

Removal of Cysteine (an Aliphatic Amino Acid) by Natural Clinoptilolite

Faghihian, Hossein*⁺

Department of Chemistry, University of Isfahan, Isfahan, I.R. IRAN

Nejati-Yazdinejad, Massoud

Department of Chemistry, University of Zabol, Zabol, I.R. IRAN

ABSTRACT: Amino acids have been extensively used in several processes of the pharmaceutical and food industries. Treatments for the recovery and reuse of the wastewaters generated from these processes are few and little known. This work aims at studying the influence of different variables on the sorption of L-cysteine, contained in aqueous solutions, on clinoptilolite mineral. L-Cysteine (Cys) was sorbed by Clinoptilolite (Clino) from aqueous solutions of variable concentrations, temperatures and pHs. The powder X-Ray Diffraction (XRD), InfraRed (IR) spectroscopy and thermal analysis (TG, DTG and DSC) techniques were applied for characterization of materials. The results indicated that the sorption process depends strongly on pH, temperature and concentration of amino acid. Cysteine is specifically sorbed on clinoptilolite surface sites, probably through its NH_3^+ moiety. The pH dependence suggests that these sites may be silanolate groups ($\equiv\text{Si-O}^-$). The experimental data of sorption isotherms obtained and analyzed with Langmuir model showed that this model fitted the sorption data. Calculated thermodynamic parameters (ΔH° , ΔS° , and ΔG°) indicate that the sorption processes were exothermic.

KEY WORDS: Clinoptilolite, Sorption, Amino acid, Cysteine, Isotherm, Thermodynamics.

INTRODUCTION

Natural zeolites are crystalline aluminosilicates, the primary building units of which are $[\text{SiO}_4]^{4-}$ and $[\text{AlO}_4]^{5-}$ tetrahedra linked together by corner-sharing, forming bent oxygen bridges. The electrical imbalance arising from the substitution of Si^{4+} by Al^{3+} in the structure of zeolites is compensated by balancing cations held electrostatically within the zeolite. These cations are not an integral part of the zeolite Si/Al-O framework and are usually called exchangeable cations, since they are fairly mobile and readily replaced by other cations. A variety

of cations can be adsorbed on zeolites by the cation exchange mechanism. Therefore, natural zeolites are known as efficient sorbents for water cationic pollutants. Numerous applications of natural zeolites arise from their properties of molecular sieves and cation exchanges. The availability and low cost of natural zeolites has stimulated further research for new applications [1-6].

Zeolites can be modified by introduction of new functional groups in order to improve its activity and selectivity for the removal of several substances [7,8].

* To whom correspondence should be addressed.

+ E-mail: h.faghihi@sci.ui.ac.ir

• Other address: Department of Chemistry, Shahreza Branch, Islamic Azad University, Shahreza, I.R. IRAN
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Many authors studied the use of zeolites as such (natural) in environmental applications mainly to remove ions from wastewater by ionic exchange processes [9,10]. Among them, clinoptilolite has received much attention due to its widespread occurrence, high selectivity for Cs, and Sr, as well as its effectiveness in removing these radioisotopes from wastewaters [3,11]. The composition, purity and mineralogical characteristics of clinoptilolite may vary widely from one deposit to another and even within the same deposit. The most common cations in clinoptilolite are Na^+ , K^+ , Ca^{2+} , and Mg^{2+} , therefore the selectivity and ion exchange rate greatly dependent on the type, number and the location of these cations.

Amino acids like cysteine are used in several sections of chemical, pharmaceutical, and food industries, where the treatment of industrial wastewaters is little practiced. Besides, cysteine is a major components of Metallo Thioneins (MTs), a family of Cys-rich, low molecular weight (MW ranging from 3500 to 14000 Da) proteins. MTs have the capacity to bind both physiological (Zn, Cu, Se,...) and xenobiotic (Cd, Hg, Ag,...) heavy metals through the thiol group of its cysteine residues, which represents nearly the 30% of its amino acidic residues [12,13]. Furthermore, it is the principal metal binding unit of metallothioneins. Metallothionein proteins participate in the uptake, transport, and regulation of zinc in biological systems. Metallothionein detoxifies mercury and heavy metals by binding to the metal before it can cause harm. Cysteine residues from metallothioneins can capture harmful oxidant radicals as hydroxide radical. In this paper we report a study of the sorption of L-cysteine ($\text{HS-CH}_2\text{CH}(\text{NH}_2)\text{COOH}$) by clinoptilolite under a range of solution conditions to provide further insight into the sorption kinetics, thermodynamics and capacity. The present data will provide information about the amino acid retention by clinoptilolite. This study is also a part of a wider investigation of the effect of this amino acid on the sorption of heavy metals on clinoptilolite mineral.

EXPERIMENTAL SECTION

Materials and methods

The natural clinoptilolite used as the sorbent in the present study was clinoptilolite tuff from the deposits of the Semnan region, Iran. It was crushed and washed with dilute HCl solution (0.1 M) to remove dirt and other

Table 1: Chemical, mineralogical and physico-chemical characteristics of the natural sorbents.

Chemical composition (%)	
SiO_2	68.98
Al_2O_3	11.41
Fe_2O_3	1.11
CaO	2.44
K_2O	1.61
MgO	1.26
Na_2O	1.50
TiO_2	0.18
MnO	0.02
SrO	0.13
LOI	11.38
Mineralogical composition (%)	Clinoptilolite (44.5)
	Cristobalite (36)
	Albite (17.3)
	Quartz (2.1)
Specific surface area (m^2/g)	16.3
CEC (meq/g)	1.41

LOI: loss on ignition.

water-soluble impurities, such as calcite, and then was air dried, ground and only the particle size lower than $71 \mu\text{m}$ was used for adsorption experiments. L-cysteine (Merck) had a purity of $> 99\%$. All other chemicals were of analytical grade and were purchased from available commercial sources and used as obtained. Water used in the experiments was deionised.

Chemical analysis of natural clinoptilolite was carried out by X-Ray Fluorescence (XRF) using a Bruker S4 PIONEER spectrometer. powder X-Ray Diffraction (XRD) patterns were collected on a Bruker D8 ADVANCE X-ray diffractometer ($\text{Cu K}\alpha$). Cation Exchange Capacity (CEC) was determined by back titration method [14] based on the saturation of the sample by H^+ ion. Specific surface area was determined by the BET method, using a Monosorb surface area analyzer. Chemical, mineralogical and physico-chemical characteristics of the sample is summarised in Table 1.

Cations in solution were analyzed by a Shimadzu atomic absorption spectrophotometer (AA-670).

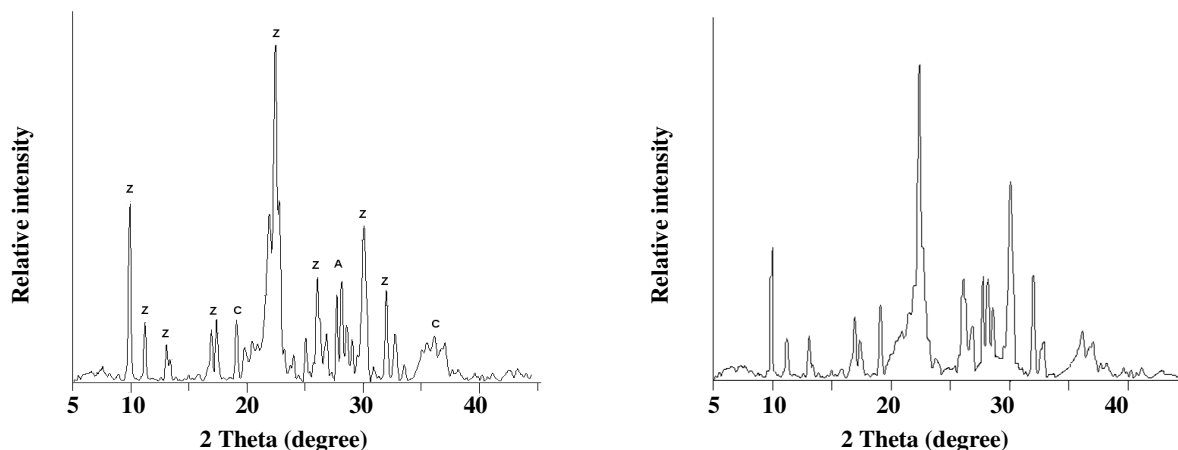


Fig. 1: a) XRD pattern of clinoptilolite (Z =clinoptilolite, C = cristobalite, A = albite). B) XRD pattern of modified clinoptilolite.

IR spectra were recorded in air at room temperature on a Shimadzu infrared spectrophotometer (IR-435). The spectra were measured in KBr pellets. TG, DTG and DSC were performed under nitrogen flow (10 mL/min) and with a heating rate of 25 °C/min on a Mettler instrument (TA 4000). The HPLC system employed in this work consisted of a Unicam liquid chromatograph (Crystal 200) and an injector with a 50 µl sample loop. The column used was a Spherisorb 250 mm x 4.6 mm I.D., from Unicam with a solution of 0.01 M phosphate buffer (pH=4.0) as mobile phase. The Cation Exchange Capacity (CEC) of the material was 190meq/100g measured with the ammonium acetate method, with Na⁺, K⁺, Ca²⁺, and Mg²⁺ being the exchangeable cations in cavities [15].

Sorption experiments

Sorption of amino acid by clinoptilolite was studied by batch technique. In a typical sorption experiment, 1.0 g of the mineral was added to 100 mL of an aqueous solution of cysteine of the suitable concentration and stirred at room temperature for 24 h. After sorption, the solid was separated by centrifugation and dried under room temperature. For the experiments carried out at variable pH, the pH of the solution was adjusted using appropriate amounts of aqueous solutions of HNO₃ (0.1M) or NaOH (0.1 M).

The amount of sorption of cysteine can be determined in terms of distribution coefficient (K_d), percentage of sorption or amount of amino acid sorbed per unit weight of the sorbent (q_e). The distribution coefficient, K_d is defined as the concentration of a species sorbed per gram

of the sorbent divided by its concentration per mL in liquid phase:

$$K_d = \frac{(C_i - C_e)}{C_e} \left(\frac{V}{m} \right)$$

$$q_e = (C_i - C_e) \left(\frac{V}{m} \right)$$

Where, C_i and C_e are the initial and equilibrium concentration of amino acid respectively, m is the mass of the sorbent in g, and V is the volume of the solution used for equilibration in ml. The concentration of the amino acid and the exchanged cations in the supernatant solution were determined by HPLC and Atomic Absorption Spectrophotometric (AAS) method, respectively [16,17].

RESULTS AND DISCUSSION

X-ray diffraction

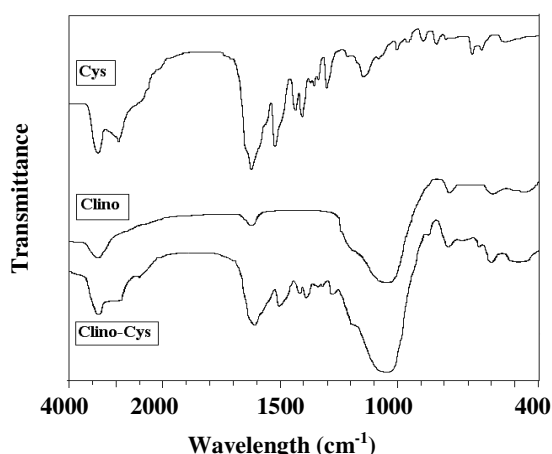
The X-ray diffraction pattern of natural clinoptilolite is given in Fig. 1a. The figure indicates that the sample is composed primarily of clinoptilolite, in addition to cristobalite and albite. It must be noted that under the applied cysteine concentrations, we did not observe a considerable change in d_{001} of clinoptilolite with increasing concentration (Fig. 1b).

InfraRed (IR) spectroscopy

Wavenumber and vibration type of clinoptilolite is given in Table 2 [18]. The IR spectra of the samples are shown in Fig. 2. The Cys-Clino spectrum is dominated by the bands of the host material, but the presence of the amino acid is also observed. Nevertheless, closer

Table 2: IR vibrations of clinoptilolite

Wavenumber (cm ⁻¹)	Vibration type
~ 3600	O–H stretching (Si, Al)–OH
~ 3400	O–H stretching H–OH
~ 1650	H–O–H deformation
~ 1150	Si–O stretching
~ 1050	Si–O–Si stretching
~ 520	Si–O–Si deformation

**Fig. 2: IR spectra of cysteine, clinoptilolite and Cys-Clino.**

inspection of the 1700-1300 cm⁻¹ of the Cys-Clino and comparison with the same wavenumber interval of the amino acid and that of the host material reveal that guest species are sorbed by the sample.

In relation to the interaction between clinoptilolite and cysteine, the peaks at 3500-2500 cm⁻¹ are probably N-H stretching vibration. The wide O-H band (~ 3500 cm⁻¹) belonging to the carboxyl in the structure is invisible because it fits into the O-H stretching of amino acid and water in clinoptilolite. The spectrum of Cys-Clino showed that the bands at 1340 (symmetric deformation of NH₃ group), 1520 (deformation of N-H group), 1740 (stretching of C=O), 680 (stretching of C-S) and 2560 cm⁻¹ (stretching of S-H) decreased in intensity [19,20]. Thus, all these results may be caused by the interaction between clinoptilolite and the NH₂ group of cysteine.

Thermal Analysis

The TG/DTG curves of clinoptilolite, cysteine and Cys-Clino are shown in Figs. 3 and 4. The DSC curve of Cys-Clino is shown in Fig. 5. The weight loss peak

observed below 200°C was a function of the loss of free water in clinoptilolite. Between 200 and 550°C, clinoptilolite did not undergo any thermally induced changes. Therefore, the peaks in this region for organo-clinoptilolite were attributed to the decomposition of the amino acid [21,22].

The mass-loss over the temperature from 550 to 650°C is ascribed to the loss of dehydroxylation of the structural OH units of the clinoptilolite. Except the loss of water from clinoptilolite, the Cys-Clino remained intact up to 250-300°C. The distinct exothermic peak above 600°C is solely due to combustion of the amino acid. These curves also demonstrate the sorption of amino acid onto the clinoptilolite.

Effect of pH

The most important parameter influencing the sorption of cysteine is the pH of the medium. Sorption of cysteine (25 mM) by sorbent as a function of pH is shown in Fig. 6. This was studied by varying the pH of aqueous solution of clinoptilolite suspension from pH 2 to 8 at 25°C. Maximum sorption yield was observed at pH_{max} 4-5 which could be due to cysteine (isoelectric point (pI = 5.07), pK (-COOH) = 1.96, pK (-SH) = 8.18, pK (-NH₃⁺) = 10.28) [23] and clinoptilolite nature. At this pH, the amine group of cysteine mainly exists as protonated form.

The point of zero charge (pHpzc) is the pH at which there are equal numbers of cations and anions present so that the surface has effectively no charge. This pH for clinoptilolite is generally reported as being around 3 [24]. Hence, the charge on the clinoptilolite surface would be close to zero at pH 3 and become increasingly negative as the pH increases, explaining the increasing amounts of sorbed cysteine. However the amino acid sorption decreases sharply when the pH increases from 5 to 8. The decline in adsorption at pH > pH_{max} is caused by reduction in concentration of cationic form of amino acid (pI=5.07). In pH < pH_{max} the competition between amino acid and hydrogen ions for active sites is further evident from Fig. 6.

Effect of contact time

The effect of contact time was studied at constant amino acid solution concentration (25 mM) and solid ratio 100 mL/g, at 25°C and pH 4. The results are shown in Fig. 7.

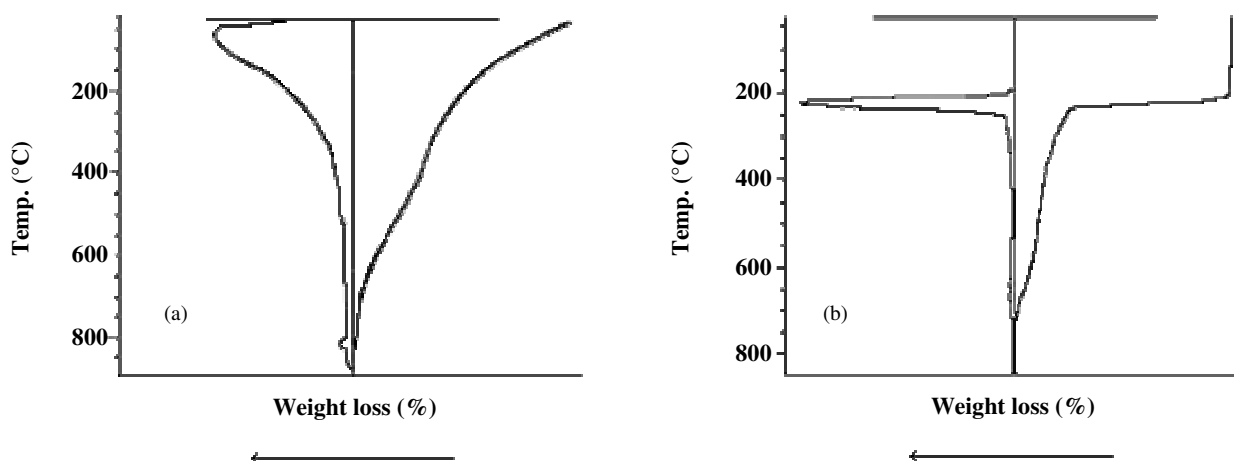


Fig. 3: TG and DTG curve of clinoptilolite (a) and Cysteine (b).

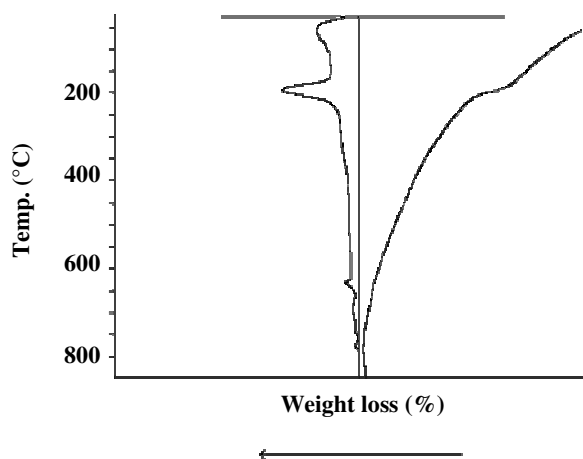


Fig. 4: TG and DTG of Cys-Clino.

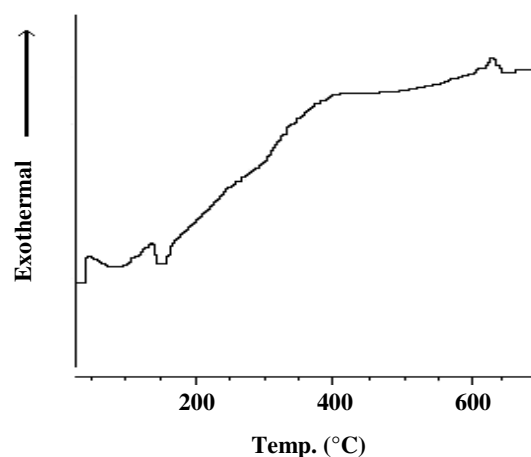


Fig. 5: DSC curve of clinoptilolite (a) and Cys-Clino (b).

It is seen that there was only a small difference between 24-30 hours which assumes that the sample has reached equilibrium with the solution. Maximum amino acid removal values were observed at this interval contact time.

Effect of temperature

Effect of temperature was also studied for the amino acid in the range of 20-50°C (Fig. 8). These results illustrates the effect of temperature for amino acid uptake on clinoptilolite at pH 4. Room temperature was found to be the most suitable for maximum amino acid sorption. This figure also shows that the amount of sorption decreased with increasing of temperature, so, the sorption of the amino acid on clinoptilolite is an exothermic process.

Thermodynamic parameters

The values of ΔH° and ΔS° were calculated from the slope and intercept of the linear variation of $\ln K_d$ with the reciprocal of the temperature, $1/T$, by using the relation [25]:

$$\ln K_d = -(\Delta H^\circ/RT) + (\Delta S^\circ/R) \quad (1)$$

The thermodynamic values are given in Table 3. The free energy of the specific sorption, ΔG° is calculated from:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (2)$$

The Gibbs free energy change is the driving force and the fundamental criterion of spontaneity. The value of $\Delta G^\circ = -5.53$ kJ/mol at 25 °C was calculated for this system. The negative value of ΔG° imply that the sorption of this

Table 3: Thermodynamic values for sorption process.

ΔH° (kJ/mol)	ΔS° (J/mol)	ΔG° (kJ/mol)			
		293 K	303 K	313 K	323 K
-15.48	-33.41	-5.53	-5.36	-5.03	-4.69

Table 4: Langmuir parameters for sorption process.

Solute	Adsorbent	K (L/mmol)	q° (mmol/g)	Degree of fit (r^2)
Cysteine	Clinoptilolite	0.533	0.17	0.9955

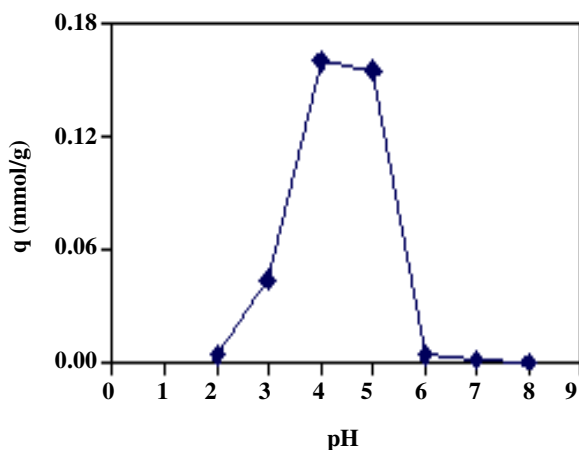


Fig. 6: Effect of pH on cysteine sorption ($C_i = 25$ mM, at 25°C , equilibrium time 24 h).

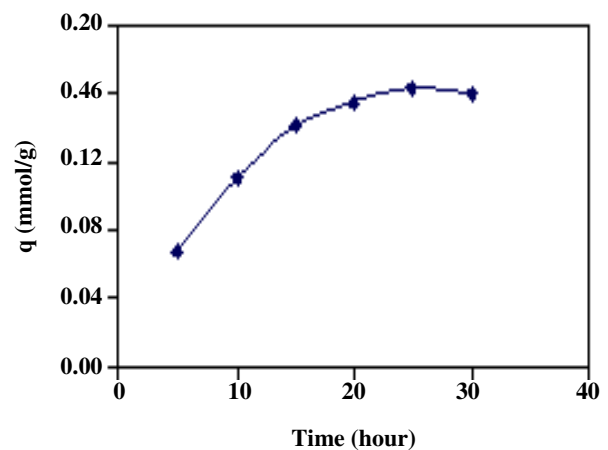


Fig. 7: Effect of contact time on cysteine sorption ($C_i = 25$ mM, at 25°C).

amino acid by the clinoptilolite mineral is spontaneous and becomes more favorable with temperature decrease. It has been reported that ΔG° up to -20 kJ/mol is consistent with electrostatic interaction between sorption sites and the sorbent [26]. The values of ΔS° is 33.41 J/mol K. The negative value of ΔS° indicates the decrease in randomness as a consequence of sorption. The value of ΔH° is -15.48 kJ/mol indicating that the sorption process is exothermic.

Sorption isotherm

The sorption isotherm was obtained at constant temperature (25°C) at various amino acid concentrations (0.5-100 mM) while keeping all parameters constant. Several common sorption models including Langmuir were considered to fit the data for sorption of amino acid on clinoptilolite. Langmuir isotherm was tested in the following linearized form for solid-liquid systems:

$$\frac{C_e}{q_e} = \frac{C_e}{q^\circ} + \frac{1}{Kq^\circ} \quad (3)$$

where K is affinity or binding constant (L/mmol) and q° is the maximum sorption capacity (mmol/g). At low concentrations the Langmuir equation reduces to a linear relationship, while the maximum sorption capacity, q° is attained at concentrations corresponding to monolayer coverage. The sorption parameters of the sorbent (q° , K and r^2) are shown in Table 4.

The observed linear plots of C_e/q_e versus C_e indicates that the sorption of Cys on clinoptilolite proceeds in a monolayer fashion according to Langmuir model. The regression coefficient ($r^2 = 0.9955$) confirms good agreement between the theoretical model and experimental results. Values of q° and K were calculated from the intercept and slope of the linear plot. The value of q° (0.17 mmol/g) are in consistency with the values experimentally obtained. The value of the Langmuir binding constant (K) was 0.533 L/mmol.

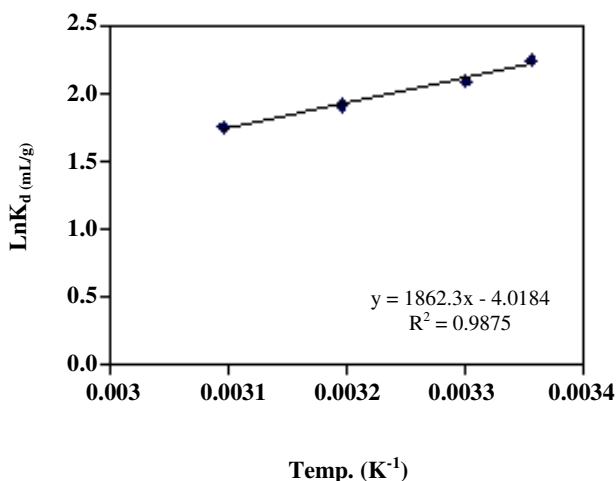


Fig. 8: Effect of temperature on cysteine sorption ($C_i = 25$ mM, equilibrium time 24 h).

CONCLUSIONS

The present study indicates that the removal of cysteine from dilute aqueous solution by clinoptilolite depends on amino acid concentration and some other sorption parameters such as pH, contact time and temperature. The uptake and cation exchange capacity of clinoptilolite were determined. The temperature variation has been used to evaluate the values of ΔG° , ΔH° and ΔS° . The uptake equilibrium is best described by Langmuir sorption isotherm. Values of ΔG° and ΔH° show the spontaneous and exothermic nature of the sorption process. The great advantage of clinoptilolite is economical sorbent for this amino acid. We report this study as part of a subsequent wider investigation of the effect of this amino acid on the sorption of heavy metals to the modified clinoptilolite.

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