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Thermodynamics, Kinetics and Adsorption Properties of Some Biomolecules onto Mineral Surfaces

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1. Introduction

Developments in biotechnology have led to an enormous increase in the exposure of proteins and enzymes (biomolecules) to non-biological solid surfaces in applications such as artificial implants, purification strategies, biosensors, and drug delivery systems. Biotechnological and biomedical applications requiring adhesion, adsorption, biocompatibility, permeability and/or other properties depend on the surface properties of the material significantly (Arica et al., 2010; Karagoz et al., 2010; Nakanishi, 2001; Luo and Andrade, 1998).

Besides the practical importance of gaining knowledge on biomolecule structures in the adsorbed state, it is also important to understand the adsorption-induced structural changes from a more fundamental view-point as rearrangements in the biomolecule structure are seen as an important driving force for adsorption to take place. The adsorption of a biomolecule to a surface usually produces a change in its physicochemical properties, which may affect the biological functioning of the molecule. Biomolecule adsorption is a complex process in which the structural stability of a biomolecule, the ionic strength and the pH of the solution and surface properties of sorbent are known to influence the affinity of a biomolecule for a given interface (Giacomelli and Norde, 2001; Norde, 2000).

Soil extracellular enzymes, like other proteins, are adsorbed on many surfaces, particularly on clay minerals, which have high specific surface areas, and so are likely to be predominantly in an adsorbed state for example, the system of bovine serum albumin (BSA) and silica particles has extensively been investigated. There are a number of studies related to the biomolecule adsorption on oxide surfaces such as silica particles, montmorillonite, hydroxyapatite, TiO₂ particles, chitosan, stainless steel surface or mica. However, understanding the mechanism of biomolecule adsorption and evaluating the impact on practical applications is very important (Kondo et al., 1992 and 1993a; Buijs et al., 1996). On the other hand, many protocols for enzyme immobilization involve irreversible binding to a functionalized support. In the reversible enzyme immobilization, the supports could be regenerated using a suitable desorption agent, and they be recharged again with a fresh enzyme. In the reversible enzyme immobilization, the supports could be regenerated using a suitable desorption agent, and they be recharged again with a fresh enzyme. On the other hand, when the covalently immobilized enzyme becomes inactivated upon use both the

enzyme and the support should be eliminated as wastes (Bayramoglu et al., 2010; Zhou, 2010; Wang et al., 2010; Bolivar et al., 2009).

Sepiolite is an oxide mineral with a unit cell formula $\text{Si}_{12}\text{O}_{30}\text{Mg}_8(\text{OH},\text{F})_4(\text{H}_2\text{O})_4 \cdot 8\text{H}_2\text{O}$ (Ahlrichs et al., 1975). In some aspects sepiolite is similar to other 2:1 trioctahedral silicates, such as talc, molecule formula is $\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$ (Rytwo et al., 2002; Grim, 1968). Sepiolites, which form an important group of clay minerals, are a magnesium silicate and currently used in a number of different applications such as many industrial, catalytic and environmental applications, most of which are similar to those of the more traditional clays. Because of their structural morphology, sepiolites have received considerable attention with regard to the adsorption of organics on the clay surfaces and to their use as support for catalysts (Frost and Ding, 2003). The abundance and availability of sepiolite mineral reserves as a raw material source and its relatively low cost guarantee its continued utilization in the future, and most of the world sepiolite reserves are found in Turkey. Sepiolite is a good adsorbent for organic species because it exhibits a variety of attractive properties such as high specific surface area, high porosity (50.8%) (Göktas et al., 1997), and surface activity. Sepiolite is therefore used in a spectrum of areas where sorptive, catalytic, and rheological properties are exploited. The sorption ability of sepiolite is mainly ascribed to its high surface area. Thus, it is commonly used in oil refining; wastewater treatment; the removal of drug, and pesticide carriers; paper and detergent; etc. (Sabah and Çelik, 2002). Investigations on sepiolite have so far focused more on its sorptive properties and attempts have been made to increase its surface area.

Caseins are frequently used as an additive in food, paint, glue etc. (Walstra and Jenness, 1984) where their amphiphilic properties are utilised to modify various types of interfaces. The main proteins in renneted milk comprise α_{s1} -, α_{s2} -, β -, and para κ -casein. Of these four caseins, para- κ -casein is known to be bound closely to the surface of casein micelles. The hydrophobic plots of amino acid residues in α_{s2} - and κ -casein suggest that they have surface active properties (Dalglish, 1993) and that these proteins may also be located on the micellar surface. This being the case, α_{s2} -, β -, and para κ -casein are likely candidates for binding to the surface of fat globules. β -casein (together with α_{s1} -casein) is the most abundant protein among the four caseins present in cow's milk (Dickinson, 1999). The biological role of casein molecules includes the sequestration of amorphous calcium phosphate to form stable complexes in milk (Holt, 1998; Holt and Sawyer, 1988; Holt et al., 1996; Walstra and Jenness, 1984). Caseins are also frequently used as additives in food, paint, glue and coating colours for paper (Walstra and Jennes, 1984). Knowledge of the mechanisms by which caseins adsorb is therefore of great interest in many colloid-related industries.

Catalase (hydrogen peroxide oxidoreductase) is an enzyme normally found in the peroxisomes of nearly all aerobic cells and serves to protect the cell from the toxic effects of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without production of free radicals. The reaction of catalase in the decomposition of hydrogen peroxide is: $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$. Herein, catalase has useful applications in various industrial fields, such as food industry, textile industry, and in the analytical field as a component of hydrogen peroxide or glucose biosensor systems (Horst et al., 2006; Jürgen-Lohmann and Legge, 2006; Yoshimoto et al., 2007). However, there are many limitations for the free enzymes. Enzymes are unstable, having a short lifetime in the circulation, and causing toxic reactions (Jiang and Zhang, 1993; Shentu et al., 2005; Özlem Alptekin et al., 2009; Ran et al., 2009). These drawbacks can be partially eliminated by immobilizing enzyme

onto solid carriers. Hence, immobilization is an indispensable step in the preparation of the biocatalyst for industrial application. It has many well known advantages, all affecting the production cost (Çetinus and Nursevin Öztop, 2003; Choi and Yiu, 2004; Liu et al., 2009). With immobilized or adsorbed enzymes, improved stability, reusability, continuous operation, possibility of better control of reactions, high purity and product yields and hence, more favorable economic factors can be expected (Kennedy and Melo, 1990). In this study, the adsorption, electrokinetic properties of biomolecules such as casein and catalase onto sepiolite surface were studied as a function of incubation time, concentrations of biomolecules, pH of the solution and temperature. In addition, thermodynamics, some parameters of kinetics and activity of catalase were studied.

2. Material and methods

Catalase (hydrogen peroxide oxidoreductase) from bovine liver, was obtained from Sigma Chemical Co. and casein was obtained from Merck. Sepiolite was obtained from Aktas Luletasi Co. (Eskisehir, Turkey). The chemical composition of the sepiolite determined by XRF is given in Table 1. Sepiolite sample was treated before using in the experiments as follows (Alkan et al., 2006). All other chemicals used were of analytical grade. All water used was of MilliQ quality or doubly distilled.

Constituent	Percentage present
SiO ₂	53.47
Al ₂ O	0.19
CaO	0.71
Fe ₂ O ₃	0.16
MgO	23.55
NiO	0.43
LoI	21.49

Table 1. Chemical composition of sepiolite

The cation exchange capacity (CEC) of the sepiolite used was determined by ammonium acetate method, the density by the picnometer method (Alkan et al., 2006) and the specific surface area by BET N₂ adsorption by Micromeritics Flow Sorb II-2300 equipment. The results obtained are summarised in Table 2.

CEC (meq/100 g)	Density (g mL ⁻¹)	Specific surface area (m ² g ⁻¹)
22	2.2	318

Table 2. Some physicochemical properties of sepiolite used in this study

2.1 Zeta potential measurements

The effects of biomolecules and concentration, pH on zeta potential were evaluated using a Zeta Meter 3.0 (Zeta Meter Inc.) equipped with a microprocessor unit (Alkan et al., 2005). The instrument automatically calculates the electrophoretic mobility of

the particles and converts it to the zeta potential using the Smoluchowski equation as follows:

$$\zeta = 4\pi V_t E / D \quad (1)$$

where E is electrophoretic mobility; V_t is viscosity of the suspending liquid; D is dielectric constant; π is constant and ζ is zeta potential. In this procedure, the solid samples were transferred to 250 mL polyethylene bottle in which 100 mL of an aqueous solution was added yielding final solid concentrations of 3 g L⁻¹ for sepiolite. The samples were dispersed by a thermostatic shaker bath. The pH was measured with a combination electrode (Orion 920A pH meter calibrated with pH 4.00, 7.00 and 10.00 standards) and adjusted by dropwise addition of HCl or NaOH solutions. After shaking overnight (24 h), the samples were allowed to stand for 5 min to let larger particles settle. An aliquot taken from the supernatant was used to measure the zeta potentials. The average of 15 measurements was taken to represent the measured potential. The applied voltage during the measurements generally was varied in the range of 50–150 mV.

2.2 Adsorption experiments

Adsorption experiments were carried out by shaking 0.3 g sepiolite samples with 100 mL aqueous solution at desired concentrations of biomolecules, various pHs (3–10), ionic strengths (1x10⁻⁴ – 2x10⁻¹ mol L⁻¹) and sodium phosphate buffer concentrations (1x10⁻⁴ – 2x10⁻¹ mol L⁻¹) for 24 h (the time required for equilibrium to be reached between biomolecules adsorbed and biomolecules in solution and equilibrium pHs for the suspensions). A thermostatic shaker bath was used to keep the temperature constant. All adsorption experiments were performed at 25 °C. At the end of the adsorption period, the solution was centrifuged for 15 min at 5000 rpm and then the concentrations of the residual biomolecules, C_e , were determined by Bradford method (Bradford, 1976), using a Cary 1E UV-Visible Spectrophotometer (Varian). The amounts of biomolecules adsorbed were calculated from the concentrations in solutions before and after adsorption.

2.3 Activity of catalase

The activity of catalase was determined spectrophotometrically by direct measurement of the decrease in the absorbance of hydrogen peroxide at 240 nm due to its decomposition by the enzyme. Hydrogen peroxide solutions (40–200 mM) were used to determine the activity. 0.3 g of catalase adsorbed sepiolite particles were mixed with hydrogen peroxide solution in 20 mM phosphate buffer (pH 7.0) at 22 °C. The absorbance of the reaction mixture was determined and the kinetic parameters of adsorbed catalase, K_m (Michaelis constant) and V_{max} (maximum reaction rate) for free and adsorbed catalase were determined by varying the concentration of hydrogen peroxide in the reaction medium.

3. Results and discussion

3.1 Isoelectric point (iep) of sepiolite

The potential determining ions are those ions that establish or change the surface charge of a solid. Since potential determining ions control the surface reactions, it is an important parameter for identifying the adsorption mechanisms. For silicate type minerals potential determining ions are generally H⁺ and OH⁻ ions. In addition, the H⁺ ions in solution

undergo exchange with some of the cations in the solid lattice leading to the consumption of H^+ ions in suspension.

For an oxide or clay, there is usually only one ionisable surface group, but that is amphoteric so it can take up either a proton or an OH^- ion depending on the pH (Hunter, 2002):



at isoelectric point (iep),

$$[SOH_2^+] = [SO^-] \tag{4}$$

where “S” denotes the surface.

When pH is equal pH_{iep} , the net charge is zero. This pH corresponds to the presence of equal numbers of oppositely charged groups on the biomolecules. Such a neutral structure of charged groups is called a dipolar ion, or zwitterions. The iep of the sepiolite was determined by measuring the zeta potential as a function of pH (Fig. 1). The zeta potential is about 0 mV for sepiolite at around pH 6.6 in this studied pH range of 1.5–10.

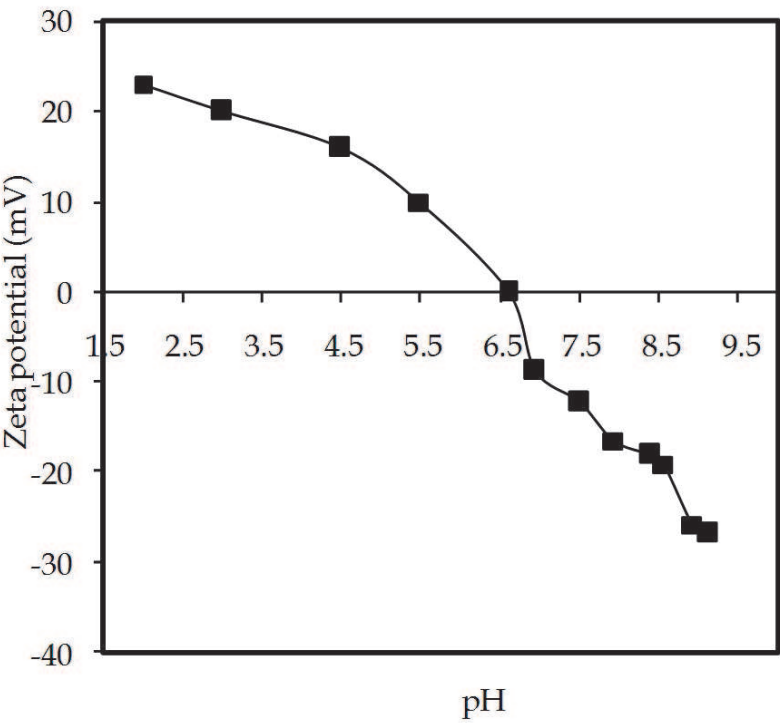


Fig. 1. The variation of zeta potential with pH of the sepiolite mineral.

3.2 Adsorption capacities as a function of incubation time of sepiolite

The adsorptions of casein and catalase on sepiolite were carried out in 0.001 M NaCl and 0.02 M sodium phosphate buffer at pH 7.0 and 22 °C. The initial concentrations of biomolecules were 1.0 mg mL⁻¹ and the solid concentration was 3 g L⁻¹ for sepiolite. Measuring the concentration of biomolecules in solution at different incubation times

generated in a time course of the adsorption. The results are shown in Fig. 2. According to Fig. 2, the time required to reach a stationary concentration is about 10 h.

3.3 Adsorption of biomolecules onto sepiolite and zeta (ζ) - potential values

Adsorption experiments were carried out at the same conditions with zeta potential experiments in order to correlate the adsorption performance with ζ -potential of sepiolite minerals. Fig. 3 shows the variation of zeta potential with biomolecule concentrations of sepiolite minerals in 0.02 M phosphate buffer and 0.001 M NaCl at pH 7.0 and 22 °C and Fig. 4 the adsorption data obtained at the same conditions above. As seen in 4, the adsorption capacity of sepiolite has increased with increase in initial concentration of biomolecule, and the zeta potential of them has decreased with increase in initial concentration of biomolecule (less negative, see Fig.3). This means that the adsorption of biomolecules on solid increases when more biomolecules are available in solution. In fact that, the negative surfaces of solid are covered with biomolecules and they have less negative surface charge than their initial conditions. As a result, the ζ -potential of solid particles is decreased (less negative) but not positive at pH 7.0 because isoelectric points of biomolecules are lower than pH 7.0. The adsorbed biomolecules on sepiolite have negative charge so measured ζ -potential values must be negative.

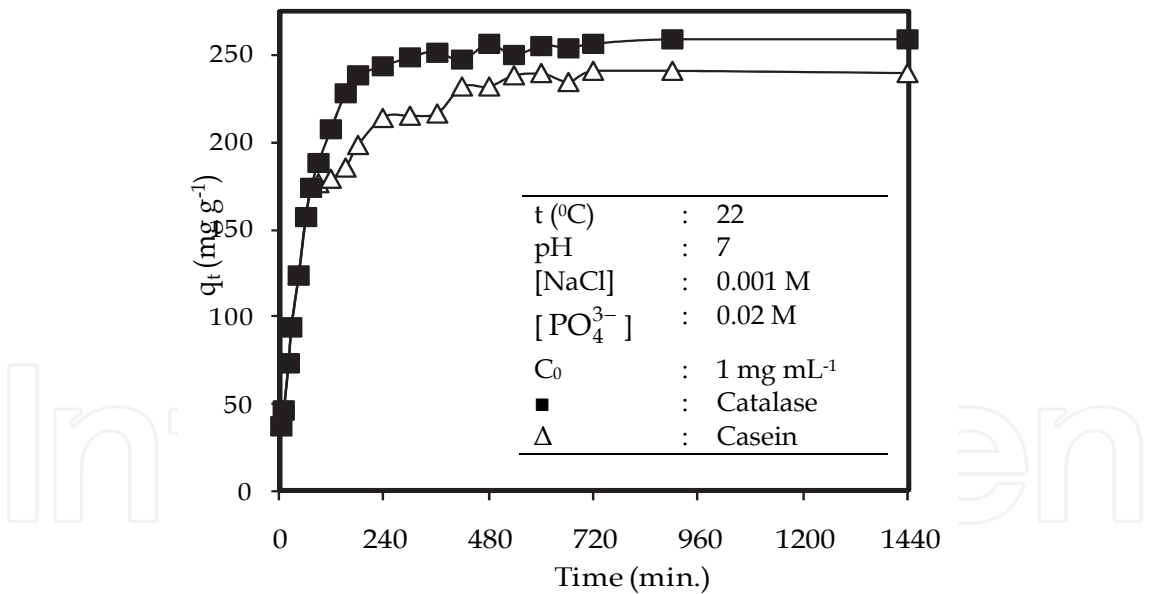


Fig. 2. The adsorption of biomolecules onto sepiolite as a function of incubation time.

Fig. 5 has shown the variation of zeta potential with equilibrium pH of various solid suspensions in 0.001 M NaCl, 0.02 M PO_3^{-4} and 1.0 mg mL⁻¹ biomolecule concentrations at 22 °C, and Fig. 6 the variation of the biomolecule adsorption with equilibrium pH onto sepiolite in 0.001 M NaCl, 0.02 M PO_3^{-4} concentrations at 22 °C. Liquid phase pH has an important effect in the biomolecule adsorption on adsorbent (Patwardhan and Atai, 1997). As seen in Fig. 6, they were found that the maximum casein and catalase adsorption

occurred at about pH 7 and pH 5.8, close to the isoelectric point of biomolecules. Since biomolecules are also negatively charged at pH values higher than about 5.8, the electrostatic repulsion between solid surface and biomolecules may prevent the binding of molecules onto sepiolite, leading to the decrease of biomolecule adsorption in the pH range of 6.0–10.0.

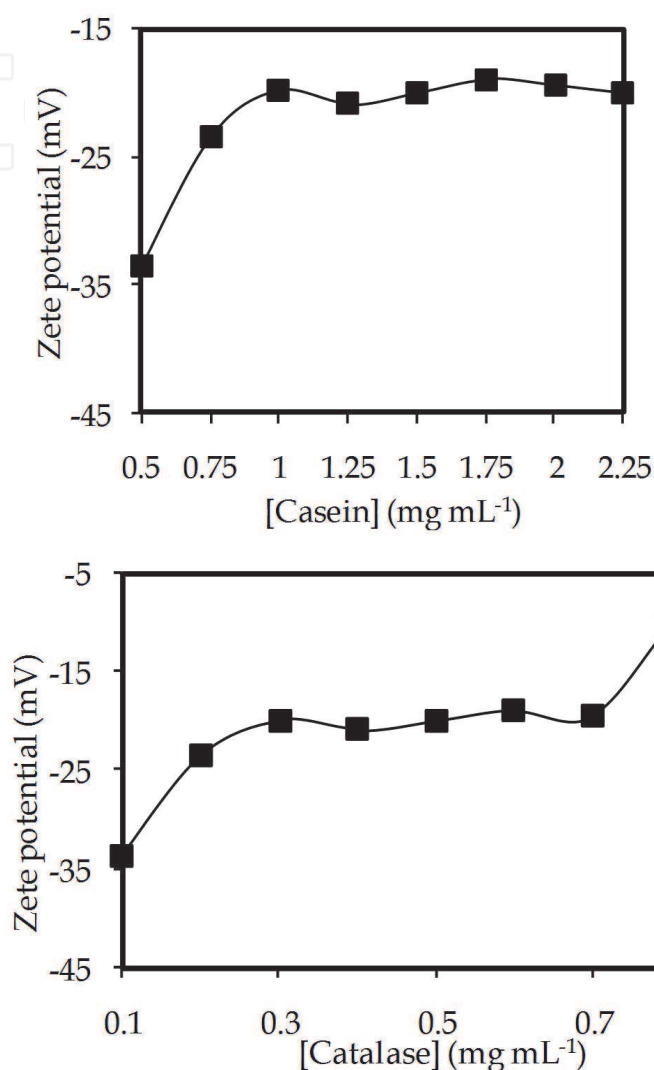


Fig. 3. The variation of zeta potential with biomolecule concentration of sepiolite minerals in aqueous solutions. (Experimental cond. 22°C, pH 7, [PO₄³⁻]: 0.02 M, [NaCl]: 0.001 M)

Addition of protein to the solution has a profound effect on the electrokinetic properties of solid particles (Giacomelli et al., 1997). Iep of sepiolite with catalase have been shown in Fig. 5. Catalase adsorption has caused a change in the ζ -potential or electrophoretic mobility in the entire pH range and shifted the iep of the solid particles from approximately 6.1 for sepiolite; which is the iep of catalase in solution. The casein-covered sepiolite samples have no isoelectric point in the studied pH range of 7–10. Because casein molecules doesn't solve in solution when the solution pH was acidic solutions and measure the ζ -potential or electrophoretic mobility and etc. correctly. A fairly similar trend has been reported by Giacomelli et al., 2001 and they found iep of the BSA-covered TiO₂ particles as pH 4.5; Shi et

al. investigated for the adsorption of BSA on chitosan-coated silica and found a high adsorption at pH 5.0; Sharma and Agarwal found a maximum adsorption at pH 8.0, for the adsorption of wheat germ agglutinin (pH 8.7) to tris(2-aminoethyl)amine-Ni(II) on Sepharose CL-6B; On the other hand, Barroug et al. reported the cooperative delivery of OH⁻ due to the adsorption of acidic proteins. Thus, it can be state that the increase in OH⁻ concentration with the increasing pH value will reduce the adsorption capacity of biomolecules.

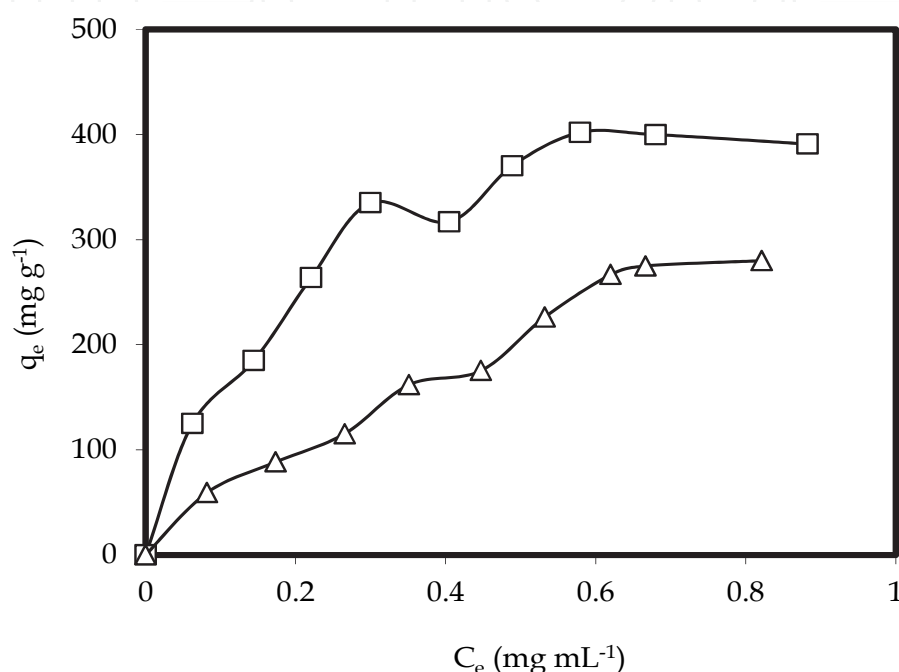


Fig. 4. The adsorption isotherms of biomolecules (△:casein, □:catalase) onto sepiolite. (Experimental cond. 22°C, pH 7, [PO₄³⁻]: 0.02 M, [NaCl]: 0.001 M)

In the present study, the effect of temperature on adsorption was studied in the range 15 – 45 °C and the results are shown in Fig. 7 and 8, which indicate that adsorption increases from 15 to 45 °C. The amount of biomolecule adsorbed on sepiolite increased by increasing temperature. This indicated that sorption on sepiolite is not a simple physical adsorption. This observation can be explained with the help of the following facts: (i) the ongoing adsorption process is endothermic, i.e., entropically driven. (ii) Sorption with dissociation and increased availability of sites due to higher penetration of adsorbing molecules into the pores with an increase in temperature may explain this behaviour. Thus, the adsorption increases with increasing temperature. This indicates that the adsorption process becomes more favourable with increasing temperature

3.4 Adsorption isotherms

Adsorption isotherm describes a number of theories describing the adsorption equilibria. Two isotherm equations in the present study have been tested: Freundlich and Langmuir models.

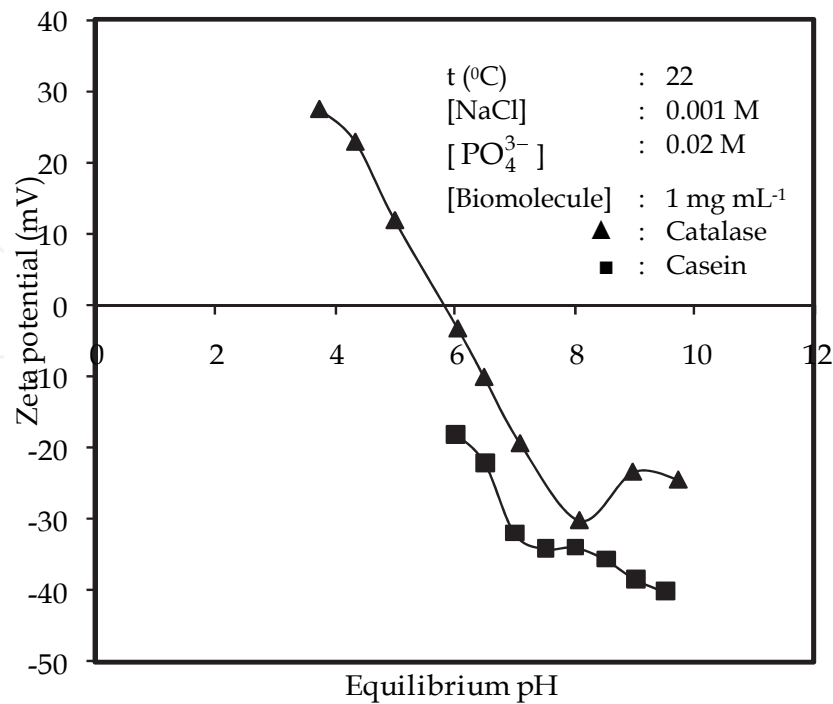


Fig. 5. The variation of zeta potential with equilibrium pH of sepiolite suspensions.

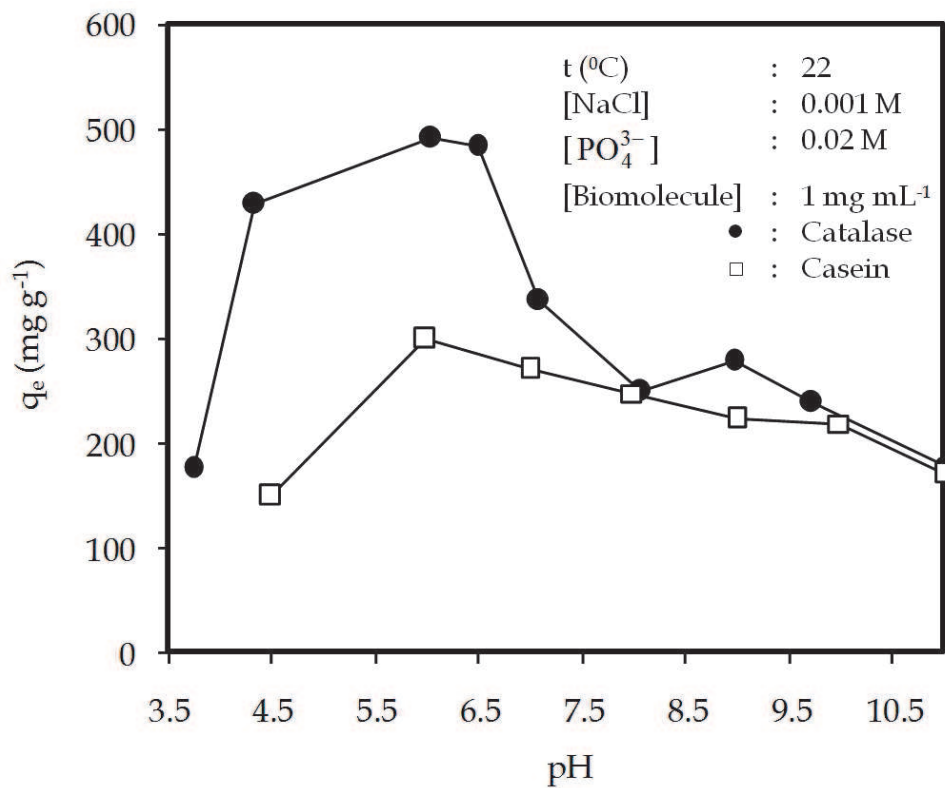


Fig. 6. The variation of biomolecule adsorption with equilibrium pH onto sepiolite.

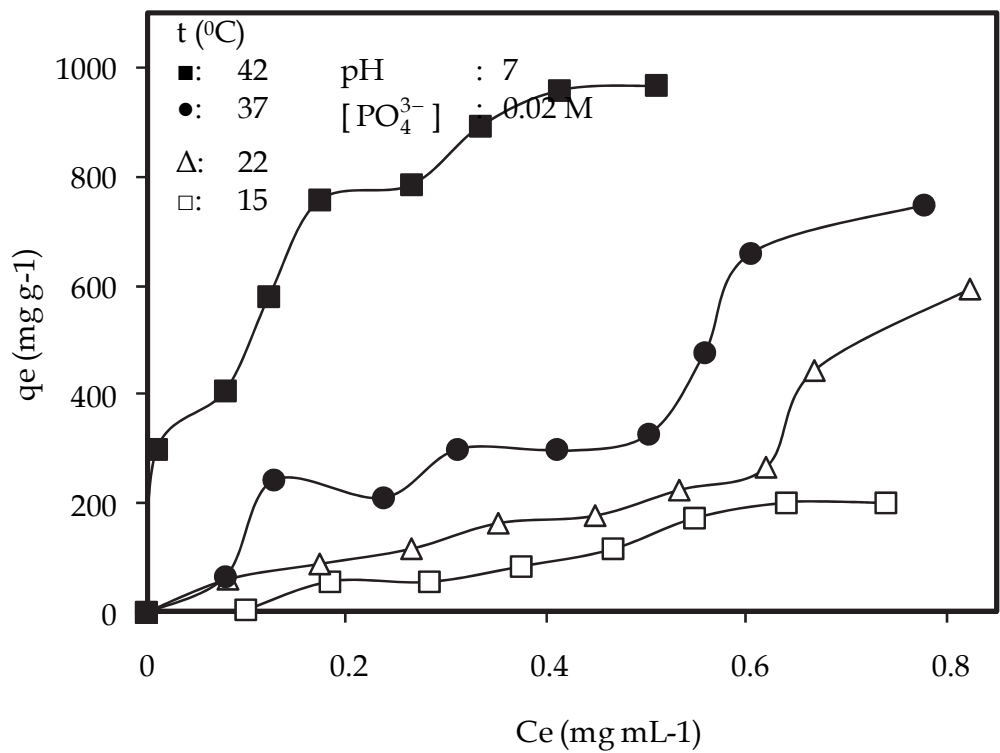


Fig. 7. The effect of temperature on casein adsorption onto sepiolite

3.4.1 Langmuir adsorption isotherm

The Langmuir isotherm theory assumes monolayer coverage of adsorbate over a homogenous adsorbent surface. Therefore, at equilibrium, a saturation point is reached where no further adsorption/desorption can occur.

The Langmuir equation is commonly expressed as (Langmuir, 1918):

$$\frac{C_e}{q_e} = \frac{1}{q_m K} + \frac{C_e}{q_m} \tag{5}$$

where C_e is the equilibrium liquid phase concentrations of protein solution (mg mL⁻¹), q_e is equilibrium protein concentration on adsorbent (mg g⁻¹), K is a direct measure for the intensity of the adsorption process, and q_m is a constant related to the area occupied by a monolayer of adsorbate, reflecting the adsorption capacity. From a plot of C_e/q_e versus C_e , q_m and K can be determined from its slope and intercept. Table 3 presents the correlation coefficient results for Langmuir isotherm, which has not a satisfactorily good correlation between the model predictions and the experimental data.

3.4.2 Freundlich adsorption isotherm

The Freundlich expression is an empirical exponential equation and therefore, assumes that as the adsorbate concentration increases so too does the concentration of adsorbate on the adsorbent surface. Theoretically, using this expression, an infinite amount of adsorption can occur (Freundlich, 1906).

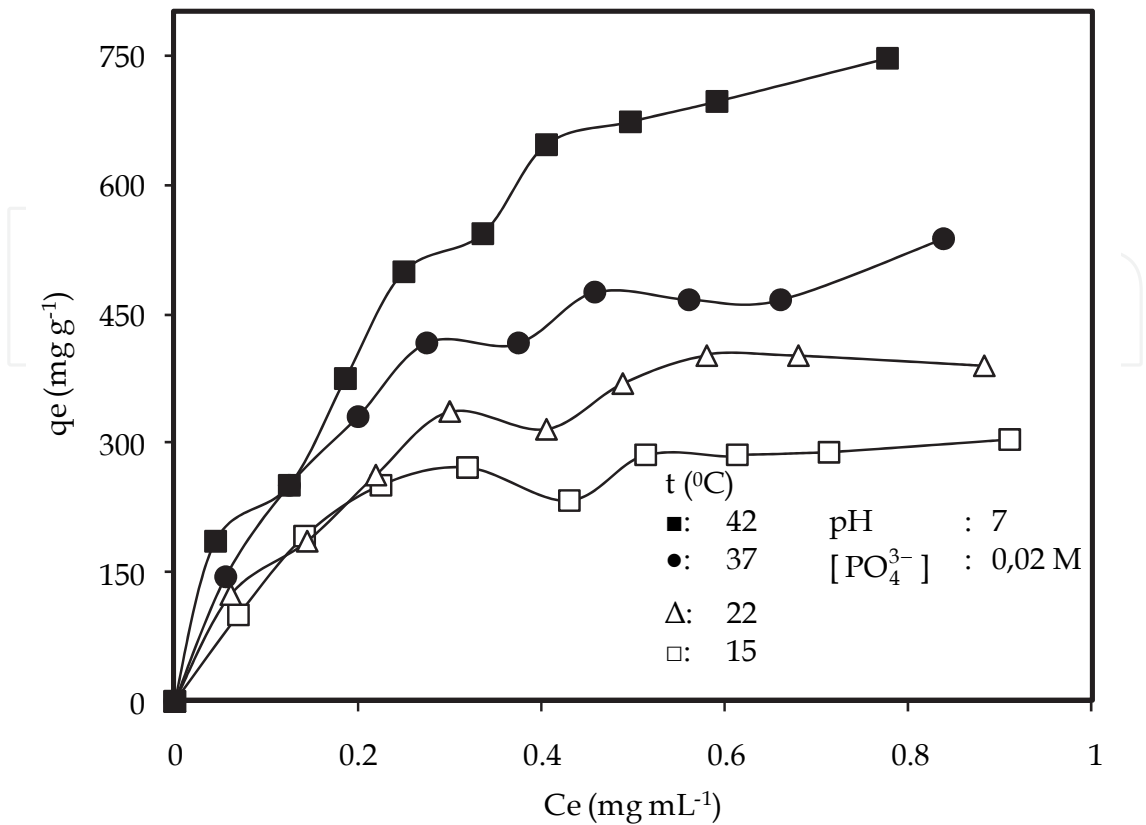


Fig. 8. The effect of temperature on catalase adsorption onto sepiolite

$$q_e = K_F C_e^{1/n} \tag{6}$$

In this equation, which is commonly given, K_F and n are the Freundlich constants. This expression is characterized by the heterogeneity factor, n , and so the Freundlich isotherm may be used to describe heterogeneous systems. To determine the constants K_F and n , the linear form of the equation (not shown) may be to produce a graph of $\ln q_e$ versus $\ln C_e$.

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e \tag{7}$$

K_F is related to the bonding energy. K_F can be defined as the adsorption or distribution coefficient and represents the quantity of protein adsorbed onto adsorbent for a unit equilibrium concentration. The slope $1/n$, ranging between 0 and 1, is a measure of adsorption intensity or surface heterogeneity, becoming more heterogeneous as its value gets closer to zero. Table 3 presents the results of the Freundlich isotherm for biomolecules adsorption on sepiolite. Freundlich isotherm appears to the experimental data better than the Langmuir isotherm, as reflected with correlation coefficients (R^2) in the range of 0.98–0.99.

3.5 Adsorption kinetics

In order to examine the controlling mechanism of sorption process, several kinetic models were used to test the experimental data. From a system design viewpoint, a lumped analysis of sorption rates is thus sufficient for practical operation.

Sample	Freundlich isotherm parameter			Langmuir isotherm parameter		
	K _F	n	R ²	q _m (mg g ⁻¹)	K (L mg ⁻¹)	R ²
Casein	2.55	2.87	0.99	595,15	5.16	0.79
Catalase	2.43	2.16	0.98	386.15	9.55	0.87

(Experimental cond. 22°C, pH 7, [PO₄³⁻]: 0.02 M, [NaCl]: 0.001 M)

Table 3. Isotherm constants for adsorption of biomolecules onto sepiolite.

3.5.1 Pseudo-first-order equation

The pseudo-first-order equation is generally expressed as follows:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \tag{8}$$

where q_e and q_t are the amounts of biomolecules adsorbed at equilibrium and time t (mg g⁻¹), respectively, and k_1 is the rate constant of pseudo first-order adsorption (min⁻¹). The half-adsorption time of the biomolecule, $t_{1/2}$, is the time required for the oxide samples to take up half as much biomolecule as it would at equilibrium. This time is often used as a measure of the rate of adsorption and given by

$$t_{1/2} = \frac{\ln 2}{k_1} \tag{9}$$

The values k_1 and $t_{1/2}$ are given in Table 4.

3.5.2 Pseudo-second-order equation

If the rate of adsorption is a second-order mechanism, the pseudo-second-order equation is expressed as

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \tag{10}$$

where q_e is the amount of biomolecule adsorbed at equilibrium (mg g⁻¹) and, k_2 is the equilibrium rate constant of pseudo-second-order sorption (g(mg min)⁻¹). The half-adsorption time of the biomolecule, $t_{1/2}$,

$$t_{1/2} = \frac{1}{k_2 q_e} \tag{11}$$

The values k_2 and $t_{1/2}$ are given in Table 4.

3.5.3 Intraparticle diffusion

The fractional approach to equilibrium changes according to a function of $(D_t/r^2)^{1/2}$, where r is the particle radius and D the diffusivity of solute within the particle. The initial rate of the intraparticle diffusion is the following:

$$q_t = f(t^{1/2}) \tag{12}$$

The rate parameter (k_{int}) for intraparticle diffusion can be defined as:

$$q_t = k_{int}t^{1/2} + C \tag{13}$$

where k_{int} is the intraparticle diffusion rate constant ($\text{mg}(\text{g min}^{1/2})^{-1}$) and given in Table 4.

Sample	First-order kinetic equation			$t_{1/2}$ (min)	Second-order kinetic equation			Intraparticle diffusion equation	
	$k_1 \times 10^3$ (min^{-1})	q_e (calculated) mg g^{-1}	r		k_2 g (mg min)^{-1}	q_e (calculated) mg g^{-1}	r	$k_{int} \times 10^2$ $\text{mg (g min}^{1/2})^{-1}$	r
Casein	6.1	248.2	0.99	125	0.02	285.1	0.77	10.3	0.89
Catalase	5.8	260.4	0.99	105	0.08	298.2	0.81	11.7	0.82

Table 4. Some kinetic values calculated for biomolecules adsorption onto sepiolite. (All values are not shown)

3.6 Thermodynamic parameters

Because the k_1 values have been determined, several thermodynamic parameters including the Arrhenius activation energy (E_a), activation free energy change (ΔG^*), activation enthalpy change (ΔH^*), and activation entropy change (ΔS^*) can be calculated by using the following equations and given in Table 5.:

$$\ln k_1 = \ln A - E_a/RT \tag{14}$$

$$\ln k_1 = k_b T K^*/h \tag{15}$$

$$\Delta G^* = -RT \ln K^* \tag{16}$$

$$\Delta H^* = E_a - RT \tag{17}$$

$$\Delta G^* = \Delta H^* - T\Delta S^* \tag{18}$$

where A is the Arrhenius factor, k_B and h are Boltzman’s and Planck’s constants, respectively, R is the gas constant, and K^* is the equilibrium constant at temperature T . A linear plots of $\ln k_1$ versus $1/T$ for the adsorption of casein and catalase onto sepiolite are constructed to generate the E_a value from the slopes.

Sample	E_a (kJmol^{-1})	ΔG^* (kJmol^{-1})	ΔH^* (kJmol^{-1})	ΔS^* ($\text{Jmol}^{-1}\text{K}^{-1}$)
Casein	40.16	248.8	38.01	-711.8
Catalase	43.12	247.1	40.67	-699.7

Table 5. The thermodynamics parameters of biomolecules onto sepiolite surface for 295K

3.7 Kinetic parameters of catalase

K_M and R_{max} are characteristic to an enzyme and are quite sensitive to the conditions under which the reaction takes place. According to the following equation, derived from Michaelis – Menten rate law:

$$\frac{1}{R} = \frac{K_M}{R_{\max}} \frac{1}{[S]} + \frac{1}{R_{\max}} \tag{19}$$

A plot of 1/R versus 1/[S], called a Lineweaver-Burk plot, would be a straight line with a slope equal to K_M/R_{\max} and intercept equal to $1/R_{\max}$. Kinetic constants of adsorbed and pure catalase are given in Table 6.

Kinetic constants	Pure	Adsorbed
K_m (mM)	172,09	24,21
R_{\max} (mM min ⁻¹)	10,10	1,25

Table 6. Kinetic constants of pure and adsorbed catalase

As seen from Table 6, the K_m and R_{\max} values of the adsorbed enzymes are lower than pure enzymes.

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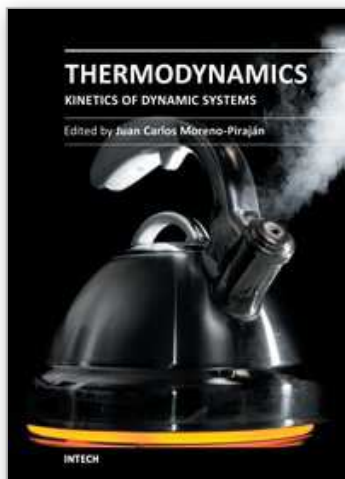
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