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Preparation and Characterization of Hard and Biocompatible Interpenetrating Polymer Networks (IPNs) of Gelatin and Polyacrylonitrile

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The present paper reports findings on the preparation of full IPNs of gelatin and polyacrylonitrile (PAN). Various compositions of glutaraldehyde-crosslinked gelatin and N,N'-methylene bisacrylamide (MBA)-crosslinked PAN were prepared and characterized by IR, DSC, and SEM techniques. The IPNs were also investigated for their water-sorption behavior and the effect of chemical composition on the extent of water uptake was evaluated. The microhardness of IPNs were determined and studied as a function of chemical architecture of the IPNs. The prepared IPNs were also assessed for in vitro blood compatibility by methods such as protein (BSA) adsorption, blood-clot formation and percent hemolysis measurements.

Keywords gelatin, polyacrylonitrile, water uptake, microhardness

Introduction

Hydrogels are three dimensional structure functional polymers that imbibes enormous amounts of water into their molecular structure without undergoing dissolution or rupture of network bonds. In order to keep the spatial structure, the polymer chains are usually physically or chemically crosslinked (1). The high water content of hydrogels accounts for their several unique biophysical properties such as soft and rubbery nature, living tissues like resemblance, low interfacial energy, unusual stability in biological fluids, permeability to metabolites, etc. All these properties of hydrogels qualify them to serve as a potential biomaterial in the biomedical and pharmaceutical community (2–7). Unfortunately, the same water content, which makes hydrogels such a special class of material, is also responsible for their biggest disadvantages, the poor mechanical properties.

An effective route to design mechanically strong polymeric matrices has been through the preparation of interpenetrating polymer networks (IPNs), which are defined as a

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physical mixture of at least two polymers which have been synthesized or crosslinked, one in the other presence with no significant degree of covalent bonds between them (8).

The literature cites numerous studies where both synthetic and natural polymers have been used together to produce IPNs of distinct properties. Fan and coworkers (9) synthesized poly(vinyl alcohol)-lactose blends and studied their thermal and mechanical behaviors. The blends were formed due to hydrogen bonding between the polymer and lactose. Several thermal and mechanical properties were also determined. Nakane et al. (10) prepared poly(vinyl alcohol)-silica composites and noticed that increasing silica content in the composite caused more stiffness and brittleness in the end product. They also reported that decomposition temperature of polymer increases with increasing silica contents. Gayet and coworkers (11) synthesized bioartificial polymeric materials by crosslinking poly(ethylene glycol) with albumin and investigated their mechanical and thermal properties, such as compressive stress, deformation, glass transition temperature (T_g) etc. In a novel study by Nishiyama et al. (12) ceramics of hydroxyapatite, titanium and silica were prepared and biocompatibility of the material was evaluated. Poly(vinyl alcohol) was crosslinked and mechanical and thermal properties of the cross-linked polymers were studied by Krumova et al. (13) A polyelectrolyte complex of chitosan, a natural biopolymer, and poly(acrylic acid) was prepared by Nge and coworkers (14) by UV-radiation exposure technique and the structure of the resulting polymer complex was confirmed by FTIR and Raman spectroscopy. A hydrogel with improved biocompatibility was prepared by Bajpai and Shrivastava (15) by developing an interpenetrating polymer network (IPN) of poly(ethylene glycol) and poly(2-hydroxyethyl methacrylate-co-acrylamide). The authors noted that the prepared hydrogel surface were fairly antithrombogenic.

Thus, realizing the significance of IPNs in materials science, the present paper aims at developing a well-characterized IPN of gelatin and polyacrylonitrile and investigating water sorption capacity, microhardness and *in vitro* blood compatibility of the polymer matrix. We have chosen gelatin, a hydrophilic protein fragments derived from collagen (16), as a model protein backbone in the construction of the IPN, due to its ability to be modified at the level of amino acids, its low level of immunogenicity and cytotoxicity, and its FDA approval as a clotting agent and exudate-absorbing construct (17–19). Gelatin can be chemically crosslinked with glutaraldehyde, thus producing covalent cross-links between the ϵ -amino group of the lysine residues of gelatin (20). Glutaraldehyde has been employed extensively for crosslinking proteins and polysaccharides in controlled drug-delivery systems and in other biomedical devices such as tissue grafts (21). The other component of the proposed IPN is polyacrylonitrile, which is a semi-crystalline vinylic homopolymer with the repeating unit $-(CH_2-CH_2CN)-$ usually in atactic form. PAN is one of the versatile polymers that are widely used for making membranes and offers good resistance (22) to a wide range of solvents. PAN shows good mechanical strength as film and is more thermally stable.

Experimental

Materials

Gelatin (Type A, isoelectric point 7.6) used as a pre-formed biopolymer was obtained from Qualigens, Mumbai, India and used as received. Acrylonitrile was purchased from Research Lab, Mumbai, India and freed from the inhibitor by successive washing the monomer thrice with 5% NaOH, 5% H_2SO_4 and distilled water and finally distilling the

washed monomer under vacuum. Glutaraldehyde and N, N'-methylene bisacrylamide were obtained from Loba Chemie, Mumbai, India and used as crosslinkers for gelatin and polyacrylonitrile, respectively. Potassium persulphate (KPS) and potassium metabisulphite (KMBS) employed as polymerization initiator, and activator respectively were obtained from Loba Chemie, India and used as received. Triple distilled water was used throughout the investigation.

Preparation of IPN

IPNs were prepared by redox polymerization method as described in our earlier communications (23). In a typical experiment, into a 10 ml of gelatin solution (4.0 g) were added 30.9 mM of acrylonitrile (AN), 0.13 mM of MBA, 1.06 mM of glutaraldehyde and 1 ml each of potassium metabisulphite (0.01 M) and potassium persulphate (0.001 M). The reaction mixture placed in a rectangular glass pellet (60 mm × 35 mm × 5 mm) was kept at room temperature for 48 h. The IPN so prepared was taken out carefully and allowed to swell for 72 h so that the unreacted monomer and other chemicals were leached out. The IPN film was dried at room temperature for one week, and cut into equal sized square pieces. The dried IPNs were stored in airtight polyethylene bags.

IR Spectra

The IPNs were characterized by IR spectral analysis (Perkin_Elmer, 1000 Paragon).

Scanning Electron Microscopy

The SEMs of the pure gelatin and the prepared blend were recorded on a scanning electron micrograph (STEREO SCAN, 430, Leica SEM).

DSC Measurements

Differential scanning calorimetry measurements of prepared IPNs were recorded on a DSC instrument (2100, DuPont) in the temperature range 25° to 400°C under N₂ atmosphere and at a heating rate of 10°C/min.

Water Sorption Measurements

In order to evaluate water uptake potential of IPNs, a gravimetric procedure was adopted as described elsewhere (24). In brief, pre-weighed pieces of IPN was placed in a water reservoir and allowed to swell till equilibrium swelling. The swollen pieces were then taken out, gently pressed in between two filter papers to remove the excess water and finally weighed in a sensitive electronic balance (No. APX-203 Denver Instruments, GmbH Germany). The swelling ratio was calculated by the following equation:

$$\text{Swelling Ratio} = \frac{\text{Weighted of Swollen IPN}}{\text{Weight of Dry IPN}} \quad (1)$$

Network Parameters

One of the most important structural parameters characterizing a crosslinked network is the average molecular mass between crosslinks (M_c) and crosslink density (q). The

magnitude of M_c and q significantly affects the physical and mechanical properties of the end polymer. Equilibrium swelling is widely used to determine M_c and q . The values of these structural parameters may be calculated by the following equations as given by Flory and Rehner,

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

$$q = M_o/M_c \quad (3)$$

where V is the molar volume of water (ml mol^{-1}), d_p is the IPN density; V_s is the volume fraction of polymer in the IPN approximately equal to $1/(1 + \text{Swelling Ratio})$ (25); χ is the Flory-Huggins interaction parameter between IPN and solvent (water); and M_o is the molar mass of repeat unit of polyacrylonitrile.

In the present case, the network parameters have been calculated for crosslinked polyacrylonitrile only. The values of χ for polyacrylonitrile were taken from the literature (26).

Some authors defined crosslink density in terms of the number of elastically effective chains given by the following equation:

$$V_e = d_p \frac{N_A}{M_c} \quad (4)$$

where N_A being the Avogadro number.

The values of M_c , q and V_e have been calculated for different IPN compositions and are summarized in Table 1.

Microhardness Measurements

The microhardness of the IPNs was quantified in terms of the Vicker's hardness number (H_v) determined with a mhp 160 microhardness tester equipped with a Vicker's diamond pyramidal indenter having a square base and 136° pyramidal angle attached to a Carl Zeiss NU2 universal research microscope (27). The values of H_v was calculated by the following relation:

$$H_v = \frac{1.854 \times L}{d^2} \text{ kg/mm}^2 \quad (5)$$

Table 1
Network parameters of the IPNs of different compositions

S. no.	Gelatin (g)	AN (mM)	MBA (mM)	S. R.	M_c	$q \times 10^{-4}$	$V_e \times 10^{19}$
1.	4	15.4	0.13	8.8	11056.1	51.14	2.39
2.	4	23.1	0.13	7.9	7882	68.51	3.36
3.	4	30.9	0.13	7.0	4323.14	124.91	6.13
4.	4	30.9	0.096	8.0	11940.29	45.23	2.22
5.	4	30.9	0.064	10.0	7818.69	69.07	3.39

where L is the load in kg and d is diagonal of indentation in mm. Indentations at each load were obtained in replicate number and an average hardness number was calculated.

Statistical Analysis

All measurements were performed in triplicate and the graphs were plotted taking mean values of the measurements.

Results and Discussion

IR Spectra

The IR spectra of pure gelatin, pure PAN and IPN are depicted in Figures 1(a, b, and c), respectively. The spectra of uncrosslinked gelatin shows two distinct absorption bands, the C=O stretching at 1629 cm^{-1} and the N-H stretching at 3462 cm^{-1} as also reported in the literature (28). The IR spectra of the IPN shown in Figure 1(b) not only confirms the presence of gelatin and crosslinked PAN but also provides significant information about the nature of the network formed. The peak observed at higher frequency (3567 cm^{-1}) due to N-H stretching of gelatin suggests formation of weak hydrogen bonds between N-H and C=N of PAN. Moreover, the peak observed at 1489 cm^{-1} clearly suggests an aldimine group of crosslinked gelatin. In addition to the commonly observed peaks of PAN, i.e., 2260 cm^{-1} (C≡N), 970 cm^{-1} (CH₂ twisting) and 440 cm^{-1} (C-CN wagging), a strong band is seen at 1703 cm^{-1} , thus indicating the presence of carbonyl stretching. What actually happens is that the presence of air

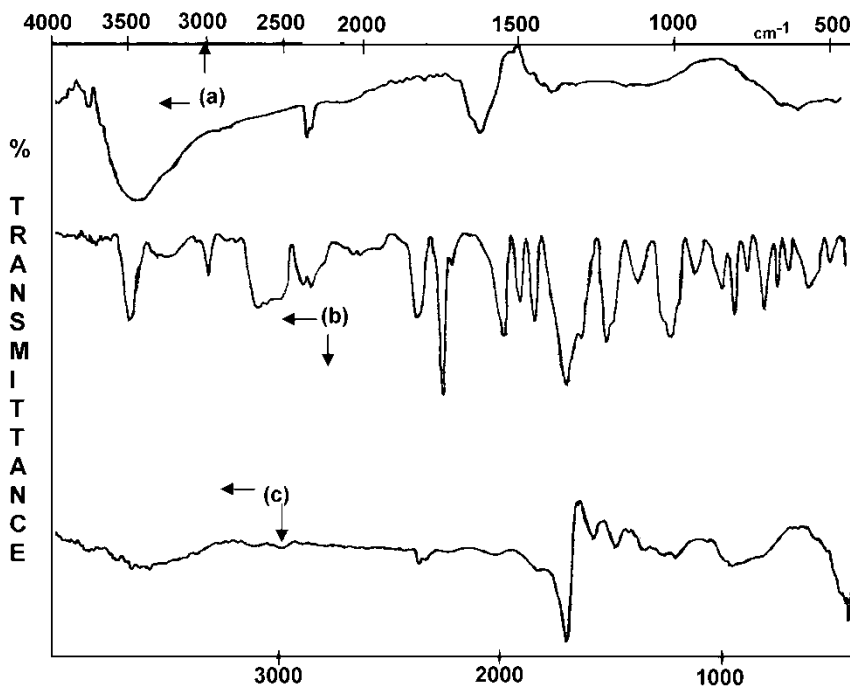


Figure 1. IR spectra of (a) native gelatin, (b) PAN, and (c) gelatin-polyacrylonitrile IPN.

during polymerization reaction hydrolyzes the nitrile groups present along the polymeric chain (29) and converts them into amide ones. Thus, based on the above discussion, the structure of the IPN may be imagined as an entangled macromolecular matrix in which crosslinked gelatin and crosslinked PAN chains are held to each other via physical type of forces.

SEM Analysis

The micrographs (Figure 2) clearly depict that, whereas in the SEM image of gelatin (a), there appears clusters of the aggregated gelatin molecules on the surface, thus forming a heterogeneous surface. On the other hand, the image of the IPN surface (b) indicates a more homogeneous surface with hydrophobic moieties scattered on the hydrophilic gelatin matrix.

DSC Studies

The thermal studies provide information not only about the thermal properties of the material, but also gives clues about its structure. In order to gain insight into the structure of the IPNs, DSC curves of IPNs of various compositions were constructed as discussed below.

Effect of Gelatin. The DSC curves of the IPNs containing varying amounts of gelatin in the range 1.0 to 4.0 g are shown in Figure 3(a, b, and c). Generally, an endotherm located at a T_g indicates time-dependent thermal relaxation in the polymer. As shown in the figure, two glass transition temperatures for gelatin are observed in each thermogram. The block copolymer model for the amino acid content of the gelatin has explained this occurrence (30). The first glass transition temperature is a minor one, located around 80–100°C and associated with the glass transition of α -amino acid blocks in the peptide chain. Detection of this is not easy with a hydrophilic material, as water is also lost in the same temperature range. The second, more intense, glass transition temperature is located around 180–200°C, and represents the blocks of imino acids, proline, hydroxyproline, with

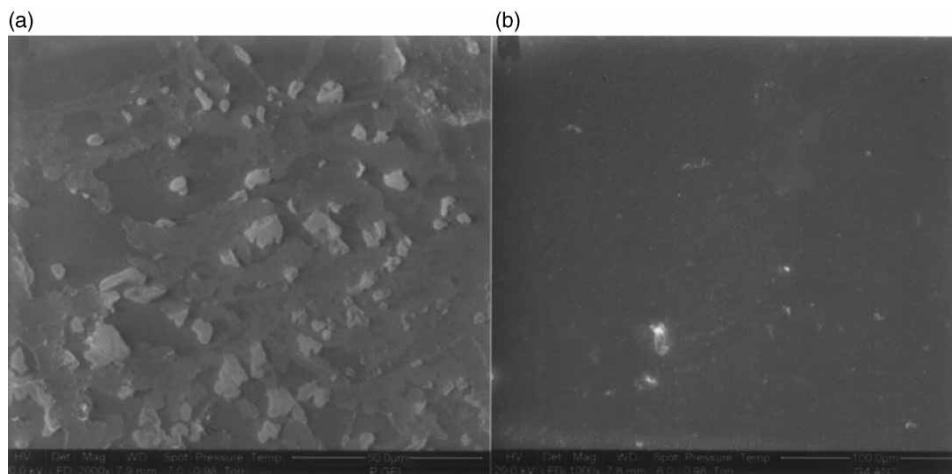


Figure 2. Scanning electron micrographs (SEMs) of (a) gelatin, and (b) IPN.

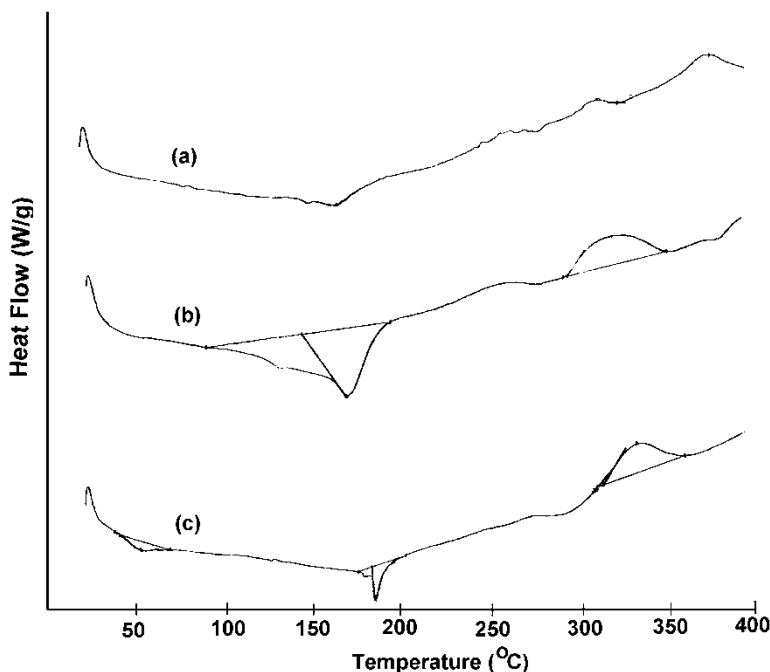


Figure 3. DSC thermograms of the IPNs of varying gelatin content for a definite concentration of [AN] = 30.9 mM, [GA] = 1.06 mM, and [MBA] = 0.13 mM, gelatin (a) 1.0 g, (b) 2.0 g, (c) 4.0 g.

glycine. The second T_g is apparently responsible for the overall physical behavior of gelatin and is one of the most cited and studied.

Another remarkable feature displayed by the thermograms is the influence of increasing gelatin on the second T_g. It is clear from the curves that at 1.0 g gelatin, T_g is 166°C, at 2.0 g it shifts to 170°C, while at 4.0 g of gelatin, T_g appears at 186°C. Thus, a shift in T_g of gelatin also confirms the increasing degree of crosslinking in the IPN as reported elsewhere (31). Although the thermograms do not show a sharp endotherm at T_g of PAN (which is 105°C), however, melting endotherm of PAN are clearly seen in the range 300 to 350°C.

Effect of PAN. DSC thermograms of the IPNs at 15.4 and 30.9 mM of AN are shown in Figure 4. The curves clearly point out that with increasing AN content in the IPN the endotherms have become quite broad and display a large peak area. This obviously suggests that with increasing PAN in the IPNs crystallinity also increases. The thermograms also reveal that at higher PAN content a small endotherm appears at 125°C, which may be assigned to the glass transition temperature of the PAN.

Effect of MBA. In the present study, the PAN has been crosslinked with MBA and its influence on the DSC thermograms has been depicted in Figure 5(a, b) which shows two curves at 0.064 and 0.13 mM of MBA. It appears from the thermograms that as the concentration of crosslinker increases, the T_g of IPN slightly increases from 324 to 332°C, which is also expected. Another feature displayed by the melting endotherms of PAN is that the peak area substantially decreases from 84 to 49 J/g, which indicates a reduction in the crystallinity of the IPN.

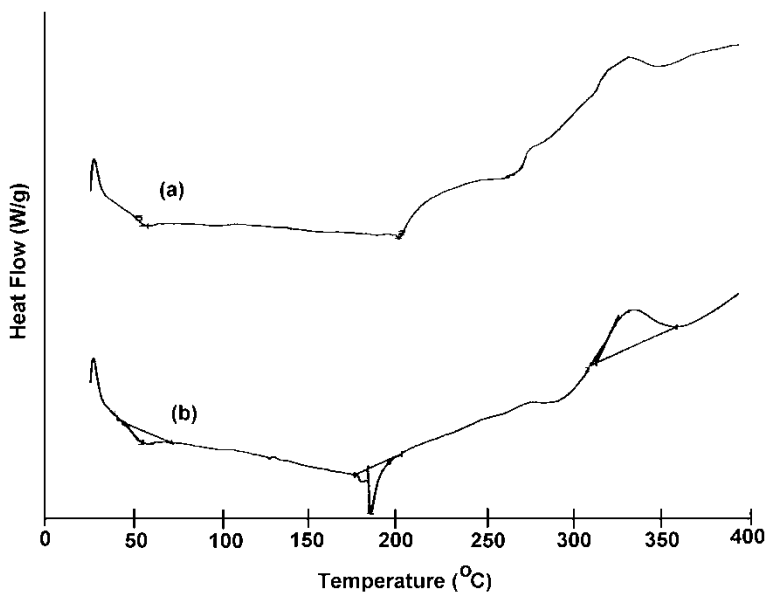


Figure 4. DSC thermograms of the IPNs of varying PAN content for a definite concentration of [gelatin] = 4.0 g, [GA] = 1.06 mM, and [MBA] = 0.13 mM, [AN] = (a) 15.4 mM, (b) 30.9 mM.

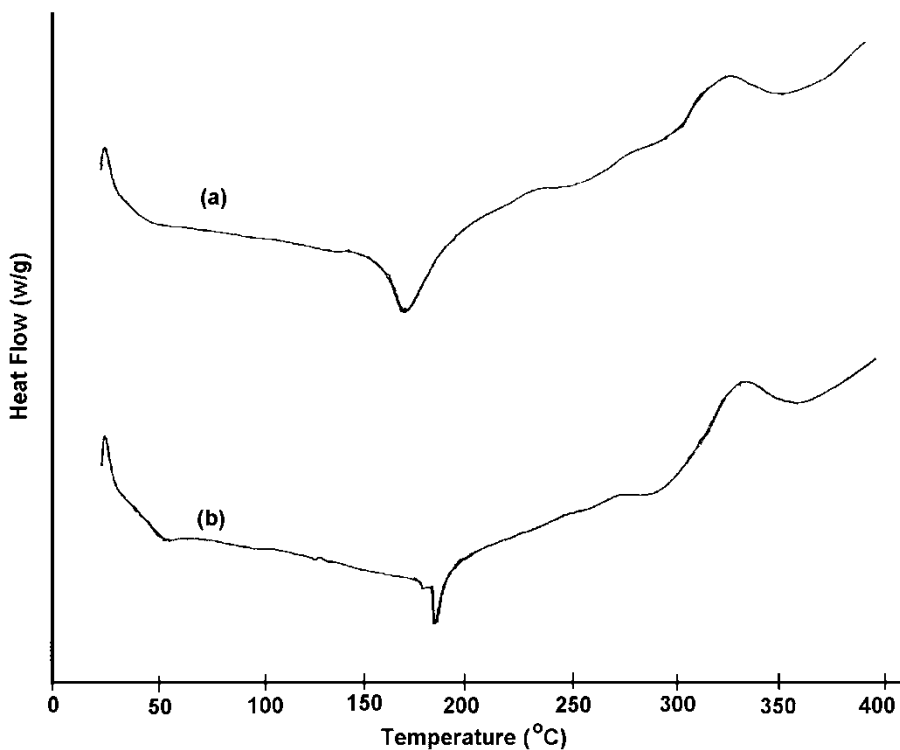


Figure 5. DSC thermograms of the IPNs of varying MBA content for a definite concentration of [gelatin] = 4.0 g, [GA] = 1.06 mM, and [AN] = 30.9 mM, [MBA] = (a) 0.064 mM, (b) 0.13 mM.

Swelling Study

As mentioned earlier, water content in a material partly determines its fate as a biomaterial and, therefore, has to be investigated as a function of the chemical architecture of the IPN.

Effect of Gelatin. Water uptake results indicate that there is a gradual fall in the swelling ratio of the IPN with increasing gelatin concentration in the range 1.0 to 4.0 g. The reason for the observed decrease in swelling ratio may be attributed to the fact that an increasing amount of gelatin in the IPN results in a greater extent of hydrogen bonding with the PAN and in the gelatin itself, thus yielding a compact network. This will obviously restrict the diffusion of water molecules into the IPN and consequently, result in a fall in swelling ratio.

Effect of PAN. Water uptake results (Table 1) clearly reveal that the swelling ratio constantly decreases when concentration of PAN is increased in the IPN in the range 15.4 to 30.9 mM. The noticed fall in water uptake may be explained by the fact that because of the hydrophobic nature of the polyacrylonitrile, its increasing amount in the IPN will result in an enhanced hydrophobic IPN, which will have less affinity for water. Furthermore, with an increasing PAN content in the IPN, the number of hydrogen bonds with gelatin will also increase and as a consequence, swelling ratio decreases. These qualitative results are further supported by the network parameter values as summarized in Table 1.

Effect of MBA. When the concentration of MBA increases in the range 0.064 mM to 0.13 mM, in the IPN, a significant fact in swelling ratio is noticed (Table 1). The observed fall in water uptake is quite obvious and has been largely reported in the literature (32). The reason for the suppressed swelling is that because of a larger concentration of MBA in the IPN, a greater number of crosslinks will be produced in the IPN, which clearly results in narrow mesh sizes of network pores. This will obviously allow a smaller number of water molecules to enter into the IPN and, therefore, the swelling ratio decreases. The network parameters data also support the above explanation.

Microhardness Study

Microhardness of a material is greatly dependent on the chemical and morphological nature of the material and, therefore, by a proper selection of the components of the material, the hardness of the material may be desirably altered. The forthcoming paragraph discusses the impact of chemical composition of the IPN on its microhardness at varying values of applied loads (10 to 100 g).

Effect of Gelatin. The influence of gelatin content of the IPN on their microhardness has been investigated by varying the concentration of gelatin from 1.0 to 4.0 g. The results are shown in Figure 6, which reveal that the value of H_v increases with increasing gelatin content of the IPN. The results may be explained by the fact that increasing gelatin tends to harden the network because of an increasing number of hydrogen bonds. The increased number of bonds causes an increase in the value of T_g with increasing the content of gelatin.

The results also indicate that the value of H_v increases with increasing load (10–100 g) on the sample. The observed increase in H_v is due to the reason that as the load

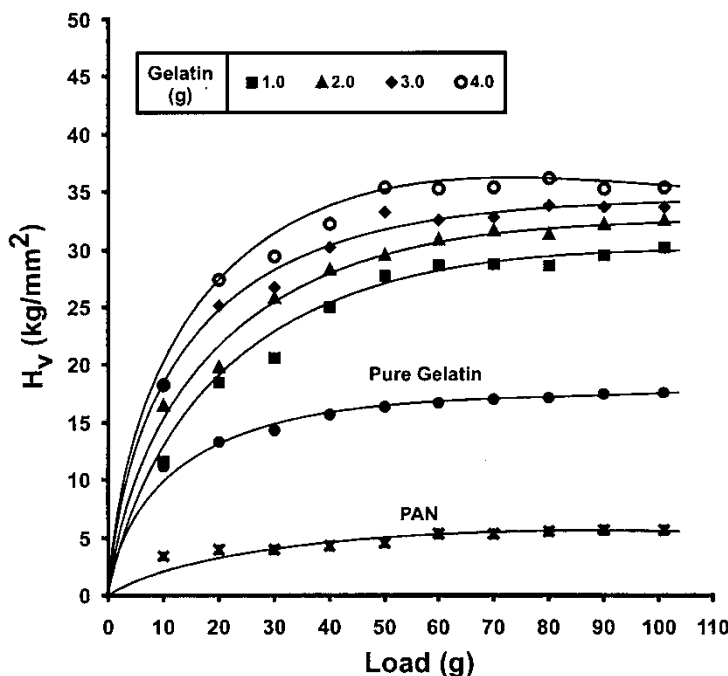


Figure 6. Influence of gelatin on the hardness number (H_v) of the IPNs for a definite concentration of $[AN] = 30.9$ mM, $[GA] = 1.06$ mM, and $[MBA] = 0.13$ mM.

increases, the IPN is subjected to a greater strain hardening (33). It is also noticed in the figure that the rate of strain hardening is greater at higher loads (20–60 g) and at higher gelatin content (3.0 and 4.0 g). The H_v attains a limiting value beyond the load of 60 g as H_v tends to be independent of the applied load, as the IPNs are fully strain hardened.

Effect of PAN. The influence of PAN content in the IPNs on their microhardness has been studied by rising the AN concentration in the range of 15.4 to 30.9 mM. The results are shown in Figure 7, which indicate that the Vickers microhardness number (H_v) increases with increasing PAN content in the IPN. The observed results are mainly expected, as PAN being a hydrophobic polymer, brings about an increase in hydrophobicity of the IPN, which directly results in increased hardness. The value of H_v also increases with an increasing load, which can again be explained by the phenomenon of the 'strain hardening effect'. Increased hydrophobicity within the IPN also induces hydrophobic interaction between the PAN chains and, thus, causes hardening of the material. The increase in crystallinity with PAN content, as detected from an increase in the T_g , also contributes to hardening of IPNs.

Effect of MBA. The number of crosslinks in an IPN is another decisive factor of hardness of the material. The effect of crosslink density of the IPN has been investigated on the H_v of the IPN by varying the concentration of MBA in the range 0.064 to 0.13 mM as shown in Figure 8. The results clearly indicate that the microhardness of the IPN increases with increasing concentration of MBA. The results are quite obvious and may be explained by the fact that due to an increase in crosslink density of the IPN, the network chains come

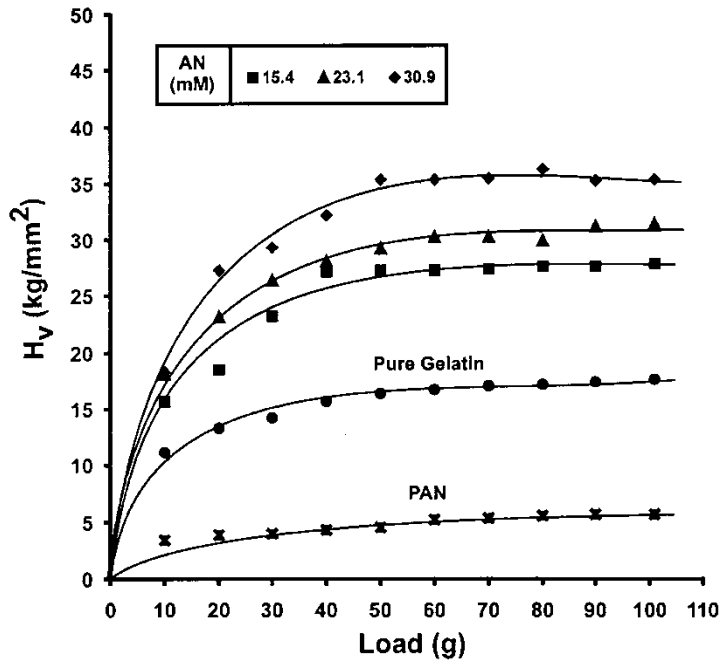


Figure 7. Effect of PAN on the hardness number (H_V) of the IPN for a definite concentration of [gelatin] = 4.0 g, [AN] = 30.9 mM, [GA] = 1.06 mM and [MBA] = 0.13 mM.

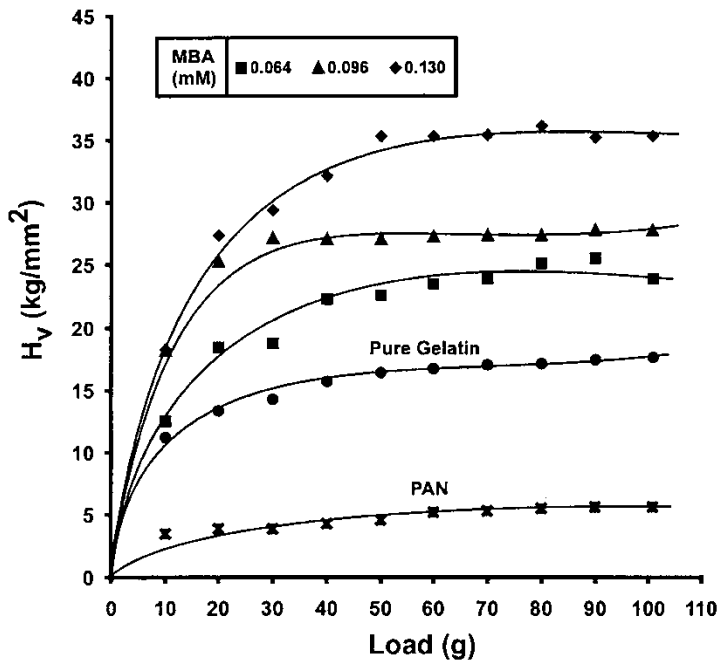


Figure 8. Influence of MBA on the hardness number (H_V) of the IPNs for a definite concentration of [gelatin] = 4.0 g, [AN] = 30.9 mM, [GA] = 1.06 mM, and [MBA] = 0.13 mM.

much closer to each other and, therefore, a dense and compact network is obtained. One of the remarkable features in this study is that for the sample with 0.096 mM MBA exhibits an almost stabilized variation of H_v with load beyond 30 g. This reveals the optimization of the content of MBA in the IPN at this value for yielding samples as far as its load dependence is concerned. The microhardness (H_v) has also been found to increase with increasing load and the observed increase in H_v , may again be due to a 'strain hardening effect'.

Blood Compatibility

Since the first attempt in 1950s to develop the blood compatible materials with negatively charged surfaces for the artificial vessels, continuous efforts to design biomaterials with superior blood compatibility have been made by various research groups (34). 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 (35), 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 the presence of specific chemical groups on the surface (36). In addition, it has been recently pointed out that the water structure on the surface of the material is one of the most important factors affecting blood compatibility (37). In spite 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, and protein (albumin) adsorption tests. The results are summarized in Table 2 and may be explained 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, non-polar, charged, uncharged parts of the proteins and the nature of the polymer surface (38). In the present study, the adsorption of BSA onto swollen IPN was determined and the results are summarized in Table 2. The results clearly indicate that the amount of BSA adsorbed

Table 2
Blood compatibility parameters of the IPNs of different compositions

S. no.	Gelatin (g)	AN (mM)	MBA (mM)	Blood clot (mg)	Protein adsorption (mg g^{-1})	% Hemolysis
1.	1	30.9	0.13	2	14	46.7
2.	2	30.9	0.13	4	2.198	25
3.	4	15.4	0.13	12	1.083	10.47
4.	4	30.9	0.13	6	1.507	7.9
5.	4	30.9	0.064	7	6.28	13.2

drastically decreases with an increasing gelatin content of the hydrogel. A decreasing protein (BSA) adsorption obviously implies an increasing blood compatibility, which is further confirmed by the observed lower values of percent hemolysis. The observed enhanced blood compatibility parameters may be attributed to the fact that increasing the gelatin content may result in formation of hydrophilic micro domains in the gel network, 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 adhesion to polymer surface (39). Thus, the above mentioned facts may be regarded as responsible factors for less protein adsorption, lower clot formation and a decreased degree of hemolysis. The observed findings may also be explained by the fact that because of the biocompatible property of gelatin, its increasing proportion in hydrogel will certainly result in a more blood compatible polymer. Another reason may be that the gelatin chains graft onto gelatin backbone via free amino groups, which are known to form polyelectrolytic complexes with acidic groups of cellular elements of blood (40). This is due to a reduction in the number of amino groups the blood compatibility of hydrogel blend will increase.

Another reason for the observed higher blood compatibility with the increasing gelatin content may be attributed to the fact that at a 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 (41). The influence of the crosslinker content of the IPN on the blood compatibility of prepared blends has been investigated by varying the concentration of MBA in the feed mixture of the blend in the range of results summarized in Table 2. This clearly indicates that whereas the amount of BSA adsorbed decreases with increasing MBA, a decrease is also noticed in the blood clot weight and percent hemolysis. The observed enhanced blood compatibility of greatly a crosslinked IPN may be attributed to the reason that because of a higher degree of crosslinking, the overall morphology of the IPN acquires compactness, thus resulting in a smooth surface hydrogel, which imparts an enhanced blood compatibility of the IPN.

On increasing the concentration of acrylonitrile (AN) in the feed mixture of the IPN, it is found that the amount of adsorbed protein slightly increases with increasing the concentration of AN in the range. It is, however, surprising that at the same time, both the blood clot weight and percent hemolysis decreases in spite of an increase observed in the adsorbed BSA. The observed increasing protein adsorption may be attributed to the fact that with an increasing PAN content in the hydrogel, the degree of hydrophobicity increases, which may favor protein adsorption. However, the observed enhanced blood compatibility may be attributed to the structured water molecules (free or bound) in the swollen IPN.

Conclusions

A full IPN of crosslinked gelatin and polyacrylonitrile results in a hard biomaterial that also displays water uptake potential. It is found that with increasing concentrations of gelatin, PAN and MBA, the IPN shows a decreasing tendency of water sorption. The

IPNs exhibit a fair degree of microhardness, which increases with increasing the content of gelatin, PAN and MBA in the IPN. This is also confirmed with the thermal studies, which reveals the increase in the value of glass transition temperature of IPNs. The IPN is found to contain crosslinked gelatin and PAN chains as confirmed by the IR spectral study. The *in vitro* blood compatibility of the prepared IPNs also depends on the chemical architecture of the network.

References

1. De, D.R., Kajiwar, K., Osada, Y., and Yamauchi, A. (1991) *Polymer Gels: Fundamental and Biomedical Applications*; Plenum Press: New York.
2. Stevens, K.R., Einerson, N.J., Burmania, J.A., and Kao, W.J.J. (2002) *In vivo* Biocompatibility of gelatin based hydrogels and IPNs. *Biomater. Sci. Polym. Ed.*, 13 (12): 1353.
3. Lopes, C.M.A. and Felisberti, M.I. (2003) Mechanical behavior of biocompatibility of poly(1-vinyl-2-pyrrolidone) gelatin IPN hydrogel. *Biomaterials*, 24: 1279.
4. Nguyen, K.T. and West, J.L. (2003) Photopolymerizable hydrogels for tissue engineering applications. *Biomaterials*, 23: 4307.
5. Matousek, J., Pouckova, P., Soucek, J., and Skvor, J. (2002) PEG chains increase as permato-genic and anti-tumor activity of RNase A and BS-RNase enzymes. *J. Control Release*, 82: 29.
6. An, Y. and Hubbell, J.A. (2000) Intraarterial protein delivery via intimately adherent bilayer hydrogels. *J. Control Release*, 64: 205.
7. Bajpai, A.K. and Bhanu, S. (2004) *In vitro* release dynamics of insulin from a loaded hydrophilic polymeric network. *J. Mater. Sci. Mater. Med.*, 15: 43.
8. Lipatov, Y.S. and Karabanova, L.V. (1994) *Advances in Interpenetrating Polymer Networks*; Technomic: Lancaster; Vol. 4.
9. Fan, X.-D., Hsieh, Y.-H., and Krochta, J.M. (2002) Thermal and mechanical behavior of poly(vinyl-alcohol)-lactose blends. *J. Appl. Polymer Sci.*, 83: 929.
10. Nakane, K., Yamashita, T., Iwakura, K., and Suzuki, F. (1999) Properties and structure of poly(vinyl-alcohol)/silica composites. *J. Appl. Polymer Sci.*, 74: 133.
11. Balsubramani, M., Kumar, T.R., and Babu, M. (2001) Skin substitutes: A review. *Burns*, 27: 534.
12. Nishiyama, K., Miyama, M., Frie, O., Osawa, T., Ishi, N., Arai, N., Kawase, T., and Saito, S. (1994) Re-evaluation of the biocompatibility of new ceramics, hydroxyapatite, titanium and silica, used in chromatographic applications. *Bull. Kangawa Dental College*, 22 (2): 139.
13. Krumova, M., Lopez, D., Benavente, R., Mijangos, C., and Perena, J.M. (2000) Effect of cross-linking on the mechanical and thermal properties of poly(vinyl alcohol). *Polymer*, 41: 9265.
14. Nge, T.T., Yamaguchi, N., Hori, A., Takemura, A., and Ono, H. (2002) Synthesis and characterization of chitosan/poly (acrylic acid) polyelectrolyte complex. *J. Appl. Polymer Sci.*, 83: 1025.
15. Bajpai, A.K. and Shrivastava, M. (2002) Water sorption dynamics of a binary copolymeric hydrogel of 2-hydroxyethylene-methacrylate (HEMA). *J. Biomater. Sci. Polymer Edn.*, 13 (3): 237–256.
16. Veis, A. (1964) *In The Macromolecular Chemistry of Gelatin*; Horecker, B., Kaplan, N.O. and Scheraga, H.A., eds.; P.S. Academic Press: New York.
17. Stevens, K.R., Einerson, N.J., Burmania, J.A., and Kao, W.J. (2002) *In vivo* biocompatibility of gelatin-based hydrogels and interpenetrating networks. *J. Biomater. Sci. Polymer Ed.*, 13 (12): 1353.
18. Olde, Damink, L.H.H., Dijkstra, P.J., Van Luyn, M.J.A., Van Wanchen, P.B., Nieuwebhuis, P., and Feijen, J. (1995) Glutaraldehyde as a crosslinking agent for collagen-based biomaterials. *J. Mater. Sci. Mater. Med.*, 6: 460.
19. Choi, Y.S., Hong, S.R., Lee, Y.M., Song, K.W., Park, M.H., and Nam, Y.S. (1999) Studies on gelatin containing article skin: II, Preparation and characterization of crosslinked gelatin-hyaluronate sponge. *J. Biomed. Mater. Res. (Appl. Biomater.)*, 48: 631.

20. Kronick, P.L., Cooke, P., and Maleeff, B. (1990) Structure, molecular biology and pathology of collagen. In *Annals of the New York Academy of Sciences*; Fleischmajer, R., Olsen, B.R., and Kuhn, K., eds.; Vol. 580.
21. Jayakrishnan, A. and Jameela, S.R. (1996) Glutaraldehyde as a fixative in bioprotheses and drug delivery matrices. *Biomaterials*, 17: 471.
22. Deepak, A.M. and Ashwini, K. (2000) Solvent and pH resistance of surface crosslinked chitosan/poly(acrylonitrile) composite nanofiltration membranes. *J. Appl. Polym. Sci.*, 77: 1782.
23. Bajpai, A.K. and Shrivastava, M. (2001) Adsorption dynamics of bovine serum albumin (BSA) onto binary interpenetrating polymer networks (IPNs) of poly(2-hydroxyethyl methacrylate) (PHEMA). *Journ. Mac. Sci., Pure & Appl. Chem.*, A38 (11): 1123.
24. Bajpai, A.K., Bajpai, J., and Shukla, S. (2003) Release dynamics of tetracycline from a loaded semi IPNs material of PVA and poly(acrylamide-co-styrene). *J. Mater. Sci. Mater. Med.*, 14: 347.
25. Finch, C.A. (1985) *Chemistry and Technology of Water Soluble Polymers*; Finch, C.A., ed.; Plenum Press: New York, Chapter 5, p. 81.
26. Brandrup, J. and Immergut, E.H., eds. *Polymer Handbook*; Wiley-Interscience: New York, 1967.
27. Awasthi, S.K. and Bajpai, R. (2001) Microhardness and X-ray diffraction studies on polymer blends of poly (ethylmethacrylate) (PEMA) and poly(ethylene oxide) (PEO): Plasticization and crystallization aspects. *Ind. J. Pure Appl. Phys.*, 39: 795.
28. Akin, H. and Hasirci, N.J. (1995) Preparation and characterization of crosslinked gelatin microspheres. *Appl. Polymer Sci.*, 58: 95.
29. Hebeish, A., ElAlfy, E.A., Abouzeid, N.Y., and Waly, A. (1980) Graft copolymerization of vinyl monomers onto modified cottons. XII Grafting of 1,1-dihydroperfluoroheptyl acrylate onto cellulose carbamate using hydrogen peroxide as initiator. *J. Appl. Polym. Sci.*, 25: 223.
30. Fraga, A.N. and Williams, R.J. (1985) Thermal properties of gelatin films. *Polymer*, 26: 113.
31. Vazquez, B., Roman, J.S., Peniche, C., and Cohen, M.E. (1997) Polymeric hydrophilic hydrogels with flexible hydrophobic chains. Control of the hydration and interactions with water molecules. *Macromolecules*, 30: 8440.
32. Bajpai, A.K., Bajpai, J., and Shukla, S. (2001) Water sorption through a semi-IPN with hydrophilic and hydrophobic chains. *React. Funct. Polymers*, 50: 9.
33. Bajpai, R., Agrawal, P., and Datt, S.C. (1994) Plasticization in poly(methyl methacrylate) and poly(chlorotrifluoroethylene) blends detected by microhardness measurement. *Polymer Testing*, 13: 103.
34. Ratner, B.D. (1996) In *Biomaterials Science*; Ratner, B.D., Hoffman Schoen, A.S. and Lemons, J.E., eds.; Academic Press: San Diego.
35. Tsai, W.B., Grunkermetzer, J.M., Horbett, T.A., and Lew, K.R. (1999) Human plasma fibrinogen adsorption and platelet adhesion to polystyrene. *J. Biomed. Mater. Res.*, 44: 130.
36. Tsuruta, T. (1996) Contemporary topics in polymeric materials for biomedical applications. *Adv. Polym. Sci.*, 126: 1.
37. Tanaka, M. and Mochizuki, A. (2004) Effect of water structure on blood compatibility—thermal analysis of water in poly(meth)acrylate. *J. Biomed. Mater. Res.*, 68A: 684.
38. Fang, F. and Szleifer, I.K. (2001) Kinetics and thermodynamics of protein adsorption: A generalized molecular theoretical approach. *J. Biophys.*, 80: 2568.
39. Grainger, D., Okano, T., and Kein, S.W. (1987) *Advance in Biomedical Polymer*; Gebelein, C.G., ed.; Plenum Press: New York.
40. Aita, S., Minoura, N., Tagudu, K., and Fujiwara, Y. (1987) Covalent immobilization of chitosan derivatives on to polymeric film surface with the use of a photosensitive hetero-bifunctional crosslinking reagent. *Biomaterials*, 8: 481.
41. Norde, W. and Lyklema, J. (1985) Surface and interfacial aspects of biomedical polymers. In *Proton and Electrokinetic Studies of Adsorbed Protein Layers*; Andrade, J.D., ed.; Plenum Press: New York.