

# Adsorption of Casein onto Alkali Treated Bentonite

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**ABSTRACT:** The adsorption measurements of casein onto alkali treated bentonite were performed at room temperature and fixed pH (11.8) with an object to study the mode of casein adsorption at the alkali treated bentonite surfaces. The adsorption isotherm was found to have a Langmuir nature. The adsorption was pH dependent and increased with a decrease in temperature. The adsorption was quite sensitive to the presence of  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{PO}_4^{3-}$  ions and showed many interesting variations with increasing concentrations of the added anions. Various kinetic and adsorption parameters such as the adsorption coefficient, rate constants for adsorption and desorption, diffusion constant, and penetration rate constant were calculated. © 2000 John Wiley & Sons, Inc. *J Appl Polym Sci* 78: 1656–1663, 2000

**Key words:** adsorption; casein; alkali treated bentonite

## INTRODUCTION

Interactions between proteins and surfaces are widespread and of great biological, medical, and technological significance. For instance, biological membranes represent the most important and most common naturally occurring surfaces that contain proteins.<sup>1,2</sup> Further, the role of protein adsorption in biocompatibility,<sup>3,4</sup> serological tests,<sup>5,6</sup> emulsification, and stability of dispersions<sup>7,8</sup> was indicated and immobilization of proteins by attachment to solid surfaces is often used for biochemical purification purposes, as well as in industrial and clinical procedures.<sup>9,10</sup> The ability of large molecules (or macromolecules) to be adsorbed onto various solid surfaces from solution led to a wide spectrum of useful applications in engineering, technology, and biology; wastewater treatment, flotation separations, and fine particle recovery by selective floccu-

lation are also important applications of the adsorption of proteins onto surfaces.<sup>11–14</sup>

Although the subject of the adsorption of proteins was frequently reviewed in the recent past<sup>15,16</sup> and many theoretical and experimental studies are cited in the literature, fewer investigations were designed to be concerned with the interactive behavior of proteins and clays. A protein molecule adsorbing on a clay surface presents a significant phenomenon that accounts for a number of important applications. Casein, a chief constituent of milk, is extensively employed as an adhesive or binder for coating with clay or other pigments. In view of the utility of casein in diversified fields where its adsorption is needed for several uses, we sought to study the adsorption of casein onto alkali treated bentonite from the alkaline aqueous solution of casein. The adsorption behavior of food proteins onto solid surfaces has not been the subject of much attention, which is also of potential importance in food processing, food preservation, leather processing, and so forth.

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**Table I Physical Characteristics of Alkali Treated Bentonite**

	Mesh Size 200–300
Average particle diameter	40 μm
Total surface area	400 m <sup>2</sup> g <sup>-1</sup>
Cation exchange capacity	90 meq/100 g
pH of 2% aqueous solution	11.53

**EXPERIMENTAL**

**Materials**

Casein (MW ca. 2 × 10<sup>5</sup> and isoelectric point 4.6) was supplied in powder form by Romali Chemicals (Bombay, India) and used without further purification. Alkali treated bentonite was used as an adsorbent and prepared by treating the bentonite powder (Loba Chemie, India) with 10% NaOH for 72 h and subsequent washing and drying at 80°C for 1 week. The physical characteristics of the treated bentonite are given in Table I.

**Method**

Adsorption experiments were carried out by the direct contact method. It involves a mild shaking of a known volume of casein solution of definite concentration (2 × 10<sup>-6</sup> mol L<sup>-1</sup>) containing 50 mg of alkali treated bentonite at fixed pH (11.8) in an electric shaker (Toshniwal, India) for 2 h. From several preliminary experiments, the time period of 2 h was found to be sufficient to attain the adsorption equilibrium. The amount of adsorbed casein was estimated by measuring the absorbance of a casein–biuret complex solution by a colorimetric method.<sup>17</sup> In brief, Cu<sup>2+</sup> in an alkaline copper reagent solution reacts with the peptide linkage of casein to form a violet colored complex, and the intensity of the color produced is proportional to the protein concentration. The amount of the adsorbed casein was calculated by the mass balance equation given as

$$\text{amount adsorbed (mg g}^{-1}\text{)} = \frac{(C_0 - C_e)V}{W} \quad (1)$$

where C<sub>0</sub> is the initial concentration of the casein solution (mg/mL), C<sub>e</sub> is the equilibrium concentration of the casein solution, V is the volume of the casein–clay suspensions, and W is the amount of adsorbent (g).

**RESULTS AND DISCUSSION**

**Effect of Casein Concentration**

The effect of the increasing concentrations of the casein solution on the adsorbed amount was investigated by increasing the concentration of the casein solution in a wide range of 2.5–25 × 10<sup>-6</sup> mol L<sup>-1</sup>. Increasing the concentration of the casein solution increases the amount of adsorbed casein, which is also quite obvious because increasing the concentration of the casein solution in the suspension causes more casein molecules to approach the surface and get adsorbed. A definite concentration of the casein solution a saturation in the adsorbed mass is noticed. Such a variation in the adsorbed amount with concentration was also reported by many workers.<sup>18</sup>

For a better understanding of the adsorption behavior of casein toward the alkali treated bentonite, the following types of adsorption isotherms can be considered.

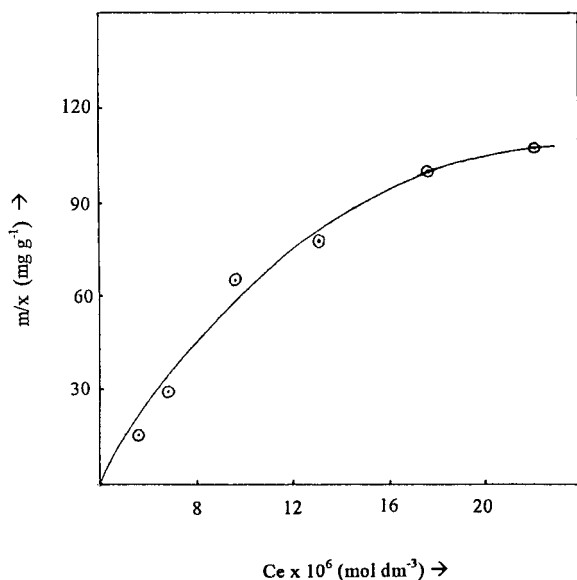
**Langmuir Isotherm**

The Langmuir equation was derived for the sorption of gases on a solid surface. Nevertheless, it has been extended to include the sorption of solutes on soils. A standard mathematical representation is

$$\frac{C_e}{a} = \frac{1}{a_s K} + \frac{C_e}{a_s} \quad (2)$$

where a is the adsorbed amount of casein (mg g<sup>-1</sup>) at any equilibrium concentration C<sub>e</sub>; a<sub>s</sub> is the adsorbed amount of casein (mg g<sup>-1</sup>) at saturation (adsorption capacity); and K = k<sub>1</sub>/k<sub>2</sub>, where k<sub>1</sub> and k<sub>2</sub> are the rate constants for the adsorption and desorption, respectively.

In the present study the Langmuir isotherm is shown in Figure 1, which clearly implies that the adsorption isotherm belongs to L2, which is the Langmuir type of isotherm that is a widely reported isotherm in most of the protein adsorption investigations.<sup>19</sup> The values of the adsorption coefficient (K) and adsorption capacity (a<sub>s</sub>) were calculated and are summarized in Table II. The large value of K (0.18 × 10<sup>6</sup> mol<sup>-1</sup> L) implies that casein has much greater affinity for adsorption. This is also true because this value is about 10<sup>4</sup> times greater than that obtained for the adsorption of gelatin onto acid treated bentonite.<sup>20</sup> The table also reveals that the graphical and experi-



**Figure 1** A plot showing the variation of the adsorbed amount of casein ( $\text{mg g}^{-1}$ ) with the concentration of casein with 0.05 g alkali treated bentonite at pH 11.8 and  $27 \pm 0.2^\circ\text{C}$ .

mental values of the adsorption capacities are almost identical.

#### Freundlich Isotherm

The Freundlich isotherm has the form

$$S = K' C_e^N \quad \text{or} \quad \ln S = \ln K' + N \ln C_e \quad (3)$$

where  $S$  is the adsorbed amount of the adsorbate,  $K'$  is the predicted quantity of sorption per gram of the treated bentonite at unit equilibrium con-

centration ( $\text{mg g}^{-1}$ ), and  $N$  is the measure of the nature and strength of the adsorption process and the distribution of active sites. If  $N < 1$  the bond energies increase with the surface density; if  $N > 1$  the bond energies decrease with the surface density; and when  $N = 1$  all surface sites are equivalent.<sup>21</sup> The values of  $K'$  and  $N$  are presented in Table II.

#### Modified Freundlich Isotherm

Solute exchange on soil adsorption sites is known to be heterogenous, not monoenergetic.<sup>22</sup> Sposito<sup>23</sup> used statistical mechanics to vigorously derive the following modified Freundlich isotherm equation:

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

where  $S_1$  is the amount of adsorbed solute,  $S_{\max}$  is the amount adsorbed at maximum, and  $C_e$  is the equilibrium concentration of the adsorbate solution. The empirical constants  $A$  and  $\beta$  can be determined through linear regression analysis. These empirical constants provide a means for visualizing the general sorption process relative to the distribution of sorptive energies by the exchange surfaces.

In accordance with eq. (4), a plot was drawn (not shown) and the values of  $A$  and  $\beta$  were calculated and are summarized in Table II. It is clear from the numerical values of  $A$  and  $\beta$  that the distribution of the adsorbate molecules is not even over the active sites of the clay surface; rather, it is widely spread, thus indicating the heterogeneity of the clay surface.

**Table II** Adsorption Parameters

Constant	Value
Adsorption coefficient ( $K$ )	$0.18 \times 10^6 \text{ mol}^{-1} \text{ L}^{-1}$
Rate constant for adsorption ( $k_1$ )	$6.66 \times 10^{-4} \text{ min}^{-1}$
Rate constant for desorption ( $k_2$ )	$0.37 \times 10^{-8} \text{ mol L}^{-1}$
Diffusion constant ( $D$ )	$19 \times 10^{-14} \text{ cm}^{-2} \text{ s}^{-1}$
Penetration rate constant ( $T$ )	$8.76 \times 10^{-2} \text{ mol L}^{-1} \text{ min}^{-1}$
Distribution coefficient ( $k_d$ )	6.02
Adsorption capacity ( $a_s$ )	
Graphical value	$1.2 \times 10^2$
Experimental value	$1.3 \times 10^2$
$A$	44.5
$N$	80.92
$K'$	6.68
$\beta$	1.14

### Distribution Coefficient

The adsorption process may also be expressed in terms of an empirical parameter  $k_d$  (distribution coefficient, mL/g), and it is defined as the equilibrium concentration of the adsorbed species in solution.

The empirical parameter  $A$  given by Sposito<sup>23</sup> can be represented as

$$A = S_{\max} k_d^\beta \quad (5)$$

The parameter  $A$  is a measure of the weighed average distribution coefficient, and the parameter  $\beta$  describes the spread of the distribution about the average distribution coefficient ( $k_d$ ).

The value of  $k_d$  in the present case was calculated and is presented in Table II. The data clearly reveals that the casein molecules have a greater tendency to remain on the clay surfaces rather than in the solution, which is evident from the average  $k_d$  value of 6.02. Also, the value of  $\beta$  is greater than unity and this implies a higher spread.

### Determination of Rate Constants

To evaluate the rate constant for adsorption ( $k_1$ ), the following linear equation may be used:

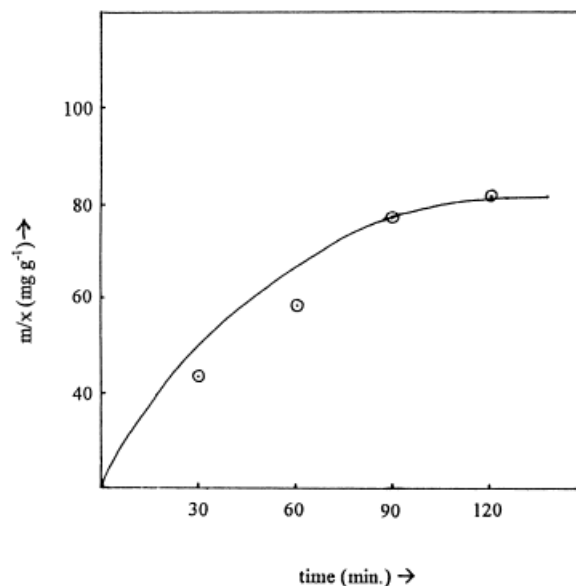
$$\frac{1}{C} = k_1 \frac{1}{C_0} t + \frac{1}{C_0} \quad (6)$$

where the terms involved have their usual significance.<sup>24</sup> It is clear that from the slope of the plot drawn between  $1/C$  and  $t$ , the value of the rate constant for adsorption ( $k_1$ ) can be calculated. Once  $k_1$  and  $K$  are known, the rate constant for desorption ( $k_2$ ) can be calculated. These values are summarized in Table II.

### Adsorption Kinetics

The progress of the adsorption process was monitored colorimetrically at different time intervals, and we found that the adsorption process arrives at equilibrium after 2 h and the rate of adsorption is nearly constant up to 30 min (Fig. 2).

In a similar type of investigation<sup>20</sup> where gelatin was adsorbed onto acid treated bentonite, the rate constant for adsorption was calculated to be  $10.7 \times 10^{-4} \text{ min}^{-1}$ , which is 1.6 times greater than that found in the present case (Table II). However, in another experiment on the adsorp-



**Figure 2** The variation in the amounts of adsorbed casein with time  $t$  for a fixed casein concentration of  $2 \times 10^{-6} \text{ mol L}^{-1}$  with 0.05 g alkali treated bentonite at pH 11.8 and  $27 \pm 0.2^\circ\text{C}$ .

tion of casein onto silica<sup>25</sup> we found that the rate constant for adsorption was about 74 times smaller than the value obtained in the present case.

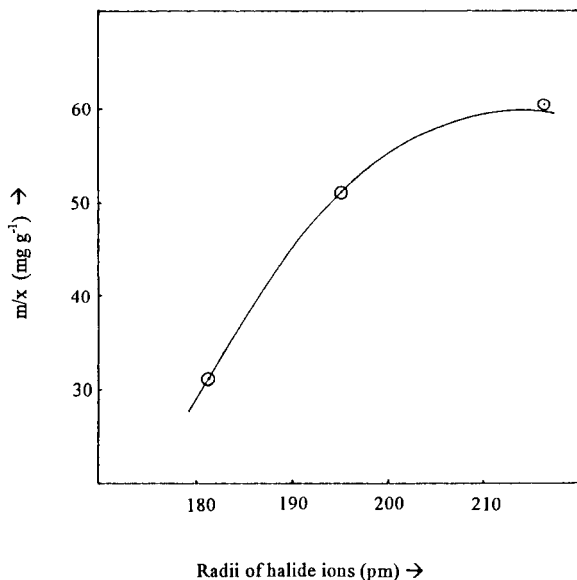
Figure 2 shows two different domains as is usually the case with polymer<sup>26</sup> and protein<sup>27</sup> adsorption. These two domains were characterized by the following two equations, respectively:

$$q = 2/\sqrt{\pi} \cdot C_0 \sqrt{Dt} \quad (7)$$

$$q = C_e [1 - \exp(-t/T)] \quad (8)$$

where  $q$  is the adsorbed mass and  $D$  is the diffusion constant of the polymer molecule. Obviously, from the linear plots drawn between the adsorbed amounts ( $q$ ) and  $\sqrt{t}$  and  $t$ , the diffusion constants ( $D$ ) and penetration rate constants ( $1/T$ ) can be calculated as presented in Table II.

Sarkar and Chattoraj<sup>28</sup> studied the adsorption of proteins such as gelatin and bovine serum albumin (BSA) onto alumina and determined their diffusion constants as in the range of  $4.0\text{--}20.0 \times 10^{-14} \text{ m}^2 \text{ s}^{-1}$ , which is in agreement with the value obtained in the present case. Also, the diffusion constant of gelatin for its adsorption onto a synthetic hydroxyapatite<sup>29</sup> was also calculated to be  $8.6 \times 10^{-14} \text{ m}^2 \text{ s}^{-1}$ , which is of the same order of magnitude as observed in the present case.



**Figure 3** The variation of the adsorbed amount of casein ( $\text{mg g}^{-1}$ ) with varying ionic radii of the added halide ions at a fixed concentration of casein of  $2 \times 10^{-6} \text{ mol L}^{-1}$  with 0.5 g of alkali treated bentonite at pH 11.8 and  $27 \pm 0.2^\circ\text{C}$ .

#### Specific Ion Effect

In the present article the effects of the addition of halide ions (i.e.,  $\text{Cl}^-$ ,  $\text{Br}^-$ , and  $\text{I}^-$ ) on the adsorption of casein was observed. We found (Fig. 3) that the adsorption gradually increases with an increase in the atomic weight and size of the added halide ions and is at maximum in the presence of  $\text{I}^-$  ions. The different halide ions obey the following increasing order of effectiveness:



The results may be explained by the fact that with the 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 of the clay. This results in greater release of the cations of the treated bentonite surface and thus facilitates adsorption.

#### Solvent Effect

The effect of the addition of water miscible aliphatic alcohols on the adsorption of casein was investigated by adding various alcohols (10% v/v) to the adsorption system. The results are shown in Figure 4, which clearly implies that the

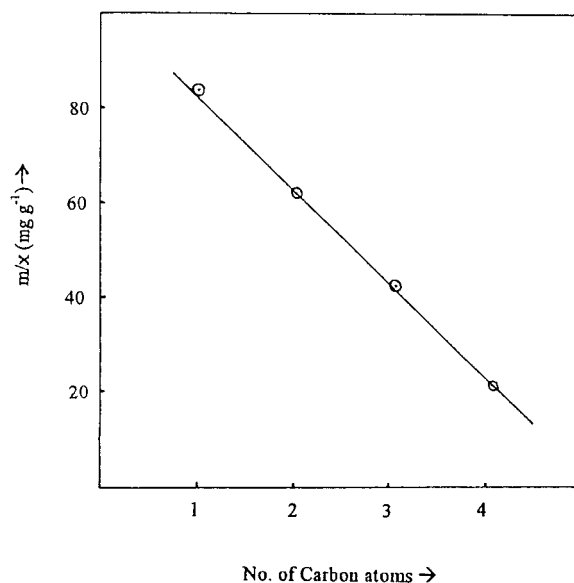
amounts of adsorbed casein constantly decreases with an increasing number of carbon atoms in the aliphatic chain of the alcohols. Thus, the order of increasing depression in the adsorbed amount of casein is as follows:



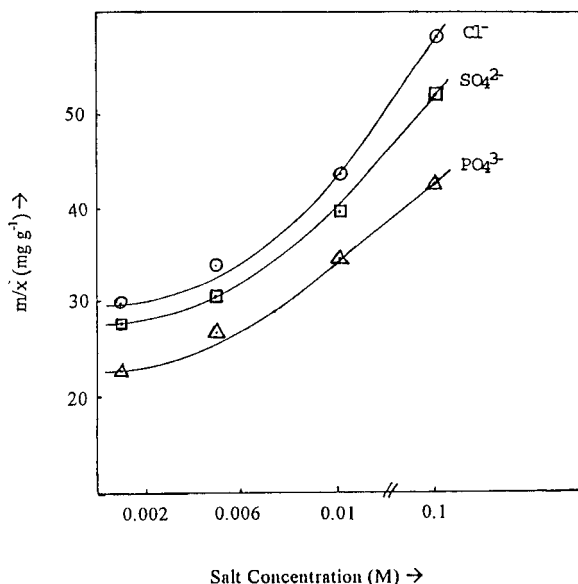
The reason for the observed decrease is that the hydrophobic interactions are mainly responsible for the adsorption of casein molecules. By the addition of aliphatic alcohols to the suspensions the hydrophobic portion of aliphatic chain interacts with the hydrophobic siloxane layer of the clay surface and may be adsorbed. Thus, because of a decrease in the number of active sites on the hydrophobic region of the surface the adsorption of casein molecules decreases. Moreover, because the hydrophobic character of added alcohols increases with increasing number of carbon atoms, the order of effectiveness of added alcohols is also justified. A similar type of preferential adsorption of alcohol molecules onto activated carbon fiber surfaces was also reported elsewhere.<sup>30</sup>

#### Salt Effect

In the present study, the effect of the addition of salts on the adsorption were studied by adding

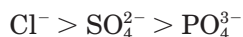


**Figure 4** The effect of the variation of aliphatic alcohols on the adsorbed amount of casein at a fixed casein concentration of  $2 \times 10^{-6} \text{ mol L}^{-1}$  with 0.5 g of alkali treated bentonite at pH 11.8 and  $27 \pm 0.2^\circ\text{C}$ .



**Figure 5** The effect of the concentration of added anions of potassium salt in the range of 0.001–0.1M at a fixed casein concentration of  $2 \times 10^{-6}$  mol L<sup>-1</sup> with 0.5 g of alkali treated bentonite at pH 11.8 and  $27 \pm 0.2^\circ\text{C}$ .

different salts of K<sup>+</sup> ions in a concentration range of 0.001–0.1M. The results are shown in Figure 5, which clearly indicates that the amount of adsorbed casein increases with increasing salt concentration and obeys the following order:



The results can be explained by the fact that the added anions screen the existing repulsive forces between the positively charged surface and similarly charged amino acid chains of the protein molecules and thus results in increased adsorption. The extent of screening is greatest for the trivalent phosphate anions and least for the univalent chloride ions. Similar results were also published elsewhere.<sup>31</sup>

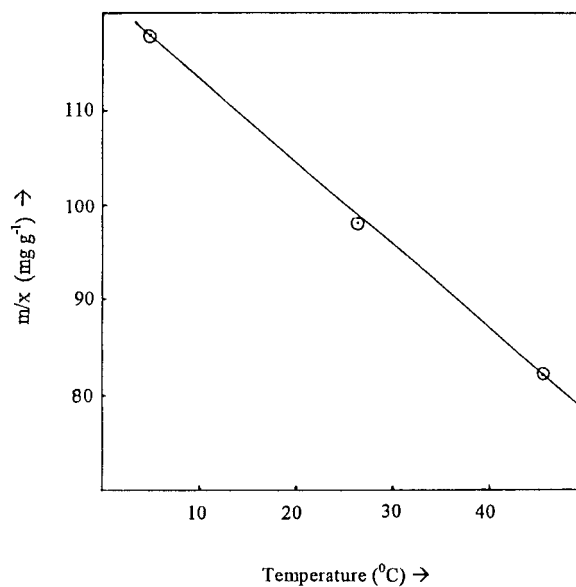
**Temperature Effect**

The effect of the increasing temperature on the adsorption of casein was studied by performing adsorption experiments in a temperature range of 5–45°C. The results are shown in Figure 6, which clearly implies that a rise in temperature causes the adsorption to gradually decrease. The greater amount of adsorption at lower temperatures may be attributed to the following facts:

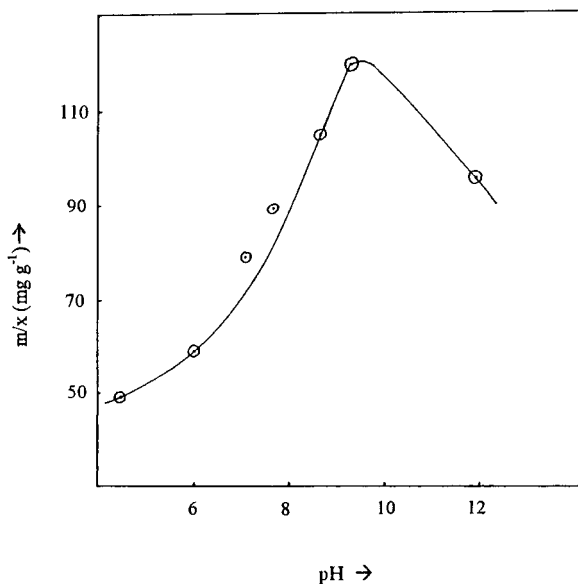
1. Because the adsorption appears to be of physical nature, the responsible intermolecular forces become prominent at lower temperatures and therefore the adsorption is greater.
2. At a lower temperature the possibility of agglomeration of charged casein molecules may not be ruled out as was reported by some authors for cationic and anionic dyes.<sup>32</sup> Obviously, greater adsorption is observed at lower temperature.
3. A greater escaping tendency of casein molecules from the solid to the bulk phase may be considered at higher temperatures, which also explains the greater adsorption at lower temperature.
4. The increased solubility of casein molecules at higher temperatures may also significantly curtail the extent of adsorption.

**pH Effect**

In the present studies the effect of pH on the adsorption of casein was investigated by varying the pH in a range of 3.5–11.8. The results are presented in Figure 7, which clearly implies that the adsorption increases initially with rising pH and attains a maximum value at pH 9.3, followed by a further decrease in the adsorbed amount. This type of adsorption isotherm at varying pH of



**Figure 6** The effect of temperature on the adsorbed amount of casein at a fixed casein concentration of  $2 \times 10^{-6}$  mol L<sup>-1</sup> with 0.5 g of alkali treated bentonite at pH 11.8.



**Figure 7** The variation of the adsorbed amount of casein ( $\text{mg g}^{-1}$ ) with the pH of the suspension at a fixed casein concentration of  $2 \times 10^{-6} \text{ mol L}^{-1}$  with 0.5 g of alkali treated bentonite at  $27 \pm 0.2^\circ\text{C}$ .

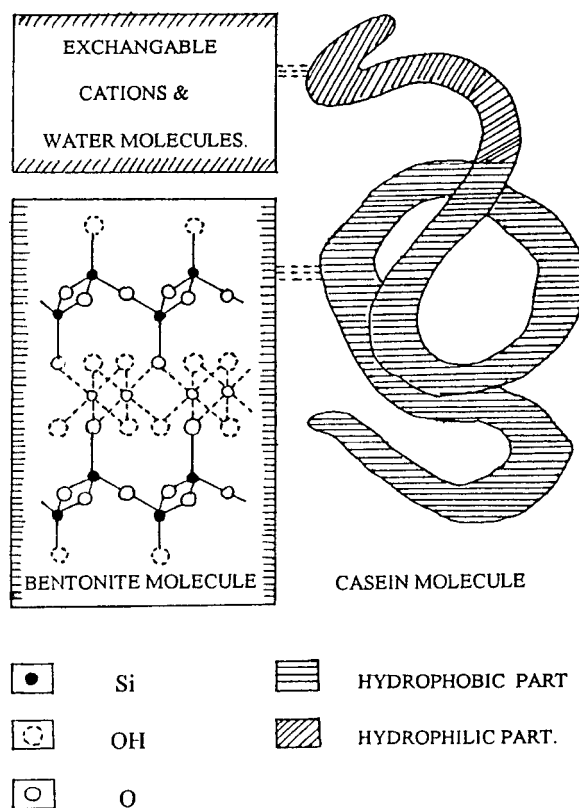
the protein–solid suspension is a common finding and is widely reported in the literature. However, a marked difference that is noticed in the present case is that whereas in other protein adsorption studies a maximum adsorption is seen at or near the isoelectric point of the protein, in the present case the maximum adsorption was found at pH 9.3, which is quite far away from the isoelectric point of casein (i.e., 4.6). Although a shift in the pH of maximum adsorption was reported in many cases,<sup>33</sup> such a large shift in the isoelectric point is not justified.

The observed results can be explained on the basis of hydrophobic interactions, which result from protein–surface interaction. In fact, in the present work the analysis of the electrostatic interaction between the casein molecule and the surface predicts a maximum adsorption at the isoelectric point of the protein because at this pH a minimum repulsion force exists between the protein molecule and sorbent surface; however, the experimental results reveal that the maximum adsorbed amount is obtained at pH 9.3, at which point the casein molecules and bentonite have negative charges. This indicates that because of electrostatic repulsion between the casein molecule and clay surfaces, the adsorbed amount should not be maximum. Therefore, it implies that the hydrophobic interaction between

the casein molecule and clay surface should be responsible for greater adsorption.

In an alkaline solution of casein the protein molecules are present as shown in Figure 8. It is also clear from the figure that the hydrophobic part is located at the inner sheet of the coil, thus shielded from the water phase. Therefore, in the lower pH range, because of less ionization of the amino acid residues, the casein molecule will remain in a compact conformational state and therefore only electrostatic forces will contribute toward adsorption.

However, upon increasing the pH beyond the isoelectric point of casein (i.e., 4.6), the number of negatively charged groups (mainly  $\text{COO}^-$ ) increases in the casein molecule and produces increasing electrostatic repulsion, causing elongation in the casein molecular dimension. Because of this expansion the hydrophobic portion of the protein molecule contacts the clay surfaces and results in greater adsorption of casein.



**Figure 8** A model depicting the adsorption of casein molecules onto alkali treated bentonite surfaces.

## REFERENCES

1. Danielli, J. F.; Davson, H. *J Cell Compos Physiol* 1985, 5, 495.
2. Wolman, M. In *Recent Progress in Surface Science*; Danielli, J. F., Raddiford, A. C., Rosenberg, M. D., Eds.; Academic: New York, 1970; Vol. 3, p 1.
3. Baier, R. E. *Adv Chem Ser* 1975, 145, 1.
4. Ratner, B. D.; Hoffman, A. S. In *Hydrogels for Medical and Related Applications*, ACS Symposium Series 31; Andrade, J. D., Ed.; American Chemical Society: Washington, D.C., 1976; p 1.
5. Singer, J. M. *Bull Rheum Dis* 1974, 24, 762.
6. Klein, F.; Bronsveld, W.; Norde, W.; van Romunde, L. K. J.; Singer, J. M. *J Clin Pathol* 1979, 32, 90.
7. Graham, D. E.; Phillips, M. C. In *SCI International Symposium*, Brunel University; Smith, A. L., Ed.; Academic: New York, 1974; p 75.
8. Graham, D. E.; Phillips, M. C. In *SCI International Symposium*, Brunel University; Akers, R. J., Ed.; Academic: New York, 1975; p 237.
9. Nishikawa, A. *Chem Technol* 1975, 5, 564.
10. Mosback, K. *Sci Am* 1971, 224, 26.
11. Sato, T.; Ruchs, R., Eds. *Stabilization of Colloidal Dispersions by Polymer Adsorption*; Marcel Dekker: New York, 1980.
12. Schwoyer, W. L. K., Ed. *Polyelectrolytes for Water and Wastewater Treatment*; CRC Press: Boca Raton, FL, 1981.
13. Napper, D. H., Ed. *Polymeric Stabilization of Colloidal Dispersions*; Academic: London, 1981.
14. Chauveteau, G.; Lecourtier, J. In *The Role of Polymers in Enhanced Oil Recovery*; Schulz, D. N., Stahl, G. A., Eds.; Plenum: New York, 1987.
15. Horbett, T. A.; Brash, J. L., Eds. *Proteins at Interfaces II, Fundamentals and Applications*, ACS Symposium Series 602; American Chemical Society: Washington, D.C., 1995.
16. Haynes, C. A.; Norde, W. *Colloids Surfaces* 1994, 2, 517.
17. Kaplan, A.; Szabo, L. L. In *Clinical Chemistry Interpretation and Techniques*; Lea & Febiger, Eds.; Philadelphia, PA, 1983; p 157.
18. Bajpai, A. K.; Dengre, R. *J Appl Polym Sci* 1996, 60, 2219.
19. Giles, C. H.; Easton, I. A.; McKay, R. B. *J Chem Soc* 1964, 4, 4495.
20. Bajpai, A. K.; Sachdeva, R. *J Sci Ind Res* 1999, 58, 791.
21. Reed, E.; Maasumore, K. *Sep Sci Technol* 1993, 28, 2179.
22. Sips, R. *J Chem Phys* 1998, 16, 490.
23. Sposito, G. *Soil Sci Soc Am J* 1980, 44, 652.
24. Bajpai, U. D. N.; Bajpai, A. K. *Polym Int* 1993, 32, 43.
25. Bajpai, A. K.; Dengre, R. *Ind J Chem Technol* 1997, 4, 113.
26. Ligoure, C.; Leibler, L. *J Phys* 1990, 51, 1313.
27. Aptel, J. D.; Voegel, J. C.; Schmitt, A. *Colloids Surfaces* 1988, 29, 359.
28. Sarkar, D.; Chattoraj, D. K. *Ind J Biochem Biophys* 1996, 33, 39.
29. Bajpai, A. K.; Sachdeva, R.; Palan, H. B. *J Ind Chem Soc* 2000, 77, 14.
30. Bajpai, A. K. *J Sci Ind Res* 1997, 56, 749.
31. Cosawa, F. *Polyelectrolytes*; Marcel Dekker: New York, 1971.
32. Giles, C. H.; Easton, I. A.; McKay, R. B. *J Chem Soc* 1964, 4, 4495.
33. Kondo, A.; Higashitani, K. *J Colloid Interface Sci* 1992, 150, 344.