Adsorption of Bovine Serum Albumin onto Glass Powder Surfaces Coated with Polyvinyl Alcohol

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ABSTRACT: The adsorption of bovine serum albumin (BSA) was carried out onto polyvinyl alcohol–coated glass powder surfaces. The adsorbed amount was about four times more than that on uncoated glass. The kinetics of the adsorption process was followed colorimetrically, and kinetic parameters, such as adsorption coefficient, rate constants for adsorption and desorption, diffusion constant, and penetration rate constant, were evaluated. The effects of experimental conditions, such as pH, presence of salts, addition of aliphatic alcohols, and variation in the dielectric constant of the medium, on the amount of adsorbed BSA were investigated. The effect of temperature on adsorption was also studied, and several thermodynamic parameters, such as standard free energy change ($\Delta G°$), heat of reaction ($\Delta H°$), and entropy change ($\Delta S°$), were evaluated.
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Key words: protein; adsorption; polymer-coated glass

INTRODUCTION

Polymers have been the first choice as biomaterials due to their good mechanical properties, chemical passivity, and, foremost, unique biocompatibility.[1–3] Interest in generating new materials for use as permanent hard tissue implants has grown considerably.[4] A variety of bioceramics, such as hydroxyapatite,[5] titanium,[6] and silica,[7] have been reported to possess reactive surfaces to form chemical bonds with bone. Thus, in view of the great application value of protein-material interactions, I have evaluated the adsorption affinity of bovine serum albumin (BSA) to polyvinyl alcohol– (PVA) coated glass powder (CGP) surfaces and investigated the kinetic nature of the adsorption process.

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EXPERIMENTAL

Materials

The protein BSA (M_w 65,000, isoelectric point 4.7) was supplied by Robert Johnson (Bombay, India) and used without further purification. The glass powder was prepared in my laboratory by finely grinding corning (borosilicate) glass pieces and subsequent multiple washing with dil hydrochloric acid and double-distilled water. The powder so prepared was dried at 120°C for 48 h and sieved to give particles of mesh size 100–200.

The polymers used for coating purposes were PVA (Loba Cheme, India; M_w 40,000), polyacrylamide (PAM; BDH, $M_w > 10^6$), and polyacrylic acid (PAA; Wilson Laboratories, India; M_w 75,000) and were used as received.

Coating Procedures

Before the glass powder was coated with PVA, it was hydrophilized by boiling a weighed amount of powder in 100 mL of concentrated HCl for 4 h and

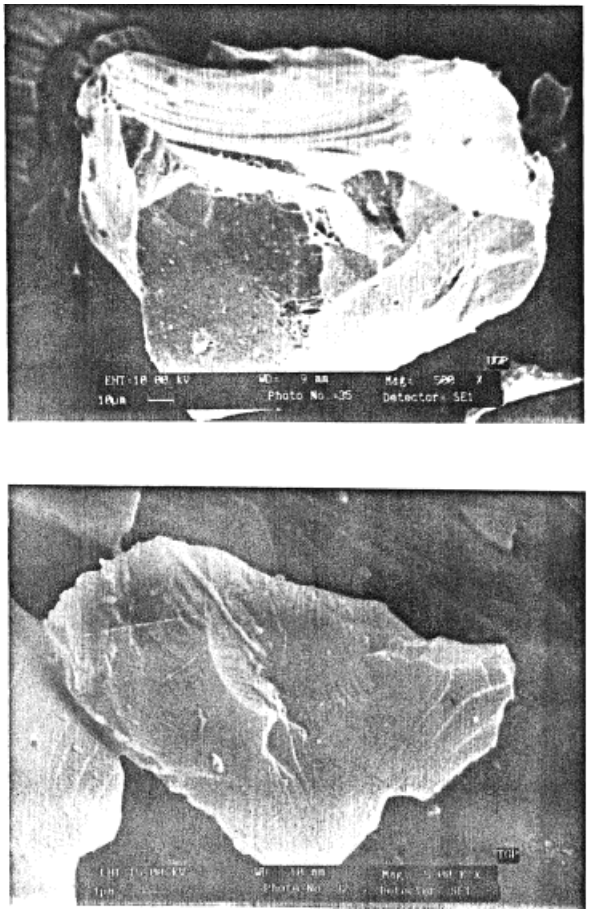

Figure 1 Scanning electron micrographs of both (a) uncoated and (b) coated glass powder surfaces.

then filtered and washed thoroughly with bidistilled water until the filtrate was at pH 7.0.

For coating purposes, a 2% (w/v) aqueous solution of PVA was prepared, and hydrophilized glass powder was kept in contact with the polymer solution for 4 days at 30°C. The glass powder was later filtered, washed gently with water, and dried at 80°C for 24 h. The CGP so prepared was coded as PVCGP for polyvinyl alcohol–coated glass powder. Glass powders coated with PAM (PAMCGP) and PAA (PAACGP) were prepared in a similar manner.

Adsorption Method

The BSA solution was prepared in a 1×10^{-3} mol dm^{-3} KCl solution of pH 6.2. The adsorption experiments were carried out by the direct contact method, which involved shaking a fixed volume of BSA–PVCGP suspension for a defined time period, then performing colorimetric estimation[8] of the remaining BSA in the supernatants. The amounts of adsorbed protein were calculated by the mass balance equation. Most adsorption experiments were duplicated, and every concentration determination was performed twice. The supernatants were also analyzed for PVA, and no polymer from CGPs was detected.

RESULTS AND DISCUSSION

Evidence for Coating

To verify that the GPSs were actually coated with the PVA film, scanning electron micrographs (SEMs) of both uncoated and coated powders were taken, as shown in Figure 1. The figure clearly shows that whereas the surface of uncoated powder appears rough and heterogeneous, the coated surfaces are smooth, due to the coating of the polymer films.

These findings provide clear evidence of the coating of the powder surfaces with PVA. In addition to the PVA coating, the powders were also coated with PAM and PAA, and the amounts of adsorbed BSA were determined, as summarized in Table I. Because the maximum adsorption was exhibited by the PVA-coated glass surfaces, subsequent studies were carried out with PVCGP only.

Mode of BSA Adsorption

Because the experimental pH of the BSA solution was 6.2, which is well above the isoelectric point of the protein, obviously at this pH the BSA molecules will have a net negative charge. It is also well known that the glass surfaces are negatively charged in aqueous suspensions. Thus in this situation the positively charged amino acid residues (—NH^+—, —NH_3^+, and ═NH_2^+) of the BSA molecules interact electrostatically with the glass

Table I Data Showing the Amounts of Adsorbed Bovine Serum Albumin (BSA) onto Various Glass Surfaces

Type of Glass	Adsorbed Amount (mg g^{-1})
Untreated	5.5
Polyvinyl alcohol–coated (PVCGP)	24.4
Polyacrylamide-coated (PAMCG)	13.3
Polyacrylic acid–coated (PAACG)	11.8

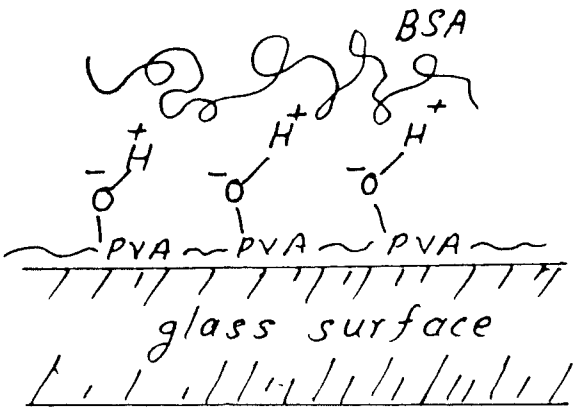

Figure 2 A model depicting the adsorption of BSA on PVCGP.

surface and get adsorbed. When the glass surface is coated with the PVA film, the negative charge of the surface is not only screened by the polymer film, but also provides hydrophilic centers to the surface, which can enhance adsorption. Thus at this moment the BSA molecules can favorably bind to the —OH groups of the PVA film. The entire situation of BSA adsorption is modeled in Figure 2.

One more possible aspect of the BSA adsorption may be considered to be due to the attachment of protein molecules to the silanol groups of the uncovered glass surface. Moreover, the presence of Lewis acid sites and their active participation in adsorption has also been confirmed by titrimetric analysis.[9]

The adsorption isotherm in the present case is shown in Figure 3, which represents a typical high-affinity–type adsorption isotherm with greater slope. The isotherm so obtained implies that the adsorption process is controlled by the Langmuir equation. The high slope of the curve indicates that the active sites on the surface are increasingly occupied by the protein molecules with increasing concentrations of the BSA solution. I also investigated the nature of the adsorption isotherm when BSA molecules adsorb on uncoated glass surface and found that the isotherm was of a lower-affinity type, as shown in Figure 3.

The adsorption coefficient evaluated from the linearized Langmuir equation was calculated to be 2.0×10^5 mol^{-1} dm^{-3}.

Kinetics of Adsorption

The adsorption of proteins from its aqueous solution onto a solid surface is normally considered to occur in three steps[10]: (1) diffusion of protein molecules from bulk to the interface, (2) attachment of protein molecules to active sites on the surface, and (3) reconformation of the structure of the protein molecule after adsorption.

Of these three steps, (3) 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 (3) 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.[11]

To verify the diffusion-controlled nature of the adsorption process, the adsorption experiments were run with varying speeds of agitation. Adsorption was found to increase with increasing speed of agitation. This confirms that the adsorption process is diffusion controlled.

The kinetics of the adsorption process was followed by determining the amounts of adsorbed BSA at various time intervals, as shown in Figure 4. It is clear from the figure that the rate of adsorption is almost constant up to 10 min and then reaches saturation after 20 min. To evaluate the rate constants for adsorption and desorption (k_1 and k_2), the kinetic scheme proposed by Bajpai et al.[12] was used.

It is also a well-recognized fact that the process of adsorption is a two-regime process.[13,14] At the initial stage, the solid surface is bare, and the kinetics of adsorption is governed by the diffusion

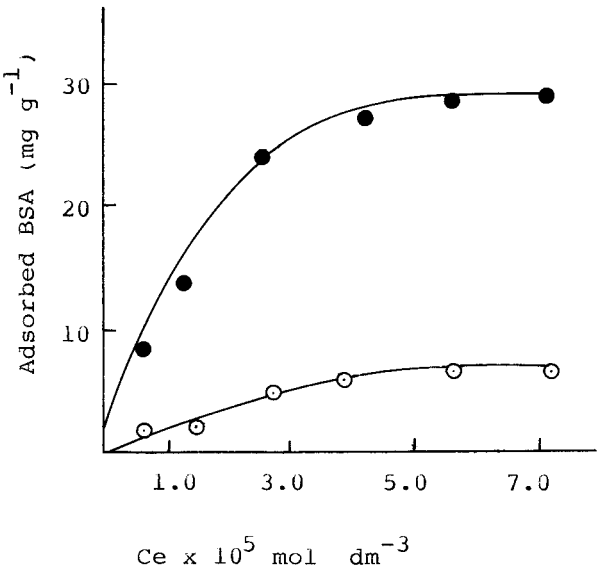

Figure 3 Adsorption isotherms of the adsorption of BSA on PVACGP (●) and uncoated (○) glass surfaces.

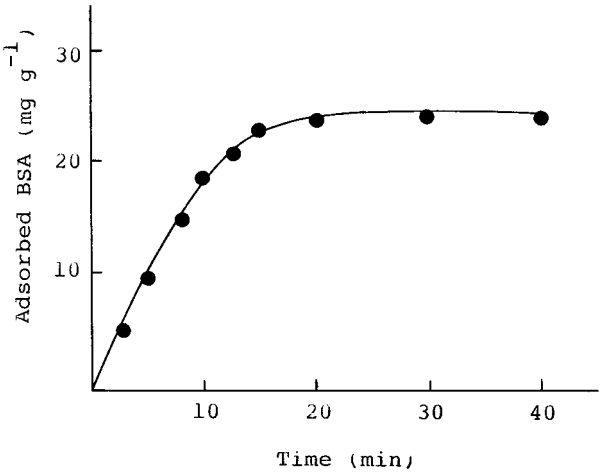

Figure 4 Plot showing the variation of the adsorbed amounts (mg g^{-1}) of BSA with time t, at fixed concentration of BSA 3.06×10^{-5} mol dm^{-3}, adsorbent 0.1 g, temperature 27°C.

of the molecules from the bulk solution to the surface. All of the molecules that arrive at the surfaces are assumed to be immediately adsorbed. The mass transport can be interpreted as a Fickian diffusion. In a simple model calculation, the diffusion constant D is obtained from the slope of the curve drawn between the adsorbed mass $q(t)$ as function of $\sqrt{t}$, which depends on the bulk concentration:

$$q(t) = \frac{2}{\pi} \text{Co} \sqrt{Dt} \tag{1}$$

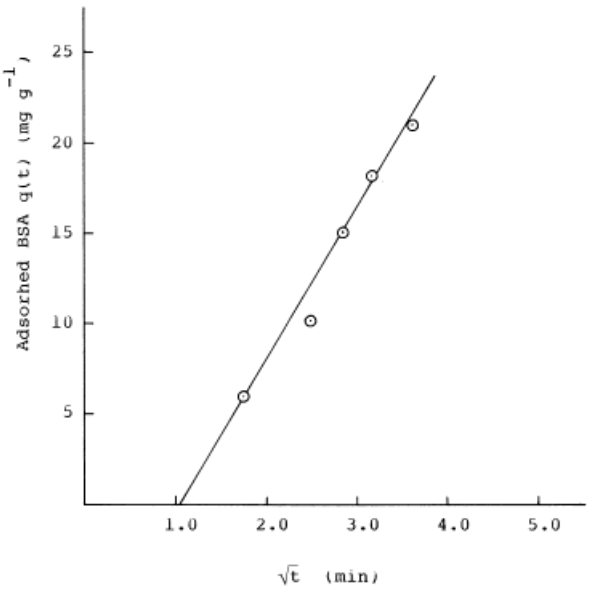

Figure 5 Plot drawn between $q(t)$ and $\sqrt{t}$ for evaluating the diffusion constant D.

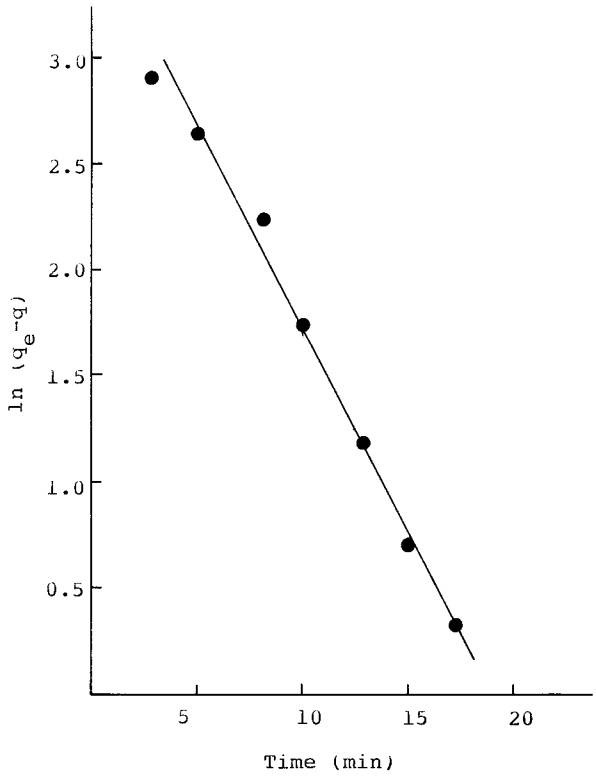

Figure 6 Plot drawn between $\ln(q_{eq} - q)$ and t for evaluating the penetration rate constant $(1/T)$.

From the slope of the curve $q(t)$ as a function of $\sqrt{t}$, the diffusion constant D can be calculated.

In the later stage, 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 Lejbler[14] predicts an exponential time dependence for the later stages:

$$q(t) = q_{eq}[1 - \exp(-t/T)] \tag{2}$$

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

With the help of Figures 5 and 6, drawn in accordance with eqs. (1) and (2), respectively, the diffusion constant D and penetration rate constants $(1/T)$ have been calculated. These are summarized in Table II.

pH Effect

Adsorption is a pH-dependent phenomenon, especially when a protein molecule adsorbs on an electrically charged surface.[15,16] In the present study, the effect of pH on the adsorption of BSA has been

Table II Data Showing the Numeric Values of Various Kinetic Parameters for the Adsorption of BSA onto PVCGP

Sr. No.	Rate Constant for Adsorption (k_1)	Rate Constant for Desorption (k_2)	Diffusion Constant (D)	Penetration Rate Constant ($1/T$)
1	$1.7 \times 10^{-4} s^{-1}$	8.5×10^{-10} mol L^{-1} s^{-1}	2.6×10^{-12} cm^2 s^{-1}	3.3×10^{-3} mg g^{-1} s^{-1}

investigated by varying the pH in the range of 2.3–11.7, as shown in Figure 7. The results obtained clearly imply that maximum adsorption is observed at pH 5.0, which is the isoelectric point of the BSA. The following interpretation may explain the results.

At pH 2.3, the BSA molecules will show the maximum structural adaptability and that will lead to the unfolding of the protein molecules, thus exposing the hydrophobic portions of the molecules to the glass surface. Because the surface is hydrophilic in nature due to the coating of PVA, this hydrophobic–hydrophilic interaction will lead to decreased adsorption. Increasing the pH of the protein solution decreases the structural adaptability, resulting in increased adsorption. This increase continues until the isoelectric point of the protein (4.7) is reached, at which point the protein shows minimum conformational change and thus adsorption becomes maximum.

Further increasing the pH of the solution causes the BSA molecule to acquire an increasing negative charge, and thus, due to electrostatic repulsive forces operating between the protein molecule and the surface, adsorption constantly decreases. This type of result, where the adsorption reaches a maximum at the isoelectric point of the protein, has been widely reported in the literature.[17]

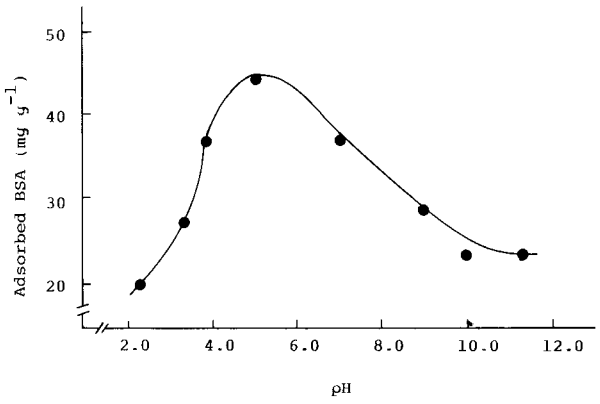

Figure 7 Effect of varying pH of the BSA solution on its adsorption onto PVCGP at fixed concentration of BSA 3.06×10^{-5} mol dm^{-3}, adsorbent 0.1 g, temperature 27°C.

In a typical study on the interaction of extracellular enzymes with the montorillonite soil surfaces, it was found that the adsorption on clay mineral brought about a shift of the optimum pH of the catalytic activity toward the alkaline value. This shift in the optimum pH was attributed to the interfacial pH hypothesis[18] and the conformational hypotheses.[19] However, no such observations were present in this case.

Salt Effect

The effect of addition of salts on the adsorption of BSA was studied by adding various anions (sodium salts) and cations (chloride salts) to the protein glass suspension in the range 0.005–0.05*M*. The results, given in Table III, clearly indicate that the addition of anions decreases the amounts of adsorbed BSA in the following increasing order of effectiveness:

$$Cl^- < SO_4^{2-} < PO_4^{3-}$$

The observed sequence of effectiveness is well justified, because the anions cause a repulsive

Table III Effect of Added Anions on the Adsorption of BSA onto PVCGP

	Adsorbed Amount of BSA (mg g^{-1})			
		Concentrations of Added Salts (M)		
Added Anions	0.005	0.01	0.03	0.05
Cl^-	16.8	11.2	8.6	7.0
SO_4^{2-}	13.4	8.2	4.4	3.6
PO_4^{3-}	9.6	3.0	1.8	No adsorption

Table IV Effect of Added Halide Ions (0.01*M*) on the Adsorption of BSA onto PVCGP

Added Halide Ions	Salt Concentration (*M*)	Adsorbed Amount of BSA (mg g^{-1})
Cl^-	0.01	11.2
Br^-	0.01	9.4
I^-	0.01	5.8

effect between the BSA molecules and glass surfaces, which obviously decreases the adsorption. It also implies that the extent of repulsion will increase with the valency of added ions, and consequently, the observed order of effectiveness of the anions is well justified.

To study the specific effect of anions, the halides of potassium were added (0.01*M*). Adsorption was found to decrease in the order, $Cl^- < Br^- < I^-$. The results, presented in Table IV, can be explained by the fact that with increasing radii on the ion, a greater steric effect is produced at the solution–glass interface. Consequently, a greater number of protein molecules is prevented from reaching the surface, and thus adsorption decreases.

In the case of addition of cations in the range 0.005–0.05*M,* an increase in adsorption is noticed, and the sequence of effectiveness of added cations is $Na^+ < Ca^{2+} < Al^{3+}$. The reason for the observed increase is clear, as the added cations cause a decrease in the electrostatic repulsion between the BSA molecules and glass surface, resulting in greater adsorption. This also explains the fact that the maximum positive charge of the Al^{3+} ion makes this the most effective of the three cations.

Solvent Effect

The nature of the medium is also one of the most influential parameters on the adsorption of proteins. In the present study, the effect of aliphatic alcohols on the adsorption of BSA was investigated by adding 10% (v/v) aliphatic alcohols to the BSA–PVCGP suspension. The results, shown in Figure 8, reveal that the adsorption decreases in the following order of effectiveness of the alcohols:

$$\text{MeOH} < \text{EtOH} < i\text{-PrOH} < t\text{-BuOH}$$

The observed decrease may be attributed to the following reasons.

1. Because the added solvent molecules and the coated surfaces are polar in nature, the preferential adsorption of alcohol molecules may reduce the number of active sites available to the BSA molecules, and thus adsorption decreases.
2. It is also likely that because of the smaller size of the added solvent molecules, they can easily arrive at the interface and get crowded, which causes a steric hindrance to the approaching BSA molecules. This evidently decreases the adsorption. Moreover, with increasing size of the alcohol molecule, the steric effect will also increase. Thus this also justifies the observed order of effectiveness of the added alcohols.

I also studied how variation in the dielectric constant of the medium affects the adsorption of BSA. For this purpose, dioxane was added in varying amounts (3–24% v/v) to the protein glass suspension, and the adsorbed amounts were determined. It was found that adsorption drastically decreases with increasing amounts of dioxane and finally becomes zero when the amount of dioxane exceeds 6%. The results are shown in Figure 9. The great decrease in adsorption may be explained by the fact that the added dioxane results in decreased dielectric constant of the me-

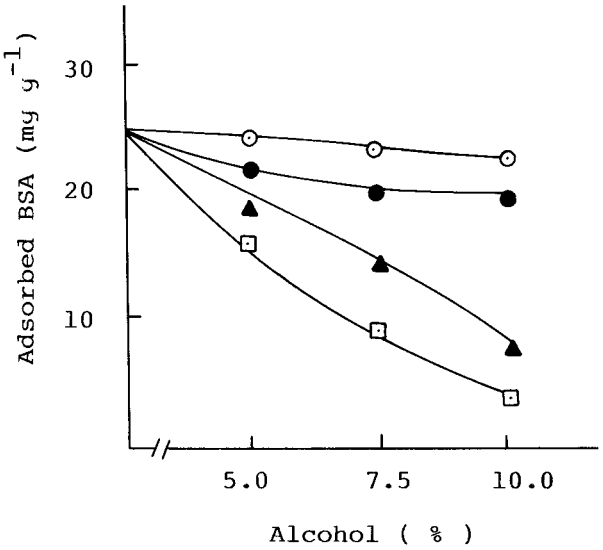

Figure 8 Effect of addition of water-miscible aliphatic alcohols on the adsorption of BSA at fixed concentration of BSA 3.06×10^{-5} mol dm^{-3}, adsorbent 0.1 g, temperature 27°C. (○) MeOH, (●) EtOH, (▲) *i*-PrOH, (■) *t*-BuOH.

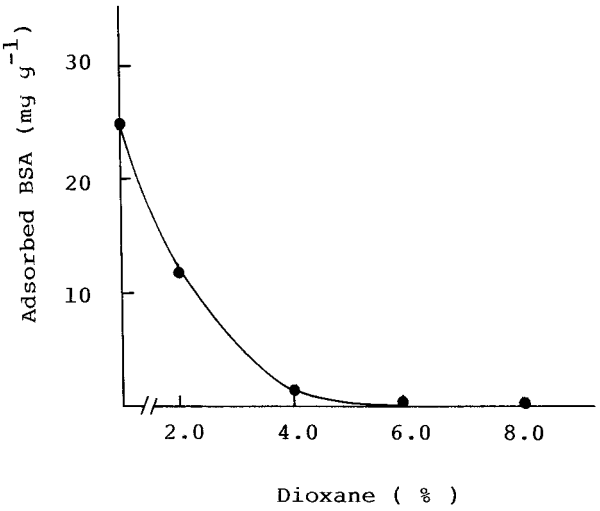

Figure 9 Effect of addition of dioxane on the adsorption of BSA at fixed concentration of BSA 3.06×10^{-5} mol dm^{-3}, adsorbent 0.1 g, temperature 27°C.

dium, which obviously causes a weakening of the attractive electrostatic forces between the BSA molecules and surfaces and leads to decreased adsorption.

Temperature Effect

The effect of temperature on adsorption of BSA has been investigated by varying the temperature in the range 5–45°C. The results, depicted in Figure 10, imply that with increasing temperature, BSA adsorption decreases. The observed decrease may be due to the following factors:

1. With increasing temperature, the electrostatic binding forces between the BSA molecules and glass surfaces weaken, and adsorption decreases.
2. At higher temperatures, the escaping tendency of the BSA molecules from the surface to the bulk solution increases, which also results in decreased adsorption.
3. At lower temperatures, the protein molecules may acquire a more compact structure, which will result in greater adsorption.

One also cannot rule out the possibility of physical agglomeration[20] of the BSA molecule at lower temperature, which also results in increased adsorption. Such agglomeration has also been postulated by several workers in the case of dye adsorption.

Several thermodynamic parameters have also been evaluated, as follows.

1. The standard free energy, $\Delta G°$, (Kcal/mol) was calculated by using the equation

$$\Delta G° = -RT \times \ln K$$

where K is the adsorption coefficient. Here $\Delta G°$ is found to be -7.32 Kcal/mol.

2. The apparent heat of reaction ΔH (Kcal/mol) was estimated by using the equation

$$\ln \frac{k_2}{k_1} = \frac{\Delta H}{2.303R}\left[\frac{1}{T_1} - \frac{1}{T_2}\right]$$

and has been calculated to be -15.9 Kcal/mol, which implies that the process is exothermic in nature.

3. The entropy $\Delta S°$ (cal/g mol/deg) of the system was calculated by the equation

$$\Delta G° = \Delta H° - T\Delta S°$$

This value was found to be 28.6 cal/g mol/deg.

CONCLUSIONS

The adsorption of BSA on GPSs increases greatly when the glass powder is coated with PVA film. A

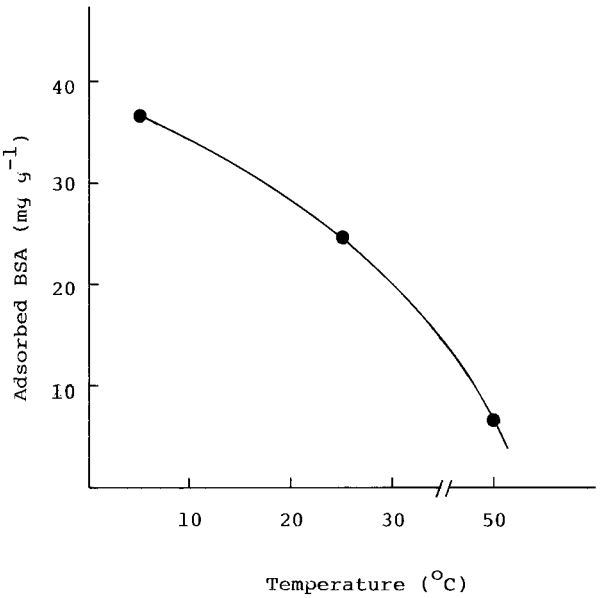

Figure 10 Effect of temperature on the adsorption of BSA at fixed concentration of BSA 3.06×10^{-5} mol dm^{-3}, adsorbent 0.1 g.

high-affinity–type adsorption isotherm is obtained, which also indicates that the adsorption is Langmuir controlled. The adsorption is significantly affected by the pH, and at the isoelectric point of the protein a maximum adsorption is noticed. With the addition of anions, the adsorption decreases; with cations, the adsorption increases. Adsorption also decreases with the addition of aliphatic alcohols to the system. In addition, adsorption decreases with decreasing dielectric constant and increasing temperature of the medium.

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