassium persulphate (KPS) to yield a homogenous reaction mixture. The whole mixture was transferred into a petri dish (diameter 7 cm, Corning glass) and kept at room temperature for 24 h, so that the whole mass solidified into a semi-transparent spongy film. The gel so prepared was equilibrated with distilled water for 48 h so that the unreacted monomer and chemicals were leached out. The fully swollen spongy IPN was cut into smaller discs (diameter 0.4 cm) and dried at room temperature for 2 days. The dried circular discs were semi-transparent and stored in air tight polyethylene bags. The photograph of dry and swollen IPNs is shown in Figure 1.

Characterization of the gel

The gels prepared as above were characterized by FTIR spectroscopy and Environmental Scanning Electron Microscopy (ESEM) techniques.

FTIR spectral analysis

FTIR spectral studies of the prepared hydrogel of definite composition gelatin (0.5 g), AM (24.7 m*M*), HEMA (28.1 m*M*), EGDMA (0.26 m*M*) were per-

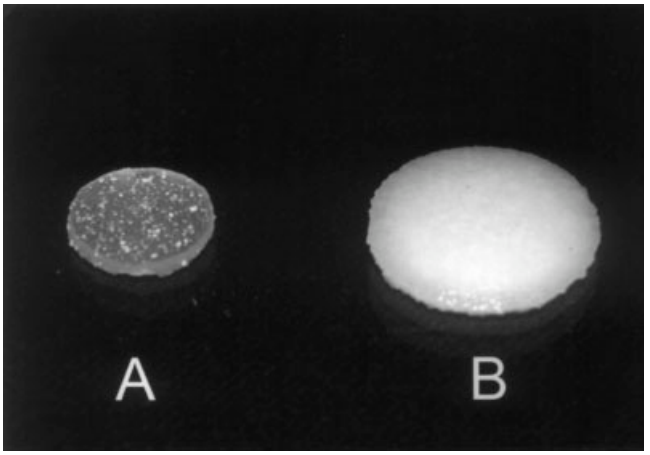

Figure 1 The (A) dry and (B) swollen photograph of the IPNs.

formed on a FTIR spectrophotometer (Perkin–Elmer, 1000 Paragon) by recording the IR spectra of a dry thin film of the gel.

Environmental scanning electron microscopy

Morphological features of dry and swollen gels were examined using an environmental scanning electron microscope (STEREO SCAN, 430, Leica, SEM, USA).

Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) measurements of prepared semi-IPNs were recorded on a DSC instrument (2100, Du Pont) in the temperature range 25–600°C under N_2 atmosphere and at a heating rate of 10°C/min.

Water uptake measurement

A conventional gravimetric procedure[16] was followed for monitoring the progress of water uptake process and the degree of water sorption was quantified in terms of the swelling ratio as calculated below:

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

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

To determine the nature of the mechanism of transport of water molecules within the hydrogel and evaluate their diffusion coefficients, the following equations were used:[17]

$$\frac{W_t}{W_\infty} = kt^n \tag{2}$$

$$\frac{W_t}{W_\infty} = 4\left[\frac{Dt}{\pi l^2}\right]^{1/2} \tag{3}$$

where W_t and W_∞ are the water intake at time t and at equilibrium, respectively; k is the swelling rate front factor, n is the swelling exponent, D is the diffusion coefficient and l is the thickness of the dry gel. The values were determined for various composition of the gels and are summarized in Table I.

Blood compatibility

In vitro blood compatibility of prepared hydrogels were evaluated by the following methods.

Thrombus formation

The antithrombogenic properties of hydrogels were evaluated with human ACD (Acid Citrate Dextrose) blood, using the method developed by Imai and Nose.[18] In brief, the gels were equilibrated with saline water (0.9% w/v NaCl) for 72 h. To these swollen gels were added 0.5 mL of acid citrate dextrose blood

TABLE I
Data Presenting the Values of Swelling Exponent "*n*" and Diffusion Coefficient "*D*" at Various Composition of Hydrogels

	Composition						
S. no.	AM (m*M*)	HEMA (m*M*)	Gelatin (g)	EGDMA (m*M*)	n	D ($\times 10^8$ cm^2 s^{-1})	Mechanism
1	28.1	24.7	0.50	0.26	0.54	29.7	Anomalous
2	28.1	24.7	0.75	0.26	0.56	8.1	Anomalous
3	28.1	24.7	1.00	0.26	0.60	7.44	Anomalous
4	28.1	24.7	1.50	0.26	0.54	17.7	Anomalous
5	28.1	32.9	0.5	0.26	0.70	23.3	Anomalous
6	28.1	16.4	0.5	0.26	0.56	31.1	Anomalous
7	28.1	8.20	0.50	0.26	0.62	23.3	Anomalous
8	10.5	24.7	0.5	0.26	0.48	8.84	Fickian
9	14.06	24.7	0.5	0.26	0.46	8.84	Fickian
10	21.1	24.7	0.5	0.26	0.50	7.43	Fickian
11	28.1	24.7	0.5	0.53	0.56	27.1	Anomalous
12	28.1	24.7	0.5	1.07	0.54	27.1	Anomalous
13	28.1	24.7	0.5	1.59	0.58	22.1	Anomalous

followed by the addition of 0.03 mL of $CaCl_2$ solution (4*M*) to start the thrombus formation. The reaction was stopped by adding 4.0 mL of deionized water and the thrombus formed was separated by soaking in water for 10 min at room temperature and then fixed in 36% formaldehyde solution (2.0 mL) for another 10 min. The fixed clot was placed in water for 10 min and after drying, its weight was recorded. The same procedure was repeated for the glass surface and respective weights of thrombus formed were recorded by a highly sensitive balance (Denver, APX-203, Germany).

Hemolysis assays

Hemolysis, defined as the release of hemoglobin into plasma due to damage of erythrocytes membranes, was determined by the method given by Singh and Ray.[19] In a typical experiment the hydrogels were equilibrated in (0.9%) normal saline water for 60 min at 37°C and human ACD blood (0.25 mL) was added on films. After 20 min, 2.0 mL of 0.9% NaCl saline was added to each sample to stop hemolysis and the samples were incubated for 60 min at 37°C. Positive and negative controls were obtained by adding 0.25 mL of human ACD blood and 0.9% NaCl respectively, to 2.0 mL of double distilled water. Incubated samples were centrifuged for 45 min. The supernatant was taken and its absorbance was recorded on a spectrophotometer at 545 nm. The percent hemolysis was calculated using the following relationship:

$$\% \text{ Hemolysis} = \frac{A_{\text{test sample}} - A_{(-)\text{control}}}{A_{(+)\text{control}} - A_{(-)\text{control}}} \times 100 \qquad (4)$$

where *A* is the absorbance. The absorbance of positive and negative controls was found to be 1.764 and 0.048, respectively.

Protein adsorption

To judge the blood compatibility of prepared hydrogels, blood protein-hydrogel interactions were investigated by adsorbing bovine serum albumin (BSA) on to the hydrogel's surface. The batch contact method[20] was used to determine the amount of adsorbed BSA. In this method, protein (BSA) solution for adsorption experiments were made in 0.5*M* PBS (Phosphate buffer saline) at physiological pH 7.4 and fresh solutions of BSA were always prepared for every adsorption experiment. Prior to adsorption experiments, the gels were equilibrated with PBS for 72 h. The adsorption was then carried out by gently shaking a BSA solution of known concentration, containing preweighed and fully swollen gels. The shaking was performed gently so that no froth was produced; otherwise an air–water interface would have formed. After a definite time period (30 min), the gels were removed and the protein solution was assayed for the remaining concentration of BSA by a spectrophotometric procedure as described elsewhere.[21] The adsorbed amount of BSA was calculated by the following mass balance equation:

$$\text{Adsorbed BSA}(\text{mg g}^{-1}) = \frac{(C_0 - C_a) \times V}{W} \qquad (5)$$

where C_0 and C_a are the initial and equilibrium concentration of BSA solution (mg mL^{-1}) and *V* the volume of protein solution (mL), *W* being the weight of the swollen gel.

Platelet adhesions

For platelet-adhesion studies, films of 1.5 × 1.5 cm^2 size were incubated for 1 h in PBS (0.1*M*, pH 7.4). Fresh human blood, anticoagulated with acid citrate dextrose (ACD), was centrifuged at 2500 rev min^{-1} for 5 min to obtain platelet rich plasma (PRP). The films were laid flat on small petri dishes, submerged with PRP and left at 37°C for 1 h in an incubator. After washing gently many times with buffer to remove nonadhering platelets, fixing was done with 2.5% buffered gluteraldehyde overnight in the refrigerator at 4°C, and examined by an environmental scanning electron microscope (ESEM).

Network parameters

One important structural parameter characterizing crosslinked polymer is M_c, the average molar mass of the chain between crosslinks, directly related to the crosslink density. The magnitude of M_c significantly affects the physical and mechanical properties of crosslinked polymer and its determination has great practical significance. Equilibrium swelling is widely used to determine M_c. Early research of Flory and Rehner[22] laid the foundation for analysis of equilibrium swelling.

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

$$M_c = \frac{-V_1 d_p (V_s^{1/3} - V_{s/2})}{\ln(1 - V_s) + V_s + \chi V_s^2} \qquad (6)$$

where, M_c is the number average molar mass of the chain between crosslinks, V_1 is the molar volume of water (mL mol^{-1}), d_p is the polymer density (g mL^{-1}), V_s is the volume fraction of the polymer in the swollen gel, and χ is the Flory-Huggin's interaction parameter between solvent and polymer.[23] In the present case χ values were calculated from those of the PHEMA and PAM, which are reported to be 0.80 and 0.48, respectively.

TABLE II
Data Showing the Values of Average Molar Mass Between Crosslinks (M_c), the Crosslink Density (q) and the Number of Elastically Effective Chains (ν_e)

S no.	Composition				M_c (g mol^{-1})	q ($\times 10^8$)	ν_e ($\times 10^{-19}$)
	AM (mM)	HEMA (mM)	Gelatin (g)	EGDMA (mM)			
1	28.1	24.7	0.50	0.26	15428	6.39	70.2
2	28.1	24.7	0.75	0.26	1198	82.3	9.04
3	28.1	24.7	1.0	0.26	1178	83.7	9.20
4	28.1	24.7	1.5	0.26	1195	82.5	9.06
5	28.1	32.9	0.5	0.26	1481	69.4	7.31
6	28.1	16.4	0.5	0.26	63968	1.45	16.9
7	28.1	8.2	0.5	0.26	15958	5.28	67.0
8	10.5	24.7	0.5	0.26	306	387	35.3
9	14.0	24.7	0.5	0.26	364	298	29.7
10	21.1	24.7	0.5	0.26	600	171	18.0
11	28.1	24.7	0.5	0.53	2590	38	4.18
12	28.1	24.7	0.5	1.07	402	245	26.9
13	28.1	24.7	0.5	1.59	547	180	19.7

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

$$q = \frac{M_0}{M_c} \tag{7}$$

where, M_0 is the molar mass of repeating unit. Some authors defined a crosslink density, V_e, as the number of elastically effective chains as given below:

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

where, N_A is the Avogadro number, d_p is the density of the gel. The values of M_c, q, and V_e of the networks have been summarized in Table II.

RESULTS AND DISCUSSION

FTIR spectral analysis

The FTIR spectra of gelatin-poly(HEMA-*co*-AM) IPN is shown in Figure 2, which clearly shows the presence of gelatin, poly(HEMA) and polyacrylamide functionals in the matrix. The IR spectrum shows strong absorption bands at 3419 cm^{-1} and 1673 cm^{-1}, which are assigned to *N*—H and C=O stretching respectively, of gelatin.[24] The presence of HEMA is confirmed by the observed bands at 1723 cm^{-1} (C=O stretching), 1158 cm^{-1} (O—C—C stretching), and 1459 cm^{-1} (O—H bending). The IR spectra exhibits a minor band at 3210 cm^{-1} due to *N*—H stretching of polyacrylamide.[25] The bands at 2961 cm^{-1} (C—H stretching) and 1280 and 1392 cm^{-1} (C—H) bending may be due to methylene groups of polymeric chains.[26]

Environmental scanning electron microscopy

Surface topography and roughness are important factors in determining the response of cells to a foreign material.[27] Surface with grooves can induce ''contact guidance,'' whereby the direction of cell movement is affected by the morphology of the substrate.[28] *In vitro* studies have demonstrated that grooves as small as 0.5 μm in depth were found to align and direct the migration of both fibroplasts and epithelial cells, and tightly spaced grooves (pitch < 30 μm) were more effective than widely spaced grooves in orienting cells.[29] von Recum and coworkers have found that the topography of an implant material (in the size range 1–3 μm) could radically alter the cellular response *in vivo*[30] and *in vitro*.[31] Sheppard et al.[32] proposed the surface roughness influences thrombogenicity more than the other surface properties by studying the results of *in vitro* protein adsorption and total blood clotting tests. Thus, the morphology of a biomaterial is of much significance and deserve attention.

In the present work also, the ESEM technique has been used to study the nature of the dry and water

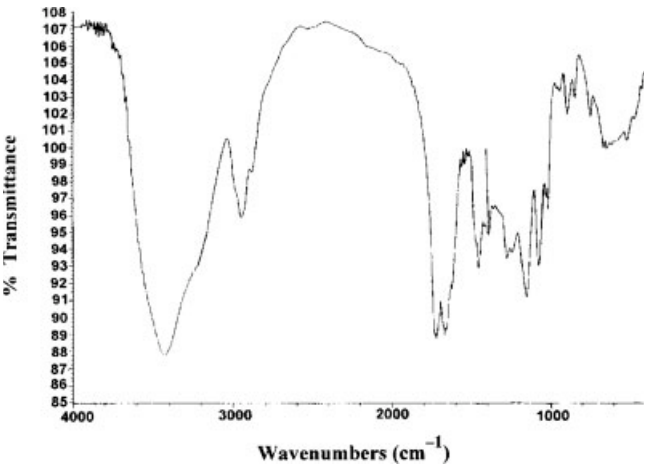

Figure 2 The FTIR spectra of gelatin-Poly(HEMA-*co*-AM) IPN.

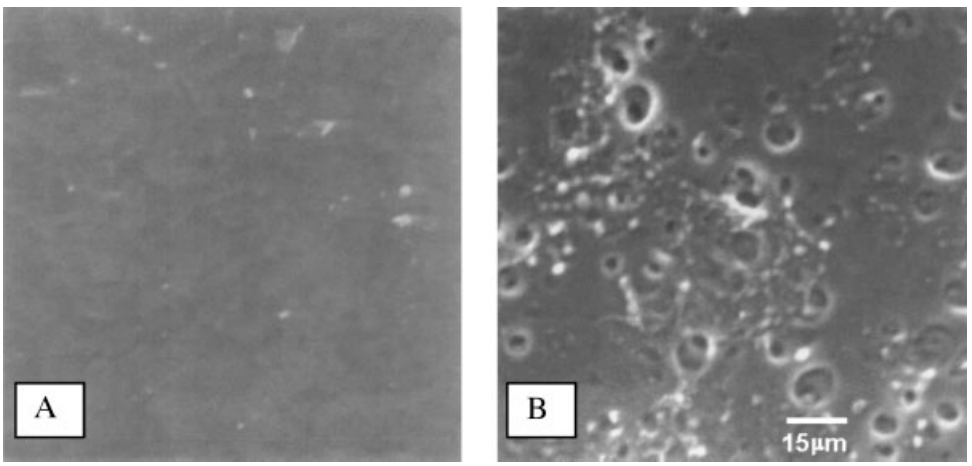

Figure 3 The ESEM images of the (A) dry and (B) water swollen IPNs surfaces.

swollen IPNs surfaces as shown in Figure 3(a,b), respectively. It is clear from the two images that whereas in dry state the IPN surface appears rather homogeneous, in the swollen state macro size pores are developed which vary in the size from 5 to 15 µm. The occurrence of phase separation during polymerization reaction could be a major cause for macroporous nature of the hydrogel. The findings have been largely reported in literature particularly in the polymerization studies of HEMA.[33]

Differential scanning calorimetry

The DSC thermogram of the prepared semi-IPN is shown in Figure 4, which presents a hybrid type of thermal response of the constituent polymers, that is, gelatin and segments of polyacrylamide and p(HEMA) of the copolymer. Generally, two glass transition temperatures of gelatin have been reported,[34] which is attributed to the block copolymer model for the amino acid content of gelatin.[35] A minor endotherm located around 67° could be attributed to the loss of moisture while the first glass transition temperature observed around 80°C may be due to the glass transition of α-amino acid blocks in the peptide chain. The second more intense glass transition temperature is located around 220°C and represents the blocks of imino acids, proline, hydroxyproline and glycine. This T_g is widely reported in the literature.[36]

The p(HEMA) blocks of the copolymer display a glass transition around 152°C and appears significantly higher than the reported value of 113°C.[37] The observed shift in T_g to a higher temperature may be attributed to the crosslinking of the p(HEMA) segments by EGDMA, as reported by some authors.[38]

The DSC curve shows a sharp endotherm around 280°C, which may be assigned to various thermal induced transitions, such as melting of polyacrylamide chains and beginning of thermal degradation.[39] A prominent endotherm located around 450°C is also indicative of the occurrence of more extensive thermal degradation processes.

An overall look of the thermogram clearly shows relatively prominent endotherms between 250 and 500°C, which is further supported by the thermogravimetric analysis (TGA) results of Xiao et al.,[40] who noticed the greatest weight loss in gelatin-polyacrylamide blend films in the temperature range of 250–400°C. Thus, DSC results provide evidence for blend type of nature of the semi-IPN.

Water sorption measurement

Effect of gelatin

Gelatin is a hydrophilic and biodegradable component of the hydrogel and has been found to exert an appreciable influence on the water sorption characteristics of the hydrogel. In the present study, the effect of gelatin on the swelling ratio of the hydrogel was investigated by varying the concentration of gelatin in the range of 0.5–1.5 g. The results are shown in Figure 5, which clearly reveal that the swelling ratio decreases as gelatin concentration is increased in the feed mixture. The observed decrease in the swelling ratio may be attributed to the fact that at higher con-

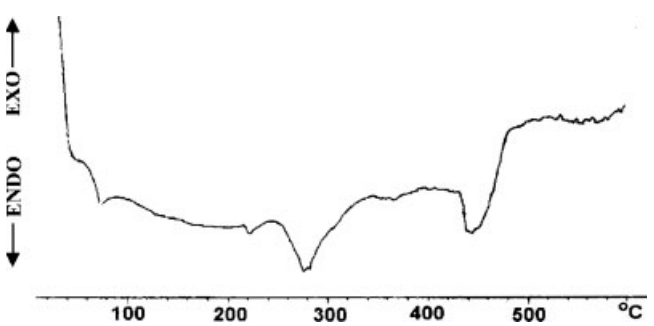

Figure 4 The DSC thermogram of the prepared semi-IPN.

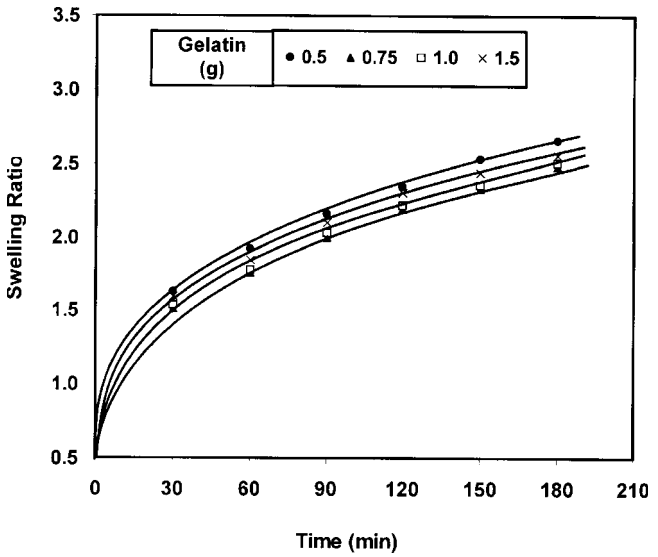

Figure 5 Effect of varying concentration of gelatin on swelling ratio of the semi-IPN of definite composition [HEMA] = 28.1 m*M*, [EGDMA] = 0.26 m*M*, [AM] = 24.7 m*M*, [pH] = 7.4, [Temp.] = (25 ± 0.2)°C.

centration, gelatin itself initiates formation of a reversible gel which develops crystalline regions in the hydrogels. This obviously results in a decrease in swelling ratio of the hydrogel.[41]

Another reason for the observed decrease in swelling ratio with increasing amount of gelatin can be explained by the fact that due to greater intermolecular attraction, the macromolecular chains become compact, thus resulting in the contraction of mesh size of the network pores. This obviously restrains the penetration of water molecules into the hydrogel and, consequently, the swelling ratio decreases.[42]

Effect of crosslinker

In crosslinked copolymeric structures the swelling process is controlled by the introduction of the appropriate amount of a second monomer with hydrophobic character. In the present study, the crosslinker is hydrophobic in nature and, therefore, the maximum hydration degree and diffusion of the swelling agent into the gel, as well as the organization of water molecules in the gel, will change depending on the chemical composition and the distribution of the hydrophobic segments along the macromolecular chains.

Ethylene glycol dimethacrylate (EGDMA), used as a crosslinker in the present study, shows a great impact on the swelling characteristics of the hydrogel. To investigate the effect of crosslinker (EGDMA) on the swelling ratio, a varying amount of EGDMA ranging from 0.26 to 1.59 m*M* were added while preparing different samples of the gel. The results are depicted in Figure 6, which clearly shows that when the EGDMA concentration is increased in the feed mixture in the range of 0.26–1.59 m*M*, the degree of water sorption decreases. The observed decrease in the swelling ratio could be attributed to the reason that high amount of crosslinker (EGDMA) produces a compact network which increases the crosslinking density and consequently reduces the pore size of the gel. This obviously results in a lower degree of swelling.

Another reason for the observed decrease in swelling ratio is quite expected as increase in crosslinker makes the hydrogel more and more compact. As a consequence, water preservation becomes increasingly difficult. As shown in Table II, the increasing amount of EGDMA decreases the value of M_c, that is, molecular mass between crosslinks which obviously reduces the mesh size of the free volumes accessible to the water molecules. Therefore, the swelling ratio decreases. Similar type of results have also been reported elsewhere.[43]

Some workers have reported an increase in the glass transition temperature (T_g) of the polymer with increase in crosslinking density. The glassy nature of the matrix does not permit loosening of the macromolecular chains which results in lower water sorption.[44]

Effect of monomers

The monomers used in the present study are HEMA and acrylamide, which are hydrophilic and neutral and have been extensively employed in hydrogel synthesis.

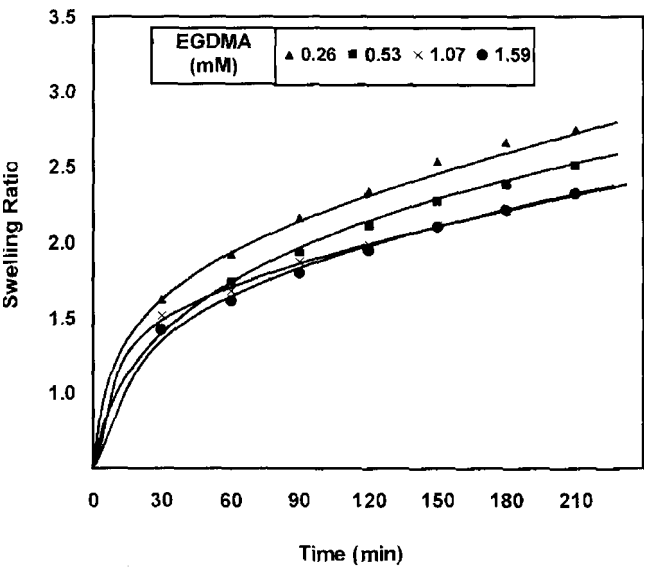

Figure 6 Effect of varying concentration of crosslinker on swelling ratio of the semi-IPN of definite composition [HEMA] = 28.1 m*M*, [AM] = 24.7 m*M*, [gel] = 0.5 g, [pH] = 7.4, [Temp.] = (25 ± 0.2)°C.

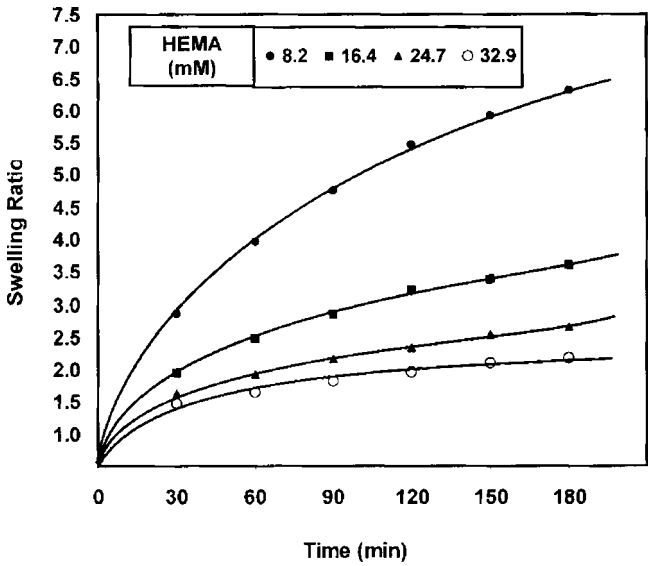

Figure 7 Effect of varying concentration of HEMA on swelling ratio of the semi-IPN of definite composition [AM] = 24.7 m*M*, [EGDMA] = 0.26 m*M*, [gel] = 0.5 g, [pH] = 7.4, [Temp.] = (25 ± 0.2)°C.

The influence of HEMA content in the gel on its swelling behavior has been investigated by varying its concentration in the feed mixture in the range of 8.2–24.7 m*M*. The results are displayed in Figure 7, which clearly indicate a constant fall in the water sorption capacity with increasing PHEMA content. The observed fall may be attributed to the fact that due to increasing polymer fraction in the gel the water molecules have to travel a longer path in the gel and, therefore, the extent of swelling decreases. Another reason for the observed fall could be that with increasing number of PHEMA chains in the gel, the intermolecular forces operating between the functional groups of chains also become stronger and consequently the gel becomes compact with reduced mesh sizes. This obviously brings about a fall in the water sorption capacity.

When the concentration of acrylamide is varied in the feed mixture in the range of 10.5–28.1 m*M*, the swelling ratio increases as depicted in Figure 8. The results are quite usual as with increase in amount of acrylamide, which is a hydrophilic monomer, the hydrophilicity of gel will increase, which consequently increases the swelling ratio of the gel.

Effect of pH

PHEMA gels are known for their resistance to high temperatures, acid and alkaline hydrolysis and low reactivity with amines.[45] Such chemical and thermal stability make PHEMA gels suitable materials for the development of controlled drug delivery systems,[46] and for other biomedical applications.[47] Since the gels of PHEMA are considered to be nonionic in nature, most of the studies reporting pH-sensitive swelling behavior involve modified PHEMA, either copolymerized with an ionic monomer[48] or partially hydrolyzed with alkali at high temperatures.[49] In the present study, the gel has been made sensitive to pH by polymerizing the monomers in the presence of gelatin, a biopolymer with pH-dependent varying charges on the macromolecules.

The influence of pH on the water intake capacity has been investigated by adjusting pH of the swelling medium in the range 1.8–9.6. The results are depicted in Figure 9, which clearly reveal that initially the swelling ratio increases with increasing pH of the medium up to 7.2 and thereafter, it decreases continuously. The observed results may be explained as below.

At low pH (i.e. 1.8) the chloride counter ions added due to HCl for the adjustment of pH of the solution cause a reduction in the solvent power of water which consequently makes polymer–solvent interaction weaker and favors hydrophobic interactions between polymeric chains. As a result, a tighter polymeric network is formed showing lower swelling levels. At this pH, the gelatin molecules of the hdyrogel will also be present with predominance of net positive charge over the macromolecules. This obviously results in an unfolding of gelatin molecules, which as a consequence exposes hidden hydrophobic domains of the protein molecule, thus giving rise to unfavorable polymer–solvent interactions leading to lower water imbibtion.

As the pH of the swelling medium increases, the solvent quality increases and the extent of unfolding

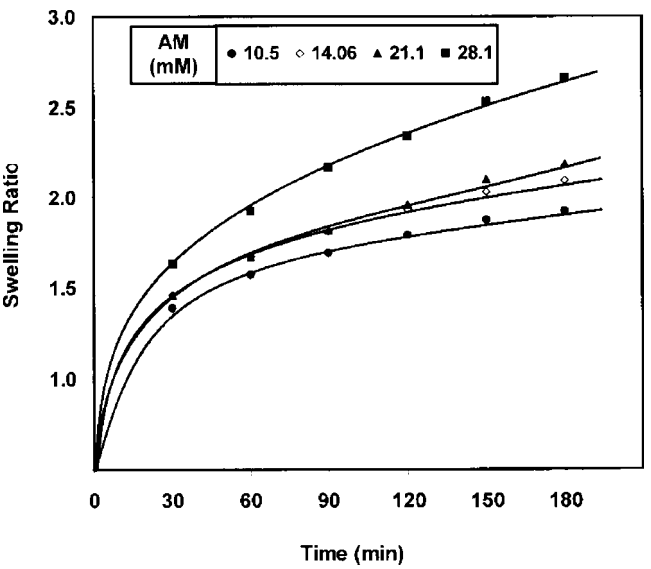

Figure 8 Effect of varying concentration of AM on swelling ratio of the semi-IPN of definite composition [HEMA] = 28.1 m*M*, [gel] = 0.5 g, [EGDMA] = 0.26 m*M*, [pH] = 7.4, [Temp.] = (25 ± 0.2)°C.

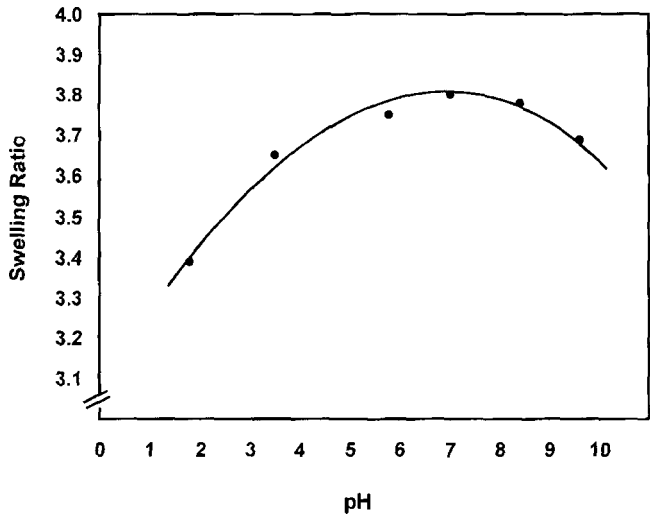

Figure 9 Effect of varying range of pH on swelling ratio of the semi-IPN of definite composition [HEMA] = 28.1 m*M*, [gel] = 0.5 g, [AM] = 24.7 m*M*, [EGDMA] = 0.26 m*M*, [Temp.] = (25 ± 0.2)°C.

decreases which together gives rise to increasing swelling. This increase in swelling is continued till the isoelectric point of gelatin (7.6) at which the protein acquires a compact conformation without overall charges on the molecules and, therefore, exhibits optimum swelling.

Beyond pH 7.0 and in the alkaline range, a decrease in swelling ratio is observed, which may be attributed to the reason that in alkaline medium the gelatin molecules acquires net negative charge which due to electrostatic repulsion results in unfolding of macromolecule which again causes a fall in water sorption capacity.

Effect of temperature

The effect of temperature on the degree of water sorption has been investigated by carrying out water sorption experiments in the range 10–40°C. The results are presented in Figure 10, which clearly indicate that the swelling ratio increases with increasing the temperature of the swelling medium. The results may be explained by the fact that when temperature is increased, both the segmented mobility of gel and diffusion of water molecules into the gel increase, which obviously results in greater swelling.

The quantitative effect of temperature on swelling ratio may also be described by the following equation:

$$\frac{d[\ln(W_\infty)]}{d(1/T)} = \frac{\Delta H_m}{R} \tag{9}$$

where ΔH_m is the enthalpy of mixing between a dry polymer and an infinite amount of water, and R is the gas constant. The value of ΔH_m has been calculated from a graph plotted between W_∞ and the reciprocal of swelling temperature ($1/T$), and found to be −18.61 kJ mol K.

Electrolyte effect

Theoretical[50] as well as experimental[51] considerations have established that a balance between the osmotic pressure and the polymer elasticity sets the physical dimensions of hydrogels. The osmotic pressure results from a net difference in concentrations of mobile ions between the interior of the gel and the exterior solution. Increasing the ionic concentration in bathing medium reduces the mobile ion concentration difference between the polymer gel and the external solutions (osmotic swelling pressure) and hence, the gel volume is reduced, that is, the gel shrinks. Although there are various theories and models which can predict the equilibrium swelling response of hydrogels to change in ionic strength, however, Donan membrane equilibrium theory can satisfactorily interpret the results. According to this theory, when a gel is placed in contact with a liquid, the solvent chemical potential μ in both the gel and the solution phase must be equal at equilibrium.

$$\Delta\mu_1^g = \Delta\mu_1^s \tag{10}$$

where the superscripts g and s represent the gel and the solution phase, respectively.

In terms of osmotic pressure, eq. (10) can be written as

$$\pi = \frac{-(\mu_1^g - \mu_1^s)}{V_1} = 0 \tag{11}$$

where V_1 is the molar volume of the solvent. Osmotic pressure π of the gel determines whether the gel will

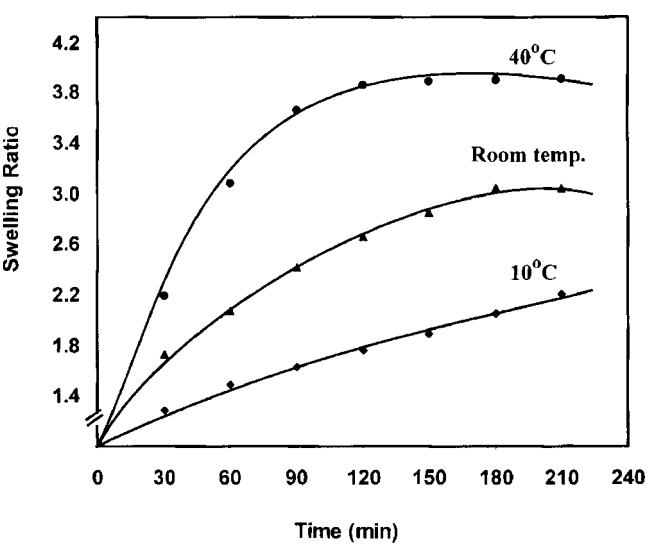

Figure 10 Effect of varying range of temperature on swelling ratio of the semi-IPN of definite composition [HEMA] = 28.1 m*M*, [gel] = 0.5 g, [AM] = 24.7 m*M*, [EGDMA] = 0.26 m*M*, [pH] = 7.4.

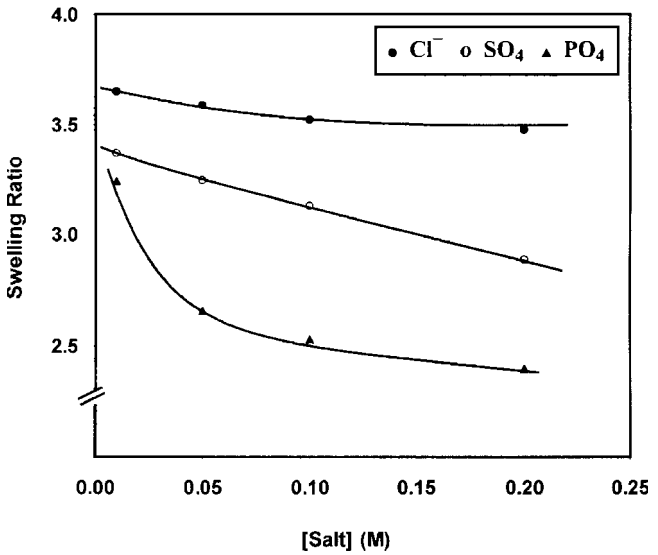

Figure 11 Effect of varying concentration of different salts on swelling ratio of the semi-IPN of definite composition [HEMA] = 28.1 m*M*, [gel] = 0.5 g, [EGDMA] = 0.26 m*M*, [AM] = 24.7 m*M*, [pH] = 7.4, [Temp.] = (25 ± 0.2)°C.

expand or shrink. In the case of an ionic system, the osmotic pressure is mainly contributed by π ions which are caused by the counter ion difference between the gel and the outer solution. Now, avoiding ion–ion, ion–solvent, and ion–polymer interaction, eq. (11) may be written as

$$\pi_{\text{ion}} = RT \sum (C_i^g - C_i^s) \tag{12}$$

where C_i is the mobile ion concentration of species i and the superscripts ''g'' and ''s'' represent gel and solution phases, respectively. The above equation implies clearly that greater the difference between the concentration of mobile ions inside and outside the gel, the larger would be the osmotic pressure and consequently greater will be the swelling of hydrogel.

In the present investigation, the relative effects of added anions on the degree of water sorption have been investigated by adding sodium salts of Cl^-, SO_4^{2-}, and PO_4^{3-} ions into the swelling system at equimolar concentration (0.1*M*) The results are shown in Figure 11, which indicate that the addition of salts brings about a fall in the swelling ratio which is quite obvious and may be explained with the help of eq. (12). The relative order of effectiveness of added anions in suppressing the swelling ratio obey the following sequence:

$$Cl^- < SO_4^{2-} < PO_4^{3-} \tag{13}$$

The observed order of effectiveness may be explained on the basis of the fact that due to smaller size, some of the added Cl^- ions diffuse into the network and enhance the ionic concentration (C_i), according to eq. (13), which obviously tends to increase the swelling ratio. However, at the same time, because of a significant increase in ionic concentration the osmotic pressure (π ion) decreases and consequently the swelling ratio will be low. Thus, the combined effect of the above two explanations would result in a slight fall in the swelling ratio of the IPN. On the other hand, in the case of SO_4^{2-} and PO_4^{3-} ions, because of their comparatively bigger sizes, the diffusion of these two ions into the IPN is less likely and, therefore, the swelling will be low. Similar type of results showing a fall in the water sorption capacity with increasing salt concentration have also been published elsewhere.[2]

Effect of simulated biofluids on swelling

The effect of the nature of the medium on the swelling behavior of gels was investigated by performing swelling study in the presence of solutes, such as potassium iodide (15% w/v), urea and D-glucose (5% w/v) and in simulated physiological fluids such as saline water (0.9% NaCl) and artificial urine. The results are presented in Table III, which show that the swelling ratio increases in the presence of KI and saline medium while presence of solute like urea, glucose and synthetic urine suppressed the swelling ratio. The maximum decrease in swelling ratio observed in the case of urea may be attributed to the fact that urea breaks hydrogen bonds between matrix and water molecules, producing a deswelling effect.

Evaluation of blood compatibility

Since the first attempt in the 1950s to develop the blood compatible materials with a negatively charged surfaces for the artificial vessels, continuous efforts to design biomaterials with superior blood compatibility have been made by various research groups.[52] Most of the studies aimed at attempting to understand the blood compatibility of foreign materials from the view point of protein adsorption and cell adhesion,[53]

TABLE III
Equilibrium Swelling Ratio of the Gel[a] in Various Simulated Physiological Fluids

Physiological fluid	Equilibrium swelling ratio
Water	3.90
KI (15% w/v)	4.57
Urea (5% w/v)	3.59
D-glucose (5% w/v)	3.66
Saline water (0.9% w/v NaCl)	5.86
Synthetic urine[b]	3.90

[a] [gelatin] = 0.5 g, [HEMA] = 28.1 n*M*, [EGDMA] = 0.26 m*M*, [AM] = 24.7 m*M*.
[b] 0.8 g NaCl, 0.10 g $MgSO_4$, 2.0 g urea, 0.06 g $CaCl_2$.

TABLE IV
Data Showing the Weights of Blood Clot Formed, Percent Hemolysis, and BSA Adsorbed on Gels of Different Composition

S. no.	Composition				Wt. of blood clot formed (mg)	Hemolysis (%)	BSA adsorbed (mg g^{-1})
	AM (m*M*)	HEMA (m*M*)	Gelatin (g)	EGDMA (m*M*)			
1	8.2	28.1	0.50	0.26	7.0	34.6	3.18
2	16.4	28.1	0.50	0.26	5.0	21.2	2.61
3	24.7	28.1	0.50	0.26	3.0	9.61	1.49
4	24.7	10.5	0.5	0.26	9.0	75.4	3.80
5	24.7	21.1	0.5	0.26	7.0	51.2	2.04
6	24.7	28.1	0.5	0.26	3.0	9.61	1.491
7	24.7	28.1	0.5	0.26	3.0	9.61	1.49
8	24.7	28.1	1.0	0.26	7.0	28.3	4.42
9	24.7	28.1	1.5	0.26	14.0	42.1	5.38
10	24.7	28.1	0.5	0.26	3.0	9.61	1.49
11	24.7	28.1	0.5	1.07	13.0	12.76	2.63
12	24.7	28.1	0.5	1.59	14.0	26.6	4.68
13	glass	–	–	–	22.8	–	–
14	polybag	–	–	–	19.0	–	–

and their investigation showed that the blood compatibility is affected by the various properties of the material surface, for example, surface charge, wettability, surface free energy, topography or roughness and presence of special chemical groups on the surface.[54] In addition, it has been recently pointed out that the water structure on the surface of the material is one of the most important factor affecting blood compatibility.[55] Inspite of the fact that a large number of investigations have been done to explore the possible factors responsible for blood compatibility of a material, a concrete conclusion has not yet emerged. In the present study, therefore, the *in vitro* blood compatibility of hydrogel has been determined in terms of blood clot formation, percentage hemolysis protein (albumin) adsorption and platelet adhesion tests. The results are summarized in Table IV and may be explained as below.

The adsorption behavior of proteins at the biomaterial surface determines the pathway and the extent of intrinsic coagulation and adhesion of platelets. Although predictions about the interaction between the biomaterial surface and the adsorbed protein can only be formulated by having an exact knowledge of the structure of the biomaterial surfaces and the conformation of the adsorbed proteins, however, the amount of adsorbed protein may be a significant parameter indicative of the blood compatibility of the surface. The adsorption of proteins onto a polymer surface is a complex process and the extent of adsorption is determined by numerous factors such as hydrophilic, hydrophobic, polar, nonpolar, charged, uncharged parts of the proteins and the nature of the polymer surface.[56] In the present study, the adsorption of BSA onto swollen hydrogels was determined and the results summarized in Table IV clearly indicate that the amount of BSA adsorbed constantly decreases with increasing PHEMA content of the hydrogel. A decreasing protein (BSA) adsorption obviously implies for an increasing blood compatibility, which is further confirmed by the observed lower values of blood clots and percent hemolysis. The observed enhanced blood compatibility parameters may be attributed to the reason that increasing PHEMA content results in increasing hydrophilicity of the gel which would result in less adsorption of protein. In fact, a large number of investigators have confirmed the observation that the composition and organization of the adsorbed protein layer can be varied by numerous factors such as hydrophobicity, sorbed water content, microphase separation and surface chemical functionality. As far as the chemistry of surface is concerned, the effect of hydrophilic and hydrophobic groups of constituent chain in polymer surface has been found to play a key role in influencing protein adsorption and subsequent platelet adsorption to polymer surface.[57] Thus, the above mentioned facts may be regarded as factors responsible for less protein adsorption, lower clot formation and decreased degree of hemolysis.

Another reason for the observed higher blood compatibility with increasing PHEMA content may be attributed to the fact that at higher PHEMA content in the hydrogel, phase separation becomes prominent and hydrophilic domains are formed to reside on the gel surface. This obviously results in an exposure of hydrophilic and ionic groups to invading protein molecules and, therefore, the adsorbed amount decreases.[58] The data summarized in Table IV also indicate that increasing concentration of AM in the hydrogel brings about a fall in protein adsorption, clot formation and percent hemolysis. With increasing PAM content, the hydrophilicity of the gel surface increases which obviously results in lower amount of

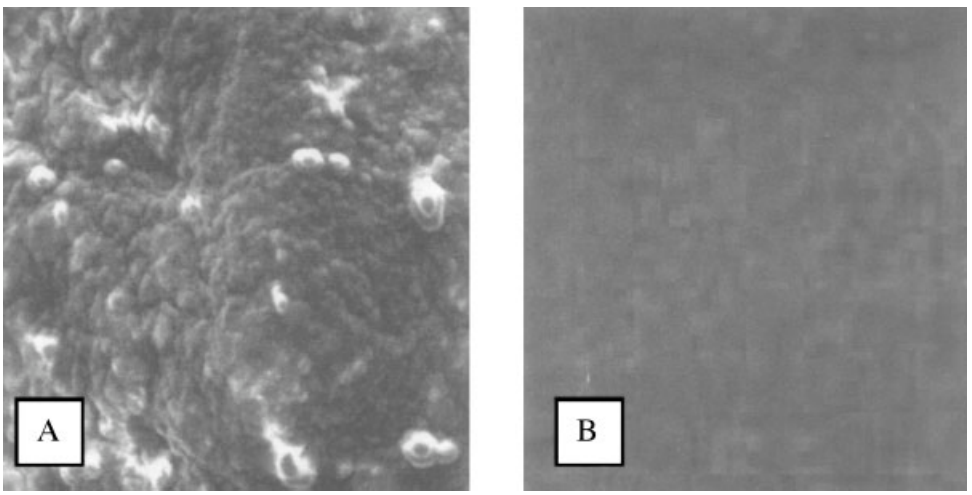

Figure 12 ESEM images depicting (A) greater number of platelets and (B) lesser number of platelets adhered to IPN films containing 1.5 g and 0.5 g gelatin, respectively.

protein adsorption. In several citations, a more hydrophilic surface has already been recognized as more antithrombogenic.[59]

The influence of gelatin on the blood compatibility of prepared blends has been investigated by varying concentration of gelatin in the feed mixture of the blend in range 0.5–1.5 g. The results summarized in Table IV clearly show that with increasing gelatin content in the gel, the blood compatibility decreases, which is clearly shown by the observed increased values of blood clot formed, percent hemolysis and adsorbed protein. The observed results are a little bit unusual as gelatin is known to be a highly biocompatible natural polymer. The obtained findings may be attributed to the reason that with increasing amount of gelatin, the intermolecular forces operative between the functional groups of the biomacromolecule also increase which results in a compact conformation of the molecule, thus, exposing hydrophobic domains to the invading protein molecules. This obviously results not only in greater protein adsorption but also in reduced hydrophilic nature of the gel which eventually results in a fall in the blood compatibility.

The influence of crosslinker (EGDMA) on the blood compatible nature of the gel's surface has been investigated by increasing its concentration in the range 0.26–1.59 m*M* in the feed mixture of the hydrogel. The results are depicted in Table IV which clearly indicate that the weight of blood clot, percent hemolysis and protein adsorption constantly increases with increasing crosslinker concentration, thus, indicating a reduced blood compatibility. The observed results are quite obvious and may be explained by the fact that due to the hydrophobic nature of the crosslinker, its increasing concentration results in an enhanced hydrophobicity of the gel surface which leads to greater protein adsorption. Thus, an increased protein adsorption gives rise to greater thrombus formation as well as larger percent hemolysis.

Platelet adhesion

Platelet adhesion to polymer surfaces offers a more direct strategy to ascertain the blood compatible nature of the material. In the present study also, the platelet adhesion studies were performed on two IPN films containing 0.5 and 1.5 g of gelatin, respectively. The results have been shown in Figure 12(a,b) as ESEM images of the two IPN films containing 0.5 and 1.5 g gelatin, respectively. It is clear from the figure that the image (a) has much greater number of platelets adhered than those in image (b), thus, indicating that the film containing 0.5 g gelatin is more blood compatible than the film with 1.5 g gelatin. The results are unusual and have been explained in the previous paragraph.

CONCLUSIONS

Incorporation of crosslinked poly(HEMA-*co*-AM) chains into gelatin results in a three dimensional spongy and macroporous semi-IPN that shows significant affinity for water.

Structural characterization of the semi-IPN by FTIR analysis confirms the presence of characteristic functional groups of gelatin, PHEMA and PAM in the resulting polymer. The presence of water during polymerization causes phase separation and consequently forms a macroporous spongy material with pore sizes varying between 5 and 15 μm.

Thermal characterization of the IPN suggests for semi-crystalline nature of the material and confirms the blend nature of the IPN as indicated by the occurrence of glass transition temperature (T_g) and crystalline

melting points (T_m) of the constituent polymers. The semi-IPN exhibits a hybrid type of thermal response implying for the blend nature of the material.

The water sorption capacity of the IPN shows a significant dependence to the chemical architecture of the IPN. The swelling ratio constantly decreases with increasing concentration of gelatin, crosslinker (EGDMA), and HEMA in the feed mixture of the semi-IPN. However, with increasing concentration of acrylamide the IPN shows an enhanced swelling.

The IPN also shows a pH dependent swelling behavior. It is noticed that at pH 7.6, an optimum swelling ratio is obtained while the swelling ratio decreases on both the sides of the optimum pH. The swelling ratio also increases with increasing temperature from 10 to 40°C while a fall is obtained beyond 40°C. The IPN also shows a decreasing water sorption capacity when placed in simulated biofluids and water reservoir containing increasing amounts of electrolytes.

The semi-IPN exhibits a fair level of blood compatibility as indicated by lower weights of blood clot formed on its surfaces as compared to glass and polybag. Moreover, the blood compatibility also varies with composition of the semi-IPN. It is noticed that whereas with increasing PAM and PHEMA content in the semi-IPN the thrombogenicity decreases while enhanced thrombogenic behavior is seen with increasing gelatin and crosslinker content in the semi-IPN.

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