Study on the Adsorption of Hemoglobin onto Bentonite Clay Surfaces

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Received 23 February 2001; accepted 25 September 2001

ABSTRACT: The adsorption behavior of a hemoglobin (Hb) solution onto bentonite powder was studied from its alkaline solution (pH 12.6) and at room temperature. Different types of adsorption isotherms are discussed and various adsorption parameters, such as the adsorption coefficient, rate constants for adsorption and desorption, diffusion constant, penetration rate constant, were evaluated. The effects of pH and temperature of the adsorption medium on the extent of adsorption and the influence of solvents were studied, and the effect of salt concentrations on the rate of adsorption were also observed. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 85: 1607–1618, 2002

Key words: adsorption; kinetics; biopolymers; interface; clay

INTRODUCTION

The adsorption of proteins onto a solid surface is a phenomenon of great biological impact.[1] For reasons which are currently not well understood quantitatively, the adsorption process can be extremely sensitive to pH, concentration of other ions and molecules, temperature, etc., and to the precise aminoacid composition of the protein. Unlike the binding of small molecules to protein receptors, which typically involves a small number of elementary reaction steps, the adsorption of proteins to surfaces may embrace a large number of parallel and consecutive reaction steps, characterized by diverse binding forces such as electrostatic, Lifshitz–van der Waals, and Lewis acid/base.[2] All these and many more pecularities involved in the protein-adsorption process put a great emphasis on a systematic investigation of the phenomenon.

By far, the greatest incentive for research in the domain of protein adsorption on solid surfaces arises from the numerous technological and medical applications that depend on this phenomenon. It plays a role in various man-made situations: biofouling,[3] thromobosis arising from medical prostheses,[4] immounological reactions on solid supports,[5] and chromatographic separation.[6] It is also a very important natural phenomenon, particularly in soil biology.[7] In fact, the adsorption of proteins on clay mineral surfaces plays a vital role in fields related to agricultural and environmental science. More specifically, the interaction of enzymes and clay minerals has important consequences in the breakdown process of soil organic matter. Moreover, the catalytic activity of enzymes may also be altered directly as a consequence of conformational changes of decreasing enzyme adsorption on soil mineral surfaces.[8] Many citations in the literature appear where BSA has been taken as a model protein to study enzyme–soil interactions. However, the adsorption of other proteins onto soil surfaces has been less investigated. Thus, in the present communication, the adsorption of hemoglobin was

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Journal of Applied Polymer Science, Vol. 85, 1607–1618 (2002)
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studied on bentonite clay surfaces, which is widely employed in the clarification of wines[9] and as an adsorbent for oil- and grease-type substances.[10]

EXPERIMENTAL

Materials

Hemoglobin (molecular weight 65,000 and isoelectric pH 6.8) was supplied in powdered form by Loba Chemie (Mumbai, India) and used without further purification. Its standard solutions were prepared by dissolving calculated amounts of hemoglobin into a 0.2*N* NaOH solution. The pH of the solution was maintained at 12.6. Bentonite, an aluminum silica hydrate, used as an adsorbent, was supplied by Loba Chemie and used as received. All other electrolytes and solvents were of analytical reagent grade.

Method of Adsorption

Adsorption experiments were carried out by the direct contact method which involves shaking (Tempstar, India) a hemoglobin solution of a definite concentration containing 50 mg of bentonite at a fixed pH of 12.6 for 2 h, which was found to be a sufficient time for equilibrium adsorption. The process of shaking was carried out so gently that no air bubbles were produced in the suspension, as this could lead to an air–water interface. After shaking, the suspensions were centrifuged in a high centrifugal force shaking machine (Remi, India) for 30 mins, which was confirmed as the required time period for complete settling of the clay particles in the centrifuging suspensions. Almost no change in the pH of the suspensions was recorded after adsorption. The remaining amount of hemoglobin in the supernatant was estimated colorimetrically (Systronics, India) by direct measuring its absorbance at 570 nm.[11] The amount of adsorbed hemoglobin was calculated by the mass-balance equation as given below:

$$\text{Adsorbed amount} = \frac{(C_0 - C)\,V}{m} \quad (1)$$

where C_0 and C are the concentrations of the initial hemoglobin solution and supernatant, respectively (mg/mL); V, the volume of the suspensions; and m, the weight of the adsorbent (g).

Table I Physical Characteristics of Bentonite Clay

Mesh size	200–300
Average particle diameter (μm)	40
Total surface area ($m^2\ g^{-1}$)	400
Cation-exchange capacity	90 meq/100 g
pH of 2% aqueous solution	12.6

Kinetics of Adsorption

The kinetic course of the adsorption process was followed by monitoring the progress of the adsorption process at different time intervals. For this purpose, several identical sets were run simultaneously and the amounts of adsorbed hemoglobin were estimated at different time intervals.

RESULTS AND DISCUSSION

Mode of Adsorption

Bentonites are highly colloidal, plastic, and exceptionally high water-adsorbing clays, which contain mainly montmorillonite as the main constituent clay. Its physical characteristics are presented in Table I. A very significant feature of bentonite-type clays is their cation-exchange property, which accounts for their many technical applications. As far as the crystal structure of montmorillonite is concerned (Fig. 1), it possesses a basal surface accounting for 99% of the total surface area with a negative charge that arises from isomorphous charge substitution in the crystal lattice, which is compensated by exchangeable cations from the external medium. These cations are usually Lewis acids like Na^+ and Ca^{2+}. In fact, montmorillonite has a composite surface. The hydrophilicity of its surface is due to the hydration properties of exchangeable cations, and, on the other hand, the hydrophobic character is due to the siloxane surface, which has been well confirmed by experimental[12] and theoretical[13] considerations.

Proteins are copolymers of some 20 natural amino acids linked to each other in a linear polypeptide chain. Along the main chain (backbone), the amino acid residues are positioned in a transconfiguration. In an aqueous environment, the polypeptide chain often adopts a specific, dense structure, forming a globular protein molecule. Some significant structural characteristics of proteins are

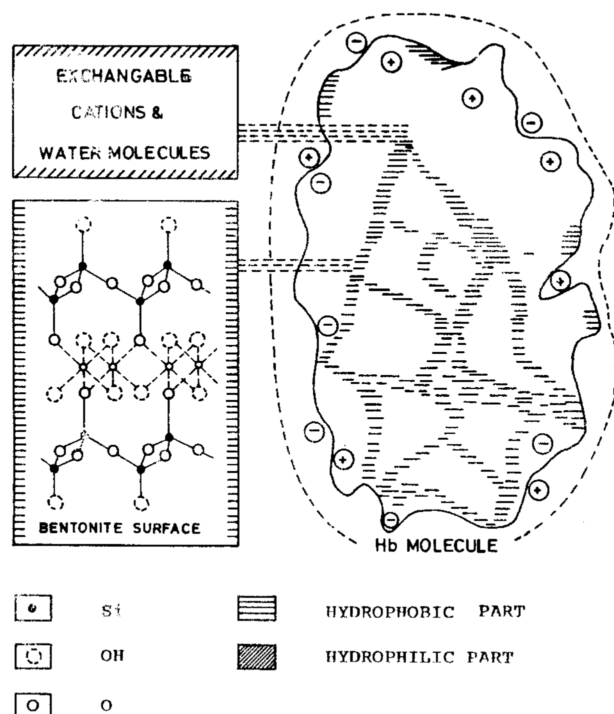


Figure 1 Model depicting the adsorption of hemoglobin molecules onto bentonite surfaces.

(i) Their molecules are more or less spherically shaped with dimensions ranging between a few to a few 10s of nanometers.
(ii) Hydrophobic side groups tend to be buried in the interior of the protein molecule, where they are shielded from contact with water. As a result, part of the polar, hydrogen-bond forming polypeptide backbone must be located in the interior also.
(iii) Charged groups are predominantly found in the aqueous periphery of the protein molecule. Any charged group in the interior occurs in ion pairs, because charge separation is stronghly opposed by the low, local dielectric constants.
(iv) Not all hydrophobic parts are hidden in the interior. Apolar atoms occupy 40–50% of the water-accessible surface of most small proteins, whereas about 60% of the interior of these proteins is apolar. For larger or more spherical proteins, the fraction of apolar atoms on the surface is typically less.

Thus, in light of the above discussion, the adsorption of proteins onto montmorillonite may be considered as due to the hydrophilic and hydrophobic interactions between the protein molecule and clay surfaces. The positively charged side chains of the protein amino acids (lysine, arginine, histidine) interact with the hydrophilic portion of the surface, which is accompanied by charge-compensating cations. Moreover, the hydrophobic amino acids of a protein come in contact with the siloxane layer and adhere to the surface by hydrophobic interactions, which have also been confirmed experimentally. The whole picture of the hemoglobin–bentonite interaction is modeled in Figure 1, which clearly depicts hydrophilic and hydrophobic interactions taking place between the bentonite surfaces and the hemoglobin molecule.

Adsorption Isotherm

To study the effect of the hemoglobin concentration on its adsorption onto bentonite, the concentration range of the hemoglobin solution was selected as from 7.69×10^{-6} to 76.9×10^{-6} mol L^{-1}. The results of increasing the concentration of hemoglobin on the extent of adsorption reveal that adsorption increases with an increasing concentration of the hemoglobin solution. The reason for the observed increase is obvious, as with an increasing concentration of the hemoglobin solution, a larger number of hemoglobin molecules approach the interface and, consequently, the adsorbed amount will increase. The results also reveal that, beyond the concentration of 76.9×10^{-6} mol L^{-1}, the adsorption reaches saturation and evidently a plateau is obtained. This state of adsorption is a common observation and has been widely reported in the literature.[14,15]

Adsorption data are often presented in adsorption isotherms, where, at a constant temperature, the adsorbed amount is plotted against the protein concentration in solution after adsorption has occurred. Fully determined isotherms can provide a convenient method to judge whether an adsorption process can be treated as reversible or not. Furthermore, the shape of the isotherm also helps in predicting the nature of the adsorbate–adsorbent interaction that is, whether a protein has a high affinity for adsorption or not or whether the interaction between the proteine and the sorbent is dominated by that between the molecules of the adsorbed proteins.

Various types of adsorption isotherms can be considered to analyze the adsorption behavior of hemoglobin toward the bentonite surfaces: According to the Langmuir isotherm equation,

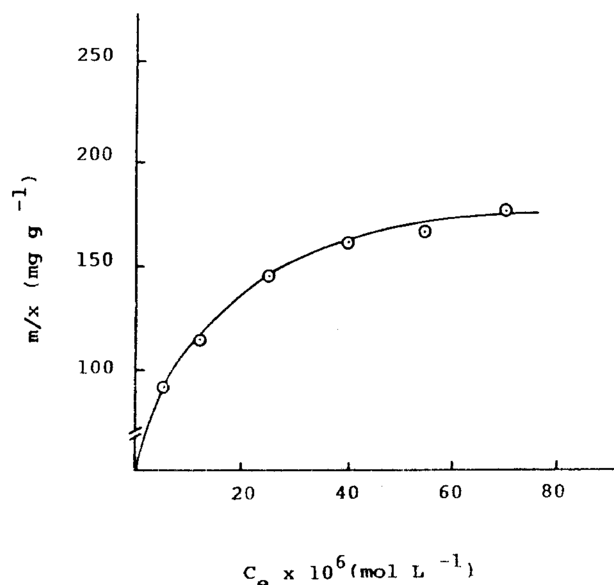


Figure 2 Plot showing variation of adsorbed hemoglobin (mg g^{-1}) with equilibrium concentration of hemoglobin; bentonite = 0.05 g; pH 12.6; temperature = 27 ± 0.2°C.

$$\frac{C_e}{a} = \frac{1}{a_s K} + \frac{C_e}{a_s} \tag{2}$$

where C_e is the equilibrium concentration of the hemoglobin solution; a, the adsorbed amount of hemoglobin (mg g^{-1}) at any equilibrium concentration C_e; and a_s, the adsorbed amount of hemoglobin (mg g^{-1}) at saturation (adsorption capacity). and $K = k_1/k_2$, with and k_1 and k_2 being the rate constants for adsorption and desorption, respectively.

The relative affinity of hemoglobin for adsorption may also be evaluated with the help of numerical values of K, which, in the present study, was found to be 0.15×10^{-6} mol^{-1} L. This clearly reveals that the adsorption affinity of hemoglobin is 8000 times greater than that observed for the adsorption of gelatin onto acid-treated bentonite[16] and twice greater than that obtained for the adsorption of gelatin onto HAP surfaces.[17] In the present case, the isotherm obtained shown in Figure 2, which is a typical Langmurian isotherm characterized by an early ascending portion followed by a well-defined plateau. Adsorption saturation is commonly found for proteins in contrast with synthetic polymers, where adsorption usually is a continuously increasing function of the concentration in solution. In some cases, protein-adsorption isotherms show kinks or steps, but such irregularities are absent in the present system. The values of the adsorption coefficient (K) calculated in accordance with eq. (2) were found to be 0.15×10^{6} mol^{-1} L.

Freundlich Isotherm

The Freundlich isotherm has the form

$$S = k\ C_e^N$$

or

$$\ln S = \ln k + N \ln C_e \tag{3}$$

where S is the adsorbed amount of the adsorbate; k, the predicted quantity of sorption per gram of bentonite at the unit equilibrium concentration (mg g^{-1}), and N, a measure of the nature and strength of the adsorption process and of the distribution of active sites. If $N < 1$, bond energies increase with the surface density; if $N > 1$, bond energies decrease with the surface density, and when $N = 1$, all surface sites are equivalent.[18]

To gain more quantitative insights into the adsorption process, the values of k [i.e., predicted quantity of sorption per gram of bentonite at the unit equilibrium concentration (mg g^{-1})] and N (measurement of the nature and strength of the adsorption process and of the distribution of active sites) were also calculated and are presented in Table II.

In accordance with the equation of the modified Freundlich isotherm as given below,[19,20]

$$\log S_1/(S_{\max} - S_1) = \beta \log C_e + \log A/S_{\max} \tag{4}$$

a plot was drawn (Fig. 3). The values of A and β were calculated and are given in Table II. The value of k_d (distribution coefficient) in mL/g was calculated using the following equation:

$$A = S_{\max}\, k_d^{\beta} \tag{5}$$

It was found to be 39.80×10^{-2} and the value of β is equal to unity and this implies a higher spread.[21]

Adsorption Kinetics

The transport of proteins onto a clear solid surface is generally a rapid process, faster than the subsequent adhesion of cells during thrombus initiation or of bacteria during dental plaque forma-

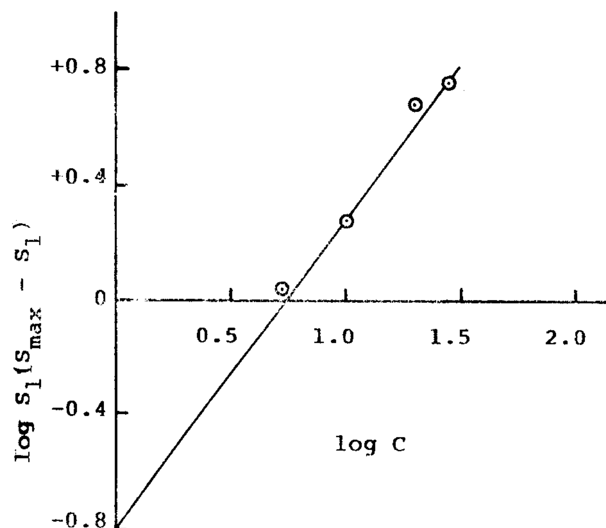


Figure 3 Plot showing variation of log C with log $S_1/(S_{\max} - S_1)$ according to modified Freundlich isotherm; bentonite = 0.05 g; pH 12.6; temperature = 27 ± 0.2°C.

tion. Numerous techniques, such as ellipsometry, protein depletion, internal reflection, infrared, spectroscopy, and radioactive labeling, have been used in earlier studies for the characterization of the proteinaceous layers at equilibrium. However, because of the complexity and the rapidity of the adsorption process, not many kinetic studies have been performed. The techniques mentioned above have been applied only under well-defined, geometrical and hydrodynamic conditions. Although numerous plausible theoretical models have been proposed, many of them predict adsorption kinetics which differ slightly from one model to another, and to test them, measurements of high precision are needed.

Table II Different Kinetic and Adsorption Parameters for Adsorption of Hemoglobin onto Bentonite

Adsorption and Kinetic Constants	Value
Adsorption coefficient (K)	0.15×10^{6} mol^{-1} L
Rate constant for adsorption (k_1)	4.86×10^{-3} min^{-1}
Rate constant for desorption (k_2)	0.032×10^{-9} mol L^{-1} min^{-1}
Diffusion constant (D)	7.1×10^{-12} cm^{-2} s^{-1}
Penetration rate constant (T)	8.76×10^{-2} mol L^{-1} min^{-1}
Distribution coefficient (k_d)	39.80×10^{-2}
A	69.88
N	0.24
k	73.69
β	1

Normally, the adsorption of a protein molecule from a bulk solution onto a solid surface is considered to occur in the following three steps,[22] namely:

(a) Diffusion of protein molecules from the bulk to the interface,
(b) Attachment of protein molecules to active sites of the surface, and
(c) Reconformation of the structure of the protein molecule after adsorption.

Of these three steps, the last one plays a significant role not only in controlling the adsorption kinetics of protein but also in modification of the surface properties of the substrate.[23] In the present case, the third step contributes little to the overall adsorption kinetics as at the experimental pH (12.6). The hemoglobin molecules do not possess as much structural adaptability as they do at lower pH.[23]

To verify the diffusion-controlled nature of the adsorption process, the experiments were also run with varying speeds of agitation and the adsorbed amount was found to decrease with a decreasing speed of the agitation. Hence, this confirms that the adsorption is diffusion-controlled. The overall progress of the adsorption process is shown in Figure 4(a), which clearly implies that the adsorption rate is almost constant 40 min, and, therefore, the kinetic scheme developed by Bajpai and Bajpai[24] can readily be applied.

Evaluation of k_1 and k_2

For evaluating the rate constant for adsorption (k_1), the following linear equation may be used:

$$\frac{1}{C} = k_1 \frac{1}{C_0} t + \frac{1}{C_0} \tag{6}$$

where the terms involved have their usual significance.[24] In the present study, the plot drawn between $1/C$ and t is shown in Figure 4(b) and the evaluated values of the rate constants for adsorption (k_1) and desorption (k_2) are summarized in Table II.

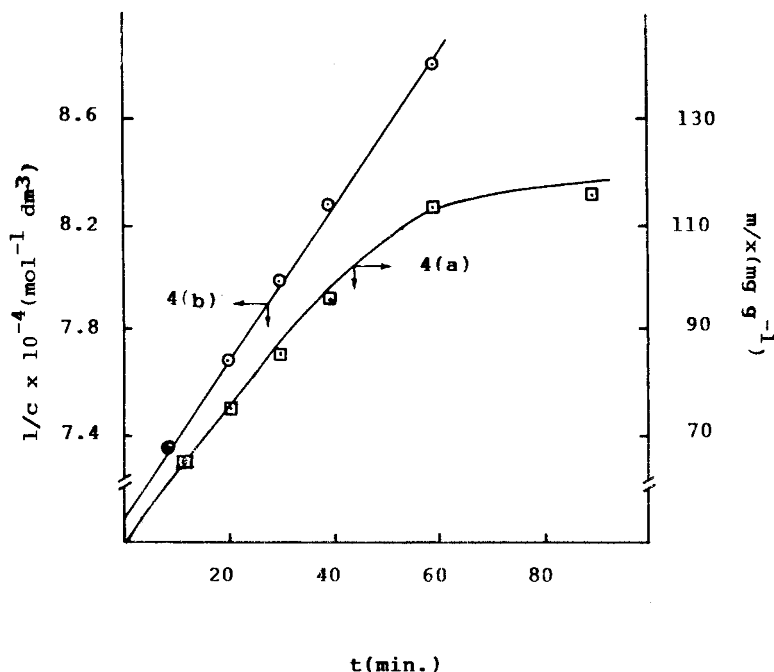


Figure 4(a) Plot drawn between 1/*C* and *t*. (b) Plot showing variation of adsorbed hemoglobin (mg g^{-1}) with time *t* at fixed concentration of hemoglobin = 15.38×10^{-6} mol L^{-1}; bentonite = 0.05 g; pH 12.6; temperature = 27 ± 0.2°C.

It is also a well-recognized fact that the process of adsorption is a two-regime process.[25,26] At the initial stages, the solid surface is bare and the kinetics of adsorption are governed by the diffusion of the molecules from the bulk solution to the surface. All the molecules that arrive at the surface 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,

$$q(t) = 2/\pi\ C_0 \sqrt{Dt} \tag{7}$$

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

In the later stages, a barrier of adsorbed molecules exists and the molecules arriving from the solution have to diffuse across this barrier. This penetration is slow and a theoretical treatment given by Ligorue and Leibeler[27] predicts an exponential time dependence for the later stages:

$$q(t) = q_{eq}\,[1 - \exp(-t/T)] \tag{8}$$

where q_{eq} is the adsorbed amount at equilibrium and $1/T$ is the penetration rate constant. With the help of the plot drawn in accordance with eqs. (7) and (8). the diffusion constant (*D*) and penetration rate constants ($1/T$) were calculated and are summarized in Table II.

pH Effect

Proteins are long chains of amino acids. Some of these amino acids carry side-chain carboxyl or amino groups, and these may remain free and exposed to the solvent when the protein is in a solution. Therefore, they can dissociate in an aqueous solution at a suitable pH, resulting in COO and NH_3^+ ions covalently attached to the protein macromolecule. The degree of ionization of these groups is not greatly influenced by their incorporation into the large molecule. The carboxyl group tends to ionize at pH values over approximately 4, and the amino group at below approximately 12. Thus, in an acid solution, a typical protein becomes positively charged because of the presence of NH_3^+ and COOH groups, and in a basic solution, it is charged negatively because of NH_2 and COO^- groups. When the pH equals the isoelectric point of the protein, the net charge is zero. This obviously corresponds to the

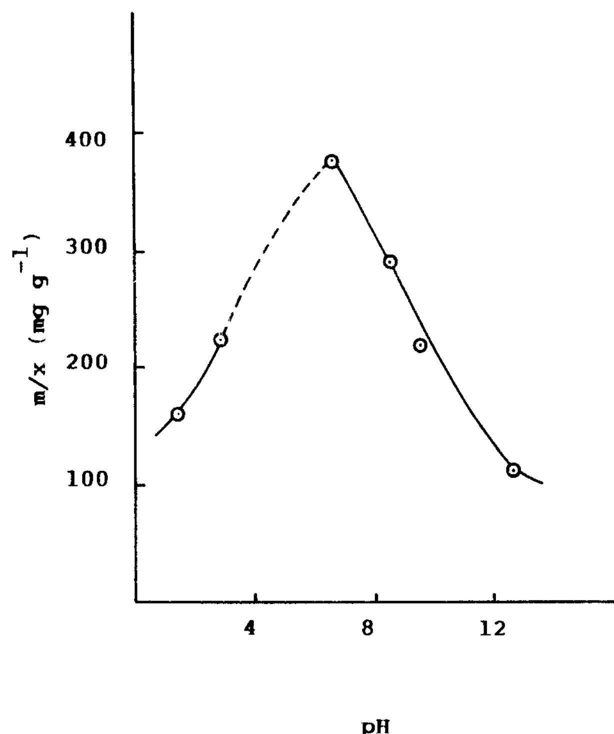
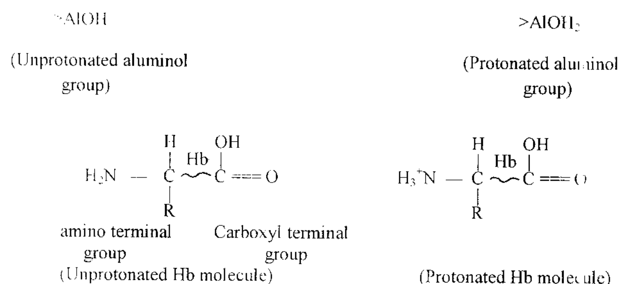


Figure 5 Plot showing variation of adsorbed hemoglobin (mg g^{-1}) with pH of the suspension at fixed concentration of hemoglobin = 15.38×10^{-6} mol L^{-1}; bentonite = 0.05 g; temperature = 27 ± 0.2°C.

presence of equal numbers of oppositely charged groups on the protein. Such a neutral structure of charged groups is called a dipolar ion or a zwitterion. At pH's near the isoelectric point, both NH_3^+ and COO^- groups are present, so that the net charge is small.

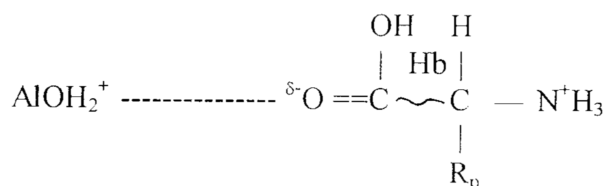
Proteins often exhibit a maximum of adsorption on electrically charged surfaces near their isoelectric point, and several explanations for this have been proposed.[27] In the present study, the effect of pH was investigated by varying the pH in the range 1.4–12.6 by using buffers. The results obtained are depicted in Figure 5, which clearly shows that, in the acidic pH range (i.e., from 1.4 to 6.6), the adsorption increases, while in the alkaline range (6.6–12.6), it continuously decreases. It is worth mentioning here that, while attempting to increase the pH above 3.0, the pH did not change much, but at the same time, hemoglobin precipitated in the solution. This is the reason why the adsorption of hemoglobin could not be studied in the pH range 3.0–6.6. Now, the behavior of hemoglobin toward the pH dependence may be explained as follows:

1. At the pH of the acidic range, such as at pH 1.4, the alumina (of the clay) and hemoglobin (Hb) molecules exist in the following forms:

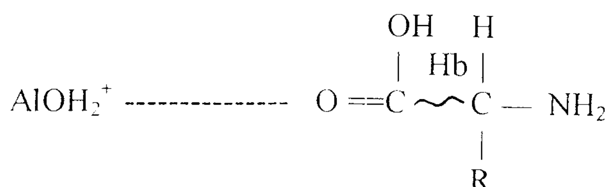


Under these conditions, the following types of interactions may lead to the adsorption of hemoglobin onto bentonite:

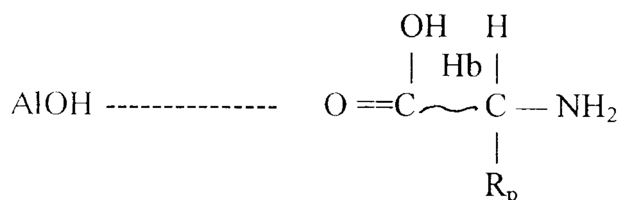
(i) Interaction between the protonated aluminol group and negatively charged oxygen atom of the carboxyl terminal group of the hemoglobin molecule:



(ii) Interaction between the protonated aluminol group and the unprotonated hemoglobin molecule:



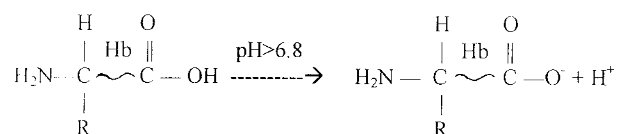
(iii) Interaction between the unprotonated aluminol group and the unprotonated hemoglobin molecule:



From the above schemes of interaction, it is clear that while in (i) both the hemoglobin and

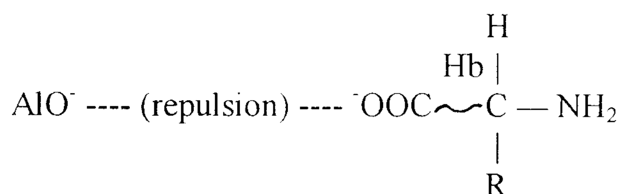
alumina are charged species, in (ii) and (iii), the hemoglobin and both the alumina and hemoglobin, respectively, are uncharged. Thus, a greater contribution toward the adsorptive forces is offered by the uncharged species. This clearly predicts an increasing adsorption with increasing pH, because as the pH is increased, the positive charge on both the hemoglobin molecules and the bentonite surface decreases, which results in a greater interaction between the two, because the electrostatic repulsion between the positively charged species is minimized.[28] This leads to an increasing adsorption, which becomes maximum at pH 6.8.

(2) Beyond pH 6.8, the ionization of hemoglobin molecules begins as follows:



In this pH range (>6.8), protonated aluminol groups ($AlOH_2^+$) keep changing to aluminol groups (A1OH), resulting in a weakening of electrostatic interaction with the negative —COO^- group of the hemoglobin molecules; consequently, the adsorption decreases. Moreover, beyond pH 7.0 (PZC of bentonite), the surface of bentonite becomes negatively charged, due to the formation of the AlO^- type of groups on it. Thus,

Now, when the hemoglobin molecules are almost fully ionized, the following electrostatic repulsion begins to operate between the bentonite surface and the hemoglobin molecules:



which obviously results in a decreasing adsorption.

Salt Effect

In all the adsorption systems, where a macroion comes in contact with an electrically charged surface, the adsorption is greatly affected by the presence of external electrolytes. In an aqueous solution, charged sorbent surfaces and protein macroions are surrounded by the counterions. In such systems, adsorption of protein molecules to the charged solid will involve a redistribution of charge in the interfacial region, which, in turn, affects adsorption. In the present case, since the pH of the solution is well above the isoelectric point of the protein, both the clay surfaces and hemoglobin molecules bear the same charge and, obviously, the adsorption will be less because of this repulsion, which will increase with a decreasing dielectric permittivity in the interfacial layer. However, coadsorption of low molecular weight counterions can substantially reduce this opposing force and enhance adsorption.[29] In principle, the following consequences are usually met when salts are present in the suspension:

1. Some authors postulated[30] that the presence of salts in a solution usually results in a poor solvent quality of the medium, which favors increased adsorption.
2. In polyelectrolyte solutions, the Coulombic potential due to the macroion is shielded by the smaller ions and the forces between the adsorbate and the surfaces are substantially affected, which ultimately affects adsorption.
3. The coadsorption of counterions either on the sorbent surface or on the protein molecule or on both can lead to enhanced or reduced adsorption.

In the present study, the effect of salts on the adsorption of hemoglobin was investigated by adding sodium salts of Cl^- and SO_4^{2-} ions to the protein–bentonite suspensions in the concentration range 0.001–0.1 *M*. The results are depicted in Figure 6, which clearly reveals that the adsorbed amount increases with an increasing concentration and valency of the anions.

The observed increase seems surprising, as at the experimental pH. (12.6), both the hemoglobin and clay surfaces are negatively charged and one has to rule out the possibility of any type of shielding by the added anions. However, the results can be explained as below:

It has been well recognized that several kinds of proteins interact with small molecules and possess good capacity to bind the ions. In the present case, at the experimental pH of 12.6, the protein molecule has a net negative charge, but, never-

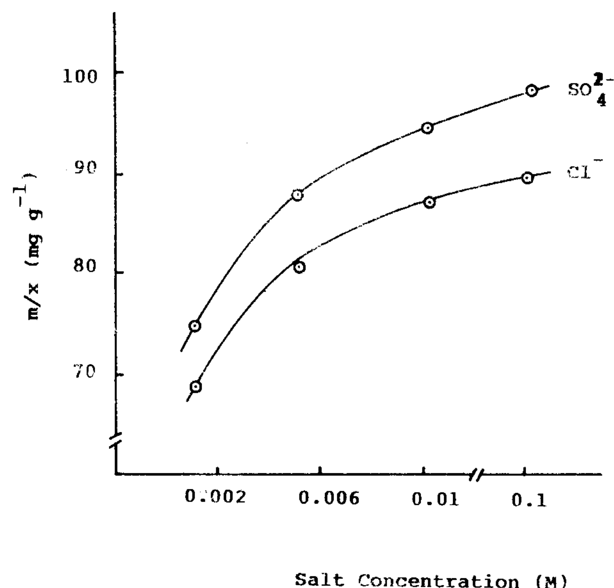


Figure 6 Plot showing variation of adsorbed hemoglobin (mg g^{-1}) with varying concentrations of the added anions at fixed concentration of hemoglobin $= 15.38 \times 10^{-6}$ mol L^{-1}; bentonite = 0.05 g; pH 12.6; temperature = 27 ± 0.2°C.

theless, its positively and negatively charged group could be considered as a binding site for the small ions.[31] Thus, K^+ interacts at the anionic groups (such as —COO, —O, and —S) and anions at the cationic groups (such as $—NH^+—$, $—NH_3^+$, and $=NH_2^+$). After binding of the added ions to various active sites on the hemoglobin molecules, electrostatic repulsion between the hemoglobin molecules and clay surfaces will be screened, which obviously results in a favorable contact between the hemoglobin and the bentonite surface. Thus, the amount of the adsorbed hemoglobin will increase. Similar binding of Cl^- and SO_4^{2-} ions was reported elsewhere.[32,33]

It was also observed that the rate of adsorption (mg g^{-1} min^{-1}) also increases with added anions in the same order of effectiveness. The reason for the increased rate is quite clear; Due to a decrease in the electrostatic repulsion between the protein molecules and clay surfaces, the diffusion of hemoglobin molecules will become relatively faster and their attachment to the surface also becomes easier. In this way, the rate of adsorption also increases. In the case of the addition of PO_4^{3-} ions to the suspension, a precipitate was observed that may be due to salt formation of the cations under alkaline conditions. In an attempt to study the effect of cations on the adsorption, various cations such as Ca^{2+}, Cu^{2+}, and Al^{3+} were added but precipitate formation was seen in the system, so that the cationic effect could not be studied.

The effect of the addition of halide ions, that is, Cl^-, Br^-, and I^-, on the adsorption of hemoglobin was observed, and it was found that with increase in the atomic weight and size of the ions, the adsorption gradually increases and it is greater in the presence of the I^- ion. The different halide ions obey the following order of effectiveness:

$$Cl^- < Br^- < I^-$$

The results may be explained by the fact that, with an increasing size of the added halide ions, the tendency of the anions to become polarized increases when they arrive at the interface and interact with the exchangeable cations. This results in a greater release of the cations of the bentonite surface and, thus, the adsorption increases.

Solvent Effect

The effect of the addition of water-miscible alcohols on the adsorption of hemoglobin was investigated by adding various alcohols (10% v/v) to the adsorption system. The results are shown in Figure 7, which clearly implies that the adsorbed

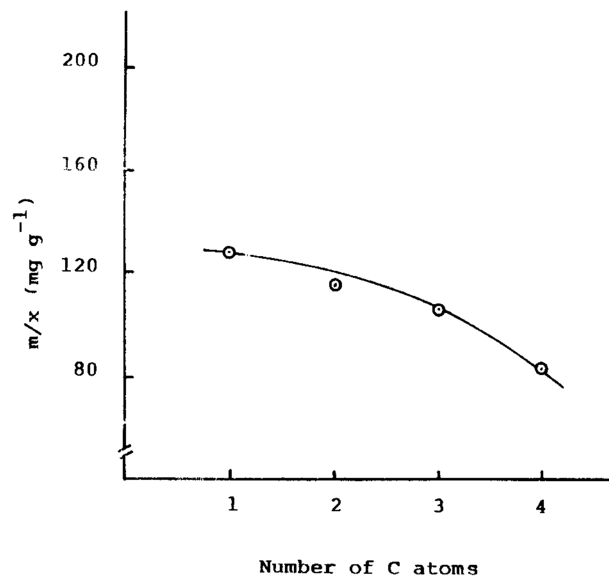


Figure 7 Effect of number of carbon atoms of added aliphatic alcohols (10% v/v) on the adsorbed hemoglobin (mg g^{-1}) at fixed concentration of hemoglobin $= 15.38 \times 10^{-6}$ mol L^{-1}; bentonite = 0.05 g; pH = 12.6; temperature = 27 ± 0.2°C.

hemoglobin constantly decreases with an increasing number of carbon atoms in the aliphatic chain of the alcohols. Thus, the order of increasing depression in the amount of adsorbed heamoglobin was

$$\text{MeOH} < \text{EtOH} < \text{iso-PrOH} < n\text{-BuOH}$$

The results can be explained as below: It is evident from the previous discussions that hydrophobic interactions are mainly responsible for the adsorption of hemoglobin molecules. Upon addition of aliphatic alcohols to the suspensions, the hydrophobic portion of aliphatic chains interacts with the hydrophobic siloxane layer of the clay surface and may get adsorbed. Thus, due to a decrease in number of active sites on the hydrophobic region of the clay surface, the adsorption of hemoglobin molecules will decrease. Moreover, since the hydrophobic character of added alcohols increases with an increasing number of carbon atoms, this accounts for the order of effectiveness of added alcohol. A similar type of preferential adsorption of alcohol molecules onto activated carbon fiber (ACF) surfaces was also reported elsewhere.[34]

Dielectric Constant Effect

In suspensions containing charged macromolecules and a charged surface, the interaction between the two is substantially affected by the polarity of the medium. Moreover, when the macromolecule is a protein, the situation becomes much more important as the conformation and the stability of the protein is greatly determined by Coulombic interaction between the charged residues of the protein molecule, which, in turn, rests upon the dielectric permittivity of the medium.[35] Obviously, a high dielectric permittivity of the solvent causes a greater unfolding of the protein molecule due to electrostatic repulsions arising from the partial ionization of the residues originally buried in the folded protein in the non-ionized form.[36]

Thus, to investigate the effect of polarity of the medium on the adsorbed amounts of hemoglobin, varying amounts of dioxane were added to the hemoglobin–bentonite suspensions. The results (Figure 8) reveal that the adsorption decreases with increasing proportions of dioxane in the medium, that is, with decreasing dielectric constants of the medium. The results may be explained by the following facts: (i) Bentonite clays are well known for their property of adsorbing organic liquids, and therefore, in the present case, also the possibility of adsorption of dioxane molecules cannot be ruled out. Obviously, due to a reduction in the number of active sites on the clay surfaces, the adsorption of hemoglobin may decrease. (ii) Looking at the experimental conditions, such as the pH of the suspensions, the isoelectric point of the protein, and the negative charge on the clay surfaces, there is no doubt that electrostatic repulsion between the protein and sorbent surface makes no contribution to the adsorption and in fact, it is the hydrophobic interaction between the amino acid residues and the siloxane surface of the clay that mainly governs adsorption. Now, upon adding dioxane to the suspensions, the low dielectric permittivity of the medium results in less unfolding of the hemoglobin molecule, which, consequently, does not expose the buried hydrophobic residues of the protein molecule to the sorbent surface for adsorption. Therefore, because of fewer hydrophobic interactions, the adsorption decreases with a decreasing dielectric constant of the medium.

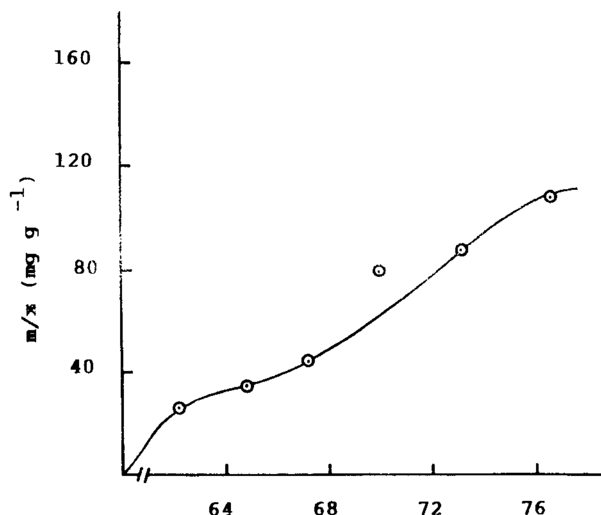


Figure 8 Effect of dielectric constant on the adsorbed hemoglobin (mg g^{-1}) at fixed concentration of hemoglobin = 15.38×10^{-6} mol L^{-1}; bentonite = 0.05 g; pH 12.6; temperature = 27 ± 0.2°C.

Temperature Effect

The effect of temperature on adsorption is important, as it not only affects the rate and extent of

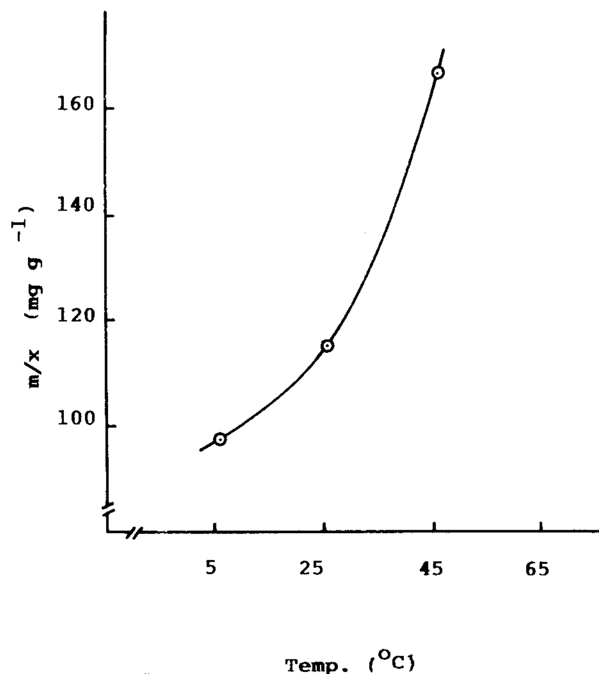


Figure 9 Effect of temperature on the adsorbed amount of hemoglobin (mg g^{-1}) at fixed concentration of hemoglobin = 15.38×10^{-6} mol L^{-1}; bentonite = 0.05 g; pH 12.6.

adsorption, but also provides information about adsorbate–adsorbent interactions. In the present study, the effect of temperature on the adsorption was studied in the range 5–45°C and the results are shown in the Figure 9, which indicates that the adsorption increases with an increasing temperature of the medium. This can be explained by the following facts: (a) As postulated by several workers,[37] the adsorbate molecules at lower temperature agglomerate, which results in increased adsorption. In a similar way, it is possible that, at higher temperature, the hemoglobin molecules unfold and cause agglomeration due to entanglement of their chains, thus resulting in an increased adsorption at higher temperature. (b) Since protein adsorption is normally a diffusion-controlled process, it may be postulated that, with increasing temperature, the mobility of the hemoglobin molecules increases and, thus, results in greater adsorption. (c) It is worth mentioning here that, at higher temperature, the electrostatic attractions are normally weakened and, therefore, a lower adsorption should be expected. However, a higher adsorption definitely points out that, besides electrostatic forces, some other forces must be contributing toward adsorption. These forces are the hydrophobic forces as suggested elsewhere.[38] Thus, at higher temperature, the hemoglobin molecules may unfold so that its hydrophobic part interacts with the hydrophobic part of the bentonite, thus enhancing the adsorbed mass of hemoglobin. Here, it is noticeable that, although the bentonite surface is known to possess hydrophobic centers (exchangeable cations), after the adsorption of hemoglobin molecules, the charge-compensating cations are exchanged with protein molecules, thus leaving the hydrophobic siloxane surface.

Thermodynamic Parameters

We also calculated the following thermodynamic parameters:

(i) Standard free-energy change (ΔG^0). The standard free energy (kcal/mol) was calculated by eq. (9):

$$\Delta G^0 = -RT \ln K \tag{9}$$

where K is the equilibrium constant of the adsorption process. The value of ΔG^0, as calculated above, was found to be −7.27 kcal/mol.

(ii) Standard enthalpy change (ΔH^0) for adsorption. The apparent heat of reaction–enthalpy, ΔH^0 (kcal/mol), was estimated using eq. (10):

$$\ln\frac{K_2}{K_1} = \frac{\Delta H^0}{R}\left[\frac{1}{T_1} - \frac{1}{T_2}\right] \tag{10}$$

The value of ΔH^0 was calculated to be 1.53 kcal/mol.

(iii) Standard entropy change (ΔS^0). It was calculated by eq. (11)

$$\Delta G^0 = \Delta H^0 - T\,\Delta\,S^0 \tag{11}$$

and the value of ΔS^0 was found to be 355.4 cal/kmol.

CONCLUSIONS

The adsorption of hemoglobin onto bentonite clay surfaces from its aqueous alkaline solution follows the Langmuir adsorption isotherm equation and belongs to the L2 category of the adsorption

isotherm. The adsorption varied with the suspension and was maximum at the isoelectric point of hemoglobin (7.0). The adsorption also increased with increasing temperature. The adsorption was found to increase in the presence of Cl^- and SO_4^{2-} anions and it also showed an increase with an increasing size of the added halide ions. Addition of aliphatic alcohols decrease the adsorbed amount with increasing lengths of alkyl chains of the added alcohol. The adsorption also decreased with increasing amounts of dioxane. The adsorption process was found to be of an endothermic nature, as it increased with an increasing temperature of the adsorption medium.

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