

# An Adsorption Study of Gelatin onto the Fuller's Earth Surfaces

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**ABSTRACT:** An adsorption study of gelatin was performed from its aqueous solution onto the Fuller's earth surfaces at room temperature. The dynamics of the adsorption process were followed spectrophotometrically; and various adsorption and kinetic parameters, such as adsorption coefficient, rate constants for adsorption and desorption, and penetration rate constant, were evaluated. The adsorption was found to vary sensitively with pH variation and

show minima in both the media, that is, in acidic and basic. It was also observed that the presence of anions reversed their impact on adsorbed amount at their high concentration. The effect of cations and temperature were also investigated. © 2005 Wiley Periodicals, Inc. *J Appl Polym Sci* 98: 42–52, 2005

**Key words:** adsorption; proteins; clay

## INTRODUCTION

The adsorption of biomacromolecules, especially of proteins, at solid/liquid interface is of considerable scientific interest as it plays an important role in biocompatibility of materials,<sup>1</sup> serological test,<sup>2</sup> and emulsification and stability of colloidal dispersions.<sup>3</sup> In addition to these applications, the immobilization of proteins on solids by the adsorption process is also an important step used in biochemical purification and some industrial and clinical procedures.<sup>4–6</sup> Although considerable studies have been done on the adsorption of proteins,<sup>7–10</sup> however, there are few investigations reported dealing with the clay–protein interaction. Therefore, the main object of the present investigation was to study the adsorption of a protein on to the Fuller's earth surfaces from its aqueous solutions.

## EXPERIMENTAL

Acid processed gelatin (MW - 65,000 and isoelectric point 7.06) in a flaky form was supplied by the Wilson Laboratories (Mumbai, India) and used without further purification. Fuller's earth (physical properties summarized in Table I) used as an adsorbent was obtained from Loba Chemicals, India, and used as received. Other chemicals employed were also of AR

grade, and doubly distilled water was used throughout the experiments.

## Method

The adsorption experiment was carried out by taking a known volume (20 mL) of aqueous solution of gelatin of definite concentration along with 0.05 g of adsorbent in a Pyrex conical flask. The solution was subjected to shaking at fixed pH 4.2 in a mechanical shaker (Toshniwal, India) for 30 min, which was found to be the equilibrium time period for the adsorption. After the shaking was over, the solutions were centrifuged and followed by estimation of residual gelatin concentration in the supernatant by measuring the absorbance of the solution at 570 nm by following the Biurette method.<sup>11</sup>

The amount of the adsorbed gelatin was calculated by using the mass balance equation reported elsewhere.<sup>12</sup>

## RESULTS AND DISCUSSION

IR spectra of native and gelatin-adsorbed clay are depicted in Figures 1a and b, respectively. The spectra of native clay marks the presence of the silanol (SiOH) group at  $3429\text{ cm}^{-1}$  (O–H stretching) and  $1062\text{ cm}^{-1}$  (Si–O stretching), respectively, and aluminol (AlOH) at  $3628\text{ cm}^{-1}$  (Al–OH). The sharp band at  $1642\text{ cm}^{-1}$  also confirms the presence ( $>\text{C}=\text{O}$ ) of organic acid moieties in the clay as reported elsewhere.<sup>13</sup>

On examination of the IR spectra of gelatin-adsorbed clay, it is found that the bands indicating the

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TABLE I  
Physical Characteristics of the Fuller's Earth

S. no.	Physical property	Numerical value
1.	Surface area	120–140 m <sup>2</sup> g <sup>-1</sup>
2.	Porosity	60–70%
3.	Cation exchange capacity	50–200 meq/100 g
4.	Mean equivalent diameters of pores	190–200 Å
5.	Point of zero charge of edge face	7.0
6.	Particle size	10 μm

silanol and aluminol groups in the clay are shifted towards lower frequency, thus suggesting the involvement of these groups in hydrogen bonding with the gelatin molecules. This fact is further confirmed by the appearance of some amide bands in the gelatin-adsorbed clay spectra at 1550 cm<sup>-1</sup> and 1459 cm<sup>-1</sup>. These bands appear at lower frequencies than their normal values, which suggests that the NH<sub>2</sub> group of gelatin plays an important role in forming H bonds with the hydroxyl groups of the clay. It is noteworthy that after adsorption of gelatin, the >C=O group is raised from 1642 cm<sup>-1</sup> to 1675 cm<sup>-1</sup>, which is due to the high concentration of >C=O onto the surfaces of the Fuller's earth.

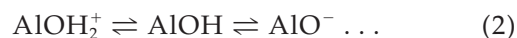
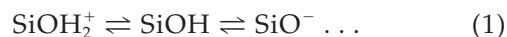
### SEM analysis

The morphological features of the native clay and gelatin-adsorbed clay are presented in Figures 2A and B, respectively. It is clear from photograph A that the average size of the clay particles varies in the range 4.3 to 71.4 μm, whereas for the gelatin-adsorbed clay, the size varies in the range 1.4 μm to 43 μm. Thus, small sized particles of protein adsorbed clay may be attributed to the fact that the molecules of gelatin flocculate the Fuller earth suspension due to a bridging mechanism, thus resulting in a formation of loose structure with well separated particle arrangement. While in the native clay, that is, without adsorption, the particles form a compact and aggregated type of structure with much narrower separation among the particles.

### Mechanism of adsorption

Before going through the explanation of the results and the mechanism of adherence of gelatin onto the Fuller's earth, it is essential to understand the basic structure of particles of Fuller's earth. The complete details of its chemical composition are given in Table II. The Fuller's earth clay, regardless of its individual origin, is basically made up of montmorillonite crystals, which have a three layered structure formed by an alumina octahedral sheet sandwiched between two inward pointing sheets of silica tetrahedra. The flat

cleavage surface is negatively charged from isomorphous replacement between SiO<sub>2</sub> and AlO<sub>2</sub><sup>-</sup>; the charge on this face is considered to be independent of pH, as stated elsewhere.<sup>14,15</sup> The middle sandwiched edge face is made up of two different functional groups, Al<sub>2</sub>O<sub>3</sub> and aluminosilicate. The edge face has a point of zero charge around pH 7; below this pH the charge is positive, and above pH 7, the charge is negative.<sup>16</sup> This charge dependence of edge face on pH of the medium may be represented as:



The adsorption of gelatin occurs mainly via H bonding between the amide group of gelatin and edge silanols and aluminols of the clay. Here, the overall mechanism is shown in Figure 3 and is well supported by the IR spectra and SEM analysis of native and gelatin-adsorbed clay as described earlier.

### Concentration effect and adsorption isotherm

The effect of increasing concentration of the gelatin solution on the adsorption has been studied by varying it in the range  $3.076 \times 10^{-5}$ – $10.76 \times 10^{-5}$  mol dm<sup>-3</sup>. The results are shown in Figure 4, which clearly implies that the adsorption of gelatin increases with increasing concentration of gelatin and no plateau region appears in the studied concentration range.

The shape of the adsorption isotherm (Fig. 5) indicates that the adsorption of gelatin follows the Langmuir model, and thus belongs to the LII category as classified elsewhere.<sup>17</sup> This type of isotherm is often reported in protein adsorption investigations.<sup>18</sup> To collect some more information on protein–soil interaction, three types of adsorption isotherms, viz. the Langmuir, Freundlich, and modified Freundlich isotherm, have been studied.

### Langmuir isotherm

A standard mathematical representation for the Langmuir isotherm is:

$$\frac{C_e}{a} = \frac{1}{a_s K} + \frac{C_e}{a_s} \quad (4)$$

where  $a$  is the adsorbed amount of gelatin (mg g<sup>-1</sup>) at any equilibrium concentration  $C_e$ ,  $a_s$  is the adsorbed amount of gelatin (mg g<sup>-1</sup>) at saturation (adsorption capacity); and  $K = k_1/k_2$ , where  $k_1$  and  $k_2$  are the rate constants for adsorption and desorption, respectively.

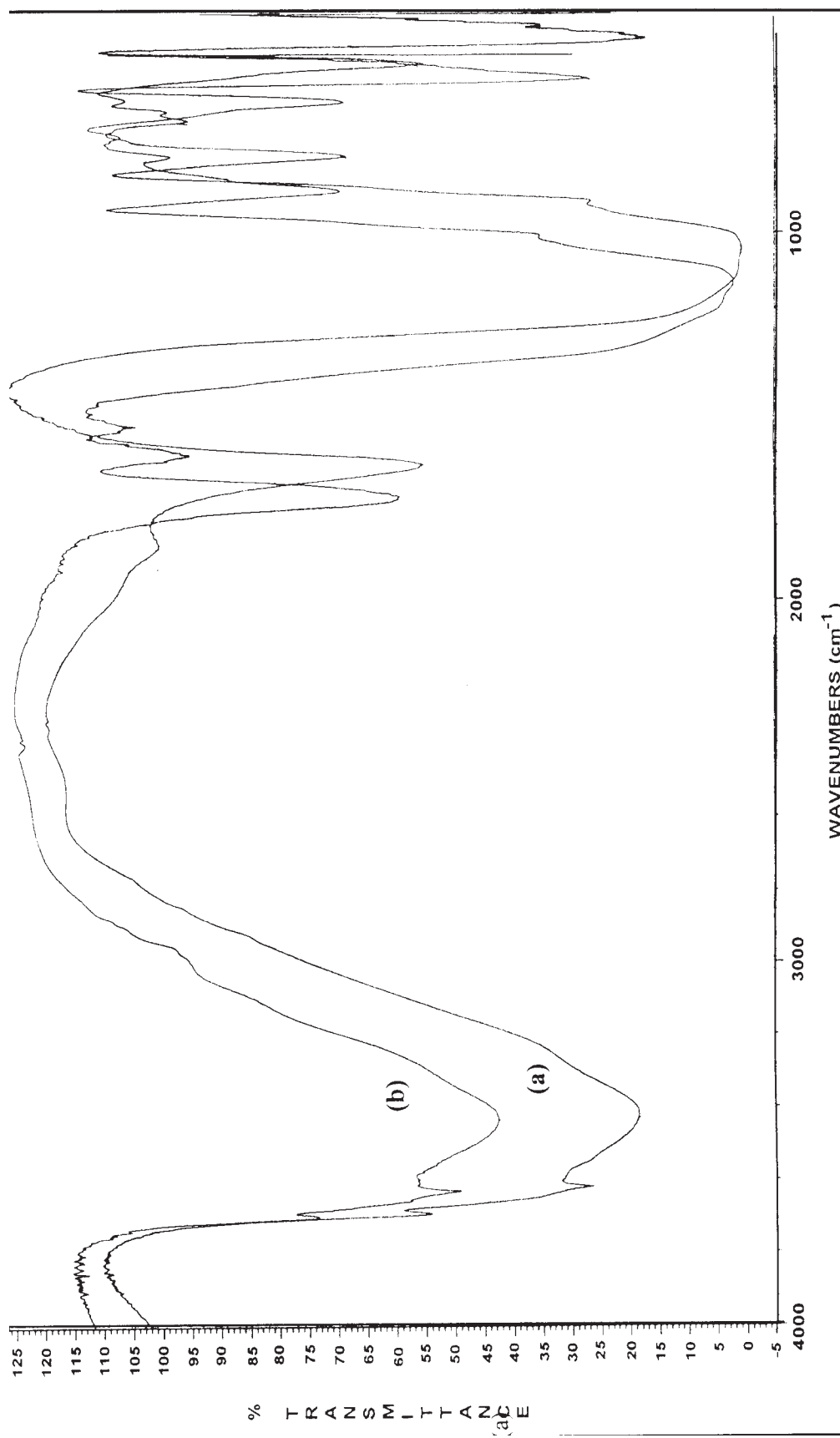


Figure 1 IR spectra of (a) native and (b) gelatin-adsorbed clay.

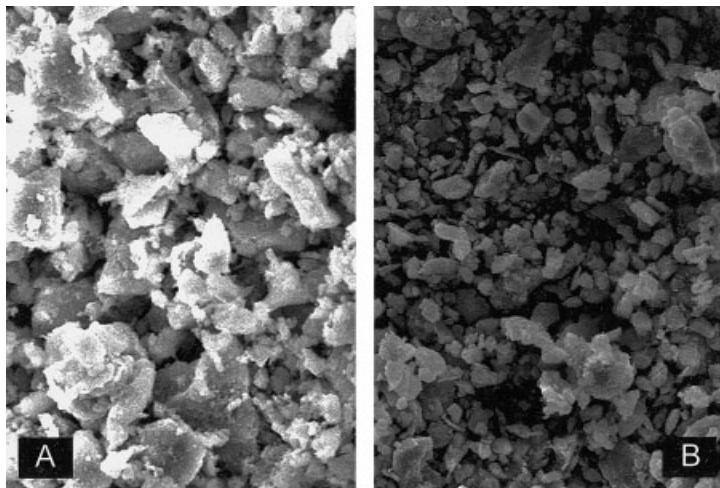


Figure 2 Scanning electron micrographs of (A) native and (B) gelatin-adsorbed clay.

According to the above equation, a plot drawn between  $C_e/a$  and  $C_e$  yields a straight line whose slope gives the value of the adsorption coefficient ( $K$ ), as summarized in Table III. The large value of  $K$  ( $60.18 \text{ mol}^{-1} \text{ dm}^3$ ) and high affinity type adsorption isotherm indicate that gelatin has great affinity for adsorption.

### Freundlich isotherm

The adsorption behavior of gelatin can also be explained by the empirical Freundlich adsorption isotherm

$$m/x = K' C_e^N \quad (5)$$

$$\ln m/x = \ln K' + N \ln C_e \quad (6)$$

where  $m/x$  is the adsorbed amount of the adsorbate,  $K'$  is the predicted quantity of sorption per gram of the adsorbent at unit equilibrium concentration ( $\text{mg g}^{-1}$ ),

TABLE II  
Chemical Composition of the Fuller's Earth

S. no.	Oxide	Percentage
1.	SiO <sub>2</sub>	71.27
2.	Al <sub>2</sub> O <sub>3</sub>	12.56
3.	Fe <sub>2</sub> O <sub>3</sub>	5.40
4.	MgO	2.13
5.	TiO <sub>2</sub>	1.94
6.	K <sub>2</sub> O	1.22
7.	CaO	0.69
8.	Na <sub>2</sub> O	0.25
9.	MnO	0.06
10.	P <sub>2</sub> O <sub>5</sub>	0.03
11.	Ba	0.04
12.	Cr	0.019
13.	Loss of ignition	18.58

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>19</sup> The values of  $K'$  and  $N$  are presented in Table III.

### Modified Freundlich isotherm

The modified Freundlich isotherm has the form:

$$\log \frac{S_1}{(S_{\max} - S_1)} = \beta \log C_e + \log \frac{A}{S_{\max}} \quad (7)$$

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. In accordance with the above equation, a plot was drawn and the values of  $A$  and  $\beta$  were calculated and are given in Table III. 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 heterogeneous nature of the clay.

### Kinetics of adsorption

The situation when a large molecule contacts a solid surface has been theoretically quantified in many ways to give numerous theories of macromolecular adsorption.<sup>20-22</sup> According to the proposed adsorption models, the adsorption of a large molecule on a solid surface occurs via a three-step mechanism:

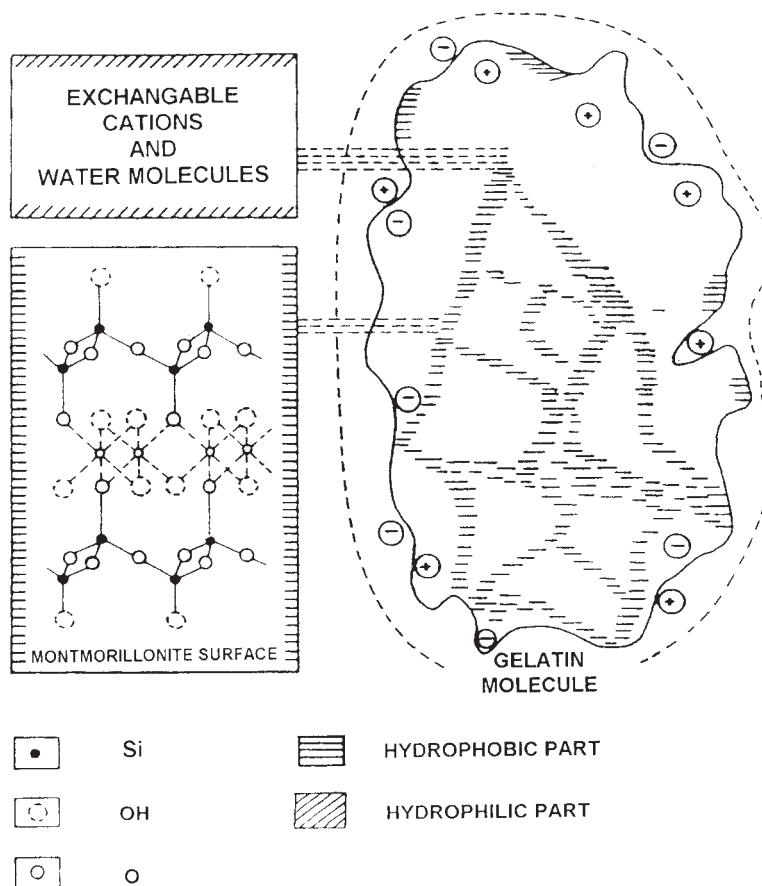


Figure 3 A model proposed for the adsorption of gelatin on to the Fuller's earth surfaces.

1. Transport of molecules towards the surface from the bulk of the protein solution.
2. Attachment of molecules at the active sites of the surface.
3. Change in the conformation of the adsorbing macromolecule.

In the case of protein adsorption, all three steps are important. To see the role of step 1, the adsorption kinetics was performed at varying speeds of shaking, and it was found that the adsorbed amount increased with increasing shaking speed.

The variation of adsorbed gelatin with time is shown in Figure 6. It is quite clear from the Figure that initially the rate of adsorption increases with time and finally attains an almost constant value. The Figure also reveals that a constant adsorption rate is noticed up to 30 min.

In the later stage of the adsorption process, the rate of adsorption decreases, which may be due to the barrier of the adsorbed gelatin chains through which the arriving molecules have to penetrate for adsorption. This penetration is slow and, therefore, the following equation suggested by Ligoure et al.<sup>23</sup> can be applied.

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

where  $q_e$  is the equilibrium adsorbed amount and  $T$  the characteristic penetration time. According to the above equation, when a plot is drawn between  $\ln(q_e - q)$  and  $t$ , the penetration rate constant ( $= 1/T$ ) may be calculated as given in Table III.

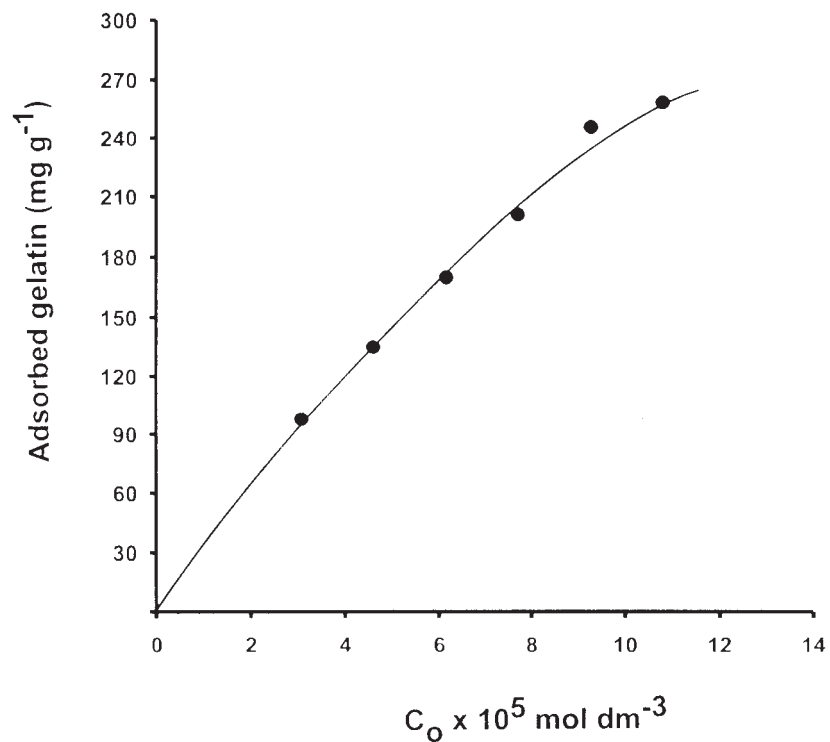
#### Rate constants of adsorption ( $k_1$ ) and desorption ( $k_2$ )

To calculate the rate constant for adsorption ( $k_1$ ), the kinetic scheme proposed by Bajpai et al.<sup>12</sup> may be applied, which utilizes the following equation:

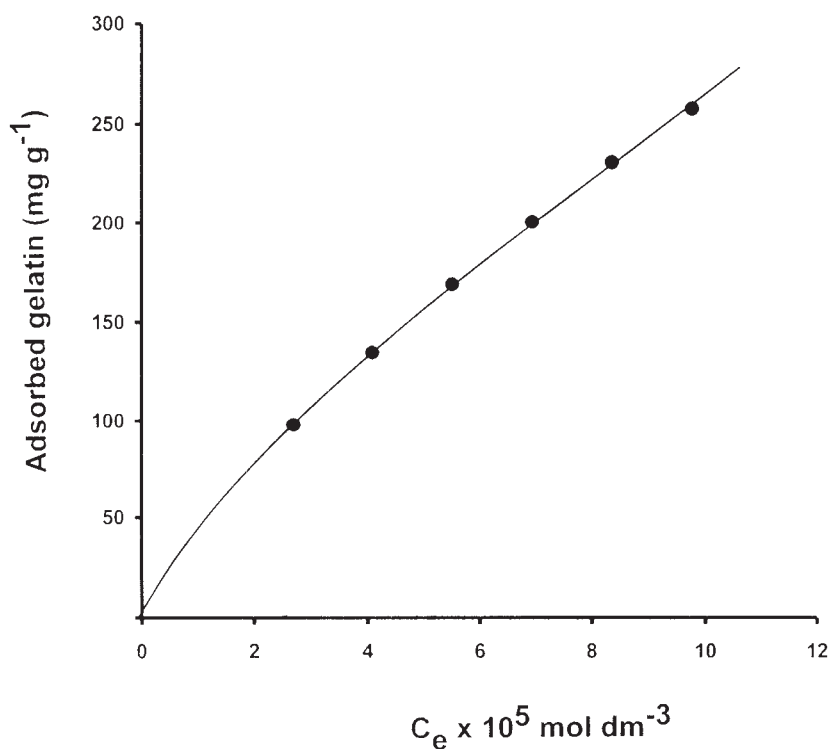
$$\frac{1}{C} = k_1 t + \frac{1}{C_0} \quad (9)$$

where  $C_0$  is the initial concentration of the gelatin solution and  $C$  is the concentration at time  $t$ , respectively.

A graph drawn between  $1/C$  and  $t$  obviously yields a straight line, from the slope of which the value of  $k_1$  may be calculated. By putting the value of  $k_1$  in the



**Figure 4** A plot showing the variation of the adsorbed amount ( $\text{mg g}^{-1}$ ) with the initial concentration of the gelatin solution at a fixed  $\text{pH} = 4.2$ ,  $[\text{KNO}_3] = 0.001\text{M}$ ,  $\text{Temp.} = 25 \pm 0.2^\circ\text{C}$ .



**Figure 5** A plot showing the variation of the adsorbed amount ( $\text{mg g}^{-1}$ ) with the equilibrium concentration of the gelatin solution at a fixed  $\text{pH} = 4.2$ ,  $[\text{KNO}_3] = 0.001\text{M}$ ,  $\text{Temp.} = 25 \pm 0.2^\circ\text{C}$ .

TABLE III  
Adsorption and Kinetic Parameters

Constant	Value
Adsorption coefficient ( $K$ )	$60.18 \text{ mol}^{-1} \text{ dm}^3$
Rate constant for adsorption ( $k_1$ )	$2.03 \times 10^{-9} \text{ min}^{-1}$
Rate constant for desorption ( $k_2$ )	$3.37 \times 10^{-11} \text{ mol dm}^{-3}$
Penetration rate constant ( $T$ )	$6 \times 10^{-2} \text{ min}^{-1}$
$A$	$6.468 \times 106$
$N$	0.774
$K'$	66.68
$\beta$	1.739

equation  $K = k_1/k_2$ , the value of  $k_2$  can also be calculated. These values have been summarized in Table III.

### Effect of pH

The effect of pH on the adsorption of gelatin has been studied in the pH range 1.8–12. The results are shown in Figure 7, which indicates that:

1. The amount of the adsorbed gelatin decreases constantly with increasing pH in the range 1.8 to 4.2.
2. In the next region, that is, from 4.2 to 7.0, it starts increasing with pH and attains a maximum value around the isoelectric point of gelatin

(7.06), and then decreases gradually with further increase in pH.

The results may be explained as below:

1. In the pH range 1.8 to 4.2, at pH 1.8, that is, at the greatest number of  $\text{H}^+$  ions in the medium, a high degree of protonation of protein molecules takes place, which leads to unfolding of the protein molecule<sup>24</sup> due to the repulsion between the charged species present in side chains, thus exposing the hydrophobic side chains of the molecule to the Fuller's earth surfaces. Therefore, a high degree of interaction occurs between the Fuller's earth and the protein molecule, and hence the adsorption increases. Now, on further increasing the pH of the protein solution beyond 1.8, the degree of protonation decreases and the protein molecule starts folding itself in tightly coiled conformation due to hydrophobic interaction between the polar side chains and, consequently, adsorption decreases.
2. In the next region, that is, from 4.2 to 7.0, the increase in adsorption observed with increasing pH may be attributed to the fact that when pH rises towards the isoelectric point of gelatin, its behavior approaches that of a neutral polymer, although due to charge neutralization at the

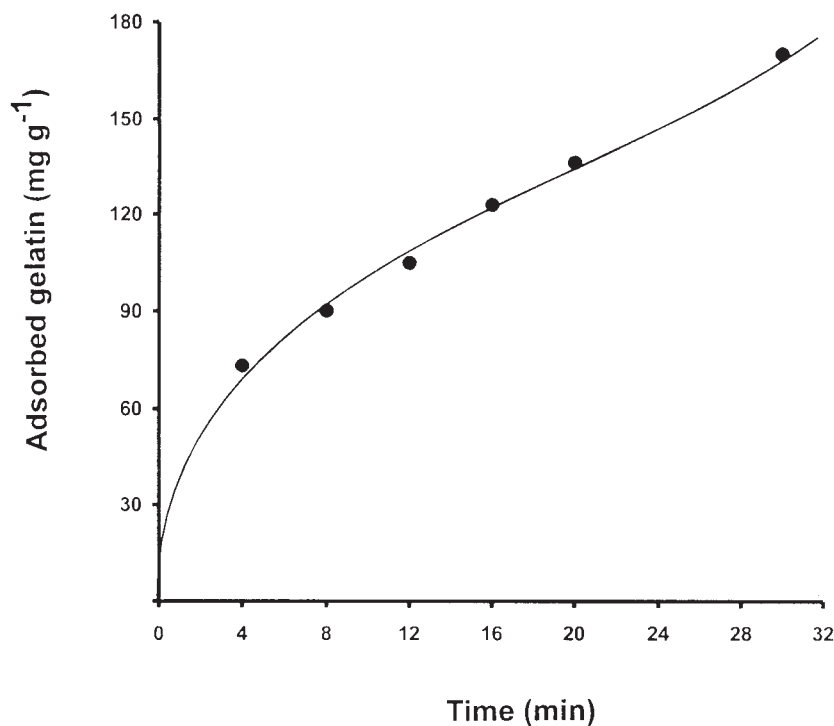
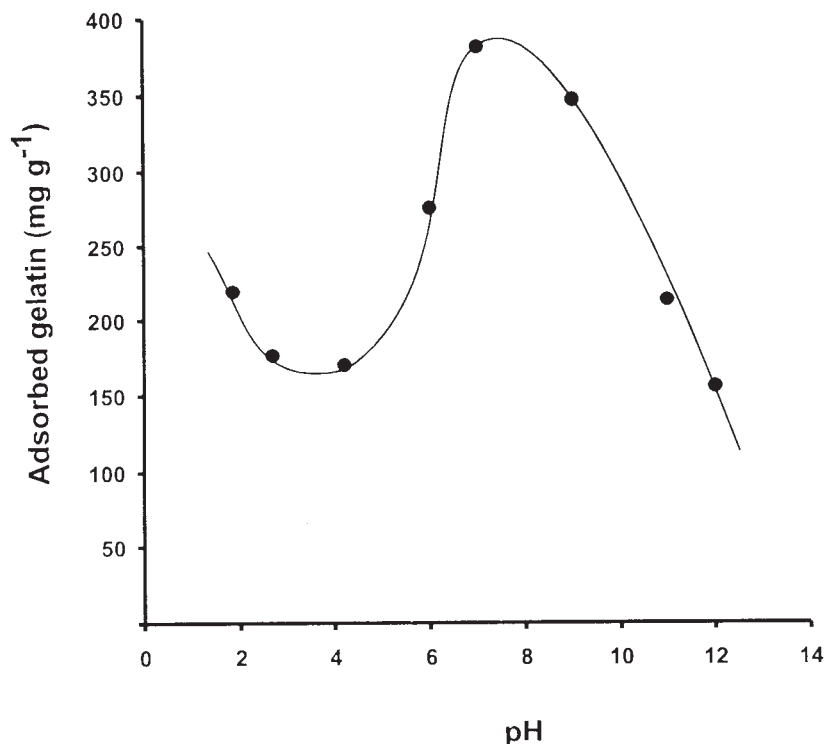


Figure 6 The variation in the amounts of the adsorbed gelatin with time  $t$  for fixed  $[\text{gelatin}] = 6.15 \times 10^{-5} \text{ mol dm}^{-3}$ ,  $\text{pH} = 4.2$ ,  $[\text{KNO}_3] = 0.001M$ ,  $\text{Temp.} = 25 \pm 0.2^\circ\text{C}$ .



**Figure 7** The effect of varying the pH of the gelatin–Fuller's earth suspensions on the adsorbed amounts of gelatin at fixed  $[\text{gelatin}] = 6.15 \times 10^{-5} \text{ mol dm}^{-3}$ ,  $[\text{KNO}_3] = 0.001\text{M}$ , Temp. =  $25 \pm 0.2^\circ\text{C}$ .

isoelectric point, its chain expansion does not take place but due to the minimum repulsion between the charged clay surface and the neutral gelatin, the adsorbed amount approaches a maximum value. These results clearly reveal that in the protein adsorption not only the conformation of the protein molecule but also the charges on the adsorbate and adsorbent play an important role. The occurrence of maxima around the isoelectric point of the protein is in agreement with the results reported in other protein adsorption investigations.<sup>25</sup>

- On further increasing pH beyond 7.0, deprotonation of gelatin starts increasing. Although this again results in an expansion of the biopolymer coil and opening of more active sites of the protein molecule for adsorption, the adsorption is greatly depressed by high repulsion working between the negatively charged edge, basal plane of the clay surfaces and negatively charged gelatin molecules. Since increase in pH of the system beyond 7.0 increases negative charges on the clay and protein molecules, electrostatic repulsion starts increasing in a similar manner, which ultimately starts depressing the adsorbed amount of gelatin.

### Effect of salts

#### Effect of cations

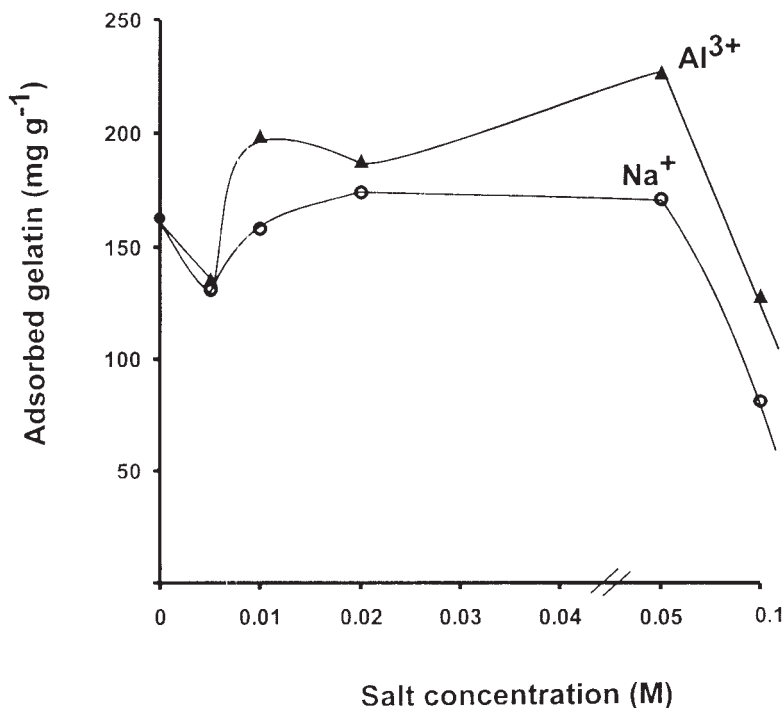
In the present study, the effect of the addition of cations on the adsorption has been studied by adding different salts of  $\text{Cl}^-$  ions in the concentration range of 0.005 to 0.1M. The results are shown in Figure 8, which clearly reveals that the amount of adsorbed gelatin increased with increasing salt concentration as well as the valency of the ion; however, beyond 0.05M concentration of salt, it is greatly depressed. The different cations obey the following increasing order of effectiveness in causing depression in the adsorbed amount of gelatin:

$$\text{Na}^+ > \text{Al}^{+3} \quad (10)$$

The addition of the calcium salt caused turbidity in the gelatin solution, so the effect of  $\text{Ca}^{2+}$  on the adsorption of gelatin could not be studied.

The observed increased adsorption in the initial range of added cations may be explained by the fact that the added cations may cause folding of the protein molecules, which results in a compact structure of gelatin. Thus, because of a reduced radii of gyration of the gelatin macromolecule, fewer active sites are occupied by the adsorbing protein molecules and, therefore, the adsorption increases. However, at higher



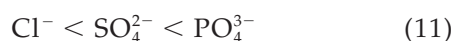


**Figure 8** The effect of the addition of cations on the adsorbed amount of gelatin at fixed  $[\text{gelatin}] = 6.15 \times 10^{-5} \text{ mol dm}^{-3}$ ,  $\text{pH} = 4.2$ ,  $\text{Temp.} = 25 \pm 0.2^\circ\text{C}$ : o - Sodium,  $\blacktriangle$  - Aluminum.

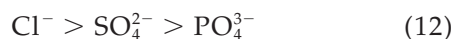
concentrations, that is, beyond 0.05M of added cations, due to enhanced repulsion between the clay particles and gelatin molecules, the adsorption decreases. It is worth mentioning that because of the larger size of the  $\text{Na}^+$  ion, a greater extent of interaction would be possible between the cation and the protein molecules. This also justifies the observed order of effectiveness.

#### Effect of anions

To study the effect of the addition of anions on the adsorbed amount of gelatin, different salts of  $\text{Na}^+$  were added to the adsorption medium in the concentration range 0.005 to 0.1M. The results shown in Figure 9 clearly imply that in the studied concentration range of 0.005 to 0.01M, the amount of adsorbed gelatin decreases with increasing salt concentration and obeys the following increasing order in bringing about depression:



However, on the other hand, in the concentration range from 0.01 to 0.05M, the added anions not only reversed their effect but also their order in causing depression. In this studied range, anions obey the following order of causing depression:



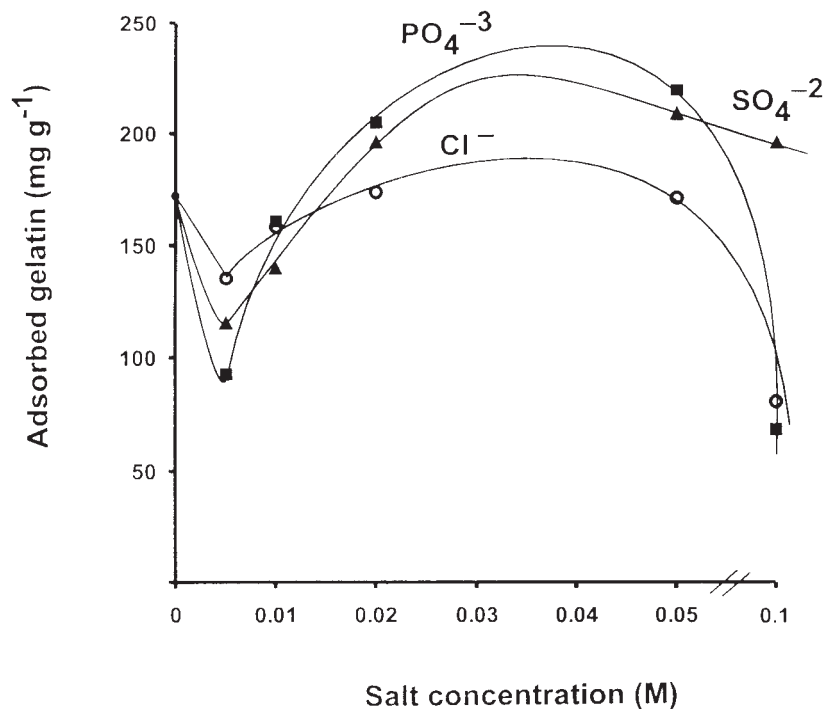
The observed influence on the adsorbed amount may be explained as follows:

Since the experiments were carried out at pH 4.2, which is well below the isoelectric point of gelatin as well as the zero point charge of the Fuller's earth, both gelatin and Fuller's earth carry positive charge at this experimental pH. Now, since the Fuller's earth surfaces are positively charged, an electrostatic repulsion will be present between the positively charged protein molecules and the clay surfaces. However, at this pH, the preferential adsorption of added anions may also take place due to an electrostatic attraction, so the greater the charge on the anion, the larger would be the depression in the adsorbed amount. Thus, the observed order of depression by the addition of salts is justified.

Now, as the concentration of anions increases beyond 0.01M, the added anions start binding themselves to the cationic groups present in the protein molecule (such as  $\text{NH}^+$ ,  $\text{NH}_3^+$ , and  $\text{=NH}_2^+$ ). This obviously increases the number of negatively charged active sites in the protein molecule and enhances the addition of protein molecules on to the positively charged clay surfaces. This type of binding properties of protein molecules for anion and cation through their  $\text{NH}_3^+$  and  $\text{COO}^-$  sites, respectively, are well documented.<sup>26</sup>

#### Temperature effect

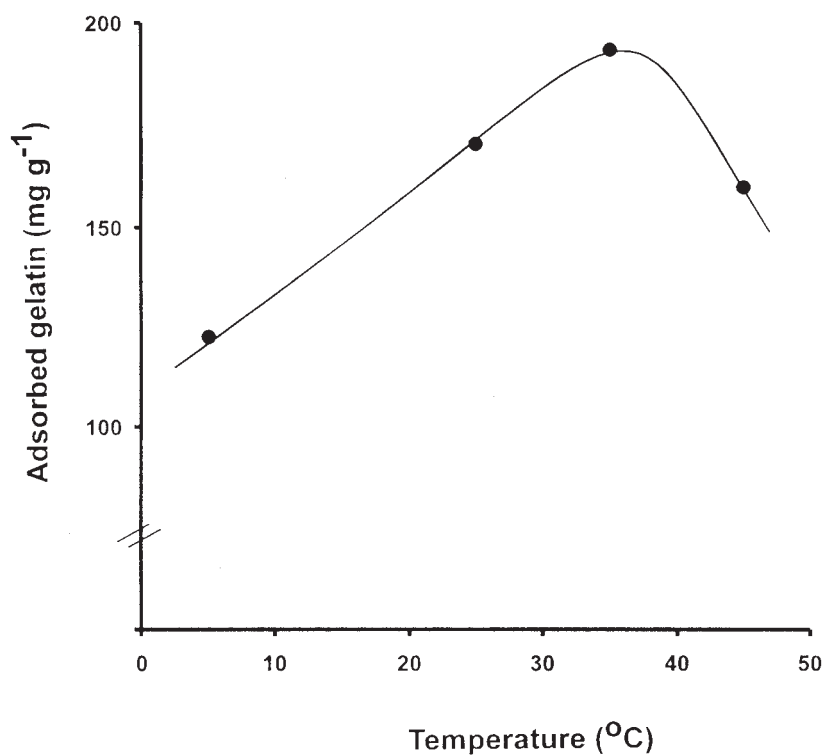
The influence of temperature on the adsorption of gelatin was studied by carrying out the ad-



**Figure 9** The effect of the addition of anions on the adsorbed amount of gelatin at fixed [gelatin] =  $6.15 \times 10^{-5} \text{ mol dm}^{-3}$ , pH = 4.2, Temp. =  $25 \pm 0.2^\circ\text{C}$ : o - Chloride, [trf] - Sulfate, ■ - Phosphate.

sorption experiments in the temperature range 5–45°C. The results are shown in Figure 10, which clearly reveals that the adsorption increases from 5

to 35°C and then decreases. The results obtained can be explained with the help of the following facts:



**Figure 10** The effect of the temperature of the adsorption medium on the adsorbed amount of gelatin at fixed [gelatin] =  $6.15 \times 10^{-5} \text{ mol dm}^{-3}$ ,  $[\text{KNO}_3] = 0.001\text{M}$ , pH = 4.2.

1. When the temperature increases, the protein molecules unfold and coil expansion takes place, which results in the opening of a greater number of active sites to the surface. Thus, the adsorption increases with increasing temperature.
2. The decrease in the adsorbed amount beyond 45°C may be attributed to the fact that as the temperature of the system increases, the intermolecular H-bonds, which are operative between the gelatin and hydroxyl groups of the clay, get weakened and, therefore, the gelatin molecules desorb into the bulk solution and the adsorption decreases.

### CONCLUSIONS

The process of the adsorption of gelatin onto the Fuller's earth surfaces is of Langmuirian type, and the protein molecules bind to the clay surfaces via electrostatic and H-bonding interactions. The adsorption is sensitive to the pH of the solution and exhibits a minimum in the acidic as well as in the basic medium. To the protein-clay suspension, the addition of very low and high concentrations of cations and anions decreased the adsorbed amount of gelatin, but their medium concentrations favors the adsorption. The increase in the temperature in the range 5–35°C results in an increase in the adsorption of gelatin, while beyond 35°C, the adsorbed amount decreases.

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