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Studies on Mechanical and Antithrombogenic Behaviors of Polyvinyl Alcohol and Gelatin Based Novel Binary Polymer Blends with Grafted Polyacrylamide Chains

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The aim of this study was to design a hydrophilic but mechanically strong macromolecular matrix to discover possible application as polymer scaffolds for tissue engineering and wound healing dressings. To achieve the objectives, acrylamide was polymerized by a redox couple in the immediate presence of polyvinyl alcohol, gelatin and a crosslinking agent (N, N′–methylene bis acrylamide). The structural and thermal characterization of the prepared hydrogels were carried out by Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC) which confirmed the formation of polyacrylamide grafted network and presented combined thermal features of the constituent polymers in their thermogram. The Environmental Scanning Electron Microscopy (ESEM) techniques were utilized for morphological characterization and the hydrogels were found to exhibit a highly porous structure. The hydrogels were also investigated for water sorption capacity and the extent of water intake was found to depend on the chemical composition of the gel. The blend hydrogels were also tested for their tensile strength and it was found that for a definite composition of the constituents of the gel the hydrogel offered optimum mechanical properties like tensile strength, percent elongation and Young's modulus. Thus, the intended objectives to design mechanically strong and blood compatible hydrogels were achieved.

Keywords: Hydrogel, mechanical properties, characterization, hydrophilic polymer

1 Introduction

Over the past 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 (1). Commonly coined as 'hydrogels' or 'smart molecules', this unique class of macromolecular family exhibits several unusual characteristics such as elasticity, softness, transparency and permeability (2–4). As a result, such hydrogels are widely used as controlled drug delivery systems (5), soft contact lenses (6), artificial implants (7), actuators (8), burn dressings (9), agrochemical release carriers (10), water purification devices (11), etc.

Biomedical polymer chemists are facing challenges in providing synthetic connective tissues that predominately serve a biochemical role in the body such as articular cartilage, semicular vascular, tendons and ligaments. However, in order to replace the natural tissues with hydrogel, a number of significant engineering questions should be addressed such as the provision of low surface friction and wear, a suitable elastic modulus and high mechanical strength, both *in vivo* and *in vitro*. For instance, an articular cartilage that is a gel containing 70% water exhibits little wear under a loading as high as several to a hundred mega Pascal's and millions of cycles with a wide range of sliding velocity (12). It has been shown (13) that if a gel has free dangling polymer chains on its surface, its frictional coefficient becomes as low as 10^{-4}. From this viewpoint, gels have of high potential as an artificial articular cartilage (14–15).

In recent years, there has been felt of an ever–increasing need to engineer tissues and organs as the population continues to age and the requirement for organs and tissue continues to outpace the donor availability (12). One tissue engineering approach to address the tremendous demand for organs and tissues relies on polymer scaffolds

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to create an environment that closely mimics the natural environment of a developing tissue. In this sense, cells are isolated from a small, healthy tissue sample and seeded onto a polymer scaffolds designed to facilitate and direct the growth of a fully functional tissue (13). Although a variety of both synthetic and natural polymers such as poly (ethylene oxide), collagen, alginate etc. have been attempted to function as polymeric scaffolds (14), however, lack of desirable mechanical strength has always been realized.

Another important area of hydrogel application lies in wound healing dressings where a number of hydrophilic polymers have been tested. Although there is no general consensus, such a polymer dressing should obviously fulfill the following requirements; absorb effectively the body fluids and prevent their loss, act as an effective barrier against bacteria, adhere well to the wound but stronger to healthy skin, exhibit high elasticity but also some mechanical strength show good transparency, enable the oxygen to penetrate through the volume of dressing to the wound surface, enable to control drug dosage, offer good handling without causing pain (15). In addition, they must have an acceptable level of biocompatibility.

Now, it is clear from the preceding discussion that a hydrogel with adequate water content and mechanical performance could prove to be a potential candidate for biomedical applications like tissue engineering scaffolds and wound healing dressings. The only problem to be addressed is that there should be maintained a proper balance between the water content and mechanical strength of the hydrogel as these two properties are quite opposite to each other. Numerous workers have made serious attempts to design such mechanically strong, hydrophilic and biocompatible materials. For instance, Banthia and coworkers (16) prepared membranes of polyvinyl alcohol and gelatin and characterized them by various techniques. The authors also studied their mechanical properties and evaluated blood compatibility of the matrix. Thus, realizing the challenges in designing a hydrogel with fair mechanical strength, the present study has been undertaken that involves preparing a polymer matrix of binary blend nature composed of polyvinyl alcohol and gelatin with grafted polyacrylamide chains. Selection of polyvinyl alcohol (PVA) rests upon the fact that it is a well-known water-soluble synthetic polymeric material with good biocompatibility (17). Its cheap cost, easy availability and neutral nature in solution bring to its credit a wide spectrum of biomedical applications like contact lenses, ophthalmic materials (18), and tendon repair (19) and drug delivery (20). Another specialty of PVA is its crosslink ability by both chemical and physical methods (21), which impart mechanical strength to this polymer. The other component of the macromolecular matrix is gelatin which is a natural biopolymer with well recognized biocompatibility, non–carcinogenicity, biodegradability and non–toxicity (22). Likewise, polymers of acrylamide are well known for their hydrophilicity and inertness that make them a material of choice in large number of applications in medical and pharmacy (23).

2 Experimental

2.1 Materials

Polyvinyl Alcohol(PVA) (hot processed, MW $\sim$ 12,000, degree of hydrolysis 98.6%) and gelatin(GT), Type A (isoelectric point 7.6) were obtained from Merck Specialties Private Ltd., 'Mumbai, India and used as received. Acrylamide was obtained from Research Lab, Pune, India and recrystallized twice from ethanol and dried in vacuum over anhydrous silica for a week. N, N′–methylene bis acrylamide (MBA) (Central Drug House, Mumbai, India) was employed as a crosslinker and used without any pretreatment.

Potassium persulphate (KPS) and potassium metabisulphite (MBS) used as initiator for polymerization were obtained from Merck Specialties Private Ltd., Mumbai, India and used as received. All other chemicals used were of analytical grade and double distilled water was used throughout in all experiments.

2.2 Methods

2.2.1. *Preparation of Blend Hydrogel*

The hydrogels were prepared by redox polymerization method as reported elsewhere (24). In a typical experiment PVA 2.0 g, gelatin 1.0 g, acrylamide 1.5 g and water (25 mL) were added into a round bottom flask and the mixture was homogenized by mechanical mixing. Dry and oxygen free N_2 gas was purged through the mixture for 30 min and to this solution were added MBA 0.06 mM, KPS 0.36 mM and MBS 4.04mM. The resulting mixture was poured into a Petri dish and kept at 35°C for a week so that the whole mixture was converted into a soft rubbery mass. It is worth mentioning here that the hydrogel was attempted with higher molecular weights PVA also, but the hydrogel obtained with the mol.wt.12,000 was quite smooth and strong. On the other hand, the hydrogels with higher molecular weights were little bit brittle and, therefore, difficult to handle.

2.2.2. *Purification of Hydrogel*

The gel was then left in a water reservoir to equilibrium swelling so that all the remaining reactants were leached out and the gel became pure. The unreacted monomer (acrylamide), gelatin and PVA were estimated quantitatively by double bond estimation and UV spectrophotometer methods. The washing was continued till no tests were obtained which indicated for complete purification of the hydrogel.

The hydrogel film was dried at 35°C for a week and cut into smaller pieces of definite dimensions. The dried and purified hydrogel films were stored in polyethylene bags.

2.3 Characterization of Hydrogels

2.3.1. *FTIR Spectral Analysis*

The structural characterization of the prepared hydrogel was performed on a FTIR spectrophotometer (Paragon 1000, Perkin-Elmer Inc., Massachusetts, USA).

2.3.2. *DSC Measurements*

Differential scanning calorimetry measurements of prepared hydrogels were recorded on a DSC instrument (2100, DuPont, USA) in the temperature range 30 to 400°C under N_2 atmosphere and at a heating rate of 10°C/min.

2.4 Environmental Scanning Electron Microscopy (ESEM)

The morphology of the prepared blend hydrogel was studied by ESEM instrument (STEREO SCAN, 430, Leica ESEM) at Indian Institute of Technology, Pawai, Mumbai (India).

2.5 Mechanical Properties

The mechanical properties such as tensile strength, percentage elongation at break of the hydrogels were determined on the Automated Material Testing System (Instron Corporation Series LX, USA). The dumb–bell shaped (20 mm × 6 mm × 1.0 mm) specimens were used for the measurement. The temperature during the experiment was 25°C and relative humidity was 80%. Testing of hydrogels was carried out under tension at a crosshead speed of 100 mm/min. The final mechanical properties were evaluated from at least four independent measurements. Different specimens having varying chemical composition in respect to PVA, gelatin, acrylamide and MBA were tested for mechanical properties.

2.6 Blood Compatibility Tests

2.6.1. *Protein (BSA) 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 surfaces, following the batch–contact method (25). In a typical experiment, 20 ml BSA solution (0.4% w/v), prepared in phosphate buffer saline (PBS), (pH 7.4) containing pre–weighed and pre–swollen hydrogel pieces was mildly shaken for 30 min so as to avoid foam formation at the solution–air interface. After shaking, the supernatant solution was analyzed for residual BSA concentration by recording its absorbance at 277 nm (UV–visible, Double Beam Spectrophotometer, Systronics, Model No. 2201, Ahmedabad, India). The amount of BSA remaining in the solution was calculated by constructing a calibration plot. The amount of adsorbed BSA (mg g^{-1}) was calculated using the following equation:

$$\text{Adsorbed BSA}\,(\text{mg g}^{-1}) = \frac{(C_o - C_a)}{w} \times 20 \qquad (1)$$

Where C_o and C_a being the BSA concentration (mg ml^{-1}) before and after adsorption respectively and w is the weight of swollen gel (g).

2.7 Blood Clot Formation Test

The anti–thrombogenicity of hydrogel surface was evaluated by recording the weights of the clots as a result of surface blood interaction as described elsewhere (26). In brief, the hydrogels under investigation were hydrated in 0.9% saline water at 30°C for 24 h in a constant temperature bath. To these swollen gels was added 0.5 ml of acid citrate dextrose (ACD) blood followed by the addition of 0.03 ml of 4M $CaCl_2$ solutions, to start the thrombus formation. The thrombus formed was dried at 35°C for 48 h.

2.8 Haemolysis Assay

Haemolysis is defined as the release of hemoglobin into plasma due to damage of erythrocyte membrane (29). In a typical experiment, the hydrogel were equilibrated in 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 solution was added to each sample to stop

$$\text{Hemolysis}\,(\%) = \frac{\text{A test sample} - \text{A}\,(-)\,\text{control}}{\text{A}\,(+)\,\text{control} - \text{A}\,(-)\,\text{control}} \qquad (2)$$

haemolysis 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 spectrophotometer at 545 nm. The percent haemolysis was calculated using the relationship given above.

3 Results and Discussion

3.1 Scheme of Polymerization

The preparation of polymeric blend of poly (acrylamide–g–PVA) and poly (acrylamide–g–gelatin) may be modeled via the scheme of polymerization reaction shown in Figure 1.

3.2 FTIR Spectral Analysis

The infrared spectra of uncured reaction mixture of hydrogel and cured hydrogel film are shown in Figure 2(a) and (b), respectively, which clearly depict a significant difference between the two spectra. Whereas in uncured hydrogel spectra the peaks obtained are quite broad because of the

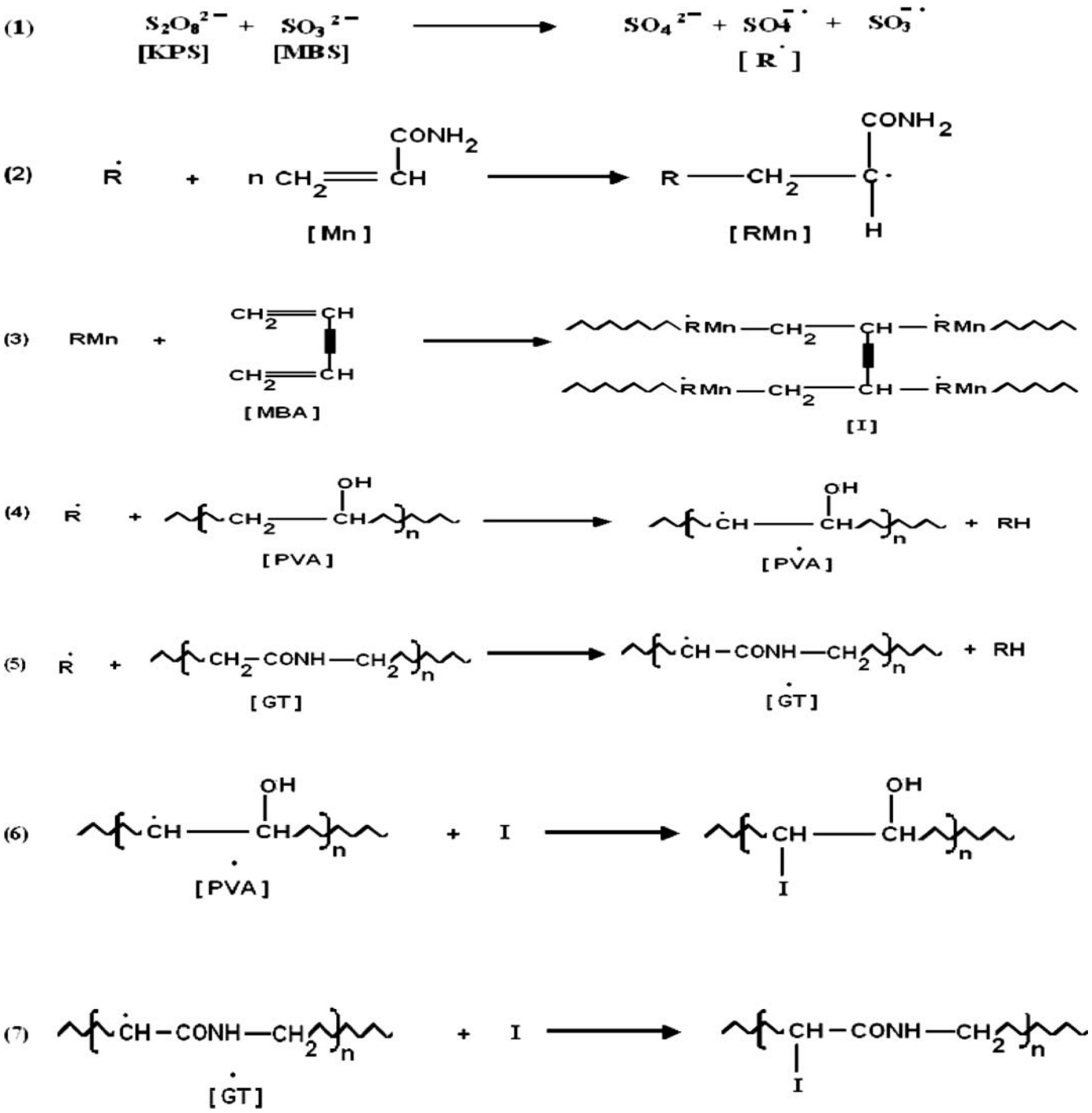

Fig. 1. The preparation of polymeric blend of poly (acrylamide–g–PVA) and poly (acrylamide–g–gelatin) may be modeled via the scheme of polymerization reaction.

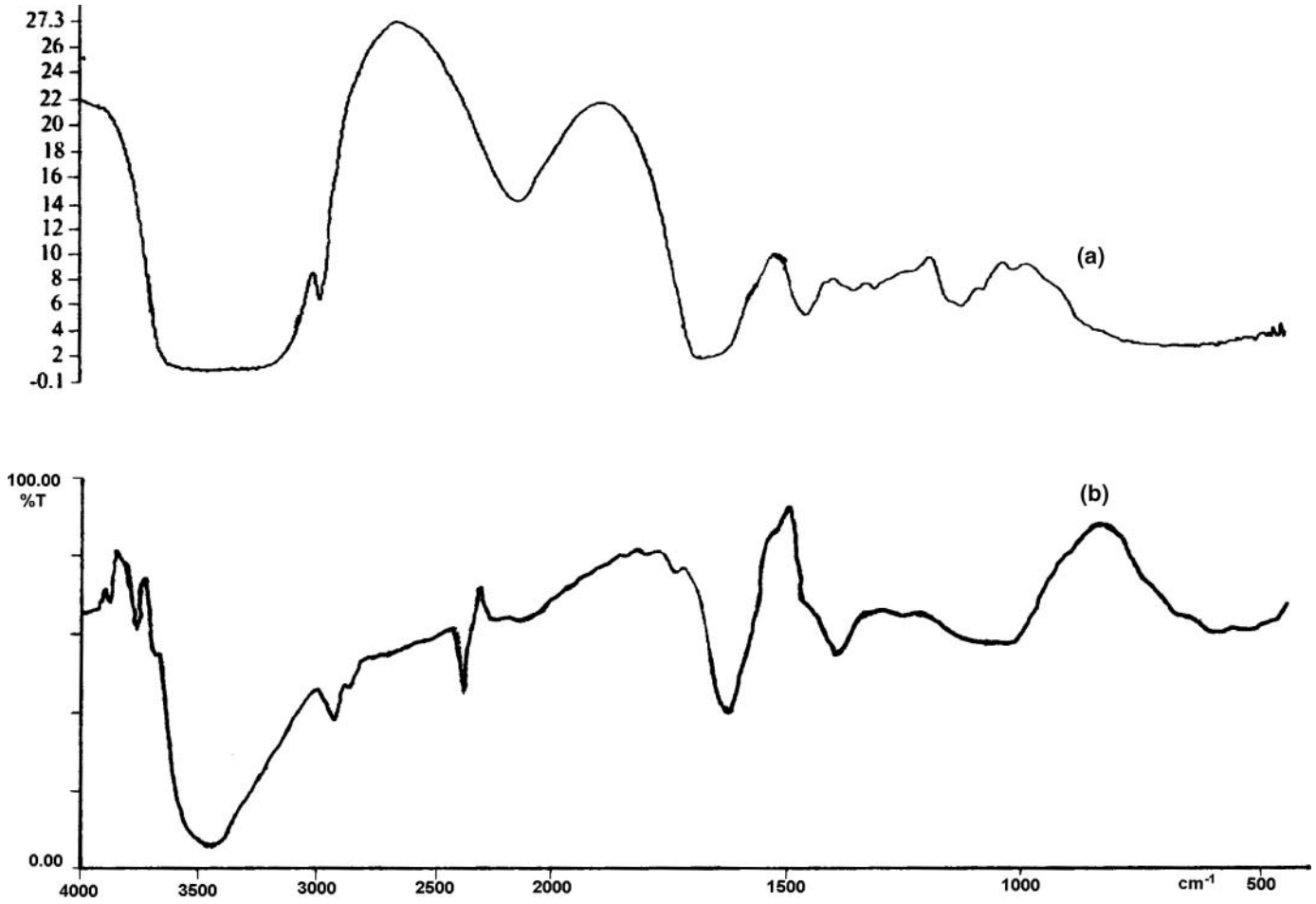

Fig. 2. FTIR spectra of (a) uncured and (b) cured hydrogels.

presence of water molecules in the feed mixture, the bands appearing in the spectra of cured hydrogel are quite sharp. Moreover, some new absorption peaks are also visible in the later spectra.

The spectra of hydrogel clearly marks the presence of both PVA and gelatin as evident from the observed peak at 3448 cm^{-1} which could be assigned jointly to O–H stretching of hydroxyl of PVA and N–H stretching of gelatin. It is observed that because of simultaneous occurrence of N–H and O–H stretching, separate appearance of respective peaks is rather not visible in the spectra. The spectra also shows characteristic absorption peak at 1630 cm^{-1} which is indicative of C=O stretching of gelatin and polyacrylamide. Other significant peaks shown in the spectra are at 2929 cm^{-1} (C–H stretching of PAM and PVA), 1399 cm^{-1} (CH_2 twisting of PVA and PAM), and 600–700 cm^{-1} (broad N–H out of plane bending of amide). It is also observed in the spectra of cured hydrogel that there is a disappearance of vinyl group peak in the cured hydrogel spectra as is evident from the band observed at 1434 cm^{-1} in the spectra (a), while it completely disappeared in the spectra (b). A prominent peak appeared at 2300 cm^{-1}in the spectra (b) suggests for the presence of water molecules which is obvious due to the hydrophilic nature of the gel.

In the present study, it has also been attempted to visualize the progress of the hydrogel formation by a continuous recording of IR spectra as shown in Figure 3. The spectra clearly reveal that as the hydrogel formation progresses, the spectral peaks become increasingly sharp and ultimately a well-defined spectrum is obtained.

3.3 DSC Analysis

The thermal behavior of prepared hydrogel was monitored by constructing DSC thermograms of pure gelatin and hydrogel as shown in Figure 4(a) and (b), respectively. The thermogram of pure (uncrosslinked) gelatin shows three prominent endotherms at 66, 176 and 195°C, which is an unusual observation. The first endotherm at 66°C may be attributed to the loss of moisture while the other two sharp endotherms observed at 176° and 195°C may be attributed to the block copolymer model for the amino acid contents of gelatin (24). These two glass transition temperatures

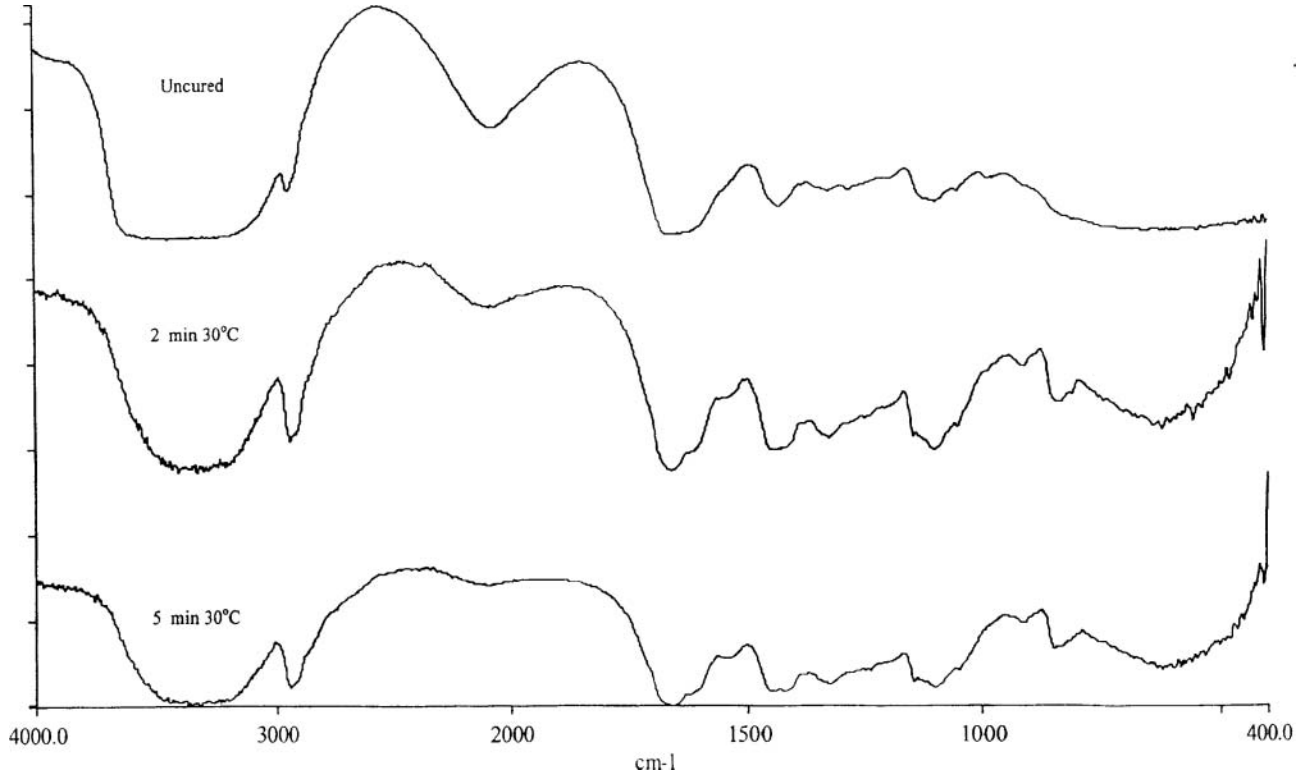

Fig. 3. FTIR spectra of hydrogel depicting various stages of curing.

represent the block of amino acids, proline, hydroxyproline and glycine.

However, the DSC thermogram of hydrogel shown in the curve (b) differs greatly from that of the pure gelatin. The broad endotherm of hydrogel suggests hydrophilic and amorphous nature of the end polymer. A close examination of the curve depicts weak endotherms around 60–70°C due to first glass transition temperature (Tg) of gelatin and

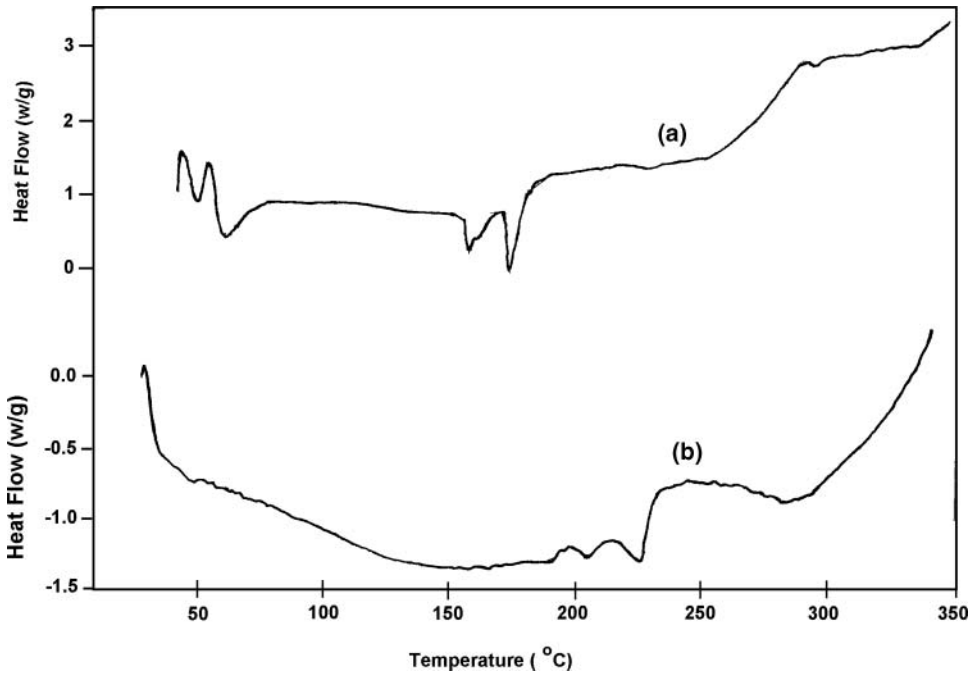

Fig. 4. DSC thermograms of (a) pure gelatin, and (b) cured hydrogel.

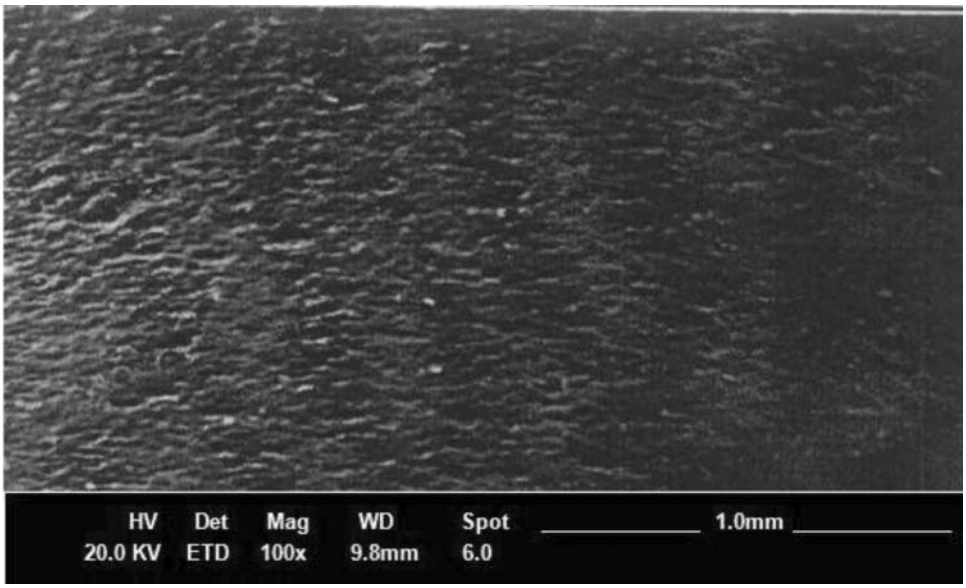

Fig. 5. ESEM image of dry hydrogel.

70–80°C because of glass transition of PVA. It is remarkable that the endotherm at 203 and 223°C are actually the enhanced Tg's of gelatin (see curve (a)) which because of interpenetrating nature of hydrogel chains appear at higher temperatures. It is interesting to see here that both the Tg's of pure gelatin (176° and 195°C) were increased by 28°C in the hydrogel thermogram (203° and 223°C, respectively). The thermogram also indicates a broad melting endotherm around 250°C, which may be attributed to the melting of PVA.

The thermograms, however, show a sharp melting endotherm at 280°C which may be assigned to melting of polyacrylamide. It is, therefore, clear that a significant change in thermal behavior is brought about due to hydrogel preparation.

3.4 ESEM Analysis

The morphological features of the hydrogel have been investigated by examining environmental scanning electron micrographs of the hydrogel as shown in Figure 5. The photograph clearly reveals that the graft hydrogel has a porous surface with pore sizes varying approximately between 1μ m to 4μ m. The large pore sized of the obtained network is attributed to the reason that because of grafting of crosslinked polyacrylamide chains onto PVA and gelatin macromolecules, there are large voids formed, surrounded by grafted PVA and gelatin chains. The ESEM observation obviously supports the grafted nature of the hydrogel.

3.5 Mechanical Properties

In order to study the influence of chemical composition on the mechanical properties of the hydrogels ,overall 16 polymer samples were prepared and the mechanical properties such as tensile strength, percentage elongation at break and Young's modulus were determined. The results are summarized in Table 1 and may be discussed as below:

3.6 Effect of PVA

It is clear from the data that the polymer sample without PVA (sample I) has the least tensile strength, percentage elongation and Young's modulus among all the synthesized samples. However, when the increasing amounts of PVA are added in the range 0 to 2.5 g, the mechanical properties increase. It can also be seen that the sample IV has 2.5 times more tensile strength, 4 times more percent elongation and 4.5 times more Young's modulus then sample I which contains no PVA. The observed increase in mechanical properties may be explained by the reason that due to an increased amount of PVA its chains cause greater entanglement within the polymer gel and thus lead to the observed increase in mechanical properties.

3.7 Variation in Gelatin

The sample V has no gelatin and shows lower values of mechanical properties. When increasing amounts of gelatin are added in the range 0.5 to 2.0 g the mechanical properties increase. It may be noted that the sample VII containing 1.0 of gelatin has 1.7 times more tensile strength,

Table 1. Mechanical properties of binary blends of varying compositions

Sample No.	*PVA (g)*	*Gelatin (g)*	*Acrylamide (g)*	*MBA (mg)*	*Tensile strength (MPa)*	*Percentage elongation (%)*	*Young modulus (MPa)*
I.	0.0	1.0	1.5	20	20.6	46.4	98.8
II.	1.5	1.0	1.5	20	30.1	58.3	134.6
III.	2.0	1.0	1.5	20	53.6	127.5	401.4
IV.	2.5	1.0	1.5	20	56.4	106.6	421.5
V.	2.0	0.0	1.5	20	29.2	78.3	160.5
VI.	2.0	0.5	1.5	20	34.2	95.8	117.2
VII.	2.0	1.0	1.5	20	53.6	127.5	401.4
VIII.	2.0	2.0	1.5	20	39.9	51.6	398.0
IX.	2.0	1.0	0.0	20	33.1	70.0	147.3
X.	2.0	1.0	1.0	20	38.0	49.1	280.7
XI.	2.0	1.0	1.5	20	53.6	127.5	401.4
XII.	2.0	1.0	2.0	20	42.0	94.1	315.3
XIII.	2.0	1.0	1.5	00	29.0	56.0	143.8
XIV.	2.0	1.0	1.5	10	37.1	61.6	219.2
XV.	2.0	1.0	1.5	20	53.67	127.5	401.4
XVI.	2.0	1.0	1.5	30	40.24	100.0	277.9

1.6 times more percent elongation and 2.4 times more Young's modulus than sample V. The increasing values of mechanical properties may be attributed to the reason that due to an increased amount of gelatin, the electrostatic attraction and hydrogen bonding increase between gelatin chains, which eventually result in an enhanced mechanical properties. However, beyond 1.0 g of gelatin there is a noticed drop in mechanical parameters which may be due to the hydrophilic nature of the gelatin. It is also worth mentioning here that there is no regular change in the modulus on increasing the content of gelatin and it is higher (approximately 400 MPa) for higher content of gelatin (1.0 g and 2.0 g). This obviously confirms the hard and brittle nature of the hydrogel at higher content of gelatin. It is also notable that at this highest content of gelatin (2.0 g) in the hydrogel, there is less % elongation with high Young's modulus. This shows increase in the brittle characteristics of semi–IPN on increasing the gelatin and can be attributed to crazing phenomenon. It was suggested by Argon (27) that the craze formation is a consequence of the events: (i) thermally activated production of stable microporosity under stress, (ii) formation of a craze nucleus by plastic expansion of holes in a small region while elastically unloading the surrounding. The crazing eventually develops microcracks in the specimens enhancing the failure probability leading to the breaking of specimens at the ultimate stress.

3.8 Effect of AM

The effect of AM content on the mechanical behavior is also shown in Table 1. It is clear from the table that both tensile strength and modulus, increases with increase in AM content in the semi–IPNs up to 1.5 g and thereafter, no significant changes are observed in both the properties. It is also clear from the table that the sample IX contains no AM and, therefore, has a low value of mechanical properties. However, the sample with 1.5 g of AM, the tensile strength becomes 1.4 times more, percent elongation becomes 8 times more and Youngs modulus attains 2.8 times higher value in comparison to those of sample IX. It is also clear from the data that on increasing the content of AM in semi–IPNs from 1.5 g to 2.5 g, percentage elongation decreases approximately 8 times and less change is observed in the Young's modulus which shows the brittle nature of the acrylamide. This change in the mechanical property can also be explained by the crazing phenomenon.

3.9 Effect of MBA

The data summarized in Table 1 reveal that the sample XII contains no crosslinker and, therefore, shows low values of mechanical properties. The effect of MBA on the mechanical properties of semi–IPN is shown in Table 1. It is clear from the table that maximum percentage elongation (127.5%), tensile strength (53.67 MPa) and Young's modulus (401.4) occurs in semi–IPN having 20 mg MBA content. On increasing the MBA content up to 20 mg, tensile strength and Young's modulus both increase and on further increasing the MBA content, both the values decrease. The increase in tensile strength and modulus is due to increase in crosslink density. However, after a certain amount of MBA (20 mg), further increase in crosslinker content segmental motion of the chains becomes less due to high degree of crosslinking and specimen can break earlier due to crazing phenomenon.

Table 2. Data showing the *in-vitro* blood compatibility parameters of the prepared hydrogels

	Composition						
S.No	*AM (mM)*	*Gel (g)*	*PVA (g)*	*MBA (mM)*	*Amount of protein adsorbed (mg g^{-1})*	*Weight of blood clot (g)*	*Percent haemolysis*
1	10.2	1.0	2.0	0.12	8.12	0.006	2.19
2	14.0	1.0	2.0	0.12	8.41	0.006	8.72
3	21.1	1.0	2.0	0.12	7.96	0.004	10.62
4	28.1	1.0	2.0	0.12	3.14	0.002	4.93
5	21.1	0.0	2.0	0.12	8.19	0.023	65.13
6	21.1	0.5	2.0	0.12	7.98	0.013	51.92
7	21.1	1.0	2.0	0.12	7.96	0.004	10.62
8	21.1	1.5	2.0	0.12	8.69	0.011	62.51
9	21.1	1.0	1.5	0.12	4.76	0.027	21.79
10	21.1	1.0	2.0	0.12	7.96	0.004	10.62
11	21.1	1.0	2.5	0.12	6.49	0.002	8.73
12	21.1	1.0	3.0	0.12	9.46	0.004	14.11
13	21.1	1.0	2.0	0.06	8.86	0.006	58.80
14	21.1	1.0	2.0	0.12	7.96	0.004	10.62
15	21.1	1.0	2.0	0.19	7.66	0.002	6.68
16	21.1	1.0	2.0	0.25	9.93	0.006	27.38
17	-Glass Surface				0.210		
18	-Poly Bag				54.14		

3.10 Blood Compatibility

The importance of hydrogel as biomaterials was first realized in the late 1950's with the development of poly (2-hydroxymethyl methacrylate)(PHEMA) gels as a soft contact lenses material (28). Since then, the hydrogels are used in numerous biomedical applications including ophthalmologic devices, biosensors, biomambranes and carriers for controlled delivery of drugs and proteins (29–30). Materials employed in biomedical technology are increasingly being designed to have specific and desirable biological interactions with their surroundings rather than the older common practice of trying to adapt traditional materials to all biomedical applications.

Many polymeric materials have already been used in devices in contact with blood as constituents of blood bags, catheters, large vascular grafts, artificial hearts, oxygenerators, renal dialyzers and micro particles for therapeutic immobilization[31]. Most of them are not intrinsically blood compatible and they induce many reactions, which can be linked to the presence of leachables or surface contaminants of diverse origins, in addition to the reactions linked to the polymer itself. Most of these studies attempted to understand the blood compatibility of foreign materials from the viewpoint of thrombosis, protein adsorption and cell adhesion (32). Several efforts of various research groups showed that the interaction of polymeric materials with blood components is very complex in nature and not very much predictable sometimes in a meaningful way (33).

Investigations showed that the blood compatibility of polymeric materials (hydrogels) is mainly affected by various surface and bulk properties of material surface, for example, surface charge, wettability, surface free energy, presence of specific groups on the surface, topography or roughness and hydrophobicity/hydrophilicity of materials etc. (34). The present study is, therefore, an attempt to study the blood compatible nature of synthesized hydrogels *in vitro*.

The results are summarized in Table 2, which revealed that with increasing AM content in hydrogel blends the blood compatibility increases. It is due to hydrophilic and biocompatible nature of PAM (35). The hydrophilicity increases the binding of water molecules onto the surface of hydrogel and does not leave a sufficient number of valencies for foreign materials. It is also very important that the state of water largely decides the protein adsorption and subsequent platelet adhesion (thrombosis) (36). Generally, high water content in swollen gels restricts the protein adsorption and coagulation of human blood on the surface. This study is further supported by a decrease in hemolysis in percentage when the rapture of blood cells is carried out mechanically in the presence of biomaterial. An increasing amount of gelatin shows an increase in biocompatibility by in large as shown in Table 2.11. Due to the well known biocompatible nature of gelatin (37), the amount of blood clot and protein adsorbed on polymeric surfaces decreases, which is further supported by a remarkable decrease in percentage haemolysis. When the amount of gelatin increases sufficiently in hydrogel blend, there is a significant fall in blood compatible nature of the hydrogel. It is suggested that high crosslinking density and negative charged groups of gelatin and AM attracts the anionically charged sites of blood platelets. It is quite evident that increased gelatin contents are characterized by a significant decrease in swelling

ratio, thus the phase separation no longer remains prominent and the blood compatibility decreases. An increasing amount of PVA in hydrogel matrix projects increased blood compatibility of hydrogel blends, a highly water loving polymer is well known for its blood compatibility for many years (38).

As shown in the results, the concentration of PVA in hydrogel blends exhibits high blood compatibility. It may arise due to lower compactness and high water contents in the swollen networks, which do not permit moderate attraction of blood components on the materials surface, MBA is difunctional and hydrophilic in nature. Therefore, as reported elsewhere (39), the increasing amount of hydrophilic crosslinker increases the antithrombogenicity of polymeric material. At high crosslink density (MBA = 0.25 mM), is due to the presence of NH_2^+ groups in PAM, as well as gelatin backbones in hydrogel blends offer anionically charged protein segments of blood to interact with hydrogel surface and hence, the amount of protein adsorbed and percentage of hemolysis increases in the presence of different hydrogel blends.

4 Conclusions

Redox initiated graft polymerization of acrylamide in the immediate presence of PVA, gelatin and crosslinker results in a hydrophilic matrix that has potential to be used in biomedical applications especially as tissue engineering scaffolds and wound healing dressings. The FTIR spectral analysis of the hydrogel confirms the presence of PVA, gelatin and polyacrylamide in the binary blend. The DSC thermograms of the resulting blend presents combined features of the three polymeric components, viz. PVA, gelatin and polyacrylamide. Moreover, interpenetrating nature of polymeric chains results in an enhanced glass transition temperature of gelatin. The ESEM image of the hydrogel suggests for a porous morphology of the end polymer.

The mechanical properties of the prepared hydrogels are found to show a significant dependence on the chemical architecture of the polymer matrix. It is noticed that upon varying the concentrations of PVA, gelatin, polyacrylamide and crosslinker in the studied range, the tensile strength, percent elongation and Young's modulus increase in the initial range of increasing concentrations and show a gradual fall after reaching at an optimum value.

The synthesized hydrogel blends display a fair degree of blood compatibility as judged from protein (BSA) adsorption, blood clot formation and percent haemolysis tests conducted in vitro. It is observed that the values of above parameters increase with increasing concentrations of PVA, Gelatin, AM and crosslinker (MBA) particularly in a lower concentration range, in the feed mixture of hydrogel blends.

The prepared hydrogels display a fair level of mechanical strength and *in vitro* blood compatible behavior which enables them to be used in a variety of biomedical applications such as artificial implants, tissue engineering etc. The presence of gelatin and polyvinyl alcohol offers numerous opportunities for functionalization of the polymer matrix so as to tailor it to the desired properties and specific applications.

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