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Chemical Engineering Journal 87 (2002) 181–186

Chemical Engineering Journal

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Impacts of the surface charge property on protein adsorption on hydroxyapatite

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Received 26 February 2001; received in revised form 13 August 2001; accepted 12 September 2001

Abstract

Knowledge of the adsorption mechanism of biological molecules on hydroxyapatite (HAP) is essential to the application of HAP chromatography and to the development of HAP-based biomedical materials. Centered on the surface charge of HAP and its impacts on adsorption of proteins, the present study started with the characterization of ζ-potential of HAP as a function of the chemical properties of solution in terms of concentration of ions of different types, ionic strength and pH. Then the adsorption of bovine serum albumin (BSA) on HAP was carried out at the same conditions to elucidate the effects of ζ-potential on BSA adsorption and the replacement of BSA with PO_4^{3-} in acidic buffer. A Langmuir isotherm was obtained, indicating a single layer adsorption of BSA on HAP. Finally, the apparent activation energy and the adsorption heat were interpreted from the adsorption at different temperatures. The low magnitude of both the apparent activation energy and the adsorption heat indicated that the fast adsorption of BSA on HAP was a physical adsorption process.

Keywords: Hydroxyapatite; ζ-Potential; Bovine serum albumin; Adsorption; Kinetics

1. Introduction

Since the introduction of hydroxyapatite (HAP) to separation of proteins by Tiselius et al. [1], HAP has been successfully applied to separation of proteins, enzymes, nucleic acid, virus and other biological molecules. As the mineral prototype for bones and teeth and with proven compatibility, HAP has also been extensively used to make medical implants [2–4]. The adsorption of biological molecules plays the fundamental role in both the separation performance of HAP chromatography and the function of HAP-based biomedical. Thus, great efforts have been made on the adsorption mechanism of biological molecules on HAP [5–7]. It is well established that biological molecules interact with HAP crystallite surface by exposing a certain section of their structure against the crystallite surface of HAP and thus arrange their conformations most suitable for the adsorption. In general, acid molecule is adsorbed on C-site and basic molecule is on P-site [8–10]. In some cases, it have been proven that electrostatic interaction between HAP and protein molecules is the dominating factor in adsorption process [11–13]. However, as pointed elsewhere [14], there is still a need of the investigation of the adsorption mechanism, especially the quantitative studies of both thermodynamics and kinetics.

The present study, being in the context of the elucidation of adsorption mechanisms of biological molecules on HAP, focused on the surface physicochemical property of HAP and its impacts on the adsorption of biological molecules. ζ-Potential of HAP was examined as function of the liquid composition in terms of concentration of cooperative anions and cations, i.e., PO_4^{3-} and Ca^{2+}, pH, and ionic strength of solution indicated by NaCl concentration. The adsorption of bovine serum albumin (BSA) on HAP was conducted at the same conditions to illustrate the role of ζ-potential and other factors on BSA adsorption. The apparent activation energy and the adsorption heat were determined from experiments of different temperatures and thus provided the basic information of the adsorption kinetics.

2. Experimental

2.1. Materials

Chemicals used in the present study included HAP (Merck, USA) and BSA (Boehringer Mannheim GmbH, Germany), the p*I* of which is 4.9. Other chemicals of

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1385-8947/02/$ – see front matter
PII: S1385-8947(01)00248-0

analytical grade were purchased from domestic companies and used without further purification.

2.2. Methods

Micrograph of HAP was obtained by transmission electron microscopy (JEM-200CX, Japan). Surface charge of HAP in terms of ζ-potential was measured with Zeta Master 3000 (Malvern, UK).

The adsorption of BSA on HAP was carried out at room temperature (18.5 °C) or in a water bath at a given temperature. First, 0.1 g HAP was weighed in a 5 ml plastic tube, then 3 ml protein solution of a certain concentration was added. Adsorption was performed for about 1 h. Then the solution was centrifuged for 10 min at 8000 rpm. Finally, the supernatant was collected and subjected to the spectrophotometric detection at 280 nm to determine BSA concentration according to the calibration curve. The experimental data were plotted with the software entitled Sigmaplot, developed by SPSS.

3. Results and discussion

3.1. Transmission electron micrography of HAP

The photograph of the transmission electron micrography (TEM) of HAP used in the present study is shown in Fig. 1, from which it is seen that HAP used in the present study is a kind of crystallite with an average cross area of $80 \times 20\,nm^2$. The molar ratio of Ca/P of HAP is 1.67. The molecular size of BSA is 4 nm × 4 nm × 14 nm and its equivalent diameter is 5.38 nm [15]. Thus, the adsorption of BSA occurred at the surface of the HAP particle.

3.2. ζ-Potential of HAP as a function of the solution properties

ζ-Potential is an indicator of the surface charge property of colloid or particle in solution. Knowledge of ζ-potential of HAP, from the viewpoint of surface charge property, is essential to establish a comprehensive understanding of adsorption mechanism. In the present study, ζ-potential of HAP was examined as a function of solution properties in terms of ionic strength, pH, and ion composition. The results are shown in Fig. 2a–d.

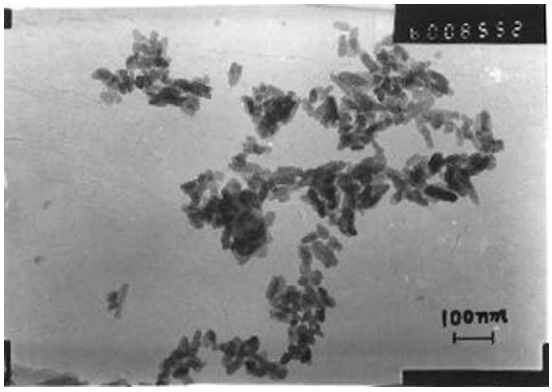

Fig. 1. TEM of hydroxyapatite (magnification 60,000×).

Fig. 2a shows ζ-potential of HAP in pH 5.82, 0.005 M NaAc–HAc buffer containing NaCl of different concentrations. Here, the increase in ionic strength leads to an increase in the magnitude of the negative ζ-potential at the beginning stage, indicating the adsorption of anions, such as Ac^- and Cl^-, on HAP surface. When the concentration of NaCl is over 0.01 M further increase in NaCl concentration results in a reduction in the magnitude of the negative ζ-potential. The diffusion of Na^+ into electric double layer at HAP surface is enhanced by the increased NaCl concentration in solution. Thus, the electric double layer is compressed and this leads to the reduction of the magnitude of the negative ζ-potential.

Fig. 2b shows ζ-potential of HAP in pH 7.0 sodium phosphate buffer of different concentrations. Similar to results shown in Fig. 2a, here the magnitude of the negative ζ-potential increases in response to the increase in phosphate concentration at the beginning stage and decreases when phosphate concentration is over 0.1 M. However, the significant change in the magnitude of ζ-potential in response to a tiny increase in phosphate concentration at the beginning stage, compared to that in Fig. 2a, indicates that PO_4^{3-} is the potential-determining ion of HAP.

ζ-Potential of HAP in pH 5.82, 0.005 M NaAc–HAc containing $CaCl_2$ of different concentrations is shown in Fig. 2c. Here, the presence of Ca^{2+} of low concentration results in the polarity change of ζ-potential from negative to positive, indicating that Ca^{2+} is also a kind of potential-determining ion for HAP. In the present study, a continuous increase in the magnitude is obtained in response to the increase in Ca^{2+} concentration. When Ca^{2+} concentration is over 0.005 M, further increase in Ca^{2+} results in a tiny increase in the magnitude of ζ-potential, indicating the adsorption of Ca^{2+} by HAP approaches a saturated level.

ζ-Potential of HAP at different pH values is shown in Fig. 2d. These solutions were prepared with HCl, 0.05 M HAc–NaAc, NaOH, and their electroconductivity were maintained at about 400 μs/cm, which was adjusted by the addition of NaCl solution if necessary. As shown in Fig. 2d, a sharp increase in the magnitude of the negative ζ-potential of HAP is achieved when pH value increases from pH 4.0 to 7.0, indicating the enhanced adsorption of anions on HAP surface. When pH of the buffer is over 7, further increase in pH results in a tiny increase in the magnitude of the ζ-potential. The increase in the magnitude of the negative ζ-potential is mainly contributed by the adsorption of OH^-. Thus, a declined increase in the OH^- concentration by pH increases from pH 7 to 9.5 results in a declined increase in the magnitude of the negative ζ-potential, as shown by Fig. 2d.

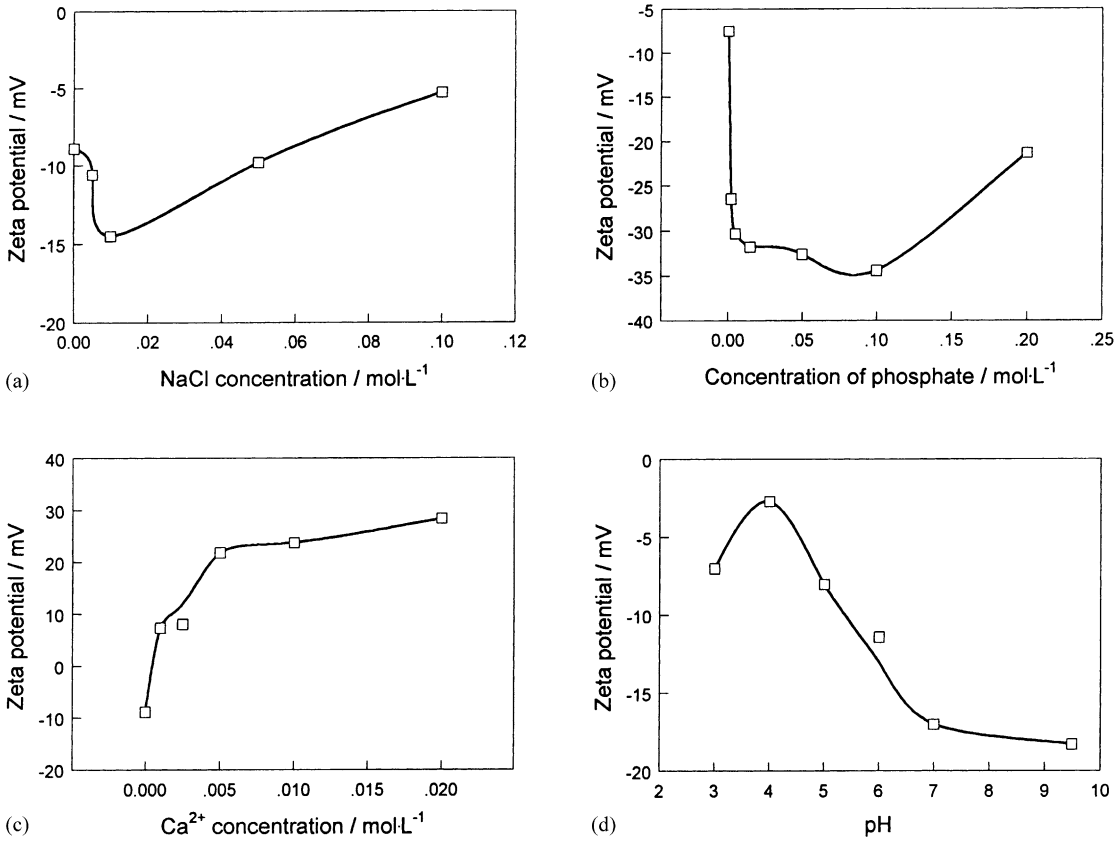

Fig. 2. ζ-Potential of HAP as a function of the physiochemical properties of the solution: (a) ζ-potential as a function of ionic strength; (b) ζ-potential as a function of buffer concentration; (c) ζ-potential as a function of Ca(II) concentration; (d) ζ-potential as a function of pH.

3.3. Adsorption of BSA on HAP

To elucidate the role of ζ-potential in the adsorption of protein on HAP, BSA was selected as a model system. Adsorption experiments were carried out at the same conditions as the prior study on ζ-potential in order to correlate adsorption performance with ζ-potential. If not stated otherwise, the initial concentration of BSA solution was 1 mg/ml. The experimental results are shown in Fig. 3a–d.

Fig. 3a shows adsorption capacity obtained at different BSA concentrations in sample prepared with pH 6.8, 0.002 M phosphate buffer. Here, the increase in the adsorption capacity is related to the increase in BSA initial concentration. This indicates that the adsorption of BSA on HAP shifts to the adsorption when more BSA molecules are available in solution.

The adsorption of BSA buffered with pH 6.80 phosphate of different concentrations is shown in Fig. 3b. A sharp decrease in the adsorption capacity is in response to the increase in phosphate concentration from 0 to 0.1 M. The increase in phosphate concentration leads to more PO_4^{3-} in the diffusion layer of the electric double layer at HAP surface. This enhances the electrostatic repulsion force between HAP and BSA and thus results in a reduction in the adsorption of BSA. However, the more important reason is that as a potential-determining ion, PO_4^{3-} has a higher affinity to HAP compared to BSA. Thus, the adsorption of BSA reduced to the increase in the PO_4^{3-} concentration. At high concentration of phosphate, almost all adsorption sites on HAP are occupied by PO_4^{3-}. That is the reason why high concentration phosphate can be used to elute BSA from HAP chromatographic column.

Fig. 3c shows the adsorption of BSA buffered with pH 6.8, 0.002 M phosphate buffers containing NaCl of different concentrations. An increased adsorption is obtained in response to the increase in NaCl concentration. It is known from Fig. 2a that the increase in the ionic strength leads to the reduction of the magnitude of the negative ζ-potential of HAP. Thus, the electrostatic repulsion force between HAP and BSA is reduced and more BSA molecules are adsorbed. At high concentration of NaCl, further input of NaCl contributes little to the reduction of magnitude of the negative ζ-potential, as shown by Fig. 2a. This leads to the declined increase in the adsorption capacity of BSA at high concentration of NaCl, as shown in Fig. 3c.

Fig. 3d shows the adsorption of BSA at different pH values in phosphate buffer. Here BSA samples of 2.0 and 3.0 mg/ml were applied for adsorption. In both cases, the increase in pH results in a decrease of adsorption capacity. As shown by Fig. 2d, the increase in pH results in an increase in the

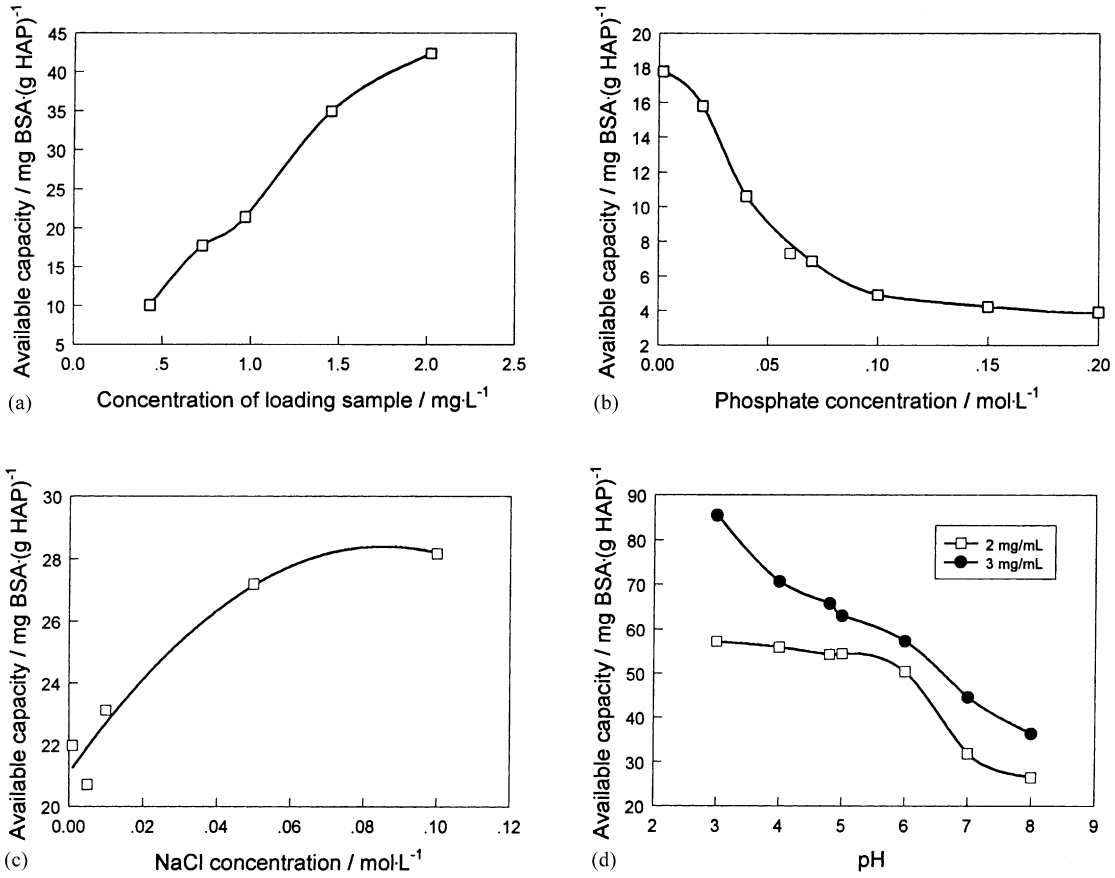

Fig. 3. Characteristics of the adsorption capacity of BSA on HAP: (a) adsorption capacity of BSA on HAP as a function of sample concentration; (b) adsorption capacity of BSC on HAP as a function of phosphate concentration; (c) adsorption capacity of BSC on HAP as a function of sodium chlorite concentration; (d) adsorption capacity of BSC on HAP as a function of buffer pH.

magnitude of the negative ζ-potential of HAP and also the negative charge of BSA. Thus, an enhanced electrostatic repulsion force between HAP and BSA is generated and this hinders the adsorption of BSA. On the other hand, Barroug et al. [7] reported the cooperative delivery of OH^- due to the adsorption of acidic proteins. Thus, the increase in OH^- concentration with the increase in pH value will also reduce the adsorption capacity of BSA.

3.4. Adsorption kinetics and equilibrium

The adsorption of BSA on HAP was operated at 0, 5, 10 and 15 °C, respectively. The initial concentration of BSA was 3 mg/ml. A time course of the adsorption was generated by measuring the concentration of BSA in solution at different incubation times. The results are shown in Fig. 4, which indicate that the adsorption of BSA on HAP can be described as a first-order reaction shown by the following equation:

$$-\frac{dC}{dt} = kC \quad (1)$$

Integration of the above equation gives

$$C = C_0 \exp(-kt) \quad (2)$$

Hence adsorption capacity is

$$q_t = q_e[1 - \exp(-kt)] \quad (3)$$

where t is the incubation time in terms of min, C_0 and C refers to starting and equilibrium concentration of BSA, respectively, in terms of mg/ml. q_e and q_t stands for the

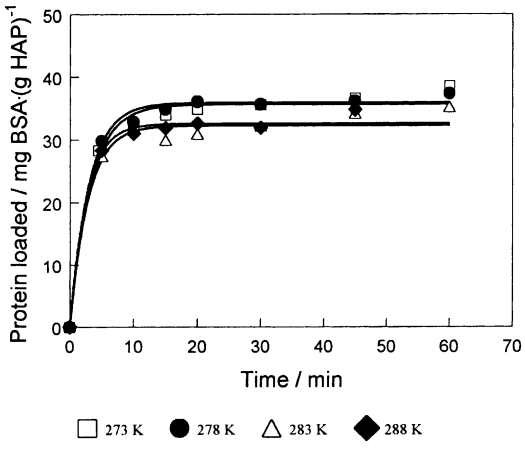

Fig. 4. Adsorption capacity as a function of incubation time.

Table 1
Apparent reaction rate constant at different temperatures

	0 °C	5 °C	10 °C	15 °C
k (min^{-1})	0.3154	0.3387	0.3557	0.3886

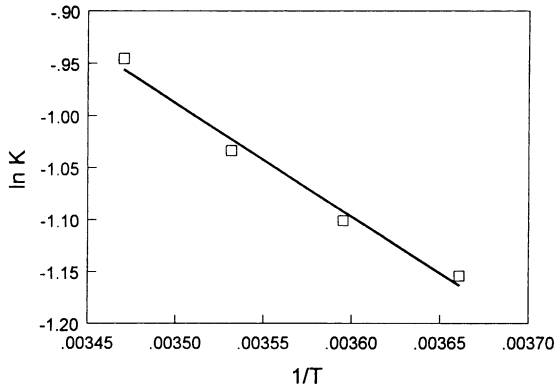

Fig. 5. Regression results of $\ln k$ and $1/T$.

equilibrium adsorption capacity and the adsorption capacity at a certain time in terms of mg protein/g HAP. k is the apparent reaction constant in terms of min^{-1}.

The apparent reaction constants at different temperatures are thus calculated according to Eq. (3) and listed in Table 1.

A correlation of k and T shown by Eq. (4) is thus established by using the data shown in Table 1, on the basis of Arrhenius equation, as shown by Fig. 5

$$k = 16.83 \exp\left(-\frac{9052}{RT}\right) \tag{4}$$

Thus, the apparent activation energy for the adsorption of BSA on HAP is 9.05 kJ/mol, which is much less than 40 kJ/mol, indicating that the adsorption of BSA falls into the category of fast reaction [16].

Adsorption isotherms of BSA on HAP at 18.5 and 30 °C are shown in Fig. 6, which can be described by Langmuir's

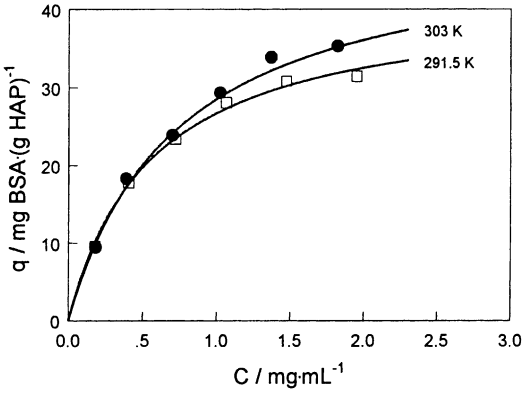

Fig. 6. Adsorption isotherm of BSA on HAP.

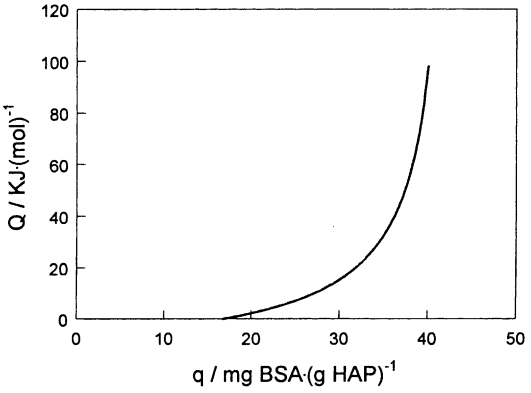

Fig. 7. Calculated adsorption heat for the adsorption capacity of BSA on HAP.

isotherm equation

$$q = \frac{q_{\max} K_a C}{1 + K_a C} \tag{5}$$

where $q_{\max}$ is the maximum adsorption capacity in terms of mg BSA/g HAP, K_a the adsorption equilibrium constant. The $q_{\max}$ interpreted from the isotherm is 41.49 and 49.06 mg BSA/g of HAP for the adsorption at 18.5 and 30.0 °C, respectively. Introducing the results above into the Clausius–Clapeyron equation, as shown by the following equation:

$$Q = RT^2 \left(\frac{\mathrm{d}\ln C}{\mathrm{d}T}\right)_N \tag{6}$$

Integration of Eq. (6) gives

$$Q = \frac{RT_1T_2 \ln(C_2/C_1)}{T_2 - T_1} \tag{7}$$

where C_1 and C_2 represent BSA equilibrium concentration at T_1 and T_2, corresponding to a certain BSA adsorption capacity (q). The adsorption heat Q is thus a function of q shown by Fig. 7.

Fig. 7 shows that the adsorption heat increases in response to the increase in adsorption capacity. When the adsorption of BSA is not more than 15 mg protein/g HAP, the adsorption heat is nearly negligible. In this study, the adsorption heat is generally less than 100 kJ/mol. This indicates that the BSA adsorption is a physical adsorption that takes much less adsorption heat compared to the chemical adsorption. The latter also requires higher activation energy in most cases.

4. Conclusions

The TEM shows that HAP studied in the present work is a kind of solid fine particle. The investigation on ζ-potential of HAP has shown that adsorption of anions and cations on the HAP surface results in changes in both the charge polarity and the magnitude of ζ-potential of HAP. PO_4^{3-} and Ca^{2+} are the potential-determining ions that lead to significant

change in both the polarity and magnitude of the ζ-potential compared to other anions or cations.

Investigation on the adsorption of BSA on HAP has shown that the electrostatic interaction between HAP and BSA is the dominating factor in the adsorption process. The preferential adsorption of the potential-determining ions, i.e., PO_4^{3-} is demonstrated by the significant reduction in the adsorption of BSA in response to the increase in the PO_4^{3-} concentration. The potential-determining ions can thus be used to elute the adsorbed biological molecules. The apparent activation energy for the adsorption of BSA is interpreted from the time course of the adsorption at different temperatures and the adsorption heat is calculated from the adsorption isotherms at different temperatures. The low magnitude of the apparent activation energy and the adsorption heat indicate that the fast and reversible adsorption of BSA on hydroxyapatite is a physical adsorption in nature. The results presented above are of fundamental importance to the application of HAP for the separation of biological molecules and the development of HAP-based biomedicals.

Acknowledgements

The supports from Natural Science Foundation, PR China (Grant No. 29676022) and Beijing Natural Science Foundation, PR China (Grant No. 2992016) are gratefully acknowledged.

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