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Evaluation of water sorption property and in vitro blood compatibility of poly(2-hydroxyethyl methacrylate) (PHEMA) based semi interpenetrating polymer networks (IPNs)

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Abstract pH responsive smart biomaterials of gelatin and poly(2-hydroxyethyl methacrylate-co-acrylic acid) were synthesized by redox polymerization and characterized by FTIR, Environmental Scanning Electron Microscopy (ESEM). The prepared environmental responsive biomaterials containing polyelectrolyte segments were assessed for their water sorption potential under varying experimental conditions. The diffusion mechanism of transport of water molecules arising due to solvent-polymer interaction was also analysed to predict the behaviour of continuously relaxing macromolecular chains. The in vitro blood compatibility of the prepared polymeric hydrophilic materials was evaluated by methods such as blood clot formation, platelet adhesion, percent haemolysis and protein-adsorption study on the surface of the prepared biomaterials.

1 Introduction

Polymers play an important role in biomedical application. These polymers include polymeric hydrogels, biodegradable polymers, tissue friendly functional polymers, and biocompatible polymers. The development and applications of polymeric materials have greatly increased as polymers offer a better balance between chemical, physical, mechanical and weight properties than other materials such as metals and ceramics [1].

Polymers have been used for the development of many prosthetic devices such as heart valves, contact lenses, replacement arteries and veins, artificial kidneys, artificial lungs and cosmetic implant materials, fixation materials (such as sutures, bone cement and surgical adhesive) and drug delivery system [2]. Polymers for biomedical applications can be naturally occurring, synthetic or combination of both. Whereas the naturally derived polymers are biodegradable, the synthetic polymers offer a wide range of properties by merely altering the chemical composition of the polymer. Crosslinked polymers, capable of imbibing large volume of water have found widespread application in bioengineering, biomedicine, food industry and water purification and separation process [3]. Although many naturally occurring polymers may be used to produce variety of material, the structural versatility available in synthetic polymers has given them distinctive properties which, in turn, have enhanced their practical utility [4–6].

Hydrogels are water swollen macromolecular matrices, consisting of crosslinked polymeric chains and insoluble in aqueous and biofluids at physiological temperature, pH and ionic strength [7]. Hydrogels have also been suggested to be useful as a model for cellular behaviour (Kiser et al. 1998) since they mimic the internal cytoskeleton structure of biological cells better than bilayer vesicles and other semipermeable membrane mimic. This is also suggested from similarities between the mechanical characteristics in hydrogels and cellular bodies, e.g. (density, mechanical strength and diffusion rate within hydrogels) [8]. These physiochemical properties of hydrogels enable them to serve as "potential biomaterials" in biomedical engineering and allied fields [9], whereas "a biomaterial is a synthetic or natural polymer whose surface is in direct contact with biological system", the biocompatibility may be defined as, "the ability to perform with an appropriate host response in a specific application" [10]. A foreign material when dipped in blood or tissue fluid, leads to

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adsorption of biomolecules, cells and usually proteins on its surface, just within a few seconds of immersion, followed by secondary interactions like cellular response and thrombus formation [11]. Surface, as well as bulk properties such as surface free energy, morphology, composition, degradability, permeability and mechanical resistance play a vital role in determining biocompatible nature of the material [12].

Thus, having acknowledged the utility of macromolecular structures in biomedical field, we in the present investigation report the preparation and characterization of semi interpenetrating polymer networks (IPNs) of gelatin and poly(2-hydroxyehtyl-methacrylate-co-acrylic acid). An effort has also been made to correlate water sorption capacity and in vitro blood compatibility of the prepared semi-IPN.

The selection of HEMA as one of the components in the IPN rests upon its unique swelling property and good oxygen permeability. PHEMA based hydrogels have been used for soft tissue prosthesis due to their biocompatible characteristics, high permeability to small molecules (i.e. tissue metabolites), high hydrophilicity, their soft consistency [13] and resistants to adhesion of blood proteins and blood cells [14].

The wide interest in gelatin is mainly due to its most frequent uses in the biomedical field that includes hard and soft capsules, sealants, microsphere, wound dressing and absorbent pad for surgical use as well as three dimensional tissue regeneration [15]. Since gelatin is soluble in aqueous solutions due to the formation of specific intermolecular interaction through hydrogen bonding, enhances the mixing properly of the gel. In long-term biomedical applications, mixing property improves both the thermal and mechanical stability of the biopolymers [16].

To increase the bonding strength and control degradation rate of hydrogel, gelatin should be chemically improved with materials with sufficient adhesion strength and a crosslinking agent before it is used as a surgical glue or as a biomaterial in biomedical field [17]. Acrylic acid was selected for this purpose because polyanions increase the concentration of carboxyl groups which leads to the shortening, the gelation rate of gelatin and improves the biomedical properties of the biomaterials [18]. Moreover, polyacrylic acid is well recognized for its polyanionic nature and has been extensively employed in designing pH-responsive macromolecular architectures mainly used in targeted drug delivery.

2 Experimental

2.1 Materials

Acrylic acid (AA) used as an ionic monomer (molecular weight 72.06, Merck (India) Limited, Mumbai) was purified and freed from inhibitor by distilling under reduced pressure. The distilled monomer was stored in a dark bottle at 4 °C. HEMA (2-hydroxyethyl methacrylate) was obtained from Sigma Aldrich Co. USA and freed from the inhibitor by prescribed method. The crosslinker used in polymerization was ethyleneglycol dimethacrylate (EGDMA) obtained from Merk (Germany) and used as received. Potassium persulphate and metabisulphite were of Loba Chemie (India) and used without any pretreatment. Gelatin (Merck, India Ltd.) type A (isoelectric point 7.6) was used as supplied. Ethylene glycol (EG) (Merck, India) was used as a cosolvent. All other chemicals used were of analytical grade and bi-distilled water was used throughout the experiments.

2.2 Preparation of semi-IPN

IPN hydrogels were prepared by a conventional redox polymerization method. A typical procedure for the copolymerization may be described as follows : Gelatin 6.5 wt% (w/w) was dissolved in 5 mL of distilled water at 60 °C and to this solution were added calculated amounts of AA, EG, HEMA, EGDMA (as a crosslinker) and the redox initiator comprising of potassium metabisulphite (KMBS) and potassium persulphate (KPS) to yield a homogenous reaction. 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 Fig. 1.

2.3 Characterization of the gel

The gel prepared as above were characterized by FTIR spectroscopy and Optical microscopy.

2.3.1 FTIR spectral analysis

FTIR spectral studies of the prepared hydrogel of definite composition were performed on a FTIR spectrophotometer (Perkin Elmer, 1000 Paragon) by recording the IR spectra of a dry thin film of the gel.

2.3.2 Water uptake measurement

A conventional gravimetric procedure [19] was followed for monitoring the progress of water uptake process and the

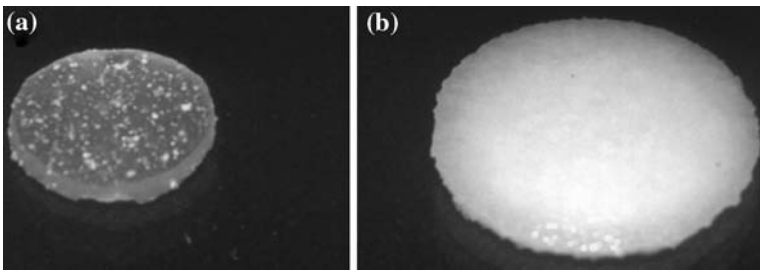

Fig. 1 The (**a**) dry and (**b**) swollen photograph of the IPNs

degree of water sorption was quantified in terms of the swelling ratio as calculated below,

$$\text{Swelling ratio} = \frac{W_s}{W_d} \quad (1)$$

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

In order 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 [20].

$$\frac{W_t}{W_\infty} = k\, t^n \quad (2)$$

$$\frac{W_t}{W_\infty} = \left(\frac{Dt}{\pi l^2}\right)^{1/2} \quad (3)$$

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 1.

2.4 Blood compatibility

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

2.4.1 Thrombus formation

The antithrombogenic properties of hydrogels were evaluated with human ACD (Acid Citrate Dextrose) blood, using the method developed by Imai and Nose [21]. 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 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 (distilled water) and the thrombus formed was separated by soaking in water for 10 min at room

Table 1 Data presenting the values of swelling exponent 'n' and diffusion coefficient 'D' for various composition of hydrogels

S. No.	Composition				n	$D \times 10^8$ ($cm^2\ s^{-1}$)	Mechanism
	Gelatin wt% (w/w)	HEMA (mM)	AA (mM)	EGDMA (mM)			
1.	6.56	16.4	21.8	0.26	0.44	1.67	Fickian
2.	9.53	16.4	21.5	0.26	0.52	4.15	Anomalous
3.	12.32	16.4	21.5	0.26	0.52	3.84	Anomalous
4.	17.41	16.4	21.5	0.26	0.54	3.39	Anomalous
5.	7.64	8.20	21.5	0.26	0.64	1.67	Anomalous
6.	6.56	16.4	21.5	0.26	0.44	1.67	Fickian
7.	5.75	24.7	21.5	0.26	0.36	2.84	Fickian
8.	5.12	32.9	21.5	0.26	0.60	1.38	Anomalous
9.	7.61	16.4	7.9	0.26	0.50	2.71	Fickian
10.	7.06	16.4	14.3	0.26	0.50	2.46	Fickian
11.	6.56	16.4	21.5	0.26	0.44	1.67	Fickian
12.	6.14	16.4	28.77	0.26	0.48	5.53	Fickian
13.	6.56	16.4	21.5	0.26	0.44	1.67	Fickian
14.	6.52	16.4	21.5	0.53	0.60	2.22	Anomalous
15.	6.43	16.4	21.5	1.07	0.62	3.39	Anomalous
16.	6.34	16.4	21.5	1.59	0.64	2.22	Anomalous

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).

2.4.2 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 [22]. 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 samples 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.

$$\%\,\text{Hemolysis} = \frac{\text{A test sample} - \text{A}\,(-)\,\text{control}}{\text{A}\,(+)\,\text{control} - \text{A}\,(-)\,\text{control}} \times 100 \quad (4)$$

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

2.4.3 Protein adsorption

In order 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 [23] was used to determine the amount of adsorbed BSA. In this method, 0.5 M protein (BSA) solution for adsorption experiments were made in 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 so gently that no froth was produced otherwise it would have formed an air–water interface. 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 [24]. The adsorbed amount of BSA was calculated by the following mass balance equation:

$$\text{Adsorbed BSA}\,(\text{mg g}^{-1}) = \frac{(\text{C}_\text{o} - \text{C}_\text{a}) \times \text{V}}{\text{W}} \quad (5)$$

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

2.4.4 Platelet adhesions

For platelet-adhesion studies, films of 1.5×1.5 cm^2 size were incubated for 1 h in PBS (pH 7.4). Fresh human blood, anticoagulated with acid citrate dextrose (ACD), was centrifuged at 2,500 rev. min^{-1} for 5 min to obtain platelet rich plasma (PRP). The films were laid flat on small petri dishes and submerged with PRP and left at 37 °C for 1 h in an incubator. After washing many times gently with buffer to remove non-adhering platelets, fixing was done with 2.5% buffered gluteraldehyde overnight in the refrigerator at 4 °C, and examined by optical microscopy (Olympus Optical Microscope Co. Ltd., Made in Japan, Model—CHS).

2.5 Network parameters

A significant 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 [25] laid the foundation for analysis of equilibrium swelling.

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

$$\text{M}_\text{c} = \frac{-\text{V}_1 \text{d}_\text{p} \left(\text{V}_\text{s}^{1/3} - \text{V}_{\text{s}/2}\right)}{\ln(1 - \text{V}_\text{s}) + \text{V}_\text{s} + \chi \text{V}_\text{s}^2} \quad (6)$$

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, χ is the Flory–Huggin's interaction parameter between solvent and polymer [26].

Table 2 Data presenting the values of average molar mass between crosslinks (M_c), the crosslink density (q) and the number of elastically effective chains (V_e) for varying compositions

S. No.	Composition				M_c (g mol^{-1})	Q	$V_e \times 10^{20}$
	Gelatin wt% (w/w)	HEMA (mM)	AA (mM)	EGDMA (mM)			
1.	6.56	16.4	21.5	0.26	171.72 ± 8.10	0.5059 ± 0.012	6.315 ± 0.26
2.	9.53	16.4	21.5	0.26	34.992 ± 2.24	2.4828 ± 0.083	30.982 ± 1.82
3.	12.32	16.4	21.5	0.26	34.992 ± 2.24	2.4828 ± 0.095	30.982 ± 1.82
4.	17.41	16.4	21.5	0.26	38.88 ± 2.76	2.2345 ± 0.092	27.884 ± 1.57
5.	7.64	8.2	21.5	0.26	21.060 ± 1.54	3.5607 ± 0.120	51.47 ± 2.61
6.	6.56	16.4	21.5	0.26	171.72 ± 8.10	0.5059 ± 0.012	6.315 ± 0.26
7.	5.75	24.7	21.5	0.26	51.45 ± 3.15	1.8468 ± 0.052	21.07 ± 1.16
8.	5.12	32.9	21.5	0.26	190.8 ± 9.08	0.1162 ± 0.010	5.682 ± 0.21
9.	7.61	16.4	7.9	0.26	83.31 ± 4.96	1.8270 ± 0.052	13.013 ± 0.94
10.	7.06	16.4	14.3	0.26	51.45 ± 3.15	2.8258 ± 0.089	21.07 ± 1.16
11.	6.56	16.4	21.5	0.26	171.72 ± 8.10	0.5059 ± 0.012	6.315 ± 0.26
12.	6.14	16.4	28.77	0.26	38.034 ± 2.76	2.1470 ± 0.084	28.504 ± 1.64
13.	6.56	16.4	21.5	0.26	171.72 ± 8.10	0.5059 ± 0.012	6.315 ± 0.26
14.	6.52	16.4	21.5	0.53	34.992 ± 2.24	2.4828 ± 0.093	30.982 ± 1.83
15.	6.43	16.4	21.5	1.07	29.16 ± 1.56	2.9794 ± 0.098	37.179 ± 1.91
16.	6.34	16.4	21.5	1.59	24.994 ± 1.48	3.4760 ± 0.130	43.376 ± 2.06

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

$$q = M_o/M_c \tag{7}$$

where, M_o 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 2.

2.6 Statistical analysis

All swelling measurements were done at least thrice and data summarized in Tables and shown in graphs, have been expressed as mean ± S.D.

3 Results and discussion

3.1 FTIR spectral analysis

The FTIR spectra of gelatin-poly(HEMA-co-AA) IPN is shown in Fig. 2 which provides strong evidences for IPN formation from gelatin, HEMA and polyacrylic acid. The

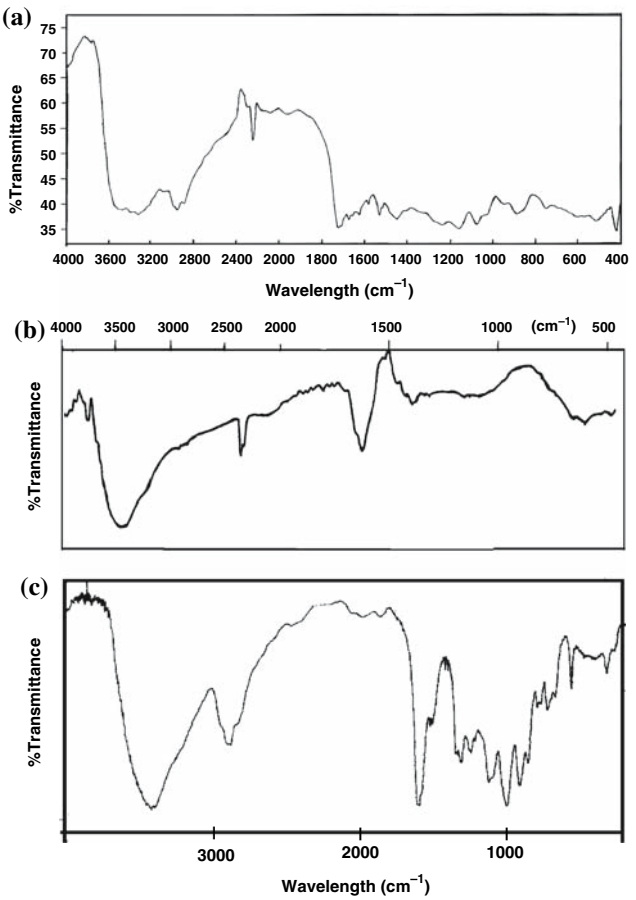

Fig. 2 The FTIR spectra of (**a**) gelatin-Poly(HEMA-co-AA) IPN, (**b**) native gelatin and (**c**) pure PHEMA

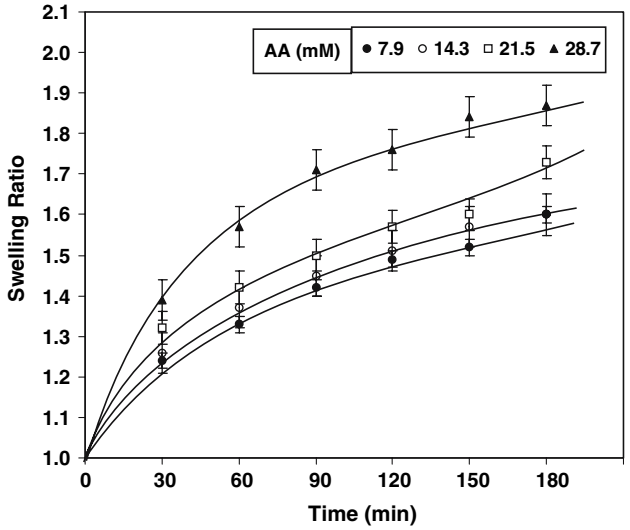

Fig. 3 Effect of varying concentration of AA on swelling ratio of the semi IPN of definite composition [HEMA] = 16.4 mM, [gel] = 6.56% (w/w), [EGDMA] = 0.26 mM, [pH] = 7.4, [Temp.] = 25 ± 0.2 °C

spectra of the hydrogel shown as Fig. 2 clearly presents a broad band between 2,450 and 3,600 cm^{-1} consisting many residual or degenerated peaks. The appearance of a broad band indicates the presence of intermolecular hydrogen-bonded O–H stretch between carboxylic group of acrylic acid and hydroxyl group of HEMA and bound water molecules in the IPN (Fig. 3).

The degenerated peak at 3,360 cm^{-1} is assigned to N–H stretching of gelatin. The IR spectra clearly marks the presence of HEMA as evident from observed residual bands at 1,729 cm^{-1} (C=O stretching), 1,143 cm^{-1} (O–C–C stretching), and 1,455 cm^{-1} (O–H bending), respectively [27]. The methylene groups (CH_2) of polyacrylic acid are quite evident at 2,947 cm^{-1} due to asymmetrical stretching mode of C–H band [28]. The characteristics peak at 1,729 cm^{-1} could be due to carbonyl group of the ester. The spectra of native gelatin and PHEMA are also shown in Fig. 2b and c, respectively.

3.2 Water sorption measurement

3.2.1 Effect of monomers on swelling

The swelling ratio (Q) of a hydrogel can be best described by Flory's swelling theory as given below:

$$Q^{5/3} = \frac{\left((i/2v_u S^{1/2}) + (1/2 - x_i)/v_1\right)^{1/2}}{(v_e/v_o)} \quad (9)$$

where i/v_u is the concentration of fixed charge with respect to unswollen network, S is the ionic concentration in the external solution, $(1/2–x_i)/v_1$ is the affinity of the hydrogel with the water and v_e/v_o is the crosslinked density of the hydrogel. Q has a relation with ionic osmotic pressure, the crosslink density and affinity of the hydrogel with the water. From the above equation, it is very clear that with increase in ionic content as well as the hydrophilicity of the composite material, the swelling ratio will increase [29].

3.2.1.1 Effect of acrylic acid The acrylic acid (AA) is an ionic comonomer and has a major impact on the swelling characteristics of the present hydrogel. To investigate the effect of AA content in the hydrogel on its swelling ratio, the AA concentration was varied over the range (7.9–28.77 mM). The results obtained are shown in the Fig. 3 which clearly reveal that increasing amount of ionic monomer increases the swelling ratio. It is important to mention here that the hydrogel of concentration greater than 28.77 mM of AA were also prepared but enhanced sorptivity of water made them much weaker for subsequent investigations and, therefore, no further increase in concentration of AA was done. The results can be explained by the well known facts that the swelling of hydrogel is induced by the electrostatic repulsion of the ionic charges of its network [30, 31]. On increasing the concentration of the monomer in the gel, the number of charged carboxylic group (COO^-) increases and due to greater repulsion among these ionic groups the polymeric chains get expanded and results in an increased swelling [32].

In addition to the role of carboxylate ions (COO^-) in enhancing swelling of the hydrogel, the contribution of cations cannot be ignored. The cations and anions enter into the gel network and get hydrated resulting in formation of solvation shells. These hydrated shells result in an increase of bulk volume of the hydrogel and, thus, cause an increase in the swelling ratio.

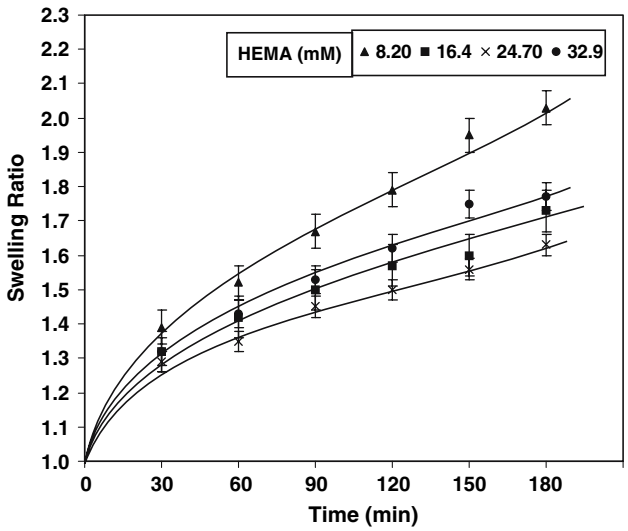

Fig. 4 Effect of varying concentration of HEMA on swelling ratio of the semi IPN of definite composition [AA] = 21.5 mM, [EGDMA] = 0.26 mM, [gel] = 6.56% (w/w), [pH] = 7.4, [Temp.] = 25 ± 0.2 °C

3.2.2 Effect of HEMA

The influence of HEMA content in the gel on its swelling behaviour has been investigated by varying its concentration in the feed mixture in the range of 8.2–32.9 mM. The results are shown in Fig. 4 which clearly indicate a fall in the water sorption capacity with increasing PHEMA content up to 24.7 mM and after that a small increase in swelling ratio is observed. The obtained fall in the swelling ratio may be attributed to the fact that with increasing number of functional groups of PHEMA (hydroxyl and carboxyl) the possibility of hydrogen bond formation will increase which may result in interpenetration and entanglements of chains. Consequently, the gel may become compact with reduced mesh sizes which will obviously bring about a fall in the water sorption.

Upon further increasing the quantity of HEMA, a small increase in the swelling ratio has been observed. The reason for this unusual increase may be due to the presence of excess amount of PHEMA which will show its own intrinsic property of hydrophilicity. At this point, any other unbonded functional group will not be present in the hydrogel matrix.

3.2.3 Effect of gelatin

Gelatin is a high molecular weight polypeptide derived from collagen and composed of amino acids with adhesion properties and has a similar structure to bond tissues. In the present study, the influence of gelatin has been examined on the swelling behaviour of the gel by adding gelatin into the feed mixture in the range 6.56–17.41 wt% (w/w). The

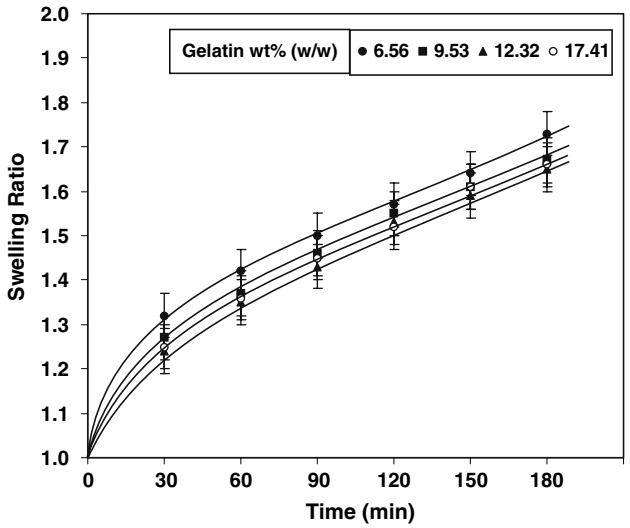

Fig. 5 Effect of varying concentration of gelatin on swelling ratio of the semi IPN of definite composition [HEMA] = 16.4 mM, [EGDMA] = 0.26 mM, [AA] = 21.5 mM, [pH] = 7.4, [Temp.] = 25 ± 0.2 °C

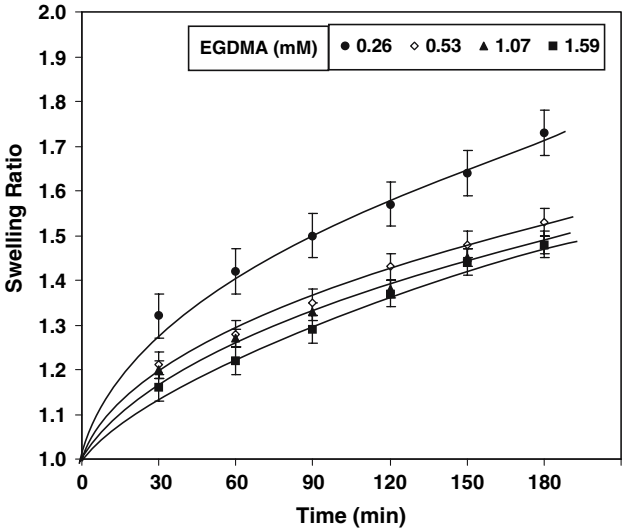

Fig. 6 Effect of varying concentration of crosslinker (EGDMA) on swelling ratio of the semi IPN of definite composition [HEMA] = 16.4 mM, [AA] = 21.5 mM, [gel] = 6.56% (w/w), [pH] = 7.4, [Temp.] = 25 ± 0.2 °C

results shown in Fig. 5 clearly indicate that the swelling ratio of the polymeric matrix is maximum at 6.56 wt% (w/w) of gelatin, and as the amount of gelatin further increases, the swelling ratio decreases.

The decrease observed in the swelling may be attributed to the fact that an increased volume fraction of the biopolymer matrix results in a reduction in the free volume available within the hydrogel network accessible to the invading water molecules. Moreover, because of an increase in the volume fraction of the polymer, the water molecules have to travel a longer path to enter the networks and this obviously results in a decrease in the swelling ratio [33].

3.2.4 Effect of crosslinker

The crosslinker has a pronounced impact on the swelling behaviour of the IPNs as shown in Fig. 6. When the concentration of EGDMA is raised into the feed mixture by varying the range from 0.26 to 1.59 mM, the degree of water sorption decreases. The decrease noticed in the IPN may be contributed to the reason that high amount of crosslinker produces a compact network with greater number of crosslink density. The pore sizes of IPN also decreases which results in a lower degree of swelling.

3.2.5 Effect of pH

pH responsive macromolecular devices have been frequently used to develop controlled release formulations for oral administration which remain the most clinically

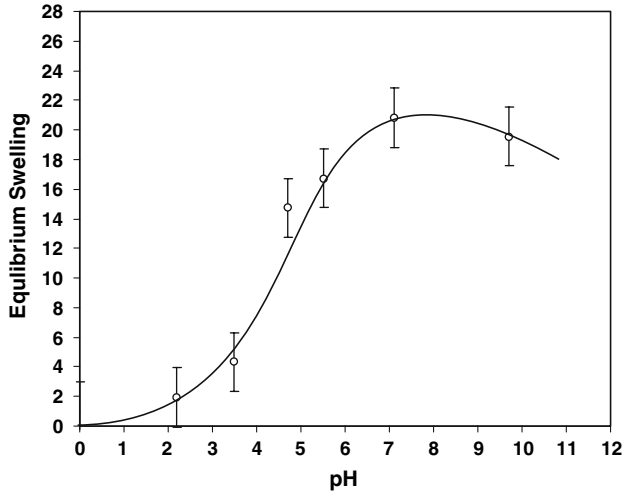

Fig. 7 Effect of varying pH on swelling ratio of the semi IPN of definite composition [HEMA] = 16.4 mM, [gel] = 6.56% (w/w), [AA] = 21.5 mM, [EGDMA] = 0.26 mM, [Temp.] = 25 ± 0.2 °C

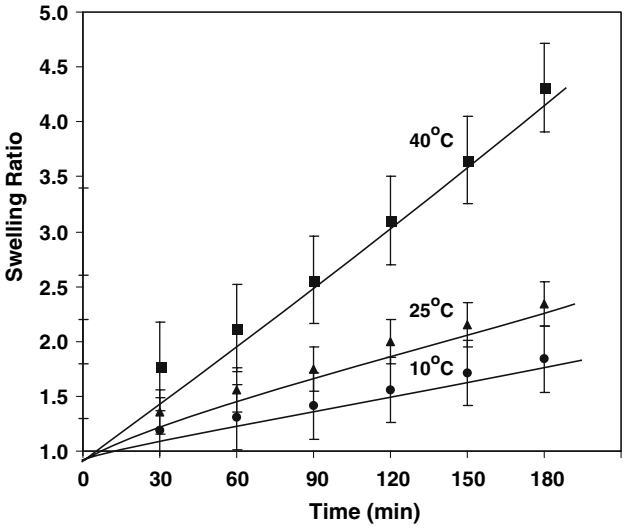

Fig. 8 Effect of varying temperature on swelling ratio of the semi IPN of definite composition [HEMA] = 16.4 mM, [gel] = 6.56% (w/w), [AA] = 21.5 mM, [EGDMA] = 0.26 mM, [pH] = 7.4

acceptable way of drug delivery [34]. The oral administration of macromolecular drugs remains a significant challenge because peptides and proteins are susceptible to hydrolysis and digestion by the acid and enzymes in the gastrointestinal tract. Also, the bioavailability of orally delivered peptide and proteins is very low because of poor membrane permeability [35]. The pH responsive swelling behaviour is basically due to ionization of the functional groups in the gel, which depends on the pH of the surrounding medium. In an ionic hydrogel, an increase in the degree of ionization contributes to electrostatic repulsion between the charged groups along the chains and, therefore, swells the gels to a higher degree. These highly swollen hydrogels contain large amount of unbonded water, which allows greater solute release. In this study, the influence of pH on the swelling ratio of hydrogel has been investigated through changes in the pH of the external medium from 1.8 to 10.8. The results depicted in Fig. 7 show the response of the hydrogel in an acidic medium and clearly reveal that the swelling ratio increases with an increase in the pH of the medium. The results can be explained by the fact that with increasing pH of the swelling medium, the carboxylic groups of PAA are ionized and generate carboxylate ions along the macromolecular chains which, because of mutual repulsion facilitate chain relaxation and cause greater swelling. Similar type of results have been noticed elsewhere [36].

The swelling profile curves also imply that the rate of swelling increases with increasing pH of the medium. The observed increasing rate of swelling may be attributed to the fact that at relatively lower pH, the network chains are firmly bonded to one another via hydrogen bonding due to an excess of –COOH groups. Thus, because of the restricted chain mobility, the swelling rate rises slowly. On the other hand, at a higher pH such as 5.0, the increased dissociation of –COOH group to carboxylate ions results in a reduction of hydrogen bonds along the network chains and thus, imparts to them faster mobility. This obviously results in an enhanced swelling rate and swelling ratio as well. The influence of an alkaline pH on the swelling profiles of the hydrogel is depicted in the Fig. 7 which clearly reveals that the swelling ratio is significantly enhanced with an increasing pH of the medium. Moreover, the swelling ratio starts decreasing after a definite time (48 h). The observed steep increase in the water sorption is quite obvious as in an alkaline medium, the –COOH groups present in both the PAA chains and gelatin molecules also undergo dissociation to yield carboxylate anions, which because of greater relaxation in the network chains cause much greater swelling of the hydrogel. The decrease observed in the swelling ratio after optimum swelling could possibly be due to the partial collapse of the swollen hydrogel. It is worth mentioning here, the swelling studies with varying pH were done under saline conditions also, but the obtained results were almost identical to those obtained without salinity.

3.2.6 Effect of temperature

To investigate the effect of temperature of the swelling medium on the water uptake potential of the prepared hydrogel, experiments were performed at different temperature ranging from 10 to 40 °C. The results are shown in Fig. 8 which clearly shows that the swelling ratio increases with increasing temperature of the swelling bath.

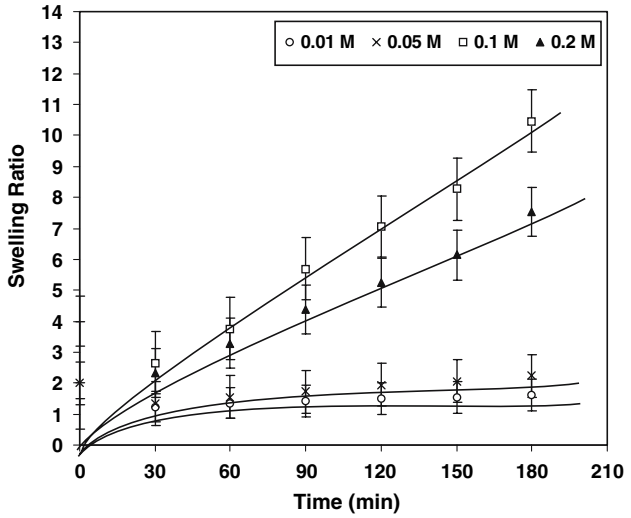

Fig. 9 Effect of varying concentration of NaCl salts on swelling ratio of the semi IPN of definite composition [HEMA] = 16.4 mM, [gel] = 6.56% (w/w), [EGDMA] = 0.26 mM, [AA] = 21.5 mM, [pH] = 7.4, [Temp.] = 25 ± 0.2 °C

The explanation of the observed results are based on the fact that with increasing temperature, the segmental mobilities of the hydrogel chains increase significantly and consequently, the water sorption capacity of the hydrogel increases. Some authors, however, have noted a decrease in swelling at higher temperature, which has been attributed to the cleavage of hydrogen bonds between the water molecules and network chains. However, no such results have been noticed in this case.

For quantitative analysis of the temperature effect, the Clausius–Clapeyron equation can be applied according to which [37]

$$\frac{d[\ln(W_\infty)]}{d(1/T)} = -\Delta H_m/R \tag{10}$$

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 –16.25 kJ/mol.

3.2.7 *Effect of ionic strength*

Theoretical and experimental considerations [38] have established that there is a balance between the osmotic pressure and the polymer elasticity that sets the physical dimensions of the hydrogels. The osmotic pressure results from a net difference in the concentration of mobile ions between the interior of the gel and the exterior solution.

According to the theory of Donan membrane equilibrium, when a gel is placed in contact with a liquid, the chemical potential of the solvent (μ) in both the gel and the solution phase must be the same at equilibrium:

$$\Delta\mu_1^g = \Delta\mu_1^s \tag{11}$$

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

In terms of the osmotic pressure (π), the equation can be rewritten as follows:

$$\pi = \frac{\left(\Delta\mu_1^g - \Delta\mu_1^s\right)}{V_1} = 0 \tag{12}$$

In the case of ionic system, the osmotic pressure is mainly contributed by π ions, which are due to the counterion difference between the gel and the outer solution. In a modified form, Eq. 12 can be written as follows:

$$\pi_{ion} = RT\,\Sigma\left(C_i^g - C_i^s\right) \tag{13}$$

where C_i is the mobile ion concentration of species i and superscripts g and s represent the gel and solution phases, respectively. From the above equation, it is very clear that larger the difference between the concentrations of mobile ions inside and outside the gel, greater would be the osmotic pressure and consequently larger the swelling of the hydrogel.

3.2.8 *Ionic strength and swelling*

With the variation of concentration of NaCl in the outer swelling bath in the range of 0.01–0.2 M, the swelling ratio is appreciably suppressed with increasing salt concentration as shown in Fig. 9. The observed depression in the swelling ratio can be attributed to the fact that the addition of ions to the outer medium results in a decrease in the net value of $(\mu_i^g - \mu_i^s)$ in Eq. 12, which consequently lowers osmotic pressure π and, thus, brings about a fall in the swelling ratio.

A remarkable feature visible in the swelling profiles of the hydrogel is the phenomenon of shrinking of the gel after a definite swelling period (24 h). The observed fall in the swelling ratio may be attributed to the fact that once the hydrogel swells to an optimum value, the pores of the gel widens, thus, allowing the cations from the swelling bath to diffuse into the bulk of the swollen gel. These cations bind to the negatively charged $-COO^-$ ions present along the network chains and obviously results in a shrinking of the network chains, bringing about a fall in the amount of the imbibed water.

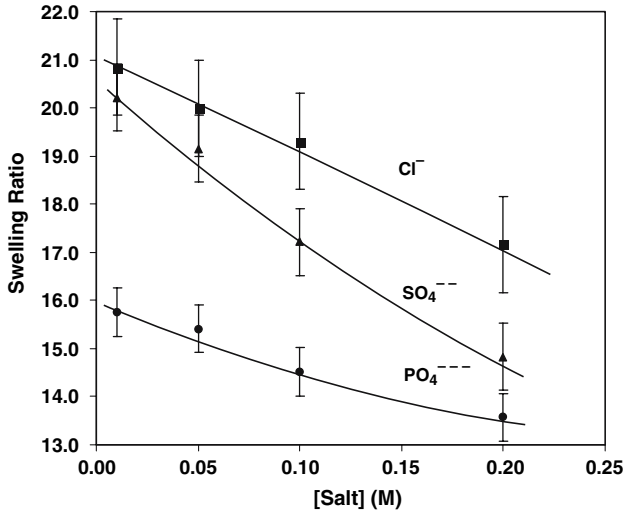

Fig. 10 Effect of varying concentration of different salts on swelling ratio of the semi IPN of definite composition [HEMA] = 16.4 mM, [gel] = 6.56% (w/w), [EGDMA] = 0.26 mM, [AA] = 21.5 mM, [pH] = 7.4, [Temp.] = 25 ± 0.2 °C

3.2.9 *Effect of anions*

The influence of the addition of anions on the swelling of the hydrogel has been investigated by the addition of sodium salts of Cl^-, SO_4^{2-}, and PO_4^{3-} ions in equimolar amounts. The results are displayed in Fig. 10 which clearly reveal that the addition of salts brings about a fall in the swelling ratio. The relative order of effectiveness of added anions in suppressing the swelling ratio obey the following sequence,

$$Cl^- < SO_4^{2-} < PO_4^{3-} \quad (14)$$

The observed order of effectiveness may be explained on the basis of the increase in sizes of the anions. The size of Cl^- ions is small so they can diffuse into the gel easily and may hinder the swelling as discussed above. 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 [39].

3.2.10 *Evaluation of blood compatibility*

The interaction between biological environment and artificial materials are likely to be dominated by the material's surface properties [40]. When a foreign material is exposed to blood, plasma proteins are adsorbed on to the material surfaces, followed by the activation of platelets and finally the formation of a fibrin network [41]. Hence, a great deal of efforts have been focused on understanding the relationship between polymer surface and thrombogenicity [42].

In the present study, an assessment of biocompatibility has been made on the basis of three in vitro tests, viz. BSA adsorption, blood clot formation and percent haemolysis assay as discussed below:

3.2.10.1 Protein adsorption Protein adsorption onto biomaterial surfaces is believed to be the earliest event after implantation. It has been largely noticed that the composition and organisation of the adsorbed protein layer can be varied by numerous factors relating to the substrate such as hydrophobicity, sorbed water content, microphase separation, and surface chemical functionality. As far as the chemistry of surfaces is concerned, the effect of hydrophilic and hydrophobic balance of constituent chains in polymer surface has been found to play a key role in influencing protein adsorption and subsequent platelet adhesion to polymer surfaces [43].

Thus, looking to the significant consequences of protein adsorption on biomaterial surfaces. The adsorption of bovine serum albumin (BSA) onto the surface of the prepared hydrogel surfaces has been studied. The protein chosen for in vitro adsorption study was BSA that is among the most abundant proteins in vertebrates and is readily commercially available. BSA has also been widely used in biochemical work as a generic protein.

The changes of BSA adsorption with the varying amount of gelatin are shown in the Table 3 which indicate that the amount of adsorbed BSA decreases with increasing amount of gelatin in the feed mixture of the gel. The observed findings may be explained on the basis of the facts that gelatin has lower affinity towards protein

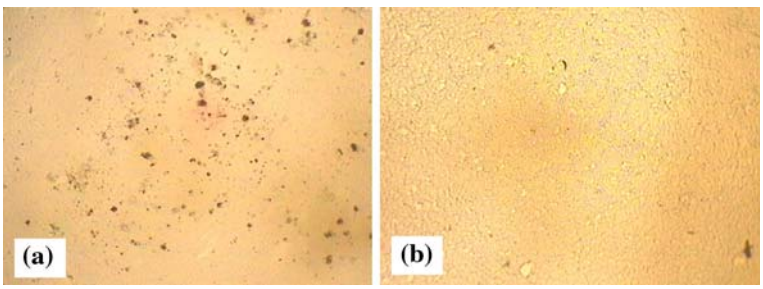

Fig. 11 Optical microscopic images depicting (**a**) greater number of platelets and (**b**) lesser number of platelets adhered to IPN films containing 0.5 and 1.5 g gelatin, respectively

Table 3 Data showing the weight 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})
	Gelatin wt% (w/w)	HEMA (mM)	AA (mM)	EGDMA (mM)			
1.	6.56	16.4	21.5	0.26	16 ± 1.3	68.74 ± 3.2	1.831 ± 0.08
2.	9.53	16.4	21.5	0.26	15 ± 1.2	66.23 ± 3.0	1.690 ± 0.06
3.	12.32	16.4	21.5	0.26	15 ± 0.98	65.50 ± 2.8	1.631 ± 0.062
4.	17.41	16.4	21.5	0.26	14 ± 1.2	63.3 ± 2.6	1.458 ± 0.043
5.	7.64	8.20	21.5	0.26	19 ± 1.6	70.38 ± 4.2	1.901 ± 0.082
6.	6.56	16.4	21.5	0.26	16 ± 1.3	68.74 ± 3.83	1.831 ± 0.08
7.	5.75	24.7	21.5	0.26	13 ± 1.02	63.95 ± 3.74	1.714 ± 0.065
8.	5.12	32.9	21.5	0.26	09 ± 0.6	58.46 ± 2.89	1.571 ± 0.046
9.	7.61	16.4	7.9	0.26	21 ± 1.9	78.15 ± 3.98	1.660 ± 0.056
10.	7.06	16.4	14.3	0.26	20 ± 1.8	75.8 ± 3.95	1.720 ± 0.067
11.	6.56	16.4	21.5	0.26	16 ± 1.3	68.74 ± 3.83	1.831 ± 0.08
12.	6.14	16.4	28.77	0.26	15 ± 1.02	61.8 ± 2.07	2.085 ± 0.094
13.	6.56	16.4	21.5	0.26	16 ± 1.3	68.74 ± 3.83	1.831 ± 0.08
14.	6.52	16.4	21.5	0.53	23 ± 1.83	68.98 ± 3.74	1.838 ± 0.065
15.	6.43	16.4	21.5	1.57	26 ± 1.89	72.49 ± 3.92	1.936 ± 0.078
16.	6.34	16.4	21.5	1.59	30 ± 2.01	76.36 ± 3.98	2.045 ± 0.089

adsorption due to its hydrophilic nature. Gelatin forms effective hydrogen bonds with water and thus formed structured water layer opposes protein adsorption on to the polymer surfaces. Another reason for observed decrease in protein adsorption with increasing gelatin content may be attributed to the fact that at higher gelatin content in the hydrogel, phase separation becomes prominent and hydrophilic domains are forced 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 [44].

The crosslinking agent employed in the present study was EGDMA which is a known hydrophobic crosslinker. The influence of EGDMA on the protein adsorption has been investigated by increasing its concentration in the IPNs in the range (0.26–1.59 mM). The results are shown in Table 3 which indicate that the adsorbed protein increases with increasing EGDMA concentration in the IPN. The increase in the protein adsorption may be attributed to the fact that hydrophobic domains of proteins interact with hydrophobic segments of crosslinker (EGDMA) and thus, causes adsorption of proteins on the sponge surface, as a result of entropic hydrophilic interaction and lyophilic liquid binding capabilities. Similar type of results have also been published by others [45].

The changes in the BSA adsorption with AA content are summarized in Table 3. The adsorption of BSA within hydrogels increased with the increase in AA content in the gel. It was observed that the addition of AA to the gel increased the pore size of the gel due to electrostatic repulsion among the carboxylate ions present along the polyacrylic acid chains. This obviously allows greater number of protein molecules to diffuse into the pore structure of the gel which eventually results in an enhanced adsorption [46]. On increasing the concentration of p(HEMA) in the gel, the changes observed in the adsorption of BSA are summarized in Table 3. The adsorption of BSA onto the hydrogel decreases with the increase in p(HEMA) content. The reason for the observed finding may be explained on the basis of the fact that p(HEMA) maintains low interfacial tension between the hydrogel and the aqueous solution and reduces the protein adsorption on to the gel [47].

3.2.10.2 Blood clot formation In order to use carrier systems for biological applications, blood is the only and main carrier. Therefore, the objective in the investigation of biomaterials for blood contacting application should be improved so that the effect of materials on blood constituents and the events inducing thrombus formation on artificial surfaces must be minimized [48].

In the present study, the anti-thrombogenic property of the gel has been judged by monitoring the amount of blood clot formed by performing blood clot formation test as described in the experimental section. The results are summarized in Table 3 which clearly indicate that the weight of blood clot constantly decreases with increasing the amount of gelatin, HEMA and acrylic acid (AA) in the

hydrogel. The decreased blood clot is quite expected because of the well-known biocompatible nature of gelatin and HEMA. Another reason may be that the PAA chains are grafted onto the gelatin backbone via free amino groups, which are well known to form polyelectrolyte complexes with acidic groups of cellular elements of blood. Thus, the reduction in the number of amino groups of gelatin results in an increase in the blood compatibility [49].

The influence of crosslinker (EGDMA) on the blood compatible nature of the gels' surface has been investigated by increasing its concentration in the range 0.26–1.59 mM in the feed mixture of the hydrogel. The results are depicted in Table 3 which clearly indicate that the weight of blood clot 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 thrombus formation.

3.2.10.3 Haemolysis test In the present investigation, the prepared gels were also tested for haemolytic activity and the results obtained are shown in Table 3 which clearly indicate that with increasing gelatin, HEMA and AA content, the extent of haemolysis constantly decreases while the greater amount of EGDMA, the haemolysis increases. The observed results may be attributed to the reason that with change in gelatin, HEMA and acrylic acid concentration in the gel, the surface composition favourably changes which improves the blood compatible quality of the material. The observed haemolysis data shown are well consistent to the clot formation results.

3.2.10.4 Platelet Adhesion The in vitro blood compatibility of the prepared semi IPN surfaces has also been judged by conducting platelet adhesion studies following the method described in experimental section. The results of platelet adhesion test have been shown in Fig. 11a and b which represent the optical microscopic images of the platelet adhered surfaces of the semi IPN containing 6.56 wt% (w/w) and 17.41 wt% (w/w) of gelatin. It is clear from the two images that with increase in the amount of gelatin in the semi IPN, the number of adhered platelets also decreases which indicate for an enhanced blood compatible nature of the surface. The observed results are consistent to the clot formation, protein adsorption and percent hemolysis studies which also suggest for increased blood compatibility with increasing gelatin content.

4 Conclusions

Synthesis of poly(2-hydroxyethyl methacrylate-co-acrylic acid) in the presence of gelatin results in a hydrophilic structure having remarkable water sorption potential and a fair level of blood compatibility.

The chemical composition of the prepared semi IPN significantly affects the swelling characteristics of the matrix. Whereas the increased acrylic acid content in the IPN increases the swelling ratio, the increasing HEMA and gelatin content in the polymer results in a fall in the water sorption capacity of the matrix.

The swelling capacity of the IPN also increases with increasing pH and temperature of the swelling bath while greater ionic strength of swelling medium resulting from the addition of cationic and anionic salts brings about a fall in the water sorption capacity. Water sorption data also enable to evaluate network parameters such as average mol wt.% and crosslink density.

The in vitro blood compatibility tests performed on the semi IPN surface indicate a greater dependence of blood clot formation, percent hemolysis and protein (BSA) adsorption on the chemical composition of the polymer surface. It is found that the polymer matrix with greater content of HEMA, gelatin and acrylic acid tends to make the material more blood compatible while a greater crosslinked semi IPN shows more thrombogenicity.

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