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Static Measurements on Adsorption of Gelatin onto Zinc-Powder Surfaces

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With an object to investigate the interactions between biopolymers and the metal surfaces, an adsorption experiment was carried out at room temperature and at constant $pH = 4.0$, which involved a study of the adsorption of weak polyelectrolyte biopolymer such as gelatin onto the surface of zinc powder. The adsorption isotherms were followed by estimating colorimetrically the residual concentration of gelatin in bulk of the solution after the adsorption experiment. The adsorption isotherms were found to be of medium affinity type and plateau adsorption was reached at relatively higher concentration of gelatin. The plateau adsorption was quite sensitive to the pH variation of the solution and was maximum near the isoelectric point of gelatin (4.8). The adsorption was also found to increase when the low molecular weight electrolyte like KCl was added in the concentration range of 0.01 to 0.08 mol/L. Furthermore, at higher concentration of the salt (> 0.08) the plateau adsorption decreased to a smaller degree.

INTRODUCTION

The adsorption of proteins onto surfaces from their aqueous solution is a fascinating and complex process that is known to have significant applications in various biological, medical and technological systems[1–4]. This greater tendency of protein molecules to show unusual affinity for adsorption lies in the fact that the amphipathic nature of proteins, resulting from their mixture of polar and non-polar side chains, causes them to be concentrated at interfaces which subsequently leads to their adsorption, Although protein adsorption has been widely studied for decades[5], the nature of molecular forces involved in adsorption process are still not well understood. Also, the dynamic and static mechanisms of the adsorption of protein molecules are very complicated. The protein-surface interaction appears to contain a large number of time dependent phenomena in addition to normal kinetic constraints caused by the diffusion of protein molecules to the solid surface. Furthermore, time dependent conformational changes of the protein molecules may occur due to the interaction between the surface and the molecules. Thus, looking to the diversity of problems in protein adsorption, a static adsorption investigation has been undertaken which involves a study of the adsorption of fibrous protein such as gelatin onto the surfaces of Zn-powder. It is worth mentioning here that in literature an ample number of adsorption studies are described in which a variety of proteins have been adsorbed from their aqueous solutions onto different common adsorbents such as silica, alumina, glass beads etc.[6–9] On the other hand, there have been rare attempts[10] to employ metals as adsorbents in protein adsorption experiments. The present investigation describes the results of the adsorptions of gelatin onto the surfaces of the zinc powder so as to have an understanding of the mechanism of protein-metal interactions at interfaces.

EXPERIMENTAL

Materials and methods

Gelatin in yellowish granular form was obtained from BDH (Pule, England) and used as received. Zinc powder used as adsorbent was supplied by Sisco Chemical Industries, India and was employed without any further treatment. Other chemicals used were of AR grade and all solutions required in the study were prepared in the conductivity water. For colorimetric estimation of gelatin in aqueous solution, biurate reagent was prepared by the method described in the literature[11].

For carrying out adsorption experiments a known amount of Zn powder (0.4 gm) and a definite volume of aqueous solution of gelatin (50 mL) of known concentration were taken at $pH = 4.0$ in a pyrex glass cell at room temperature. The contents were vigorously stirred by a magnetic stirrer for a period of 2 h which was found to be a sufficient time for the attainment of equilibrium. After equilibrium had been attained, the mixture was centrifuged in an electric centrifuge with a speed of 1500 rpm for 5 min. For determining the amount of gelatin adsorbed solution depletion method was used[12] which consists of knowing the concentration of gelatin in bulk solution before and after the adsorption experiment. For this pupose a colorimetric method was employed which involves to reading the absorbednce of biuret-gelatin amplex in a colorimeter (Systronics, India) by the method described in the literature[13].

The pH measurements were made using a digital pH meter (Systronics, India) and variation in the pH of the protein solution was done by the addition of 0.2 M NaOH solution to the adsorption medium.

For measuring viscosity of the protein solutions, an Ostwald type viscometer was used at room temperature. In all experiments reproducibility of the data was checked and the results were found within ±2% experimental error.

RESULTS AND DISCUSSION

(i) *Adsorption Isotherms*: The very first information is eventually offered by the shape of the adsorption isotherm for a given adsorbate-adsorbent system. If one analyzes a typical Langmuir's type adsorption isotherm then it is seen that the initial portion (slope) of the isotherm describes the rate of change of site availability with increase in solute concentration which qualitatively describes the affinity of the adsorbate for the adsorbent. Similarly, the plateau portion of the isotherm is indicative of a saturation in the adsorption process.

Fig. 1 describes the adsorption isotherm for the adsorption of gelatin onto the surface of Zn-powder at a given pH of 4.0. The isotherm was obtained by gradually increasing the concentration of gelatin solution and determining the residual concentration of gelatin in solution after the adsorption experiment was over. From the figure it is clear that the initial slope of the curve is not as steep as is commonly found in adsorption experiments[14, 15]. Thus, the observed less pronounced exponent of the isotherm clearly implies that gelatin has not much affinity for its adsorption onto zinc powder surfaces which may probably be due to the lack of specific interactions between the gelatin molecules and metal surface. It is also revealed by the poor affinity of the curve that purely physical forces must have been involved in the adsorption of gelatin molecules. It is also clear from Fig. 1 that the plateau adsorption is reached at relatively higher concentration of gelatin solution which is automatically justified on the basis of the poor affinity for adsorption.

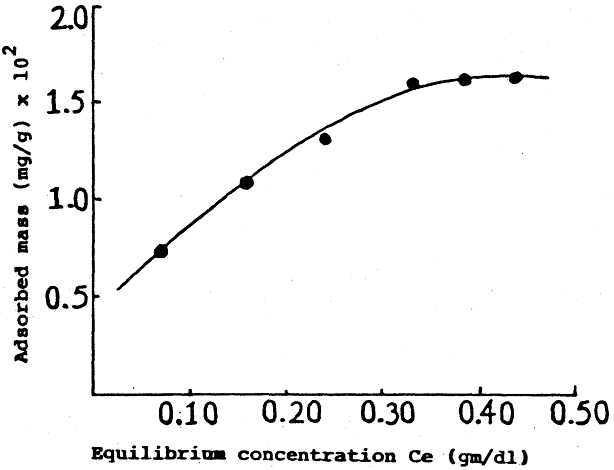

Fig. 1. Adsorption isotherm of gelatin on zinc powder surfaces at pH = 4.0.

(ii) *pH Effect*: In adsorption studies of polyectrolytes variation in the pH of the medium plays an important role in regulating the adsorption characteristics of the system. The effect of pH becomes much more significant when the polyelectrolyte is a protein because any change brought about in pH of a protein solution does bring a change in the electrostatic charge network along the macromolecular coil which subsequently leads to a number of unusual effects.

Fig. 2 summarizes results obtained for variation in plateau adsorption of gelatin at various pH of the adsorpthon medium. It is clear from the figure that on increasing the pH of the medium the plateau adsorption increases initially and attains a maximum value near the vicinity of the isoelectric point of gelatin (4.8). Further increase in pH results in a fall in the plateau adsorption. In attempting to explain the observed pH dependence of plateau adsorption one must consider the conformational change in gelatin molecules that are brought about by increasing the pH of the solution. The observed increase in plateau adsorption and subsequent fall may be explained on next page:

Since the adsorption was carried out at pH = 4.0 the gelatin molecules in the solution must bear a net positive charge as this pH lies to the acidic side of the isoelectric point (4.8). On addition of 0.20 M NaOH solution to protein solution for increasing the pH of the solution, the gelatin molecule decreases in two ways:

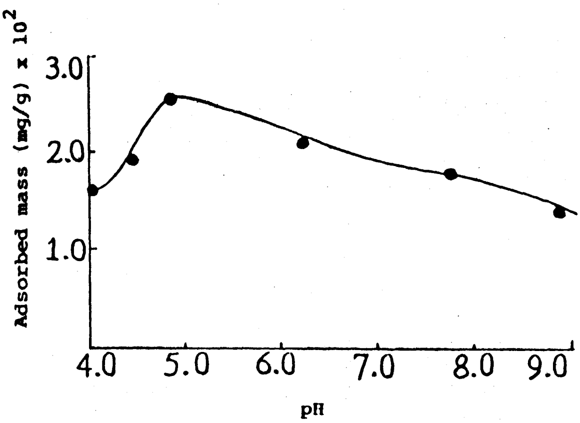

Fig. 2. pH dependence of plateau adsorption of gelatin on zinc powder surfaces at an equilibrium gelatin concentration of 0.33 gm/dl.

either the OH^- ions come in between the two positively charged centres on the gelatin molecules and thus reduce the repulsive forces by screening the positive electrostatic charges, or/and the added OH^- ions cause a partial ionization of the carboxylic groups of protein molecules and thus decrease the net positive charge on the gelatin molecule. Clearly the decrease in net positive charge results in a contraction of the macromolecular dimension and, therefore, the plateau adsorption increases. At the isoelectric point (4.8) the net charge on the gelatin molecule is zero and thus the contraction in the macromolecular coil will be maximum which is reflected by the maximum plateau adsorption of gelatin as shown in Fig. 2. Further addition of NaOH gradually increases the pH and the gelatin molecules become more and more negatively charged due to increasing ionization of the carboxylic groups. In this way, due to electrostatic repulsion between the negatively charged groups, the dimension of gelatin molecules increase and consequently the adsorption decreases. The elongation in the gelatin molecules due to the addition of OH^- ions is also evidenced by the increase in reduced viscosity of the gelatin solution as shown in Table-1. Similar type of findings have also been reported by other workers[16–18].

TABLE-1
INCREASE IN REDUCED VISCOSITY (η_{red}) OF GELATIN SOLUTION (0.40 gm/dl) WITH RISE IN pH OF THE SOLUTION

pH of the gelatin solution (0.4. gm/dl)	η_{red} (reduced viscosity of the solution) $\times 10^2$
4.0	66.5
4.5	62.5
5.0	91.5
6.0	108.2
7.6	120.0
8.4	133.7

(iii) *Salt Effect*: Small electrolytes play a significant role in influencing the adsorption behaviour of both the natural and synthetic polyelectrolytes. It is due to the simple fact that the addition of small electrolytes to a polyelectrolyte solution causes a change in the dimension of the macromolecule which directly affects the mass of the adsorbed polymer. In the present study the influence of electrolyte on the course of adsorption has been investigated by adding KCl of varying concentration to the adsorption media. It is clear from Fig. 3 that the addition of KCl in the concetraction range 0.01 to 0.08 mol/L brings about an increase in the plateau adsorption. The observed increases may be attributed to the following reasons:

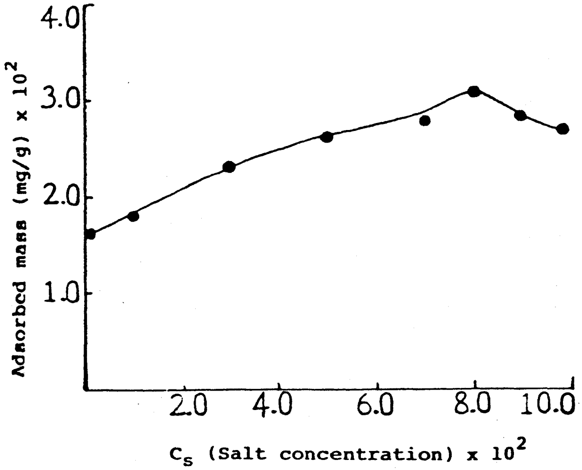

Fig. 3. Effect of KCl concentration (in mol/L) on plateau adsorption of gelatin on zinc powder surfaces at an equililirium concentration of 0.33 gm/dl.

(i) When KCl is added to the gelatin solution, a small decrease in viscosity of the solution is noticed which facilitates the diffusion of gelatin molecules towards the interface and hence the adsorption increases. The observed decrease in viscosity in shown in Fig. 4 wherein (reduced viscosity) of the gelatin solution has been plotted against ln C_s (salt concentration). The fall in viscosity is well predicted by the theory also[19] and has been experimentally observed by many workers[20, 21].

(ii) Added salt may also affect the confromation of gelatin molecules since the mobile ions screen electrostatic repulsion acting on chain segments. The consequence is some contraction of the expanded macromolecule and thus a partial recovery of entropy. The contraction in macromolecular dimension is evidenced by a decrease in the reduced viscosity of the gelatin solution.

(iii) The increase in plateau adsorption may also be viewed as due to the decrease in solvent power of the medium, as stated by Eisenberg and King[22], and is experimentally revealed by the fall in reduced viscosity of the solution. Obviously the smaller radii of gyration in the presence of electrolyte will allow

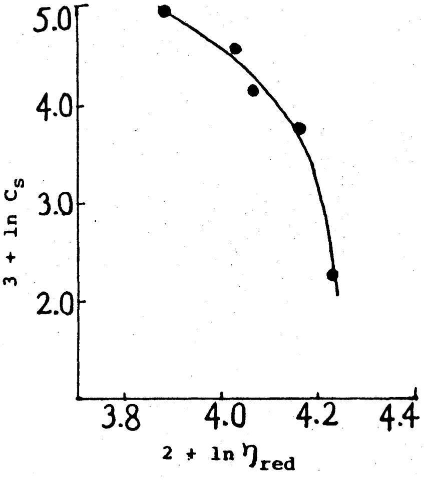

Fig. 4. Variation of ln (reduced viscosity) of gelatin solution with ln C_s (salt concentration) at gelatin concentration of 0.4 gm/dl.

a greater number of gelatin molecules to occupy the available surface sites for adsorption. Similar type of results have also been obtained by other workers[23–26]. In several adsorption investigations a decrease in plateau adsorption has also been noticed by some workers[20] while experimenting with higher concentration of polyelectrolyte solution. However, no such observation was found in the present study in any concentration range of gelatin solution. The results obtained in the present communication are in good agreement with the theoretical predictions made for polyelectrolytes adsorption[27].

At higher concentration of KCl (> 0.8 mol/L) the plateau adsorption was found to decrease slightly. The fall observed may be explained on the basis of salt exclusion effect according to which at higher surface charge density the salt ions occupy the volume at interface and thus the volume occupied by the ions is no longer available for polymer which subsequently results in a lower adsorption. In the present study, surface charge has not been directly increased but at higher salt concentration accumulation of salt ions at interface may be supposed to have resulted in an increase in the surface charge and, therefore, the adsorption decreases. The situation has been modeled in Fig. 5 where the less accessibility of gelatin molecules to the surface is shown due to the occupation of volume by the bigger chloride ions at interface.

(iv) *Effect of Surface Charge*: The electrostatic nature of adsorbent surface greatly influences the adsorption behaviour of adsorbates. In an aqueous medium, both proteins and solid surfaces are usually charged, but these charges are partly screened by counter-ion atmospheres. Where a protein molecule approaches a surface, the electrical double layers overlap, leading to a charge redistribution[28] with strong consequences for the adsorption process itself. In the present investigation it is quite reasonable to assume that in aqueous solution zinc particles must be present in the form of hydroxides of zinc and since the experiment is done in acidic medium obviously the surface of zinc particles bears

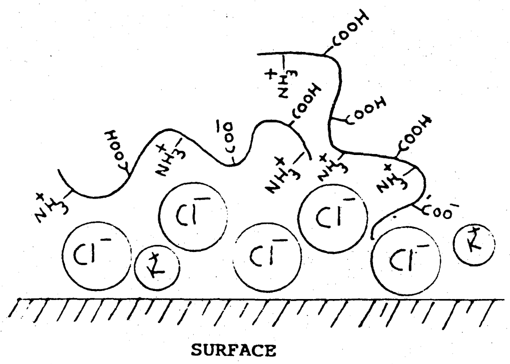

Fig. 5. Schematic representation of salt exclusion effect showing the occupation of volume by Cl^- at gelatin solution zinc powder interface.

a net positive charge. Following equilibria may be written to show the state of zinc particles in aqueous solution:

$$Zn + H_2O \xrightarrow{[O]} Zn(OH)_2 \tag{1}$$

$$Zn(OH)_2^+ \xrightarrow{H^+} ZnOH^+ + H_2O \tag{2}$$

$$ZnOH^+ \longrightarrow ZnO + H^+ \tag{3}$$

$$ZnO + H_2O \longrightarrow Zn(OH)_2 \tag{4}$$

Now it is clear from the above equations that due to the similar charges on the surface and gelatin molecules a weak electrostatic interaction occurs between them which leads to a poor affinity of gelatin molecules for adsorption. After the adsorption is over, the analysis of the centrifuged mass confirms the presence of zinc hydroxides and, therefore, supports the above equilibria.

Conclusion

The adsorption of gelatin from its aqueous solution onto the surfaces of zinc powder results due to a weaker protein-metal interaction. The adsorption isotherms follow the Langmuir's pattern and the plateau adsorption is found to increase as the solution tends to approach the isoelectric point condition. The adsorbed mass also increases with addition of low concentration of ionic salts to the gelatin solution while at relatively higher concentration of the salt the adsorption is lowered by a smaller degree. The results obtained are in fair agreement with the predictions of the theories laid down for weak polyelectrolytes.

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