JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **17** (2006) 49–61

Preparation and characterization of novel biocompatible cryogels of poly (vinyl alcohol) and egg-albumin and their water sorption study

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Polyvinyl alcohol (PVA) and egg albumin are water-soluble, biocompatible and biodegradable polymers and have been widely employed in biomedical fields. In this paper, novel physically cross-linked hydrogels composed of poly (vinyl alcohol) and egg albumin were prepared by cyclic freezing/thawing processes of aqueous solutions containing PVA and egg albumin. The FTIR analysis of prepared cryogels indicated that egg albumin was successfully introduced into the formed hydrogel possibly via hydrogen bonds among hydroxyl groups, amide groups and amino groups present in PVA and egg albumin. The gels were also characterized thermally and morphologically by DSC and SEM-techniques, respectively. The prepared so called 'cryogels' were evaluated for their water uptake potential and influence of various factors such as chemical architecture of the spongy hydrogels, pH and temperature of the swelling bath were investigated on the degree of water sorption by the cryogels. The effect of salt solution and various simulated biological fluids on the swelling of cryogel was also studied. The *in vitro* biocompatibility of the prepared cryogel was also judged by methods such as protein (BSA) adsorption, blood clot formation and percentage hemolysis measurements.

Introduction

Hydrogels are crosslinked hydrophilic polymers that can imbibe water or biological fluids. Their biomedical and pharmaceutical applications include a very wide range of systems and processes that utilize several molecular design characteristics [1]. Hydrogels are three-dimensional polymer networks in which individual hydrophilic polymer chains are connected by physical or chemical bonds. These bonds give rise to the integrity and physical stability of the networks whereas the thermodynamic compatibility of the polymer chains with water allows these materials to swell in aqueous solvents [2].

Hydrogels have become increasingly important materials for pharmaceutical and biomedical applications due to their water imbibing property. Hydrogels have been widely used in such applications because of their biocompatibility with the human body and also because they exhibit characteristics similar to natural tissue. They are used in a variety of applications including artificial skin [3], controlled release drug delivery systems [4], contact lenses [5], wound dressings [6], etc.

PVA is well known for its processability, strength and long-term temperature and pH stability. The characteristics which make it ideal for biomedical use are its biocompatibility, non-toxicity and minimal cell and protein adhesion [7] which can be easily crosslinked with irradiation [8], or bifunctional group containing chemical agents such as gluteraldehyde [9], thermal methods [10] but these usually suffer from several disadvantages. Therefore, several research groups have focussed their efforts on preparation of physically crosslinked PVA hydrogels by repeated freeze-thaw methods.

Lozinsky and co-workers [11] prepared fomed poly (vinyl alcohol) cryogel and studied influence of low molecular weight electrolytes on the swelling characteristics of PVA cryogels. Liu and co-workers [12]

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prepared cryogel of PVA and influence of cryogenic treatment on extremely dilute aqueous solution of poly (vinyl alcohol) was performed by viscometry. Li and co-workers [13] prepared poly (vinyl alcohol) nanoparticles by repeating freeze-thaw process and used them for protein/peptide drug delivery. Wu and co-workers [14] designed physically crosslinked hydrogels composed of poly (vinyl alcohol) and amine-terminated polyamidoamine dendrimers. Ariga *et al.* [15] investigated the effect of the preparation method on the mechanical characteristics of the PVA hydrogel and the stability of immobilized micro-organisms.

Kim and co-workers [16], by inclusion of biological components into the PVA hydrogel, were able to modify the PVA microstructure by affecting its crystallization process during the freezing-thaw procedure.

Thus, with these considerations in mind the authors were motivated to prepare and characterize a blend hydrogel of PVA and egg albumin by repeated freeze-thaw method and subsequently studied water sorption and blood compatibility of the prepared cryogels. The egg albumin is biodegradable, biocompatible, non-toxic and non-carcinogenic biopolymer and due to the special properties of egg-albumin, it was introduced into PVA hydrogel to form a novel physically crosslinked spongy cryogel.

Experimental

Materials

Poly (vinyl alcohol) (PVA) (degree of hydrolysis 98.8%, mol. wt. Ca. 70000 Da) was purchased from Merck, India and used without any pretreatment. Egg albumin was supplied by Loba Chemie, India and used as received. All the other chemicals were of analytical reagent grade and triple distilled water was used in preparation of all solutions.

Methods

Preparation of cryogel

The freeze-thaw method was adopted in preparing blend hydrogels of PVA and egg albumin as reported elsewhere [17–19]. In a typical experiment, 1.5 g of egg albumin and 2.0 g of PVA were dissolved in 25 ml of 1 M NaOH solution in a petri dish (diameter 4", Corning) and kept at −20 °C for 24 h. The frozen gel was then thawed for 2 h at room temperature (25 °C) and again kept at −20 °C for freezing. Such freezing-thawing cycles were repeated at least thrice so that whole mass converted into a soft spongy, bone white coloured hydrogel. The gel so prepared was purified by equilibrating it in distilled water for 72 h so that all unreacted chemicals were leached out. The swollen gel was cut into smaller discs and dried at room temperature for a week. The dried cryogel pieces (buttons) were stored in air tight polyethylene bags.

IR Spectral analysis

The Infrared spectral analysis of the prepared hydrogels were performed on an FTIR spectrophotometer (Perkin Elmer, 1000 Paragon).

DSC Measurement

The thermal properties of the cryogels were studied by constructing their differential scanning thermograms on a DSC instrument (2100, DuPont) in the temperature range 20 to 600 °C at a heating rate of 10 °C/min in the N_2 atmosphere.

SEM Studies

The morphological features of the cryogels were investigated by recording their scanning electron micrographs (STEREO SCAN, 430, Leina, SEM, USA).

Water uptake measurements

A conventional gravimetric procedure [20] was followed for monitoring progress of water uptake process. In brief, a pre-weighed dry piece of cryogel was immersed into distilled water at a definite temperature, taken out at pre-determined time intervals, gently pressed between two filter papers to remove excess water and finally weighed by a sensitive balance (APX-203 Denver, Germany). The degree of water sorption was quantified in terms of the swelling ratio as calculated below.

$$\text{Swelling Ratio} = \frac{\text{Weight of swollen gel } (W_s)}{\text{Weight of dry gel } (W_d)} \quad (1)$$

The kinetic data was analyzed in the light of the following Equation [21]

$$\frac{W_t}{W_\infty} = kt^n \quad (2)$$

and

$$\frac{W_t}{W_\infty} = 4\left(\frac{Dt}{\pi L^2}\right)^{0.5} \quad (3)$$

where W_t and W_∞ are the water intakes at time t and equilibrium time, respectively, L is the thickness of dry spongy gel, k is the swelling rate front factor and n is the swelling exponent. When $n = 0.5$, the swelling process is of Fickian nature and is diffusional controlled while the value of n between 0.5 and 1.0 suggests for non-Fickian diffusion or more specifically anomalous diffusion. When n becomes exactly equal to unity, then the diffusion is termed as Case II. In some cases, the value of n has been found to exceed unity and it has been termed as Super Case II transport. In Equation 3,

TABLE I Data showing the kinetic parameters for the swelling process of PVA-egg albumin cryogel

PVA (g)	Egg albumin (g)	n	Diffusion constant $D \times 10^5$ ($cm^2 s^{-1}$)	Penetration velocity $V \times 10^3$ ($cm\ s^{-1}$)
1.0	1.5	0.37	1.39	7.192
1.5	1.5	0.4	1.68	5.993
2.0	1.5	0.56	1.30	9.588
2.5	1.5	0.52	2.6	6.350
2.0	0.5	0.5	1.00	3.200
2.0	1.0	0.566	1.03	3.955
2.0	1.5	0.56	1.30	9.588
2.0	2.0	0.6	1.09	7.671

D is diffusion coefficient of water molecules and L being the thickness of the dry gel. The value of n and D for different blend compositions were calculated and summarized in Table I.

Penetration velocity measurement

The penetration velocity for each cryogel composition was determined by weight gain method as described by Peppas and Franson [22]. The penetration velocity was calculated from the slope of the initial portion of the penetration uptake curve from the equation given below :

$$V = \left[\frac{dWg}{dt}\right] \cdot \left[\frac{1}{\rho}\right] \cdot \left[\frac{1}{2A}\right] \tag{4}$$

where V denotes the penetration velocity, dWg/dt denotes the slope of the weight gain vs time curve, ρ is the density of the swelling solvent, A is the area of one face of disc and 2 accounts for the facts that penetration velocities calculated for different cryogel compositions are listed in Table I.

Blood compatibility studies

(i) *BSA Adsorption*

Adsorption of BSA onto the hydrogels was performed by the batch process as reported in other communications [23]. For adsorption experiment, protein (BSA) solutions were prepared in 0.5 M PBS (Phosphate Buffer Solution) at physiological pH 7.4. A fresh solution of BSA was always prepared prior to adsorption experiments. The hydrogel buttons were equilibrated with PBS for 24 h. The adsorption was then carried out by gently shaking a BSA solution of known concentration containing pre-weighed and fully swollen gels. By taking fully swollen gels, the possibility of soaking of BSA solution within the gel becomes minimum. The shaking was performed so gently that no froth was produced, otherwise, BSA would adsorb at air-water interface. After a definite time period, the gels were removed and the adsorbed protein was assayed for the remaining concentration of BSA by recording the absorbance of protein solution at 272 nm on a UV spectrophotometer (Systronics, Model No. 2201, India). The adsorbed amount of BSA was calculated by the following balance equation:

$$\text{Adsorbed BSA} = \frac{(C_O - C_e)V}{m} \tag{5}$$

where C_O and C_e being the initial and equilibrium concentrations of BSA solution (mg/ml), V is the volume of protein solution and m is the mass of fully swollen gel, i.e. the adsorbent.

(ii) *Clot formation test*

In order to evaluate the blood compatibility of cryogel, cryogel surfaces were examined for their blood compatible property by blood-clot formation test as described elsewhere [24–26]. In brief, the hydrogels were equilibrated with saline water (0.9% NaCl solution w/v) for 1 day in a constant temperature bath. To these water swollen and equilibrated samples were added 0.5 ml of ACD blood from a healthy donor and 0.03 ml of $CaCl_2$ solution to start the thrombus formation. The reaction was stopped by adding 4.0 ml of deionized water and the clot formation was separated by soaking in water for 10 min. at room temperature and then fixed in 36% formaldehyde solution (2.0 ml) for another 10 min. The fixed clot was placed in water for 10 min. and after drying, its weight was recorded. The same procedure was repeated for a PVC film (−ve control) ($2'' \times 2''$) cut from a commercial blood-bag (Eastern Medicated Ltd., India) and glass (+ve control), respectively. The blood clot data are summarized in Table II.

(iii) *Hemolysis assay*

Hemolysis assay experiments were performed on the surfaces of the prepared cryogels as described elsewhere [27]. In a typical experiment, cryogel films (4 cm^2) were equilibrated in normal saline water (0.9% NaCl solution) for 24 h at 37 °C and human ACD blood (0.25 ml) from a healthy donor was added on the cryogel films. After 20 min., 2.0 ml of 0.9% sodium chloride (saline water), was added to each sample to stop hemolysis and the samples were incubated for 60 min. at 37 °C. Positive and negative controls were obtained by adding 0.25 ml of human ACD blood and 0.9% NaCl solution, respectively to 2.0 ml of bidistilled water. Incubated samples were centrifuged for 45 min, the supernatant was taken and its absorbance was recorded on a spectrophotometer (Systronics Model No. 106, India) at 545 nm. The percentage hemolysis was calculated using the

TABLE II Data showing the biocompatibility parameters with varying composition of cryogel

PVA (g)	Egg albumin (g)	Freeze -thaw cycles	Blood clot (mg)	% Hemolysis	BSA adsorbed (mg g^{-1})
1.0	1.5	3	6.0	36.46	0.763
1.5	1.5	3	5.0	34.65	0.515
2.0	1.5	3	4.0	22.59	0.489
2.5	1.5	3	4.0	23.14	0.478
2.0	0.5	3	5.0	25.32	0.631
2.0	1.0	3	4.2	24.65	0.578
2.0	1.5	3	4.0	22.59	0.489
2.0	2.0	3	3.0	11.15	0.357
2.0	1.5	3	4.0	22.59	0.489
2.0	1.5	5	5.0	23.14	0.521
2.0	1.5	7	6.0	23.44	0.728
2.0	1.5	9	6.2	23.86	1.00
Glass surface			30.0	–	–
PVC (Blood bag)			1.0	13.75	–

following relationship:

$$\% \text{ Hemolysis} = \frac{\text{A test sample} - A(-) \text{ control}}{A(+) \text{ control} - A(-) \text{ control}} \quad (6)$$

where A = Absorbance. The absorbance of positive and negative controls were found to be 1.764 and 0.048, respectively.

Results and discussion

Mechanism of cryogel formation

A mechanism of hydrogel preparation involves "physical" crosslinking due to crystallite formation. This method address toxicity issues because it does not require the presence of crosslinking agent. Such physically crosslinked materials also exhibit higher mechanical strength than PVA gels crosslinked by chemical or irradiative techniques because the mechanical load can be distributed along the crystallites of the three-dimensional structure [28].

Although formation of an elastic gel either upon standing on aqueous solution of PVA at room temperature or successively freezing-thawing a moderately concentrated PVA solution is not a new concept [29], however, a molecular explanation for this phenomenon has yet to come. Three basic models including hydrogen bonding, polymer crystallites formation and liquid-liquid phase separation have been suggested to explain the mechanisms including the gel formation. In the present study since a mixture of PVA and egg albumin solution was taken, the above three models may be considered to become operative simultaneously, thus yielding a highly elastic gel. The reason for this supposition is that the egg albumin is a multifunctional, biopolymer and its presence in PVA solution will enhance the processes of hydrogen bond making, polymer crystallite formation and phase separation, respectively. The porous nature of cryogels produced by freezing-thawing method may be explained due to the fact that whereas the freezing of a PVA-egg albumin mixture results in formation of ice crystal domains within the polymer mixture matrix, the thawing process results in melting of the ice crystal, thus leaving wide pores in the gel. A repeated performance of the two processes wides the pore sizes and thus enhances the porous nature of the hydrogel. The formation of porous network due to freezing-thawing method is modeled in Fig. 1.

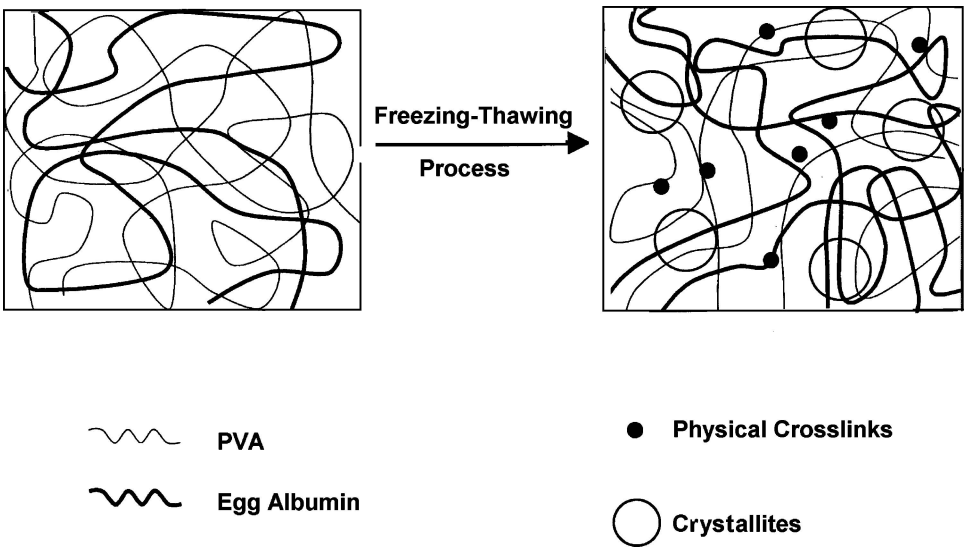


Figure 1 A hypothetical model depicting the formation of cryogel due to freeze-thaw cycles.

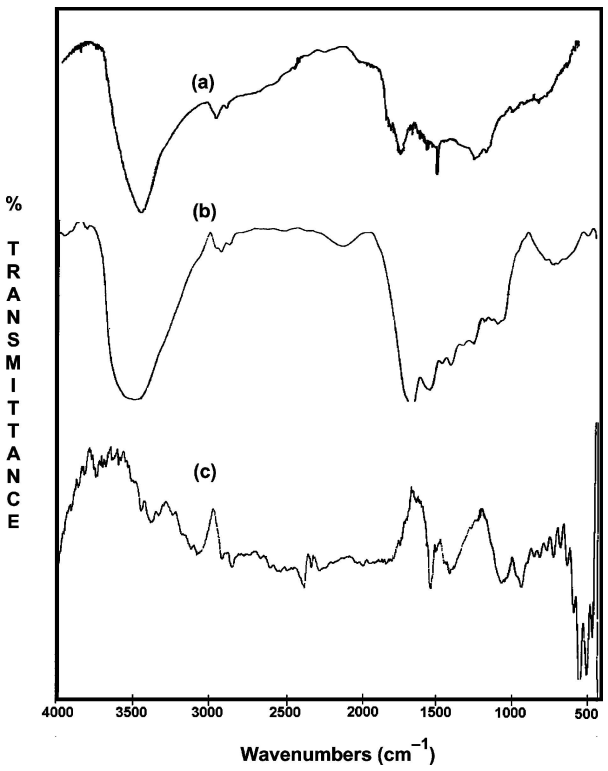


Figure 2 IR spectra of (a) PVA, (b) egg-albumin, and (c) PVA-egg albumin cryogel.

IR Spectral analysis

The IR spectra of the pure PVA, pure egg albumin and hydrogel are depicted in Fig. 2(a)–(c), respectively. The spectra 2(c) clearly marks the combined presence of hydroxyl groups of polyvinyl alcohol and amide group of egg albumin at 3389 cm^{-1}. This broad peak appears due to superimposition of N─H and O─H stretching which also indicates the formation of H─bonding in the hydrogel. In addition to the above mentioned peak, the IR spectra also confirms the presence of PVA and egg albumin in the hydrogel as evident from the observed adsorption band at 3083 cm^{-1} (due to ═C─H stretching and hydrogen banded ─N─H groups), 1545 cm^{-1} (due to symmetric bending of $-NH_3^+$ group), 1428 cm^{-1} (due to ─O─H in plane bending vibration), 1082 cm^{-1} (due to ─C─O stretching), 944 cm^{-1} (due to out of plane bending of banded ─O─H group), two sharp peaks at 547 cm^{-1} and 501 cm^{-1} (due to torsional oscillation of $-NH_3^+$ group), a strong band at 1545 cm^{-1} and a weak band at 1428 cm^{-1} (due to asymmetric and symmetric stretching of C─O group of $-COO^-$ ion) respectively.

DSC Studies

In order to gain insight into the thermal properties of the cryogel, the differential scanning calorimetric thermograms were constructed separately for PVA hydrogel (prepared by the same freezing-thawing method) and PVA-egg albumin cryogel as shwon in Fig. 3(a) and (b), respectively.

The thermogram of PVA hydrogel is shown in the curve (a) which clearly shows a sharp melting endotherm around 110 °C, which could be assigned in the melting of crystallites in the hydrogel developed due to strong hydrogen bonding established between PVA-water and PVA-chain themselves. The broad peak at approximately 110 °C represents the evaporation of residual water present in sample. The sharp peak at approximately 230 °C represent the melting of PVA [30, 31].

The thermogram of the cryogel shown in Fig. 3(b) display combined features of PVA and egg albumin. As shown in curve, a broad shape of the endotherm implies for a homogeneously distributed microcrystalline regions in the cryogels.

A sharp endotherm obtained at 219 °C, which is slightly lower than the reported value of 230 °C, may be explained by the fact that the presence of egg albumin acts as an impurity and thus, lowers the melting of PVA. A sharp endotherm obtained at 266 °C due to crosslinking in the cryogel.

SEM Analysis

The morphological features of the prepared pure PVA and PVA-egg albumin cryogel have been investigated by SEM analysis and the photographs of the image of the PVA-egg albumin hydrogel is shown in Fig. 4(a) and (b), respectively.

A close examination of the photographs of the pure PVA and cryogel clearly reveals that the prepared PVA-egg albumin cryogel has porous morphology because the surface appears quite heterogeneous and possesses a multilayer morphology due to aggregation of PVA and egg albumin molecules in the cryogel. The observed morphology, thus, supports the mechanism of pores formation in the cryogel.

Water sorption measurements

Effect of PVA

Inclusion of a hydrophilic polymer into a gel network is expected to enhance its water sorption capacity. In the present investigation, the effect of increasing PVA content in the range 1.0 to 2.5 g in the feed mixture of the hydrogel has been studied on the swelling characteristics of the hydrogel. The results are displayed in Fig. 5 which clearly reveal that the swelling ratio increases with increasing concentration of PVA up to 2.0 g while beyond it, a fall is noticed. The observed results may be explained by the fact that since PVA itself has a natural tendency to form reversible gel, its increasing concentration in PVA-egg albumin mixture lowers the concentration of egg albumin in the feed mixture and

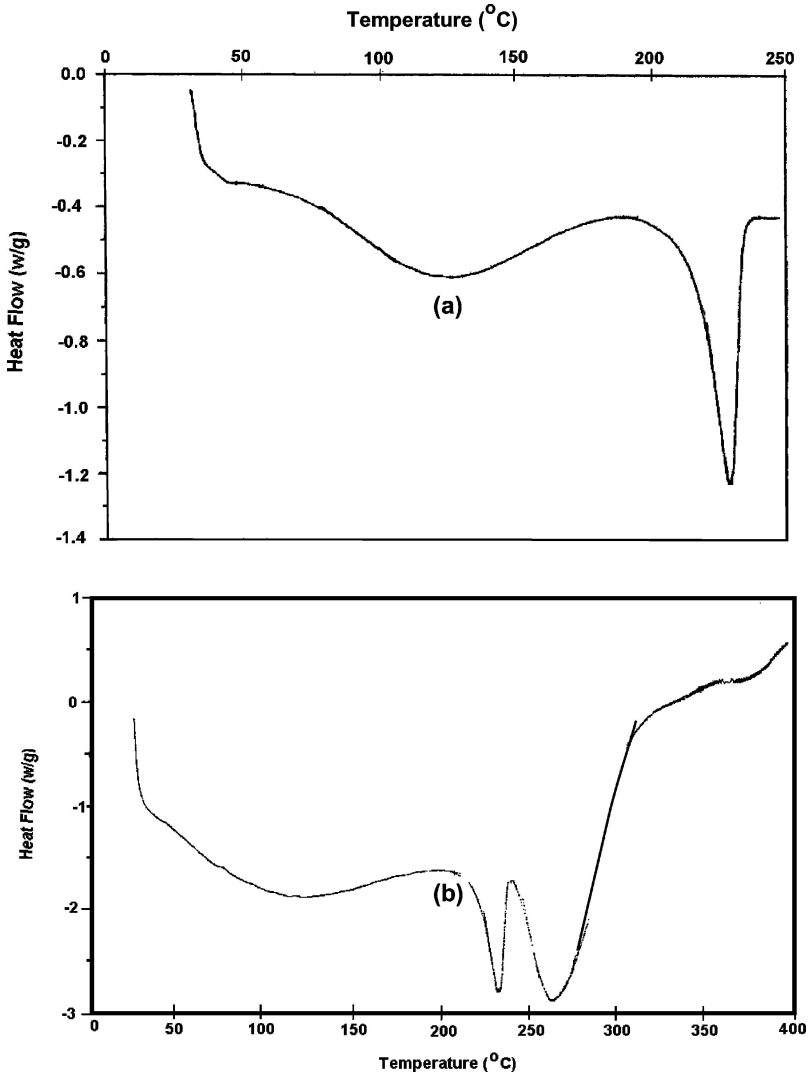


Figure 3 DSC thermograms of (a) PVA hydrogel (prepared by freeze-thaw method) and (b) PVA-egg albumin cryogel.

as a consequence upon successive freezing and thawing, the pore size increases as noticed by others also [31–33].

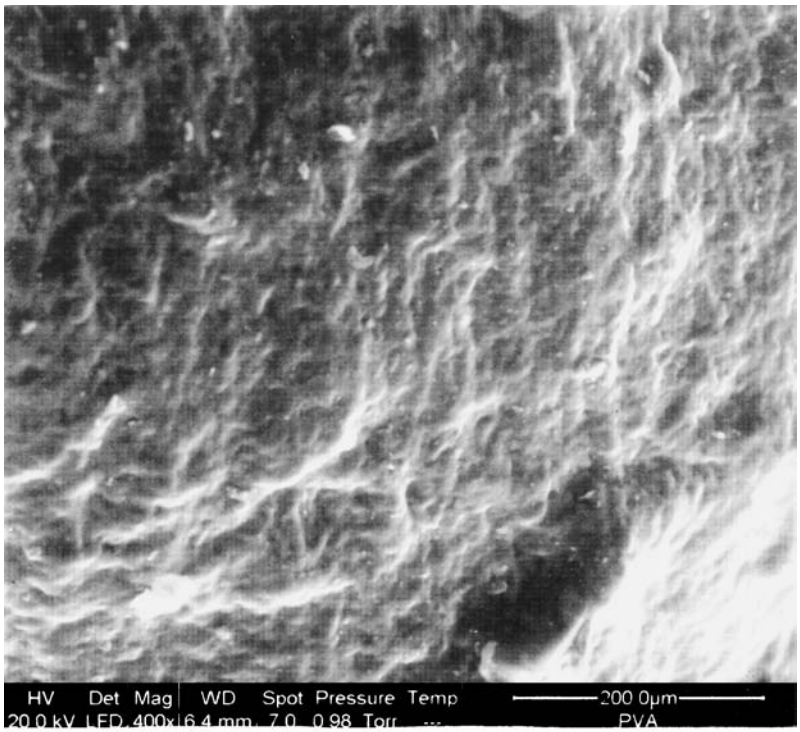
Thus, increased pore sizes result in an easier penetration of water molecules into the cryogel, thus bringing about an increase in the amount of water in the cryogel. Thus, the solution becomes increasingly poor in PVA and unfavours crystallite formation. In this way, a lower degree of crystallinity results in an enhanced swelling.

However, on further increasing the PVA concentration, i.e. beyond 2.0 g, the decrease observed in the swelling ratio may be attributed to the fact that at higher concentration of PVA, greater number of hydrogen bonds shall be established between PVA-PVA and PVA-egg albumin molecules due to increasing number of PVA molecules in PVA-egg albumin aqueous solution. In the same way, with increasing richness of PVA in the polymer mixture, the formation of PVA crystallites is favoured which results in a lesser degree of swelling.

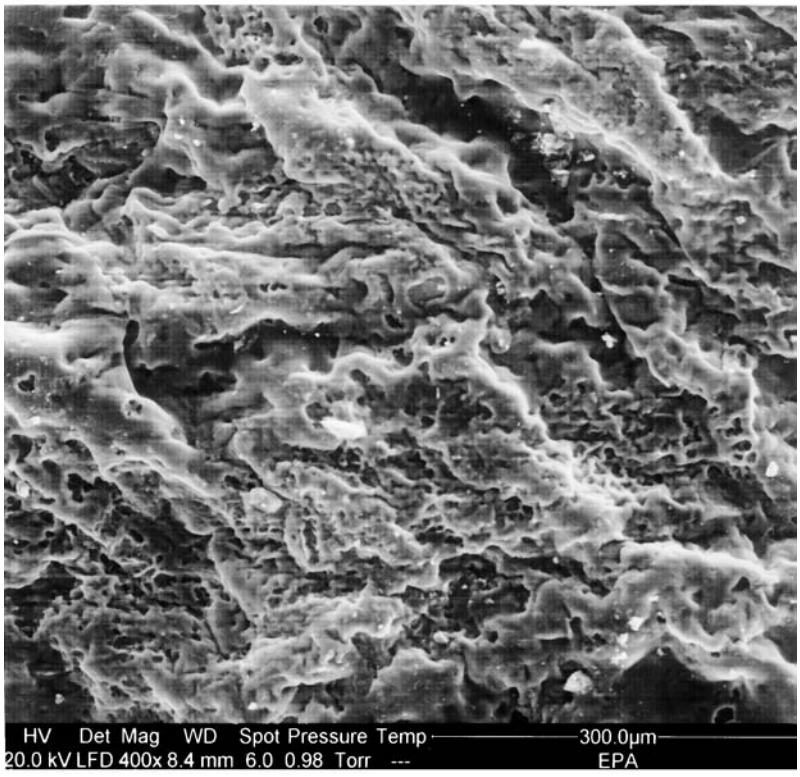
Effect of egg albumin

In the present investigation, the influence of varying concentration of egg albumin in the range 0.5 to 2.0 g in the feed mixture of the cryogel has been investigated on the swelling ratio of the hydrogel. The results are shown in Fig. 6, which clearly reveal that the swelling ratio increases with increasing concentration of egg albumin in the feed mixture up to 1.5 g while beyond it, a fall is noticed. The observed results may be explained by the fact that since egg albumin itself has a natural tendency to form reversible gel, its increasing concentration in the PVA-egg albumin mixture lowers the concentration of PVA in the feed mixture and as a consequence upon successive freezing and thawing, the pore sizes increases as confirmed by others also [31, 32].

However, on further increasing the egg albumin, i.e. beyond 1.5 g, the decrease observed in swelling ratio, may be attributed to the fact that at higher concentration, egg albumin itself initiate formation of reversible



(a)



(b)

Figure 4 Scanning electron micrographs (SEM) of (a) PVA, and (b) PVA-egg albumin.

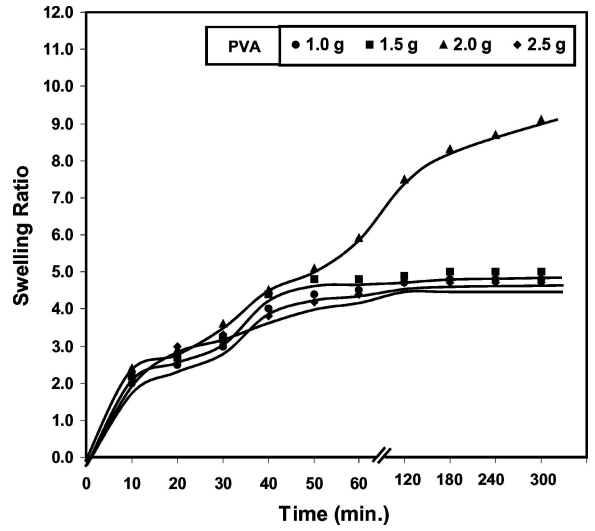


Figure 5 Effect of varying amount of PVA on the swelling ratio of the cryogel of definite composition [egg albumin] = 1.5 g, freeze-thaw cycle (FTC) = 3, Temp. = 25 ± 0.2 °C.

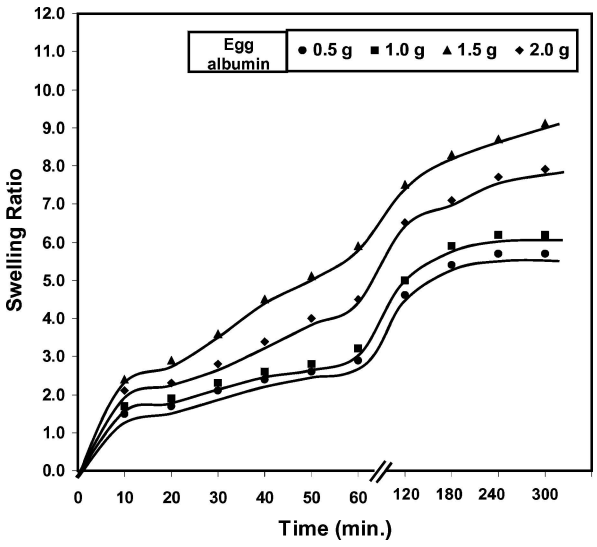


Figure 6 Effect of varying amount of egg albumins on the swelling ratio of the cryogel of definite composition [PVA] = 2.0 g, freeze-thaw cycle (FTC) = 3, Temp. = 25 ± 0.2 °C.

gel which develops crystalline regions in the cryogel. This obviously results in low swelling ratio of the blend hydrogel.

Effect of number of Freeze-Thaw Cycle (FTC)

The effect of number of repeated freeze-thaw cycles on the swelling ratio of cryogel has been studied and shown in Fig. 7. The results clearly reveal that the extent of swelling ratio decreases with increasing number of FTC. The observed results may be attributed to the fact that in the case of 3rd cycle, the cryogel does not have large crystalline regions and therefore, the chains of PVA and egg albumin keep on relaxing with increasing swelling

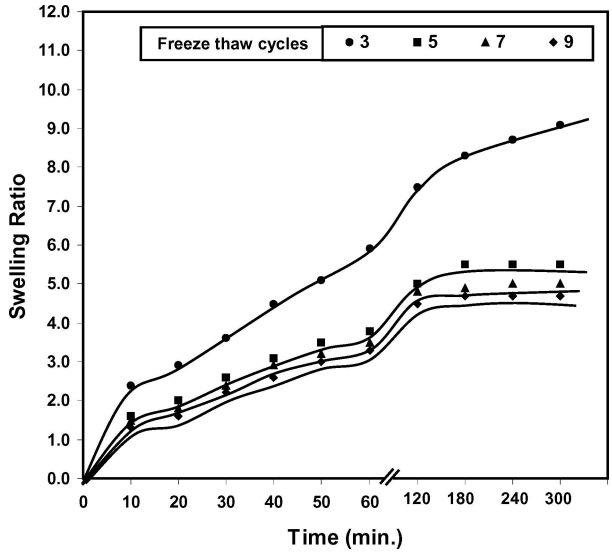


Figure 7 Effect of number of freeze-thaw cycle (FTC) on the swelling ratio of the cryogel of definite composition [PVA] = 2.0 g, [Egg albumin] = 1.5 g, Temp. = 25 ± 0.2 °C.

time. However, with increasing number of FTC, the gel acquires increasing crystallinity which restricts the mobility of PVA and egg albumin chains and, consequently, results in a suppressed value of swelling ratio. Peppas and co-workers [32] also noticed that the swelling ratio of cryogel decreases with increasing number of FTC due to an increase in crystallinity and crosslinking density within the hydrogel. An increase in mechanical strength has also been noticed by some workers [14, 34].

Effect of pH

In the present investigation, the influence of pH on the swelling ratio of the cryogel has been studied by varying the pH of the swelling medium in the range 4 to 9.2. The results are shown in Fig. 8, which clearly reveal that the swelling ratio of the cryogel increases up to pH 7 and achieve an optimum swelling and, thereafter, decreases with further increase in the pH.

The observed results may be attributed to the reason that in the lower pH range, the egg albumin molecules bear a net positive charge (due to $-NH_3^+$) and undissociated carboxyl groups (–COOH) which easily form hydrogen bonds with the hydroxyls (–OH) of PVA, thus, resulting in the formation of crystallites in the gel. This obviously favours a lower swelling ratio of the cryogel in acidic medium. However, with increasing pH in acidic medium, the number of OH^- ions goes on increasing which produce repulsions among network chains, thus causing their relaxation within the gel network. This obviously facilitates diffusion of water molecules into the gel and, therefore, the swelling increases. However, beyond pH 7, i.e. in slight alkaline range, the network chains are so much relaxed that the hydrogen bonds

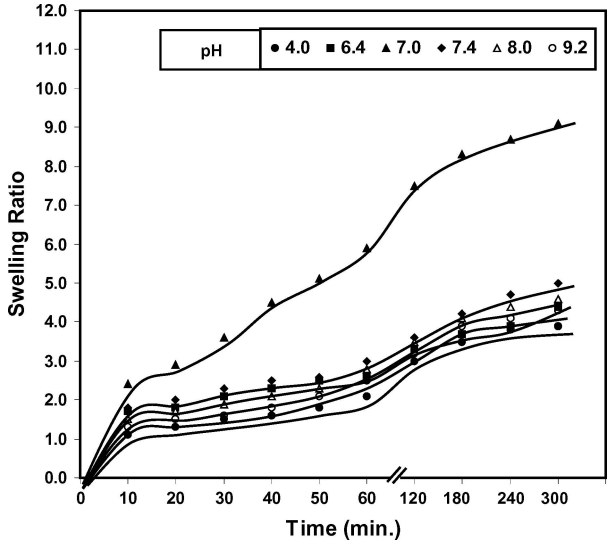


Figure 8 Influence of pH on the swelling ratio of the cryogel of definite composition [PVA] = 2.0 g, [Egg albumin] = 1.5 g, FTC = 3, Temp. = 25 ± 0.2 °C.

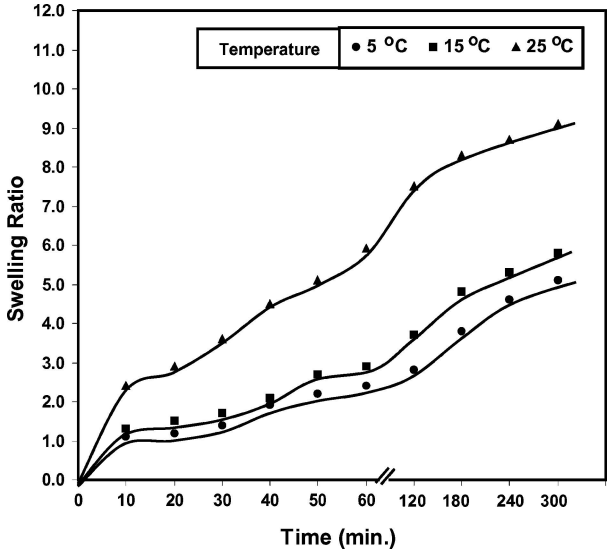


Figure 9 Effect of temperature on the swelling ratio of the cryogel of definite composition [PVA] = 2.0 g, [Egg albumin] = 1.5 g, FTC = 3.

established between the water molecules, the PVA and the egg albumin chains become weak and the entrapped water molecules are forced out, thus, resulting in a lower swelling ratio of the hydrogel. The expulsion of bound water molecules at large relaxation of macromolecular chains has also been observed elsewhere [35].

Effect of temperature

The influence of temperature on the swelling of the hydrogel is of much significance because it directly controls the diffusion of water molecules into the gel, segmental mobility of the network chains and water-polymer interaction.

In the present study, the effect of temperature on the degree of water sorption has been investigated by carrying out water sorption experiments in the range 5 ° to 25 °C. The results are present in Fig. 9 which clearly indicate that the swelling ratio constantly increases with increasing temperature of the swelling medium. The results may be explained by the facts that when the temperature is increased, both the segmental mobility of cryogel chains and diffusion of water molecules into the gel increase which obviously results in a greater swelling.

For quantitative analysis of the temperature effect, the Clausius-Clapeyron equation can be applied according to which [36]

$$\frac{dln(W_\infty)}{d(1/T)} = -\Delta H_m/R \qquad (7)$$

where R is a gas constant and ΔH_m is the enthalpy of mixing between the dry polymer and infinite amount of water. When ln W_∞ is plotted against reciprocal of temperature ($1/T$), a straight line with a negative slope is obtained (Fig. 10), which clearly implies for an endothermic process. The value of ΔH_m was calculated to be –12.48 kJ/mole which clearly suggests for an endothermic nature of the swelling process.

Effect of salts

The presence of salt in a swelling medium of a hydrogel is of importance in agriculture and biomedical applications. Therefore, in the present investigation, the influence of salt on the swelling ratio of the cryogel has been studied by adding NaCl in the concentration range

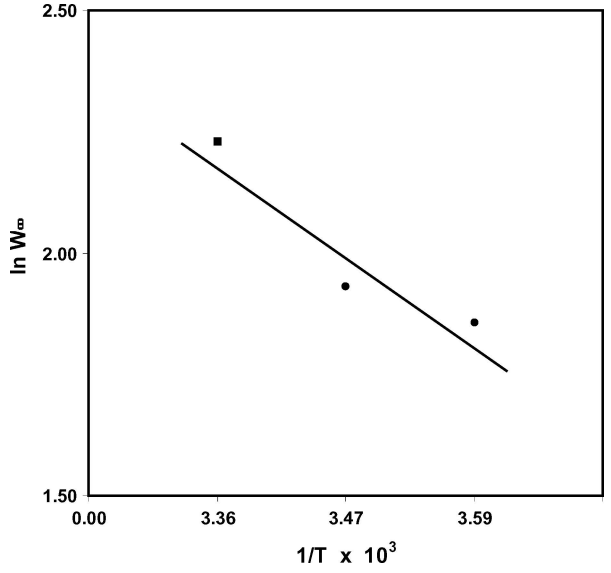


Figure 10 Plot drawn between ln W_∞ and 1/T for the cryogel of definite composition [PVA] = 2.0 g, [Egg albumin] = 1.5 g, FTC = 3.

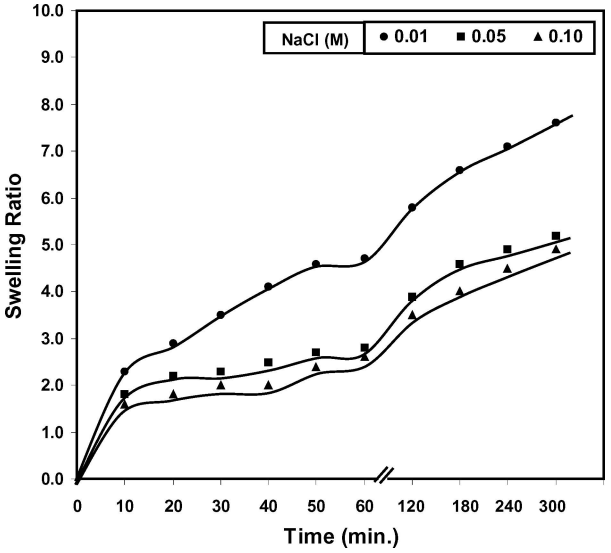


Figure 11 Effect of NaCl on the swelling ratio of the cryogel of definite composition [PVA] = 2.0 g, [Egg albumin] = 1.5 g, Temp. = 25 ± 0.2 °C.

0.01 to 0.1 M. The results are depicted in Fig. 11 which clearly reveal that the swelling ratio decreases with increasing salt concentration in the swelling medium. The results are quite obvious and may be explained by Equation (8) based on the theory given by Flory,

$$\pi_{\text{ion}} = RT\Sigma\left(c_i^g - c_i^s\right) \tag{8}$$

where C is the mobile ion concentration of species i and superscripts g and s represent the gel and solution phases, respectively. The above equation implies that greater the difference between the concentrations of mobile ions inside and outside the gel, the greater would be the osmotic pressure and larger would be the swelling of the gel. On increasing the concentration of NaCl in the swelling medium, the osmotic pressure (π_{ion}) decreases due to decrease in ($C_i^g - C_i^s$) term in equation and consequently, the swelling ratio decreases. The results are quite usual and have been published by other workers also [37].

Effect of biological fluids

It is well established that the equilibrium swelling behaviour of a polymer network in a solvent is the result of a balance between osmotic and restoring elastic pressure. The presence of salts in the surrounding aqueous medium is capable of tilting this balance, which may result in either an increase or decrease in swelling. The effect of biological fluids has been examined by performing swelling experiments in the presence of Urea, D-glucose (5% w/v), potassium iodide (KI) (15% w/v) and in phyiological fluids such as saline water (0.9% NaCl) and artificial urine. The results are summarized in Table III, which clearly show that the presence of solute suppress the swelling ratio due to a decrease in osmotic pressure of the external solution.

TABLE III Data showing the swelling ratio of the Cryogel in various simulated physiological media

Physiological fluids	Swelling Ratio (after 5 h)
Water	9.1
KI (15% w/v)	7.1
Urea (5% w/v)	7.0
D-glucose (5% w/v)	6.1
Saline water (0.9% w/v NaCl)	5.0
Synthetic Urea*	5.8

*NaCl (0.8% w/v), $MgSO_4$ (0.10% w/v), Urea (21% w/v) and $CaCl_2$ (0.06% w/v).

Effect of drying temperature on swelling

The preparative conditions have a great impact on the overall properties of a hydrogel. In the present investigation, the effect of drying temperature of the cryogel has been investigated on its swelling ratio by drying the cryogel in the temperature range 25 to 70 °C. The results, shown in Fig. 12 clearly indicate that the swelling ratio drastically decreases with increasing drying temperature. The observed results may be attributed to the fact that when the drying temperature is high, some of the crosslinks may be disrupted and this obviously allow freedom to both PVA and egg albumin chain to form crystalline phase. Thus, degree of crystallinity increases with increasing drying temperature and results in a lower water sorption due to the reason that in crystalline polymers like cellulose [38], polyvinyl alcohol [39], nylon [40], etc., swelling occurs mainly through amorphous region. This explains the lower swelling

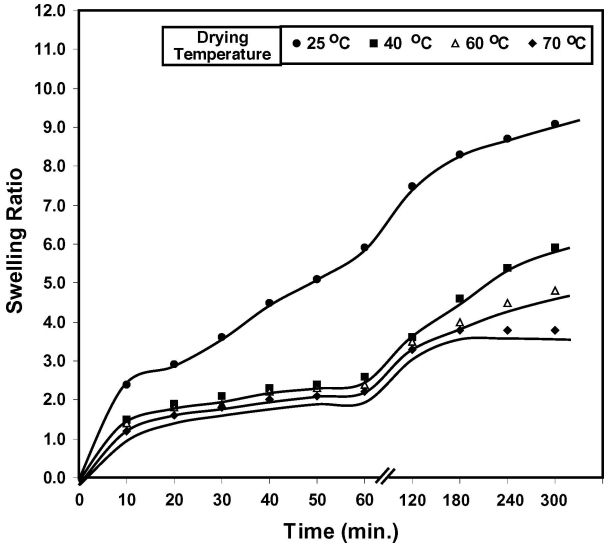


Figure 12 Effect of drying temperature of the cryogel on the water sorption capacity of the cryogel of definite composition [PVA] = 2.0 g, [Egg albumin] = 1.5 g, FTC = 3.

with increasing drying temperature. The morphology of the swelling ratio vs time curve also indicates that with increase in drying temperature, the equilibrium swelling is attained much earlier. The reason for the observed findings is that with increased crystallinity in the cryogel, the smaller amorphous phase of the gel swells rapidly and attains equilibrium swelling ratio value at earlier times.

Kinetic analysis of sorption data

A dry hydrogel when put into an aqueous reservoir absorbs water and swells which can be monitored either gravimetrically or by measuring dimensions of the swelling matrix. The swelling process, however, is known to involve a series of steps that also include interaction between the solvent and hydrogel at molecular level. In the present study, the dynamic water sorption experiments were performed and data were analyzed in terms of swelling exponent '*n*' and diffusion constant '*D*' and summarized in the Table I.

It is clear from the Table that when the amount of PVA is varied in the range 1.0 to 2.5 g in the feed mixture of the cryogel, the value of swelling exponent '*n*' is found to increase from 0.37 to 0.52 in the Fickian region, thus, suggesting a Fickian or diffusional controlled water transport process. The observed Fickian nature may be attributed to the fact that because of the formation of crystallites and hydrogen bonds in the cryogel, the diffusion of water molecules becomes slower in comparison to the network chain relaxation and, thus, gives rise to diffusion controlled swelling process.

In the case of variation in the egg albumin content in the cryogel, it is noticed that from 0.5 to 2.0 g, variation of egg albumin content in feed mixture, the value of swelling exponent '*n*' is found to increase from 0.5 to 0.6 in the non-Fickian region. This clearly indicate a shift of water transport mechanism from Fickian to anomalous type, i.e. from a diffusion controlled to relaxation controlled process. The observed shift is due to the reason that the number of egg albumin molecules and their compact arrangement in the cryogel results in a decreasing relaxation rate of the network chains.

Evaluation of biocompatibility

The selection of a given biomaterial for a given end use must be based on several criteria. These are : physicochemical properties, functions desired, nature of the physiological environment, adverse effects in case of failure, expected durability and consideration relating to cost and ease of production. Whatever the given application may be, biocompatibility is the foremost requirement for all biomaterials [41].

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

(i) *BSA adsorption*

In the present investigation, the biocompatibility of prepared cryogels has been judged by monitoring the amount of protein (BSA) adsorbed by the cryogel. The results are shown in Table II, which indicate that the amount of adsorbed BSA decreases with increasing amount of PVA and egg albumin in the feed mixture of the cryogel. The observed findings may be explained on the basis of the facts that both the PVA and egg albumin are hydrophilic polymers and do not provoke either any damage of blood cells or any change in the surface of plasma proteins which are the main requests for biocompatibility. From the Table, it is also clear that a marginal increase in adsorbed BSA noticed with increasing number of freeze-thaw cycles is due to the fact as the number of freeze-thaw cycles increases, the swelling ratio decreases, i.e. gel acquires lower degree of hydrophilicity and results in higher adsorption of BSA protein on surface.

(ii) *Blood clot formation*

In the present study, the antithrombogenic property of the cryogel has been judged by monitoring the amount of blood clot formed by performing blood clot formation tests as described in the experimental section. The results are summarized in Table II which clearly indicates that whereas the weight of the blood clot constantly decreases with increasing amount of PVA and egg albumin in the hydrogel, a marginal increase in blood clot is noticed with increasing number of freeze-thaw cycles. The results may be explained on the basis of the facts that both PVA and egg albumin are hydrophilic polymers and, therefore, are not expected to provoke either any damage of blood cells or any change in the structure of plasma proteins.

The results also imply that the cryogel surface becomes more and more thrombogenic as the number of freeze-thaw cycles increases. The observed results may be attributed to the reason that increased number of cycles result in a cryogel with greater pore sizes and this obviously produces a rough surface of cryogel which causes a greater clot formation.

(iii) *Hemolysis test*

In the present investigation, the prepared cryogels were also tested for hemolytic activity and the results obtained are shown in Table II. The results obtained clearly indicate that with increasing PVA and egg albumin content, the extent of hemolysis constantly decreases while with cryogel prepared with greater number of freeze-thaw cycles, the present hemolysis increases. The observed results may be attributed to the reason that with change in PVA and egg albumin concentration in the cryogel, the surface composition favourably changes

which improves the blood compatible quality of the material. The observed hemolysis data shown are well consistent to the clot formation results.

Conclusions

Repeated freezing and thawing of PVA and egg albumin solution together produces a highly elastic, water absorbing and blood compatible spongy hydrogel (cryogel).

It is noticed that the water sorption property of cryogel is greatly determined by the chemical composition of the network. When the concentration of PVA and egg albumin increases in the gel, the swelling ratio of the cryogel constantly increase and beyond a definite concentration of PVA it starts decreasing. While in the case of increasing egg albumin concentration, the degree of swelling initially increases and beyond a definite concentration, it gradually falls.

The increasing number of freezing-thawing cycles (FTC) also results in a constant fall in the amount of water sorption.

The extent of water sorption by the cryogel is found to increase from acidic to neutral pH range while a fall in the swelling ratio is noticed with increasing pH in the alkaline pH range. The swelling ratio increases with increasing temperature of the swelling medium while it decreases when the concentration of electrolyte (NaCl) is increased in the outer aqueous medium. The cryogel shows a significantly suppressed swelling when they are dried at increasing temperatures. A lower degree of swelling is also observed in simulated biological fluids like saline water, artificial urine, urea, KI solution, and *D*-glucose solutions. It is also found that with varying egg albumin composition of cryogel, the mechanism of water transport changes from Fickian to non-Fickian nature while in case of PVA, it remains Fickian in nature.

The prepared cryogels exhibit a fair degree of blood compatibility as evident from protein adsorption, blood clot formation and hemolysis assay tests. It is found that on increasing the concentrations of PVA and egg-albumin, the biocompatibility of cryogel increases while a reduced biocompatibility is noticed with increasing number of freeze-thaw cycles.

Acknowledgment

The authors gratefully acknowledge the Regional Sophisticated Instrumentation Centre, Indian Institute of Technology, Mumbai (India) for carrying out IR, DSC and SEM analysis.

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Received 9 June 2004
and accepted 25 May 2005