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ADSORPTION DYNAMICS OF BOVINE SERUM ALBUMIN (BSA) ONTO BINARY INTERPENETRATING POLYMER NETWORKS (IPN_S) OF POLY(2-HYDROXYETHYL METHACRYLATE) (PHEMA)

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ADSORPTION DYNAMICS OF BOVINE SERUM ALBUMIN (BSA) ONTO BINARY INTERPENETRATING POLYMER NETWORKS (IPNs) OF POLY(2-HYDROXYETHYL METHACRYLATE) (PHEMA)

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ABSTRACT

A binary hydrophilic interpenetrating polymer network (IPN) comprising of polyethylene glycol (PEG) and poly(2-hydroxyethyl methacrylate) (PHEMA) was prepared and the adsorption of bovine serum albumin (BSA) was carried out onto the surfaces of the IPNs. The adsorbed amounts of BSA were investigated as a function of the composition of the hydrogel, pH and ionic strength of the protein solution. The adsorption of protein was also studied kinetically and various parameters such as the rate constants for adsorption, diffusion and penetration rate constants were evaluated. For the assessment of the antithrombogenic property of the IPNs surfaces, blood-clot formation tests were performed.

Key Words: Adsorption; BSA; Hydrogel; Poly(2-hydroxyethyl methacrylate); Polyethylene glycol

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INTRODUCTION

Hydrogels are water-swollen macromolecular matrices consisting of polymeric chains crosslinked together either covalently or non-covalently and insoluble in water at physiological temperature, pH and ionic strength [1]. The water imbibing capacity of hydrogels is not only responsive to the pH, temperature and ionic strength of the swelling reservoir, but also to the chemical architecture of the network such as chain flexibility, crosslinking density, hydrophilic functionals, osmotic potential, and free volume. The high water content of the hydrogel not only imparts soft and rubbery texture to the material, but also develops antithrombogenicity in the polymer matrix because of the lowered free energy of the hydrated interface [2]. These physicochemical properties of hydrogels enable them to serve as potential biomaterials in biomedical engineering and allied fields.

At present, a wide number and great variety of clinically important hydrogels are finding applications as short and long term materials in kidney dialyzers, blood oxygenators, heart valves, vascular grafts, contact lenses, etc. [3, 4]. All of these implants and devices contain materials that are recognized by blood as foreign; the result is a process of thrombosis often followed by formation of thromoemboli. This process generally involves a sequence of protein adsorption steps followed by blood cell interactions.

In fact, protein adsorption onto biomaterial surfaces is believed to be the earliest event following implantation. This process is determined by the nature of the protein, the composition of the biological fluid, and the surface characteristics of the implanted device [5]. Conditioning with a protein layer can induce a series of consequential processes which may be beneficial or deterimental to the performance of the biomaterial. For instance, the binding of structural proteins such as fibronectin and collagen can improve the attachment of tissue cells, favoring the integration of a prosthesis into the host tissue [6]. Conversely, fibronectin and fibrinogen have been found to enhance the adhesion of bacterial species such as Staphylococcus aureus, a common cause of implant-related infection, onto polymeric biomaterials [7].

Since the first attempt in 1950s to develop blood-compatible materials with a negatively-charged surface for artificial vessels [8], continuous efforts to design biomaterials with superior blood compatibility have been made by various research groups. For instance, an extremely hydrophilic surface was found to display minimum protein adsorption and to prevent thrombus formation [9]. This concept stimulated research in the development of many type of hydrogels which have utility for constructing blood-contacting surfaces of various medical devices. Among these approaches, surface grafting with tethered brushes of hydrophilic polymers, such as polyethylene glycol (PEG), have been found to achieve minimal interaction with proteins and platelets [9]. Both flexibility and hydrophilicity are thought to play an essential role in this reduced interaction of brushed surfaces with blood components due to a steric repulsion mechanism.

It has also long been recognized that various plasma proteins offer different degree of interactions with the biomaterial surfaces and thus lead, to respective physiological events. For instance, it is known that fibrinogen has a high affinity for most surfaces [10] and that fibrinogen and γ-globulin (lgG) adsorbed to surfaces promote platelet adhesion and activation [11]. In contrast, albumin has been found to reduce biomaterial surfaces towards thrombogenic reactions [12], in part due to lack of glycoprotein subunits present on fibrinogen, IgG, and other plasma proteins that permit platelet membrane binding [13].

Thus, looking to the significant consequences of protein adsorption on biomaterial surfaces, we, in the present investigation, are reporting results on the adsorption of bovine serum albumin (BSA) onto the surfaces of an IPN of polyethylene glycol (PEG) and poly(2-hydroxyethyl methacrylate) (PHEMA). The selection of PEG as a hydrophilic polymer for synthesizing IPN rests upon the fact that it is a low cost, water soluble, biocompatible, non-toxic and non-immunogenic material and has been extensively employed in biotechnical and biomedical fields [14]. On the other hand, the HEMA polymers are well known for their vast biomedical and pharmaceutical applications such as soft contact lenses, wound dressings, surgical prostheses, blood compatible surfaes, drug-delivery vehicles, etc. [15]. The protein opted for *in vitro* adsorption study is BSA which is among the most abundant proteins in vertebrates and is commercially available at low cost. BSA has also been widely used in biochemical work as a generic protein.

EXPERIMENTAL

Materials

Polyethylene glycol (mol.wt. 600) was obtained from Loba Chemie, India and used as received. 2-Hydroxyethyl methacrylate (HEMA) was obtained from Lancaster, England and freed from the inhibitor by the method described in the literature [16]. The monomer was further purified by vacuum distillation at 67°C and 3.5 mm Hg. Hydroquinone was added to the distillation flask to inhibit polymerization during the distillation. The top and bottom 20% fractions were discarded. The middle 60% was stored in a dark glass bottle at 4°C prior to polymer synthesis. Ethylene glycol dimethacrylate (EGDMA), used as a crosslinker, was obtained from Merck, Germany and used as received. Potassium metabisulphite and potassium persulphate (both from E. Merck, India) were used to form a redox pair and their fresh solutions were always prepared prior to start the experiment. Double distilled water was used throughout the experiments.

Preparation of IPN

An IPN has been defined as an intimate mixture of two or more polymers in which at least one has been polymerized in the immediate presence of other [17].

Following the above definition, the IPN's were prepared by a redox polymerization method. In brief, into a petri dish (diam. 4', Corning) were added PEG (3.4% v/v), HEMA (34.5% v/v), EGDMA (2.3% v/v), Ethylene glycol (34.5% v/v, as a cosolvent), potassium metabisulphite and potassium persulphate (11.5% v/v of each solution of 0.4M and 0.04M, respectively). The mixture was allowed to polymerize for 72 hours and then equilibrated in bidistilled water for a week where it changed into a soft white circular disc. The swollen IPN was cut into equal sized buttons (diam. 0.5 cm, thickness 0.18 cm) which were then dried at room temperature for 15 days. Upon drying, the buttons became transparent from white.

For testing the stability of the IPNs, the IPNs were left in a water reservoir in a swollen state for two weeks and their weights were recorded every day after one week. It was found that the swollen IPNs gained constant weights after one week which indicates that neither PEG nor other reacted components of the IPN were leached-out after optimum swelling. This clearly implies a stable IPN.

Swelling Measurement

The extent of swelling was determined by a conventional gravimetric procedure. In brief, a preweighed piece (0.10 g) of hydrogel was immersed into a swelling medium (pH 7.4) and allowed to swell till the equilibrium swelling was attained by the hydrogel. The swollen piece was then taken out, pressed gently in between two filter papers to remove excess water and finally weighed. The degree of water sorption was expressed in terms of the equilibrium water content as given below:

$$\text{Equilibrium Water Content} = \frac{\text{Amount of water in the swollen gel}}{\text{Weight of the dry gel}} \quad (1)$$

Blood Compatibility Tests

In order to evaluate the blood compatibility of the IPN surfaces, a blood-clot formation method was used as described elsewhere [18]. In brief, the IPN samples were equilibrated with saline water (0.9% NaCl solution) for 24 hours in a constant temperature bath. To these water swollen and equilibrated samples were added 0.5 ml of acid citrate dextrose (ACD) blood followed by the addition of 0.03 ml of $CaCl_2$ solution (4M) to start the thrombus formation. The reaction was stopped by adding 4.0 ml of deionized water and the thrombus formed was separated by soaking in water for 10 minutes at room temperature and then fixed in 36% formaldehyde solution (2.0 ml) for another 10 minutes. The fixed clot was placed in water for 10 minutes and after drying, its weight was recorded. The same procedure was repeated for the glass surface and other IPNs and respective weights of thrombus formed were recorded.

Protein (BSA) solution for adsorption experiments were made in 0.5M phosphate buffer saline (PBS) at physiological pH 7.4. A fresh solution of BSA was always prepared for every adsorption experiment. Prior to adsorption experiments,

the IPN buttons were equilibrated with PBS for 24 hours. The adsorption was then carried out by gently shaking a solution of BSA of known concentration containing preweighed and fully swollen IPN button. After a definite time interval, the IPN button was removed and the protein solution was analyzed for the remaining concentration of BSA by a spectrophotometric procedure (Systronics, Model No. 106, India) as described elsewhere [19]. The adsorbed amount of BSA was calculated by the following mass-balance equation:

$$\text{Adsorbed BSA} = \frac{(C_o - C_e)V}{m} \tag{2}$$

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

For studying the kinetics of the adsorption process the amount of adsorbed BSA was determined at predetermined time intervals.

All the experiments were performed in replicate numbers (five) and a fair reproducibility was always obtained.

RESULTS AND DISCUSSION

Swelling of IPN Samples

For a complete adsorption investigation a series of IPNs were prepared by manipulating the feed mixture composition of the IPNs by varying the concentrations of PEG, HEMA, and EGDMA in the feed mixture. The IPNs synthesized with different compositions and respective water sorption capacities are presented in Table 1. It is clear from the data that a variation in PEG, HEMA, and EGDMA clearly and significantly brings about a change in the EWC of the IPNs.

Table 1. Data Showing the Equilibrium Water Content of the IPNs of Varying Compositions

S. No.	PEG (% v/v)	HEMA (% v/v)	EGDMA (% v/v)	EWC[a]
1.	3.45	34.5	2.3	250±6.24
2.	5.75	34.5	2.3	210±4.80
3.	9.2	34.5	2.3	180±2.12
4.	13.8	34.5	2.3	108±1.88
5.	5.75	25.8	2.3	280±3.46
6.	5.75	34.5	2.3	210±2.95
7.	5.75	43.1	2.3	162±1.76
8.	5.75	51.7	2.3	110±2.68
9.	5.75	34.5	1.15	270±1.76
10.	5.75	34.5	2.3	210±1.84
11.	5.75	34.5	3.45	166±2.26
12.	5.75	34.5	4.6	140±1.94

[a]Values represent the mean ± S.D. of at least triplicate determination.

The results indicate that when the concentration of PEG increases from 3.45 to 13.8 %v/v, the EWC significantly decreases from 250 to 108. The observed fall could be attributed to the reason that on increasing the amount of PEG in the IPN the number of network chains also increases which, in turn, enhances the density of the IPN. Thus, the IPN becomes more dense in network chains which results in an entrance of less number of water molecules into the IPN thus decreasing the EWC. In a similar way the increasing amounts of HEMA and EGDMA suppress the EWC of the IPN's. Water sorption results have already been discussed elsewhere in detail [20].

Concentration Effect and Adsorption Isotherms

To observe the influence of initial concentration of BSA solution on the adsorbed amounts of protein, the concentration of BSA was varied in the range from 1.0 to 6.0 mg/ml. The results clearly indicate that the adsorbed amount gradually increases with increasing BSA concentration and finally it attains a limiting value which is indicative of a formation of a monolayer on the IPN's surfaces. The observed findings are quite usual and may be explained by the fact that with increasing bulk concentration of the protein solution, a greater number of BSA molecules appear at the interface and get adsorbed over the IPN surfaces. This is a very common result and has been widely reported.

More quantitative information about the protein-surface interaction can be obtained by constructing an adsorption isotherm by plotting the adsorbed amount of BSA against the residual concentration of the protein solution. The isotherm so plotted is shown in Figure 1, which implies that it belongs to a typical Langmuir type curve which is characterized by an initial rising portion followed by a plateau portion. A similar type of isotherms have been frequently reported in the literature [21].

The affinity of BSA for the IPN surfaces was quantitatively evaluated by applying the following linear form of the Langmuir equation:

$$\frac{C_e}{q_e} = \frac{C_e}{Q^o} + \frac{1}{bQ^o} \tag{3}$$

where C_e is the equilibrium concentration of the protein solution, q_e is the amount of adsorbed BSA, Q^o is defined as the mass of the protein adsorbed per unit mass of adsorbent in forming a complete monolayer (monolayer capacity), and b is a constant known as the adsorption coefficient or affinity constant. The value of b has been calculated to be 0.45 $cm^3\ mg^{-1}$ from the linear plot drawn in accordance with Equation 3). When we compare this value of adsorption coefficient with the values determined for the same protein adsorbing on polystyrene and poly(styrene-co-ethylene oxide) [22], it is found that a much lower value of b is obtained in the present system which indicates a low protein adsorbing nature of the IPN's surfaces.

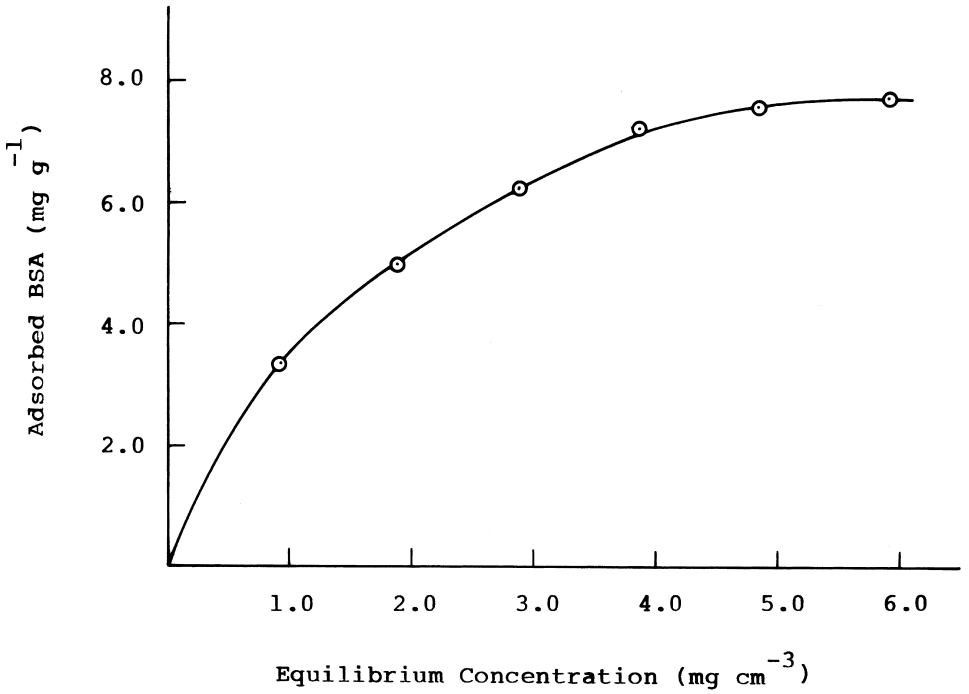

Figure 1. Adsorption isotherm of the BSA on the IPN surfaces of fixed composition [PEG] = 5.75 %v/v, [HEMA] = 46.0 %v/v, [EGDMA] = 2.3% v/v, Ionic strength = 0.01 M KCl, pH = 7.4, Temp. = 27 $\pm$ 0.2°C.

Effect of pH and Ionic Strength

The influence of pH on adsorption of proteins onto ionic surfaces is of prime significance as a variation in pH of the system affects the charge profiles of both the protein and surfaces. In the present investigation, since the adsorbent is an IPN of non-ionic nature it is, therefore, believed that the extent of BSA adsorption will be solely determined by the net charge possessed by the protein molecule. To observe the role of pH in BSA adsorption the pH of the BSA solution was varied in the range 3.6 to 10.7 and the results are depicted in Figure 2. It is clear from the results that a maximum adsorption is noticed at pH 5.0 which is near to the isoelectric point of the protein. The observed maximum adsorption at isoelectric point has been largely reported by other workers [23] and may be attributed to the fact that BSA molecules form compact structures and more molecules can adsorb in the given surface area, because the electrostatic repulsions of intramolecules and the lateral interactions between BSA molecules are minimized in the isoelectric region.

An interesting and important observation is that the shape of the adsorption isotherm is appreciably affected by the ionic strength of the medium as shown in Figure 2. As the figure implies, in the region of acidic pH, the amount adsorbed increases with increasing ionic strength. For this result the possible explanation may be

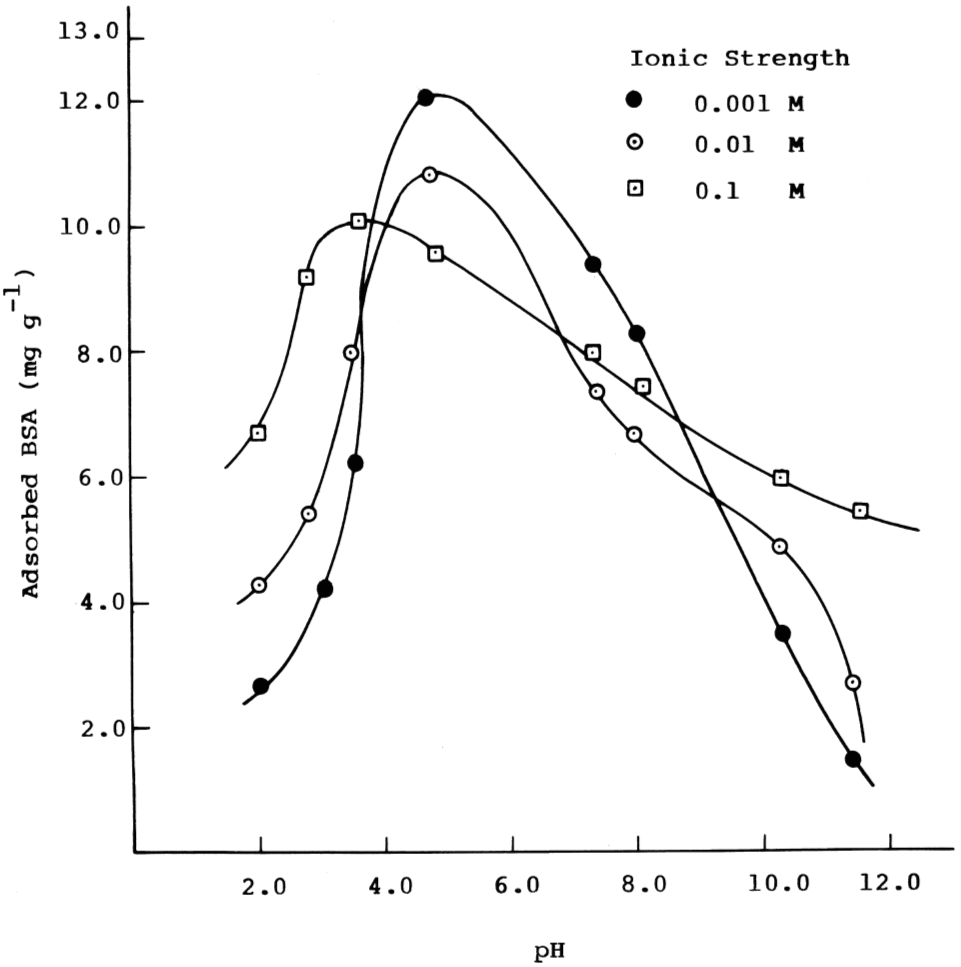

Figure 2. Adsorption isotherms of BSA onto the IPN surfaces at different pH and ionic strengths of the BSA solution at fixed composition [PEG] = 5.75% v/v, [HEMA] = 46.0% v/v, [EGDMA] 2.3% v/v, Temp. = 27 $\pm$ 0.2°C.

that with increasing ionic strength the electrostatic repulsions in the interior of protein molecules are decreased. This leads protein molecules to form more compact structures. Moreover, lateral repulsions between adsorbed protein molecules decrease with increasing ionic strength. Thus, more molecules can adsorb in the given surface area.

Another interesting feature displayed by the Figure 2 is that with increasing ionic strength of the protein solution, the optimum pH shifts towards acidic range as noticed by several other authors also [24]. A possible explanation may be that with increasing ionic strength, the isoelectric point of BSA may also shift to the acidic range as BSA is well recognized for its binding property [25]. What actually may happen is that the salt ions may interact with anionic groups (such as $-COO^-$, $-O^-$ and $-S^-$) and cationic groups (such as $-NH^+-$, $-NH_3^+$, and $=NH_2^+$) of the protein molecule and thus alter the isoelectric point of the protein.

Effects of IPN Composition on Protein Adsorption

A large number of investigations have confirmed the observation that the composition and organization of the adsorbed protein layer can be varied by numerous factors relating to the substrate, such as hydrophobicity, sorbed water content, microphase separation, and surface chemical functionality. As far as the chemistry of surfaces is concerned, the effect of hydrophilic and hydrophobic balance of constituent chains in polymer surfaces has been found to play a key role in influencing protein adsorption and subsequent platelet adhesion to polymer surfaces [26]. In general, a hydrophobic surface offers greater affinity for protein adsorption then that by a hydrophilic surface and this has been confirmed by a number of investigators also [27].

In the present study, the effect of PEG content in the IPN on protein adsorption has been studied by increasing PEG content in the feed mixture of the IPN in the range 3.4 to 13.8% v/v. The results are shown in Figure 3 which indicates that the equilibrium protein adsorption decreases with increasing PEG content in the

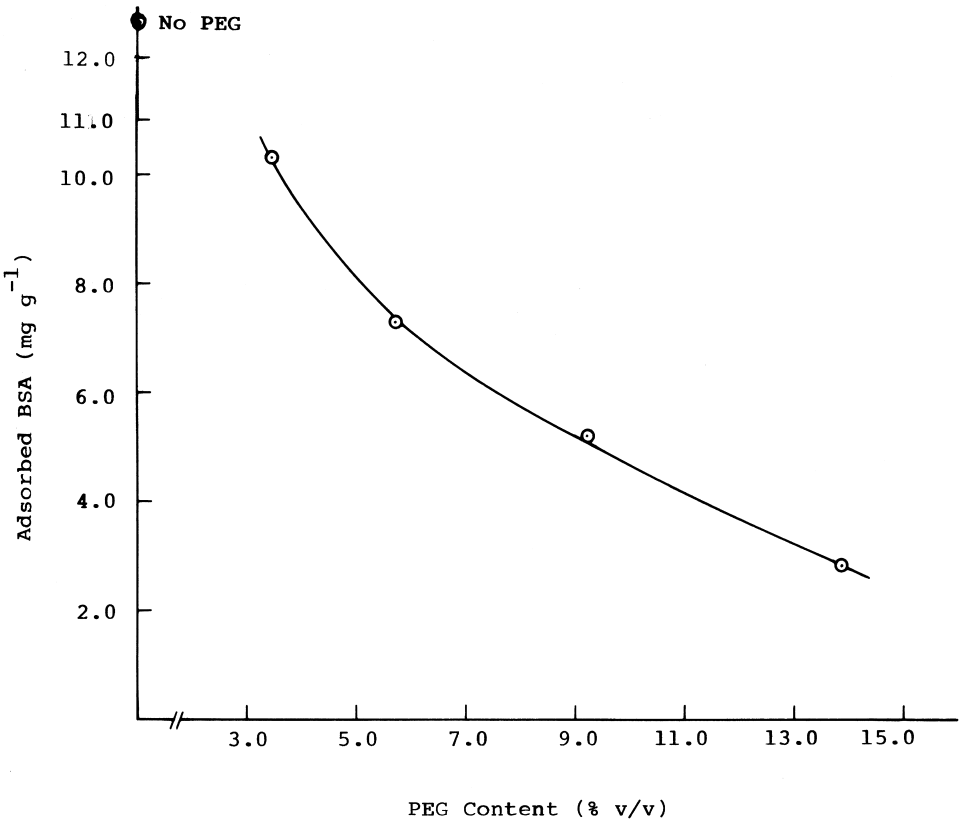

Figure 3. Effect of PEG content of the IPN on the adsorbed amount of BSA at fixed composition [HEMA] = 46.0 %v/v, [EGDMA] = 2.3% v/v, [BSA] = 4.0 mg/ml, Ionic strength = 0.01 M KCl, pH = 74., Temp. = 27 $\pm$ 0.2^{o}C.

IPN. This can also be seen from the Figure. A maximum adsorption of BSA (12.6 mg g^{-1}) is observed for that IPN which does not contain PEG. The results can be explained by the fact that PEG itself is known for its unique physiological behavior in suppressing levels of both protein adsorption and platelet adhesion [28, 29]. Another reason may be that PEG rich surfaces have extreme chain mobility or excluded volume effects [30], and for a lack of molecular binding sites on PEG for proteins [31]. The inertness of PEG towards proteins is further confirmed by the fact that PEG is used to concentrate proteins in blood. Several workers have also noticed a reduced platelet adhesion and thrombin adsorption with PEG containing hydrogel surfaces [32].

If the above results of BSA adsorption are correlated with the water content of the IPN's, then a contradiction is noticed as we have earlier said that a less hydrophilic surface should display more protein adsorption which is not the case here. It is, therefore, worth mentioning here that the water content is not only important, but also the surface mobility and volume restriction which, consequently, determine the state of water (degree of free water fraction over restricted water), a critical factor influencing blood interactions at polymer interface. Therefore, it is imperative that a conclusion is not drawn of biocompatibility based only on water content of polymers. Similar types of observations have also been reported by other workers [33].

The influence of HEMA content of the IPN on the adsorption of BSA has also been studied by increasing HEMA in the feed mixture in the range 34.5 to 69.0% v/v. The results are depicted in Figure 4, which reveal that the adsorption increases with increasing HEMA content in the feed mixture. The results can be explained by the fact that increasing HEMA results in less water content in the IPN and, therefore, as a consequent adsorption increases.

The crosslinker employed in the present study was EGDMA which is a known hydrophobic crosslinking agent. The effect of EGDMA on the adsorption of BSA has been investigated by increasing its proportion in the IPN in the range 1.15 to 6.9% v/v. The results are shown in Figure 5 which reveal that the adsorbed BSA increases with increasing EGDMA concentration in the IPN. The increase in the BSA adsorpton can be attributed to the fact that albumin, which is identified for containing high hydrophobic domains interact with the hydrophobic segments of the crosslinker EGDMA and thus cause adsorption of BSA on the IPN surfaces as a result of entropic hydrophobic interactions and lyophilic liquid binding capabilities. A similar type of results have been frequently published by other workers [26].

Kinetics of Adsorption

Accurate knowledge of the adsorption kinetics under a given set of conditions is a prerequisite for elucidating the mechanisms of many fundamental biological processes. The process of adsorption of proteins onto solid surfaces

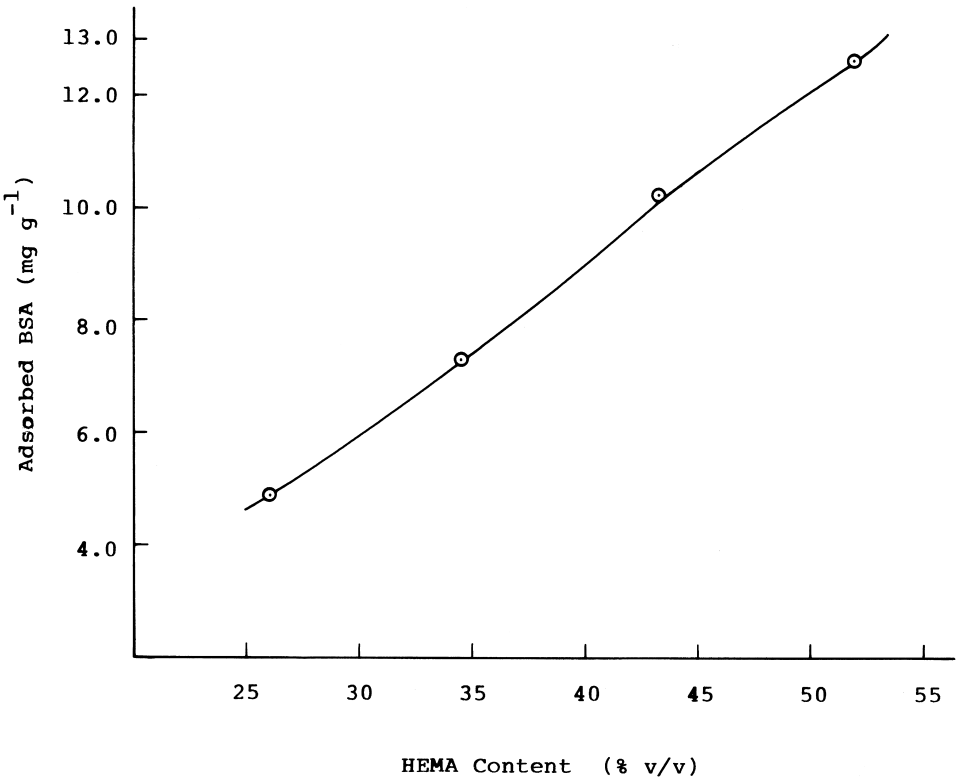

Figure 4. Effect of HEMA content of the IPN on the absorbed amount of BSA at fixed composition [PEG] = 5.75 %v/v, [EGDMA] = 2.3% v/v, [BSA] = 4.0 mg/ml, Ionic strength = 0.01 M KCl, pH = 7.4, Temp. = 27 $\pm$ 0.2°C.

involves the following three steps [34]: (a) transport of protein molecules towards the surface by diffusion and/or convection, (b) attachment of protein molecules at the active sites on the surface, and (c) changes in the conformation of the adsorbing protein molecules.

In the present study, the progress of the adsorption process is shown in Figure 6 which implies that the rate of adsorption is almost constant up to 15 minutes and then it gradually slows down with time. Actually, the adsorption kinetics of end functionalized large chains is a two regime process [35]. At the initial stages, the substrate surface is bare and the kinetics of adsorption is governed by the diffusion of the chains from the bulk solution to the surface. All the chains that arrive at the interface are considered to be immediately adsorbed. The mass transport can be interpreted as a Fickian diffusion.The diffusion coefficient can be determined by the following equation:

$$q = \frac{2}{\pi} C_o \sqrt{Dt} \tag{4}$$

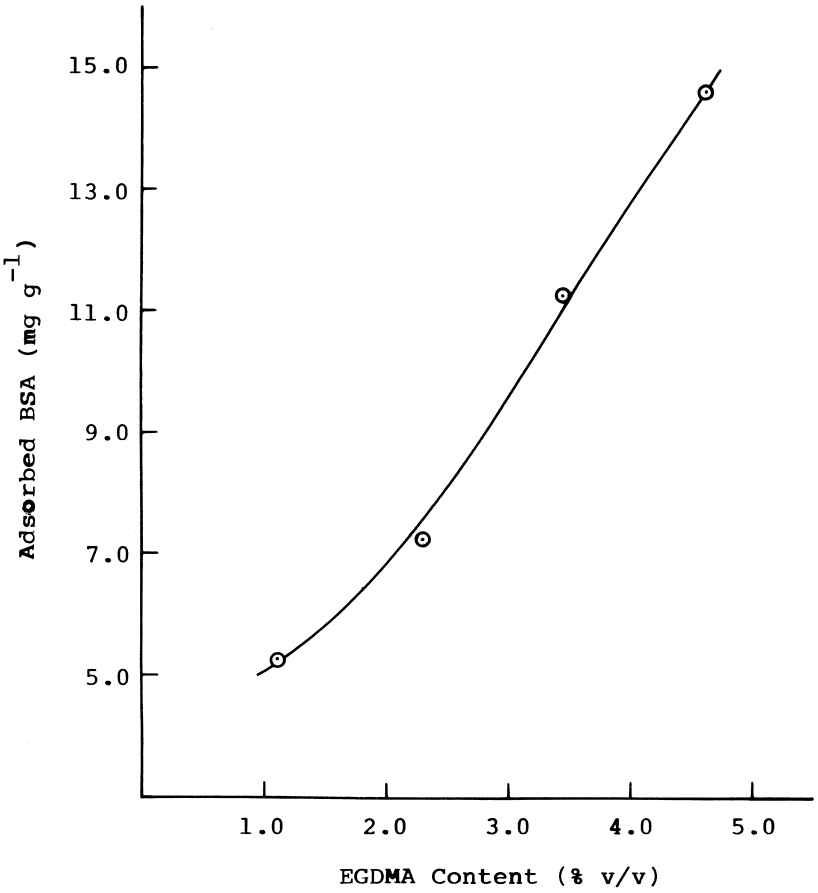

Figure 5. Effect of EGDMA content of the IPN on the absorbed amount of BSA at fixed composition [PEG] = 5.75 %v/v, [HEMA] = 46.0% v/v, [BSA] = 4.0 mg/ml, Ionic strength = 0.01 M KCl, pH = 7.4, Temp. = 27 ± 0.2°C.

From the slope of the curve drawn between q (adsorbed BSA) and √t, for the BSA solution at varying concentrations, the diffusion constant has been calculated and summarized as Table 2. It is revealed from Table 2 that with an increasing concentration of BSA solution, the diffusion constants also increase.

After the initial step, an activation barrier of adsorbed chains is formed which governs the kinetics because the chains arriving from the solution have to diffuse across the barrier. Ligowe and Leibler [36] considered a simplified model where the adsorbed amount approaches exponentially an equilibrium adsorbed amount q_e with time t, introducing a characteristic penetration rate constant (1/T):

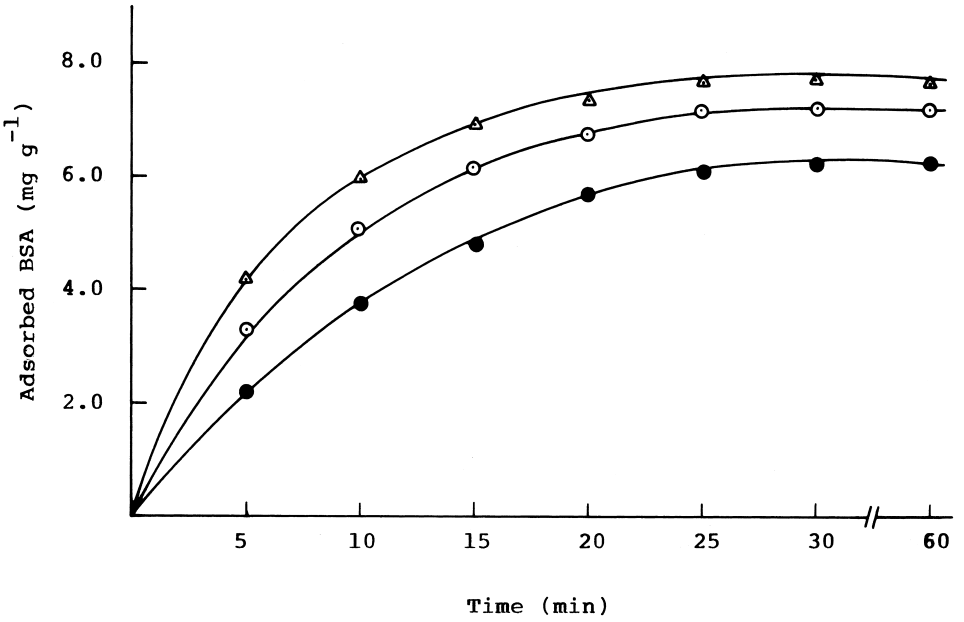

Figure 6. A plot showing the variation of the adsorbed amounts of BSA (mg g^{-1}) with time t, at varying concentratons of BSA, (●) 13 mg/ml, (○) 4 mg/ml, (△) 15 mg/ml. Ionic strength = 0.01 M KCl, pH = 74., Temp. = 27 ± 0.2°C (IPN composition as in Figure 1).

$$q=q_e[1-\exp(-t/T)] \tag{5}$$

The above equation suggests that the second process has an exponential nature, and the penetration rate may be obtained from the slope of $[\ln(q_e\text{-}q)]$ as a function of time. From the slope of the straight lines (not shown) the penetration rate constants have been calculated and summarized in Table 2.

Table 2. Various Kinetic Parameters of the Adsorption Process for Varying Concentrations of BSA Solution

Initial BSA Concentration (mg cm^{-3})	Penetration Rate Constant[a] $k_p \times 10^2$ (mg g^{-1} s^{-1})	Rate Constant for Adsorption[a] $k_1 \times 10^6$ (s^{-1})	Diffusion Constant[a] $D \times 10^7$ (cm^2 s^{-1})
3.0	12.0±0.37	3.1±0.28	6.2±0.37
4.0	14.2±0.28	3.3±0.32	8.6±0.84
5.0	15.0±0.48	3.3±0.44	10.2±1.05

[a]Values represent the mean ± S.D. of at least triplicate determination.

We also calculated the rate constants for adsorption and desorption (k_1 and k_2, respectively) from Equation 6 [37]:

$$\frac{1}{C} = \frac{k_1 t}{C_o} + \frac{1}{C_o} \tag{6}$$

and the data is summarized in Table 2. The results clearly reveal that the rate constant for adsorption (k_1) is almost independent of the BSA concentration, thus confirming the first order nature of the adsorption process.

Blood-Clot Formation

Also, as mentioned earlier, a thin layer of protein is formed at the blood-material interface within a few seonds after blood contacts a foreign surface. Subsequent cellular events, such as adhesion and aggregation of platelets that initiate clot formation, are most likely mediated by this protein layer instead of by the material surface itself. Since different IPN's show different affinity for protein adsorption, the clot formation must also be a function of the chemical architecture of the IPN's and their surface as well.

The results of blood-clot formation study are presented in Table 3 which indicate that with variation in the composition of the IPN the weight of the blood clot also varies. It can be clearly seen in the table that the weight of blood-clot increases with an increase in HEMA and EGDMA content in the IPN. While an increase in PEG content results in clot formation of lower weights. It should also be noted that an IPN without PEG produces a greater weight of blood-clot on its

Table 3. Weights of Blood Clots Formed on the Surfaces of IPN's of Varying Compositions

S. No.	PEG (% v/v)	HEMA (% v/v)	EGDMA (% v/v)	Wt. of Blood-clot (mg)[a]
1.	0.0	34.5	2.3	17.2±0.54
2.	3.45	34.5	2.3	12.4±0.78
3.	5.75	34.5	2.3	10.2±0.62
4.	9.2	34.5	2.3	8.0±0.88
5.	13.8	34.5	2.3	6.2±1.04
6.	5.75	25.8	2.3	8.6±0.86
7.	5.75	34.5	2.3	10.2±0.42
8.	5.75	43.1	2.3	12.0±0.64
9.	5.75	51.7	2.3	14.4±0.34
10.	5.75	34.5	1.15	7.2±0.41
11.	5.75	34.5	2.3	10.2±0.36
12.	5.75	34.5	3.45	12.6±0.79
13.	5.75	34.5	4.6	14.8±0.79
14.	Glass surface			19.4±0.81

[a]Values represent the mean ± S.D. of at least triplicate determination.

surface as depicted in Table 3. This is in accordance with our earlier observation that an IPN without PEG shows maximum BSA adsorption on its surface. The weights of blood clots formed on hydrophilic IPN surfaces are much less than that formed on a glass surface. These results support the hypothesis that the greater the water content, the larger would be the antithrombogenicity of the materials surface, although it is not universally true.

CONCLUSION

The polymerization of HEMA in the presence of PEG gives rise to an IPN with improved water-sorption property. The IPN's display a varying degree of swelling with changing chemical architecture of the IPN. Whereas, the swelling ratio of the IPN decreases with increasing HEMA and crosslinker (EGDMA) concentrations in the feed mixture, the IPN shows optimum swelling at 3.45% v/v content of the PEG while beyond it, the swelling decreases.

The surfaces of the IPN exhibits affinity for adsorption to BSA molecules which varies in degree with varying composition of the IPN. It is found that the adsorption of BSA follows Langmuirian nature. The adsorbed amount of protein decreases with increasing the PEG content, while it increases with increasing the HEMA and crosslinker (EGDMA) content of the IPN's. The adsorption is found to be sensitive to pH of the protein solution and becomes maximum at the isoelectricpoint (pH 4.8) of the BSA. It is also observed that the shape of the isotherm is influenced by the ionic strength of the medium and the pH at the optimum adsorption shifts towards the acidic range when the ionic strength of the protein solution is higher.

The BSA molecules adsorb at the IPN surfaces via a three step mechanism, i.e., diffusion, attachment at the surface, and reconformation. The IPN's synthesized show a good degree of antithrombogenicity which depends on the chemical nature of the surface. Thus, an IPN with higher PEG and lower HEMA and EGDMA (crosslinker) displays least weight of the blood-clot formed on its surfaces.

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REFERENCES

1. Peppas, N.A.; Korsmeyer, R.W. In *Hydrogels, Medicine and Phamacy*; Peppas, N.A., Ed.; CRC Press: Boca Raton, FL., 1987; Vol. 3, pp. 109-135.
2. Kin, S.W.; Feijen, J. CRC Crit. Rev. Biocompatibility **1985**, *1*, 229-260.

3. Honiger, J.; Couturiety, C.; Goldschmidt, P.; Maillet, F.; Kazatchkine, M.D.; Laroche, L. New Anionic Polyelectrolyte Hydrogel for Corneal Surgery. J. Biomed. Mater. Res. **1997**, *37*, 548-553.
4. Szycher, M. High Performance Biomaterials. In *A Comprehensive Guide to Medical and Pharmaceutical Applications*, Technomic: Lancaster, 1991.
5. Horbett, T.A. Principles Underlying the Role of Adsorbed Plasma Proteins in Blood Interactins with Foreign Materials. Cardiovascular Pathol. **1993**, *2*, 137-148.
6. Drumheller, P.D.; Elbert, D.L.; Hubbell, J.A. Multifunctional poly(ethylene glycol) Semi-Interpenetrating Polymer Networks as Highly Selective Adhesive Substrates for Bioadhesive Peptide Grafting. Biotech. Bioeng. **1994**, *43*, 772-780.
7. Vaudaux, P.E.; Francois, P.; Procter, R.A. Use of Adhesion-Defective Mutants of Staphylococcus aureus to Define the Role of Specific Plasma Proteins in Promoting Bacterial Adhesion to Canine Arteriovenous Shunts. Infect. Immun. **1995**, *64*, 585-590.
8. Sawyer, P.N.; Pate, J.W. Surgery **1953**, *34*, 491.
9. Ishihara, K. Blood Compatible Polymers. In *Biomedical Applications of Polymeric Materials*; Tsuruta, T.; Hayashi, T.; Kataoka, K.; Ishihara, K. (Eds.); CRC Press: Boca Raton, FL, **1993**, p. 89.
10. Lin, J.-C.; Cooper, S.L. In vitro Fibrinogen Adsorption from Various Dilutions of Human Blood Plasma on Glow Discharge Modified Polyethylene. J. Colloid Interface Sci. **1996**, *182*, 315-325.
11. Rao, G.H. Physiology of Blood Platelet Activation. Indian J. Physiol. Pharmacol. **1993**, *37*, 263-275.
12. Brash, J.L. Role of Plasma Protein Adsorption in the Response of Blood to Foreign Surfaces. In *Blood Compatible Materials and Devices Perspectives Towards the 21st Century*; Sharma, C.P.; Szycher, M.C., Ed.; Technomic Publishing Co.: Lancaster, PA, 1991, pp. 3-19.
13. Lee, E.S.; Kim, S.W. Trans. ASAIO, **1979**, *25*, 124.
14. Harris, J.M. In *Poly(ethylene glycol) Chemistry*. Harris, J.M., Ed.; Plenum Press: New York, 1992.
15. LaPorte, R.J. In *Hydrophilic Polymeric Coatings for Medical Devices*, Technomic Publishing Company Inc.: Lancaster, PA, 1997.
16. Pinchuk, L.; Eckstein, E.C.; Van De Mark; M.R. Effects of Low Levels of Methacrylic Acid on the Swelling Behaviour of Poly(2-hydroxyethyl methacrylate). J. Appl. Polym. Sci. **1984**, *29*, 1749-1760.
17. Sperling, L.H. In *Source Based Nomenclature of Polymer Blends, IPN's and Related Materials*. Division of Polymer Chemistry Nomenclature Committee Document, 1984.
18. Imai, Y.; Nose, Y. J. Biomed. Mater. Res. **1972**, *6*, 165.
19. Kaplan, A.; Szaboo. In *Clinical Chemistry: Interpretation and Techniques*. Lee, Febiger, Ed.; Philadelphia, 1987.
20. Bajpai, A.K.; Shrivastava, M. Water Sorption Dynamics of a Semi-IPN of 2-Hydroxyethyl Methacrylate. J. Polym. Materials (Communicated).
21. Brash, J.L.; Horbett, J.A. In *Proteins at Interfaces. ACS Symposium Series*, 1995, Vol. 602, Am. Chem. Soc., Washington, DC.
22. Grainger, D.W.; Okano, T.; Kim, S.W. Protein Adsorption from Buffer and Plasma onto Hydrophilic-Hydrophobic Poly(ethylene oxide)–Polystyrene Multiblock Copolymers. J. Colloid Interface Sci. **1989**, *132*(1), 161-175.

23. Revilla, J.; Elaissari, A.; Carriere, P.; Pichot, C. Adsorption of Bovine Serum Albumin onto Polystyrene Latex Particles Bearing Saccharidic Moieties. J. Colloid Interface Sci. **1996**, *180*, 405-412.
24. Koutsoukos, P.G.; Norde, W.; Lyklema, J. Protein Adsorption on Hematite (α-Fe_2O_3) Surfaces. J. Colloid Interface Sci. **1983**, *95*(2), 385-397.
25. Goldstein, A.; Aronow, L.; Katman, S.M. In *Principles of Drug Action, The Basis of Pharmacology*, Wiley: New York, 1974.
26. Grainger, D.; Okano, T.; Kim, S.W. In *Advances in Biomedical Polymers*; Gebelein, C.G., Ed.; Plenum: New York, 1987, p. 229.
27. Kataoka, K.; Ito, H.; Amano, H.; Nagasaki, Y.; Kato, M.; Tsuruta, T.; Suzuki, K.; Okano, T.; Sakurai, Y. Minimized Platelet Interaction with Poly(2-hydroxyethyl methacrylate-block–4-bis(trimethylsilyl) Methylstyrene). Hydrogel Showing Anomalously High Free Water Content. J. Biomater. Sci. Polymer Edn. **1998**, *9*(2), 111-129.
28. Hawk, G.L.; Cameron, J.A.; Dufault, L.B. Prep. Biochem. **1972**, *2*, 193.
29. Mori, Y.; Nagaoka, S.; Takiguchi, H.; Yokota, K.; Tanzawa, H.; Nishiumi, S. Polym. Prepr. **1983**, *24*, 67.
30. Mori, Y.; Nagaoka, S. Trans. ASAIO **1982**, *28*, 459.
31. Merrill, E.W.; Satzman, E.W. Polyethylene Oxide as Biomaterials. ASAIO J. **1983**, *6*, 60.
32. Wasiewski, W.; Rasio, M.J.; Martin, B.M.; Detwiler, J.C.; Fenton, J.W. Thrombin Adsorption to Surfaces and Prevention with PEG 6000. Thromb. Res. **1976**, *8*, 881.
33. Nagaoka, S.; Mori, Y.; Takiuchi,H.; Yokota, K.; Tanzawa, H.; Nishimui, S. Interaction Between Blood Components and Hydrogels with PEO Chain. Polym. Prepr. **1983**, *24*, 67.
34. Dijit, J.C.; Cohen Stuart, M.A.; Hofman, J.E.; Fleer, G.J. Kinetics of Polymer Adsorption in Stagnation Point Flow, Colloids Surfaces **1990**, *51*, 141-158.
35. Siqueira, D.F.; Reiter, J.; Breiner, U.; Stadler, R.; Stamm, M. Competitive Adsorption of Functionalized Polymers. Langmuir **1996**, *12*(4), 972-979.
36. Ligoure, C.; Leibler, L. J. Phys. (Paris) **1990**, *51*, 1313.
37. Bajpai, A.K.; Dengre, R. Dynamics of the Adsorption of Egg Albumin at the Silica-Solution Interface. J. Appl. Polym. Sci. **1996**, *60*, 2219-2225.

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