Adsorption of a blood protein on to hydrophilic sponges based on poly(2-hydroxyethyl methacrylate)

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Spongy materials of poly(2-hydroxyethyl methacrylate) were synthesized and the adsorption of bovine serum albumin was carried out onto their surfaces. The sponges were characterized by IR spectral analysis, and water sorption property. It was noticed that the chemical architecture of the sponge has a pronounced impact on both the water sorption capacity and adsorption affinity of the sponge surfaces. The adsorption was also studied kinetically and the effect of pH was also investigated. The synthesized sponges were evaluated for antithrombogenic property by performing blood–clot formation tests.

Introduction

The history of 2-hydroxyethyl methacrylate (HEMA) and its polymer (PHEMA) dates back to 1934 when Woodhouse filed their patent without judging their remarkable water–sorption property [1]. However, it was only after two decades when the polymers of HEMA were recognized as promising biomaterials [2]. The first and the most successful application of HEMA based polymers was as an occular device, the hydrophilic contact lens [3], currently a universal commodity supported by excellent clinical results. The literature is richly documented with extensive studies on HEMA because of their high water content, non-toxicity and favorable tissue compatibility, which leads to many applications as biocompatible materials. These applications include soft contact lenses [4], drug-delivery systems [5], kidney dialysis systems [6] and artificial lever support systems [7]. The presence of a hydroxyl and a carboxyl group on each repeat unit makes this polymer compatible with water, and the hydrophobic α-methyl group and backbone impart hydrolytic stability to the polymer and support the mechanical strength of the polymer matrix [8].

Synthesis conditions of HEMA polymerization exert a great impact on the ultimate properties of the end polymer as evident from a large number of publications cited in [9]. One of the most significant parameters to have influenced the morphology of polymers of HEMA has been the presence of a diluent (normally water) in the polymerization mixture. Bulk polymerization of HEMA in presence of a crosslinking agent leads to a glassy and transparent material which can subsequently swell in water or other liquid agents, thus acquiring a gel behavior. When swollen in water, these materials are known as hydrogels. On the other hand, polymerization in solution of HEMA, in the presence of a diluent in amounts exceeding a critical concentrations (around 45%) results in the formation of an opaque spongy material known as ''heterogeneous'' or ''white'' gels or ''sponges'' [10]. These highly porous and spongy materials are produced because of the reason that since water is a non–solvent for the polymer (PHEMA), its concentration in the polymerization mixture results in unfavorable thermodynamic interaction between water and the polymer network which causes phase separation. This obviously produces an opaque and porous hydrogel. Although, these white gels or sponges were seldomly used in reconstructive surgery of breasts [11] and nasal cartilages [12] because of their low mechanical strength, however, recent development work on a novel type of artificial cornea (keratoprosthesis) has greatly stimulated research work on synthesis of these polymeric sponges [13].

A biomaterial is a substance used in prostheses or in medical devices designed for contact with the living body for the intended method of application and for the intended period [14]. At present, a wide number and great variety of clinically important polymers are finding applications as short- and long-term materials in kidney dialyzers, blood oxygenators, heart valves, vascular

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grafts, etc. [15]. All 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 thromoebdi. This process generally involves a sequence of protein adsorption steps followed by blood-cell interactions.

To be biocompatible, materials used in medical applications must meet certain criteria and regulatory requirements. What actually happens is that when a material comes in contact with the flowing blood, the earliest event that happens at the solid–liquid interface is believed to be the adsorption of blood proteins which is further followed by a number of consequential processes such as the activation of intrinsic coagulation, the adhesion and aggregation of platelets and the activation of the complement system. These consequential processes are greatly determined by the composition of the adsorbed protein layer. 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 [16]. Conversely, fibronectin and fibrinogen have been found to enhance the adhesion of bacterial species such as *Staphylococcus aureus*, a common course of implant-related infection, onto polymeric biomaterials [17].

Since the first attempt in 1950s to develop blood-compatible materials with a negatively-charged surface for artificial vessels, 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 [18]. This concept stimulated research in the development of many type of polymers which have utility for constructing blood-contacting surfaces of various medical devices.

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 example, it is known that fibrinogen has a high affinity for most surfaces [19] and that fibrinogen and *y*-globulin (IgG) adsorbed to surfaces promote platelet adhesion and activation [20]. In contrast, albumin has been found to reduce biomaterial surfaces toward thrombogenic reactions [21], in part due to lack of glycoprotein subunits present on fibrinogen, IgG and other plasma proteins that permit platelet membrane binding.

Thus, realizing the significance of protein adsorption onto macromolecular surfaces, we in the present investigation are reporting results on the adsorption of bovine serum albumin (BSA) onto PHEMA sponges.

Experimental

Materials

2-Hydroxyethyl methacrylate was obtained from Sigma Aldrich Co. and the monomer was freed from the inhibitor following a method discussed in the following section. The crosslinker used in polymerization of HEMA was ethylene glycol dimethacrylate (EGDMA) obtained from Merck, Germany and used as received. Potassium persulphate and metabisulphite were of Loba Chemie, India and used as received. Ethylene glycol was used as a cosolvent. BSA was obtained from Loba Chemie, India and used without any pretreatment. All other chemicals used were of standard quality and double distilled water was used throughout the experiments. Biurette reagent was prepared according to the method described in literature [22].

Purification of monomer

Because of poor stability of HEMA, the high purity of the monomer is essentially required in hydrogels synthesis as the presence of impurities may greatly affect the swelling characteristics of the end polymer. Degradation of the monomer during transportation and storage at ambient temperatures may result in increased levels of methacrylic acid (MAA) and the natural occurring crosslinker EGDMA. As illustrated in Fig. 1, the

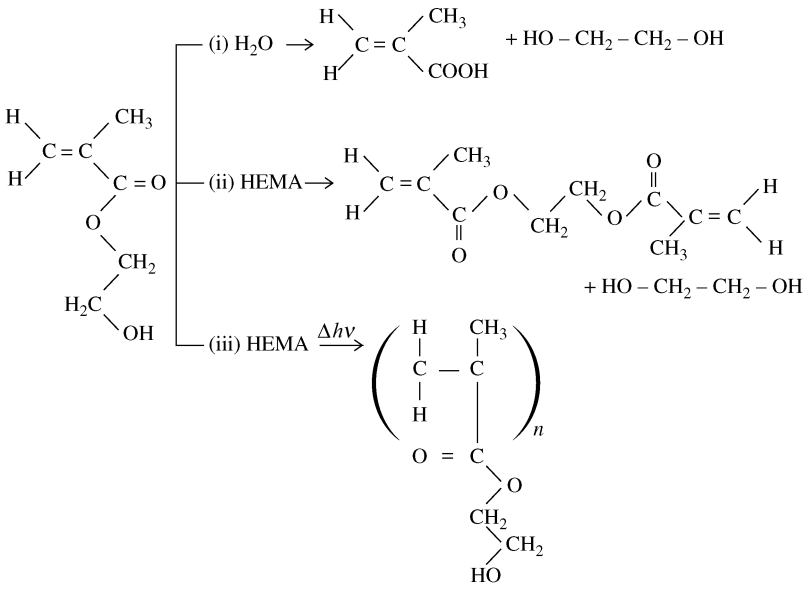

Figure 1 Synthesis of monomer HEMA.

HEMA monomer readily undergoes three common reactions:

(1) 2-Hydroxyethyl methacrylate may hydrolyze at the ester linkage to form MAA and ethylene glycol; (2) two molecules of HEMA may transesterify to form the crosslinker and ethylene glycol; (3) monomer may polymerize at the double bond resulting in oligomer or polymer. Although an inhibitor such as hydroquinone (300 p.p.m.) is normally added to minimize the later reactions, however, an ultra purity is desirable for producing reliable data.

The impurity of MAA in HEMA monomer was removed by stirring the monomer with 15% by weight of anhydrous sodium carbonate for 3 h at 24 °C, then vacuum filtering through Whatman filter papers. The yield on an initial volume of 100 ml of HEMA was 92%.

The impurity of EGDMA was then removed by first dissolving the above treated monomer in three times its volume of distilled water. Four extractions were performed with 50 ml of a 1 : 1 (volume) mixture of carbon tetrachloride and cyclohexane, allowing the layers to separate for 30 min between extractions. The organic layer containing EGDMA was discarded after each extraction. The aqueous phase was placed under vacuum to remove any remaining organic solvent. The HEMA was then salted out with 100 g of NaCl then dried with anhydrous sodium sulphate, and filtered.

The partially purified HEMA monomer was vacuum distilled in the presence of 1 g of hydroquinone (added to prevent polymerization) at 60 mm Hg. The monomer was collected at 45 °C with the distillation flask being heated in water bath at 55 °C. The collection flask was collected in an ice/acetone bath. The first and last fractions of the distillation product were discarded. After distillation, the pure HEMA was transferred to an opaque glass bottle and stored at 0 °C until use.

Purity of HEMA

The purity of distilled HEMA was determined by high pressure liquid chromatography (HPLC). A Backmen System (Cold 127) equipped with a ultraviolet (UV) detector, a 25 cm × 46 mm id separation columns ODS (C_{18}), 5 μm particle size were used. The UV detector was set at 217 nm. The mobile phase was methanol–water (60 : 40 v/v) and the flow-rate was kept at 1 ml min^{-1}. All samples were diluted with pure methanol to 1/1600. 10 μL samples were injected for each analysis. Samples of known concentrations of MAA and EGDMA were injected into the HPLC and the resultant chromatographs used to construct a standard curve of known concentrations *vs.* area under the curve. The chromatograph showed three distinct peaks. The first peak, 3.614 min was identified as (MAA). The next peak 5.503 min was the major peak due to HEMA monomer. The final peak, 15.3 min, was due to the crosslinker, EGDMA. The amounts of impurities of MAA and EGDMA in the monomer samples were found to be less than 0.01 mol % MAA and 0.001 mol % EGDMA.

Synthesis of PHEMA sponges

Poly 2-Hydroxyethyl methacrylate sponges were synthesized by a redox polymerization method as described by many workers [23]. In a typical experiment, into a petri dish (diameter, 4″, Corning) were added HEMA 32.9 mM, ethylene glycol 71.6 mM as a cosolvent, EGDMA 0.53 mM and water. The amount of water was so adjusted that the total water content always exceeded the critical concentration. The whole reaction mixture was degassed by purging dry N_2 for 30 min. For initiating polymerization reaction, a redox couple comprising of degassed solutions of potassium persulphate (0.04 M) and metabisulphite (0.3 mM) were added to the reaction mixture and polymerization was allowed to proceed for 24 h. The white spongy gels of PHEMA were formed, which were allowed to swell in bidistilled water for a week. The swollen gel was cut into small circular discs and allowed to dry at room temperature for a week. Upon drying the gels changed into transparent buttons which were stored in an air tight containers. A series of PHEMA gels of varying compositions were prepared which have been summarized in Table I.

Swelling experiments

The dry gel buttons were allowed to swell in phosphate buffer saline (PBS, pH 7.4) and taken out after seven

TABLE I Data showing the effect of composition of the PHEMA sponges on their swelling ratio

HEMA (mM)	EGDMA (mM)	Initiator ($K_2S_2O_8$) (mM)	Water content (%)	Swelling ratio
24.6	1.06	0.037	50	6.4
32.9	1.06	0.037	50	7.4
41.1	1.06	0.037	50	10.0
49.3	1.06	0.037	50	11.2
32.9	0.53	0.037	50	9.2
32.9	1.06	0.037	50	7.4
32.9	1.59	0.037	50	6.0
32.9	2.12	0.037	50	4.4
32.9	1.06	0.037	50	7.4
32.9	1.06	0.074	50	8.6
32.9	1.06	0.111	50	9.8
32.9	1.06	0.148	50	10.4
32.9	1.06	0.037	50	7.4
32.9	1.06	0.037	55	8.8
32.9	1.06	0.037	60	10.2
32.9	1.06	0.037	64	10.8

days. Upon swelling, the gels again become spongy white. The white swollen gels were gently pressed in between the filter papers to remove excess water and weighed. The swelling ratio was calculated by the following equation,

$$\text{Swelling ratio} = W_S/W_d \quad (1)$$

where W_S and W_d are the swollen and dry weights of the sponges, respectively.

Adsorption experiments

The adsorption of BSA onto the PHEMA sponges was performed by the batch process as reported elsewhere. [24]. Protein (BSA) solution for adsorption experiments were made in 0.5 M PBS at physiological pH 7.4. A fresh solution of BSA was always prepared for every adsorption experiment. Prior to adsorption experiments, the PHEMA buttons were equilibrated with PBS for 72 h. The adsorption was then carried out by gently shaking a solution of BSA of known concentration containing preweighed and fully swollen sponges. The shaking was performed so gently that no froth was produced otherwise it would have formed air–water interface. After a definite time period, the sponges were removed and the protein solution was assayed for the remaining concentration of BSA by a spectrophotometric procedure (Systronics, Model No. 106, India) as described elsewhere [22]. The adsorbed amount of BSA was calculated by the following mass-balance equation:

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

where C_o and C_e being the initial and equilibrium concentrations of BSA solution (mg ml^{-1}), V is the volume of protein solution and m the mass of the swollen sponges, 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 number (three) and less than 1% error was always obtained.

Blood compatibility tests

The antithrombogenic potential of the sponge surfaces was judged by the blood-clot formation test as described elsewhere [25]. In brief, the PHEMA sponges were equilibrated with saline water (0.9% w/v NaCl) for 72 h in a constant temperature bath. To these swollen sponges were added 0.5 ml of acid citrate dextrose blood followed by the addition of 0.03 ml of $CaCl_2$ solution (4 M) to start the thrombus formation. The reaction was stopped by adding 4.0 ml of deionized water and the thrombus formed 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 the glass surface and sponges of other compositions and respective weights of thrombus formed were recorded by a highly sensitive balance.

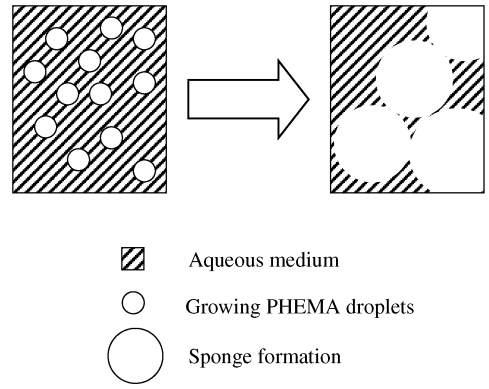

Figure 2 A hypothetical model depicting the mechanism of phase separation.

IR spectra

The IR spectra of dry sponge was recorded on a Perkin Elmer spectrophotometer (FTIR, Paragon 1000).

Results and discussion

Characterization of sponges

Mechanism of phase separation

A mechanism has been suggested for the formation of sponges due to phase separation [26]. According to this, before the polymerization of HEMA begins, the reaction mixture exists as an intimate medium of HEMA and water. However, as the polymerization starts, the reaction medium becomes increasingly richer in PHEMA which is a water insoluble polymer. This significantly reduces the solubility of HEMA, thus causing the growing polymer phase to separate as droplets in the aqueous phase. The droplets formed in this way join together and take the shape of a network consisting of spheroidal polymer particles surrounded by channels occupied with water. The whole mechanism may be modeled as shown in Fig. 2.

Appearance

During polymerization the phase separation occurs in the polymer system and as a result white, soft, spongy material is formed. While in dry state, the spongy material becomes hard and fully transparent. The dry and swollen sponges are depicted in Fig. 3.

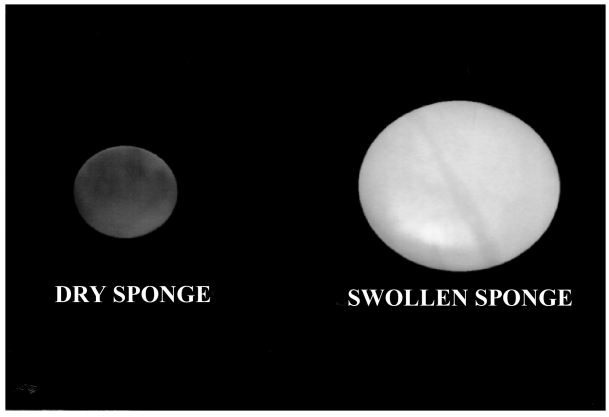

Figure 3 A photograph depicting (a) dry and (b) swollen PHEMA sponges.

IR spectral analysis

The IR spectra (Fig. 4) clearly marks the presence of HEMA as evident from the observed bands at 1726 cm^{-1} (C=O stretching), 1154 cm^{-1} (O–C–C stretching), 3668 cm^{-1} (O–H stretching) and 1411 cm^{-1} (O–H bending), respectively. The spectra also indicate for the asymmetric stretch of methylene groups at 2901 cm^{-1}.

Swelling of PHEMA sponges

Since the biocompatibility of a material is also decided by the water content in it, the PHEMA sponges of varying compositions were synthesized and allowed to swell in PBS for 72 h. The equilibrium water content of respective sponges are presented in Table I and it is clear from the swelling data that the water sorption capacity of sponges depend not only on the chemical architecture of the polymer but also on the amount of water present in the polymerization mixture.

The results summarized in Table I clearly reveal that the swelling ratio of the sponges increases with increasing water content in the polymerization mixture. The increased swelling could be attributed to the fact that increasing water content in the polymerization mixture increases the proportion of non-solvent which facilitates the phase separation process during polymerization. This obviously results in the formation of PHEMA sponges with larger interstitial void spaces which are occupied by water molecules, thus enhancing the swelling.

The swelling ratio of the sponges is also influenced by the PHEMA content in the polymer. It is clear from the Table I that when the amount of monomer (HEMA) increases from 24.6 to 49.3 mM in the feed composition, the swelling ratio also increases. The possible explanation may be that for a given content of water in the polymerization medium increasing HEMA content in the reaction mixture causes a faster phase separation, thus producing PHEMA sponges of wider pore sizes. This obviously enhances the degree of water sorption.

The crosslinker has a pronounced effect on the swelling ratio of the sponges. When the EGDMA concentration is raised in the feed mixture in the range 0.53–2.12 mM, the degree of water sorption decreases which is an usual finding. The observed decrease in swelling ratio could be attributed to the reason that high amount of crosslinker (EGDMA) produces a compact network that increases the crosslink density of the sponges. Thus, because of higher crosslink density, the pore sizes of PHEMA sponges decreases which results in a lower degree of swelling. Another explanation for the observed fall in swelling ratio of PHEMA sponges is that the introduction of crosslinker increases the glass transition temperature (Tg) of the polymer which obviously lowers the amount of water sorption. Similar type of results have been published elsewhere.

The effect of initiator concentration on the EWC of PHEMA sponges has been investigated by varying the amount of initiator in the range 0.037–0.148 mM. The results presented in the Table I indicate that with increasing initiator concentration, the swelling ratio also increases. A possible explanation may be that the gel particles occur in a larger number, but with a smaller size, at higher initiator concentration. This is due to an increase in the number of growing radicals. The resulting sponges will, therefore, contain much more

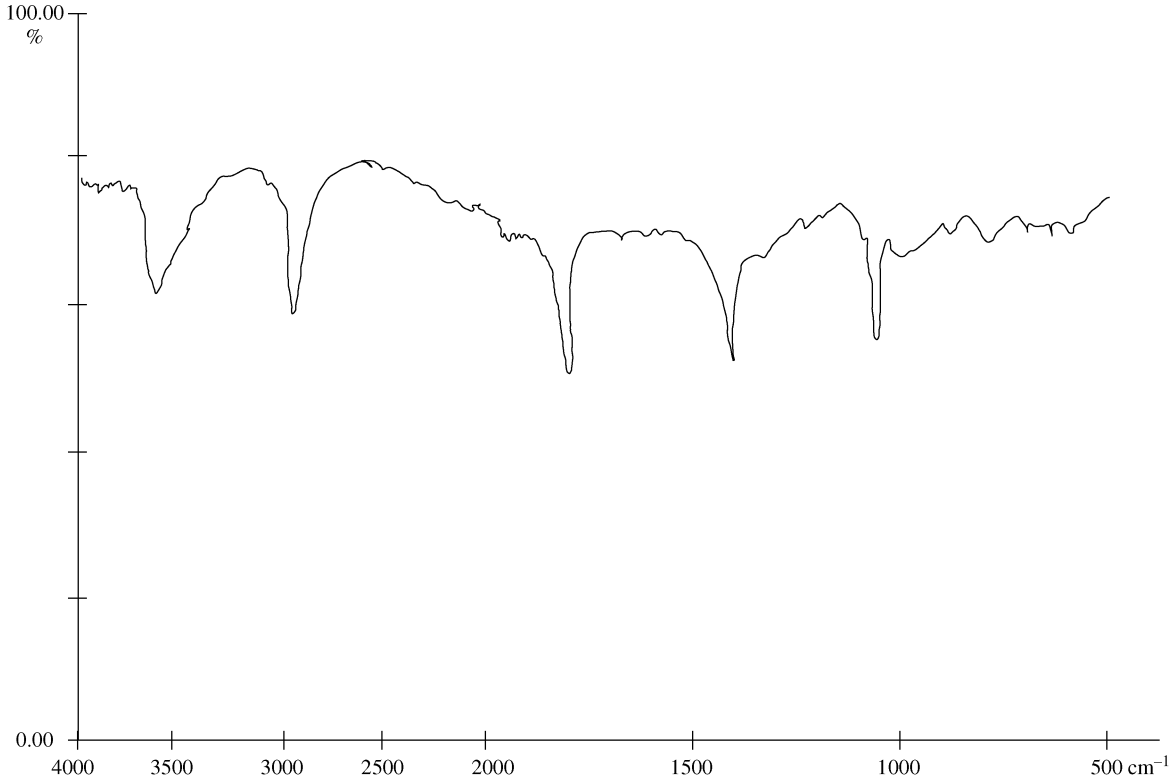

Figure 4 IR spectra of the PHEMA sponge.

''interstitial'' void space to be occupied by water molecules. This obviously enhances the swelling ratio of sponges.

Adsorption isotherms

The amount of adsorbed BSA is greatly dependent on the initial concentration of the protein solution. The effect of initial concentration of BSA solution on the adsorbed amount of BSA has been investigated by varying its initial concentration in the range 1.0–6.0 mg ml^{-1}. The results clearly indicate that the adsorbed amount gradually increases with increasing concentration of protein solution and ultimately attains a limiting value which is indicative of the formation of a monolayer on the PHEMA sponges. The observed increase may be attributed to the fact that with increasing bulk concentration of protein solution, greater number of BSA molecules arrive at the sponge–water interface and get adsorbed over the sponge surfaces.

A more quantitative information about the protein–surface interaction may be obtained by constructing an adsorption isotherm which is normally obtained by plotting the adsorbed amount of BSA against the residual concentration of the protein solution. The adsorption isotherm obtained in the present case is shown in Fig. 5 which is a typical Langmuir type of curve which is characterized by an initial rising portion followed by a plateau portion. Similar type of isotherms have been frequently reported in [27].

The affinity of protein for the PHEMA sponges could be quantitatively evaluated by applying the following linearized Langmuir equation,

$$\frac{C_e}{q_e} = \frac{C_e}{Q^o} + \frac{1}{bQ^o} \quad (3)$$

where C_e is the equilibrium concentration of the BSA solution, q_e is the amount of adsorbed BSA, Q^o is defined as the amount of protein required in formation of monolayer (monolayer capacity), and b is a constant known as the adsorption coefficient or affinity constant. The value of b in the present study has been calculated to be 0.25 from the linear plot drawn in accordance with Equation 3. The observed value of b is quite lower than those evaluated for the same protein adsorbing on polystyrene and poly(styrene-co-ethylene oxide) [28]. The lower value of adsorption coefficient ''b'' obtained with the PHEMA sponges indicates a less adsorption of BSA on the sponges and this may be attributed to the hydrophilic nature and small dimensions of the pore sizes of the sponges.

Effect of pH and ionic strength

The pH of an adsorption medium has a significant influence on the amount of adsorbed protein. The effect is much more observable particularly in those systems which involve ionic type of adsorbate and adsorbent surfaces [29]. However, in the present case since the PHEMA sponges are non-ionic in nature, the effect of pH on adsorption is solely determined by the BSA whose net charge varies with the pH of its solution. The effect of pH on the adsorption of BSA has been investigated by varying pH of the protein solution in the range 1.8–11.0. The results are depicted in Fig. 6 which clearly imply that a maximum adsorption is noticed at 5.0 which is near to the isoelectric point of BSA. The optimum adsorption of proteins at their isoelectric points have been frequently reported in the literature.

The observed optimum adsorption of BSA at the isoelectric point may be because of the following reasons that at isoelectric point the lateral interactions between BSA molecules are minimized and the protein acquires a compact conformation. Thus, greater number of BSA molecules can adsorb in the given surface area of the PHEMA sponges.

An interesting feature revealed by the Fig. 6 is that the adsorption isotherms constructed for various ionic strengths respond differently to pH of the adsorption

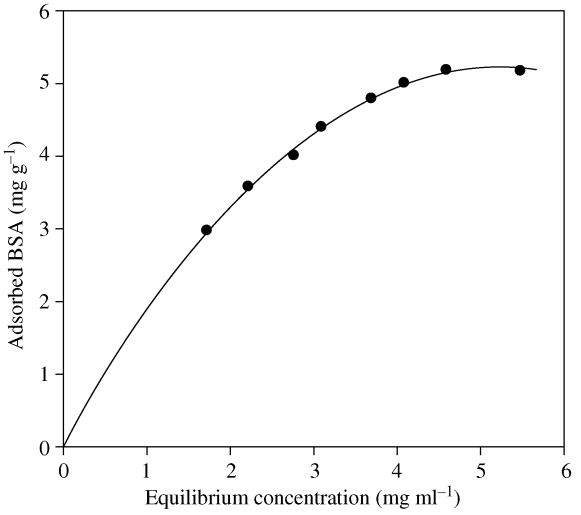

Figure 5 Adsorption isotherm of the BSA on the PHEMA sponge surfaces of fixed composition [HEMA] = 32.9 mM, [EGDMA] = 1.06 mM, [Water] = 50%, [KPS] = 0.037 mM, [MBS] = 0.40 mM, pH = 7.4, Temp. = 27 ± 0.2 °C.

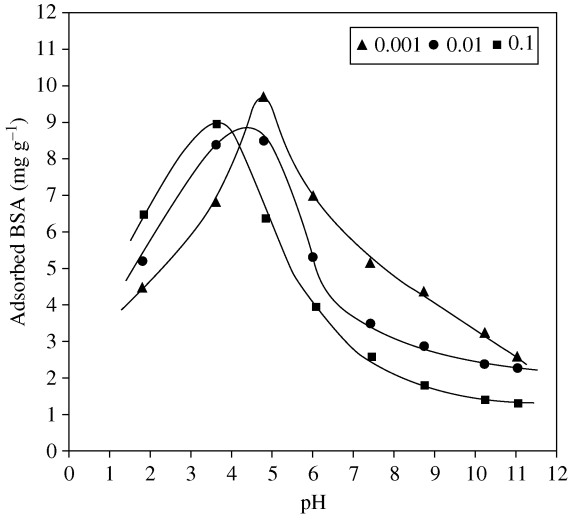

Figure 6 Adsorption of BSA onto the PHEMA sponge surfaces at different pH and ionic strengths of the protein solution at fixed composition [HEMA] = 32.9 mM, [EGDMA] = 1.06 mM, [Water] = 50%, [KPS] = 0.037 mM, [MBS] = 0.40 mM, Temp. = 27 ± 0.2 °C.

medium. It is clearly shown in the figure that as the ionic strength decreases the maxima present in the isotherm becomes more and more pronounced. The observed results may be attributed to the reason that at lower ionic strength, the density of BSA molecules at the sponge–solution interface will be low and less number of protein molecules are operative at the active sites for adsorption. Thus, the lateral interactions among the molecules are not indifferent and significant at below and above the isoelectric point of the protein. As a result of this the adsorbed BSA also does not vary appreciably, thus giving an isotherm of less pronounced maxima. On the other hand at higher ionic strength, greater number of BSA molecules cause a greater degree of lateral interactions and, therefore, result in a pronounced maxima.

Another important observation is that the shape of the adsorption is appreciably affected by the ionic strength of the medium as shown in Fig. 6. It is implied by the results shown in the figure that in the acidic range the amount of adsorbed BSA increases with increasing ionic strength of the medium. The observed increase may be due to the reason that with increasing ionic strength, the electrostatic repulsions in the interior of protein molecules are decreased which leads protein molecules to form more compact structures. Moreover, lateral repulsions between the adsorbed protein molecules decrease with increasing ionic strength. Thus, greater number of molecules can adsorb in the given surface area.

It is also revealed by the figure that the maxima at which the adsorption of BSA becomes optimum is shifted toward acidic pH range with increasing ionic strength. The shifting of the maxima has also been reported by other authors [30]. 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 [31]. What actually happens is that the salt ions may interact with anionic groups (such as $—COO^-$, $—O^-$, $—S^-$) and cationic groups (such as $—NH^+—$, $—NH_3^+$ and $=NH_2^+$) of the protein molecule and thus alter the isoelectric point of the BSA molecule. The shift of the isoelectric point of BSA to acidic pH upon increasing ionic strength was also confirmed by other workers [32].

Dynamics of BSA adsorption

Accurate knowledge of the adsorption kinetics under a given set of conditions is a prerequisite in elucidating the mechanisms of many fundamental biological processes. The adsorption of proteins from its aqueous solution onto a solid surface is normally considered to occur in three steps [33]: (i) diffusion of protein molecules from bulk to the interface, (ii) attachment of protein molecules to active sites on the surface and (iii) reconformation of the structure of the protein molecule after adsorption.

Of these three steps, (iii) plays a significant role not only in controlling the adsorption kinetics of proteins, but also in modification of the surface properties of the substrate. In the present case, step (iii) contributes little to the overall adsorption kinetics, as at the experimental pH the BSA molecules will not have as much structural adaptability as they do at other pH values [34].

The dynamics of the adsorption process was followed by determining the amounts of adsorbed BSA at various time intervals, as shown in Fig. 7. It is clear from the figure that the rate of adsorption isotherm is almost constant up to 12 min and then it gradually slows down attaining a limiting value after 30 min. The kinetic profile of the adsorption process may be explained by the fact that the adsorption of functionalized large chains (such as proteins) is a two regime process [35]. At the initial stages, the sponge 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 BSA molecules arriving 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

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

From the slope of the curve drawn between a (adsorbed BSA) and $\sqrt{t}$, for the BSA solution of varying concentrations, the diffusion constant has been calculated and summarized in Table II. It is revealed from the table that with an increasing concentration of BSA solution, the diffusion constants also increase. This is expected from the Ficks law of diffusion since the concentration gradient at the sponge–solution surface increases with increase in the initial concentration of BSA solution.

In the later stage of adsorption process a barrier of adsorbed molecules exists, and the molecules arriving from solution have to diffuse across this barrier. This penetration is slow, and a theoretical treatment given by Ligorue and Leibler predicts an exponential time dependence for the later stages [36]:

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

where q_{eq} is the adsorbed amount at equilibrium and $1/T$ is the penetration rate constant. The above equation

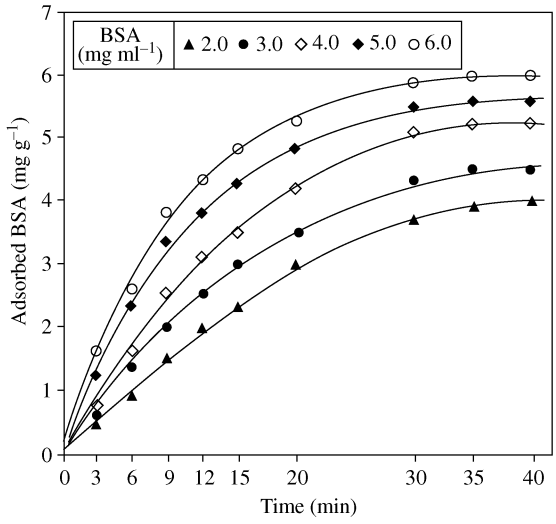

Figure 7 A plot showing the variation of the adsorbed amounts of BSA ($mg\,g^{-1}$) with time t, at varying concentration of BSA, (▲) $2.0\,mg\,ml^{-1}$, (●) $3.0\,mg\,ml^{-1}$, (◇) 4.0 mg/ml, (◆), $5.0\,mg\,ml^{-1}$ and (○) $6.0\,mg\,ml^{-1}$. Ionic strength = 0.001, pH = 7.4, Temp. = 27 ± 0.2 °C. (PHEMA sponge composition as in Fig. 6.)

TABLE II Various kinetic parameters of the adsorption of BSA onto a PHEMA sponge for different initial bulk concentration of the protein solution

BSA concentration ($mg\,ml^{-1}$)	Diffusion constant $D \times 10^5$ ($cm^2\,s^{-1}$)	Penetration rate constant $1/T \times 10^4$ (s^{-1})	Rate constant for adsorption $(k_1) \times 10^4 z$, (s^{-1})	Rate constant for desorption $(k_2) \times 10^4$ ($mol^{-1}\,l\,s^{-1}$)
2.0	0.16	8.3	1.0	4.0
3.0	0.29	11.9	1.2	4.8
4.0	0.6	13.8	1.0	4.0
5.0	2.6	15.4	1.0	4.0
6.0	4.1	20.8	1.1	4.4

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

The rate constants for adsorption and desorption (k_1 and k_2, respectively) were also calculated from Equation 4 of [37] and summarized in Table II. The results imply that the rate constant for adsorption is almost independent of the BSA concentration, thus confirming the first order nature of the adsorption process.

Effect of sponge composition on BSA adsorption

It has been largely noticed 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 [38]. 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 [39]. For example, Andrade *et al*. proposed that a surface with an extremely hydrophilic nature shows minimum protein adsorption to prevent thrombus formation due to low interfacial free energy. Similarly, surface grafting with tethered brushes of hydrophilic polymers, such as poly(ethylene glycol) have been shown to achieve minimal interaction with proteins and platelets [40]. 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. On the other hand, microarchitecture of material surface is also found to affect the protein–material interaction substantially.

As mentioned previously, the presence of water is the decisive factor in the occurrence of phase separation since the thermodynamically unfavorable interaction between water and polymer becomes dominant over other factors. This phase separation further results in a network consisting of contiguous spheroidal particles surrounded by interconnected channels occupied by water. On increasing the proportion of water in the polymerization mixture, the onset of phase separation becomes fast and, therefore, the pore sizes of the PHEMA sponges decrease whereas the equilibrium water content increases. Thus, because of the observed increase in water content of the sponges the adsorption of BSA is not favored and a decrease in the adsorbed amount is obtained as shown in Table III. The observed decrease may further be explained on the basis of the fact that on increasing the initial dilution, the pore sizes of the polymer network also decreases which obviously restricts the free diffusion of BSA molecules into the sponges thereby decreasing the amount of adsorbed protein.

In the present study the effect of HEMA content in the sponges on the adsorbed amount of BSA has been investigated by varying the concentration of HEMA in the range 24.6–49.3 mM. The results are shown in Table III which clearly imply that the amounts of adsorbed BSA significantly decreases with increasing HEMA in the feed mixture of the sponges. The observed results could be explained by the fact that because of hydrophilic nature of HEMA, its increasing concentration further enhances the hydrophilicity of the polymer which because of more water content does not favor protein adsorption. The observed results are in straightway consistent with the swelling results.

The crosslinking agent employed in the present study was EGDMA which is a known crosslinker. The influence of EGDMA on the protein adsorption has been investigated by increasing its proportion in the sponges in the ranges 0.53–2.12 mM. The results are shown in Table III which reveal that the adsorbed BSA increases with increasing EGDMA concentration in the sponge. The increase in the BSA adsorption can be attributed to the fact that hydrophobic methylene groups of albumin interact with the crosslinker EGDMA and thus cause adsorption of BSA on the sponge surface as a result of entropic hydrophilic interactions and lyophilic liquid binding capabilities. Similar type of results have also been published by other workers [38]. It is worth mentioning here that only water content is not important,

TABLE III Effect of the composition of the PHEMA sponges on the amounts of adsorbed BSA

HEMA (mM)	EGDMA (mM)	Amount of adsorbed BSA ($mg\,g^{-1}$)
24.6	1.06	8.8
32.9	1.06	5.2
41.1	1.06	4.6
49.3	1.06	3.0
32.9	0.53	3.9
32.9	1.06	5.2
32.9	1.59	7.4
32.9	2.12	9.2

but also the surface morphology 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 surfaces. Therefore, it is not imperative that a conclusion is not drawn of biocompatibility based only on water content of polymers.

Blood clot formation

A great deal of experimental work has been confined to fabricate a biomaterial that lasts long without failure when put in contact with a stream of flowing blood under *in vivo* conditions. The fundamental approach behind the above task has been to minimize the extent of thrombus formation on blood contacting devices thus to synthesize a non-thrombogenic polymer. The rationale for the development of these non-thrombogenic polymers is to prevent activation of the thrombogenic pathway by tailoring polymer surfaces to minimize blood-interaction. An alternative route to achieve a non-thrombogenic polymeric implant has been to design a material with very low affinity for protein adsorption since, as mentioned earlier also, a thin layer of protein is formed at the blood-material interface within a few seconds 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. As different surfaces show different affinity for protein adsorption, the clot formation must also be a function of the chemical architecture of the materials and their surface as well.

The clot formation data is presented in Table IV which clearly reveal that with variation in the composition of the PHEMA sponge the weight of the blood-clot also varies. It can be clearly seen in the table that whereas an increase in the initial water dilution, HEMA and polymerization initiator concentration in the reaction mixture has resulted in PHEMA sponges with lower amount of clot formed, a higher weight of blood-clot was found with increased crosslinker (EGDMA) concentration. The obtained results are consistent with our water sorption and BSA adsorption results and 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.

TABLE IV Variation in amounts of blood-clot formed on the PHEMA sponge surfaces of different compositions

HEMA (mM)	EGDMA (mM)	Initiator ($K_2S_2O_8$) (mM)	Water content (%)	Weight of blood-clot (mg)
24.6	1.06	0.037	50	29.6
32.9	1.06	0.037	50	24.2
41.1	1.06	0.037	50	19.8
49.3	1.06	0.037	50	16.2
32.9	0.53	0.037	50	19.4
32.9	1.06	0.037	50	24.2
32.9	1.59	0.037	50	26.6
32.9	2.12	0.037	50	28.9
32.9	1.06	0.037	50	24.2
32.9	1.06	0.074	50	22.4
32.9	1.06	0.111	50	18.6
32.9	1.06	0.148	50	15.3
32.9	1.06	0.037	50	24.2
32.9	1.06	0.037	55	23.4
32.9	1.06	0.037	60	20.2
32.9	1.06	0.037	64	18.8
Glass surface			—	38.6

Conclusions

Hydrophilic and spongy type of polymers are obtained when HEMA monomer is polymerized by a redox system in the presence of a crosslinking agent and a critical concentration of water. The water sorption capacity of these sponges increases with increasing concentration of water, HEMA and initiator in the polymerization mixture. While a decrease in the degree of water sorption is noticed with increasing concentration of crosslinker (EGDMA).

The adsorption of BSA onto the sponge surfaces follows a Langmuirian process and occurs in two steps, viz. diffusion of BSA molecules into the pores of the sponges and adsorption into the internal surface. The adsorption is greatly influenced by pH and ionic strength of the protein solution and an optimum adsorption is noticed at the isoelectric point of the BSA which further shifts into acidic region with increasing ionic strength. The adsorption is further affected by the chemical nature of the sponges. Whereas the adsorbed amount of BSA is found to decrease with increasing amount of water, HEMA and initiator in the reaction mixture, it increases with increasing crosslinker content in the sponges.

The PHEMA sponges exhibit a fair antithrombogenic potential to act as a biomaterial. It is confirmed from the blood-clot formation tests that a PHEMA sponge synthesized at higher water, HEMA and initiator concentration yields much lower weights of blood-clot on its surface than that formed with more crosslinker content.

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