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Batch Studies on Biosorption of Ciprofloxacin on Freshwater Macro Alga *Lemna minor*

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ABSTRACT

Objectives: Ciprofloxacin (CIP) antibiotic adsorption from aqueous solution unto *Lemna minor* (LM) was studied. The aim of the experiment was to utilize the LM as a cheap alternative adsorbent compared to more expensive and relatively less effective techniques for the removal of CIP from solution. **Methods:** The experiment was performed using batch adsorption technique. Thermodynamic parameters such as the changes in enthalpy, entropy and Gibbs free energy was determined, showing adsorption to be an endothermic yet spontaneous process. Pseudo-first-order, pseudo-second-order and intraparticle diffusion models were considered to evaluate the rate parameters. **Results:** The experimental data fitted the pseudo-second-order kinetic model, with qe between 4.31 to 19.62 mg/g at concentrations between 10 to 50 mg/L. also due to the inter-particle diffusion, It can be stated that mechanism of the adsorption process is probably a combination of boundary layer and pore diffusion which contribute to the rate determining step. **Conclusion:** The results indicate that LM biomass could be employed as a low-cost in wastewater treatment for the removal of antibiotics.

Key words: Ciprofloxacin, *Lemna minor*, Thermodynamics, Kinetics.

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INTRODUCTION

Contamination of surface and even ground water is becoming an increasingly serious problem worldwide because of the growing population and wide use of chemicals, including pharmaceuticals.[1,2] Pharmaceuticals are emerging contaminants and have been used extensively in human and veterinary medicine.[3] Most antibiotics are incompletely metabolized; thus, their residues and degradation products are excreted and can enter water environments in various ways.[4] Exposure to the residues and metabolites of antibiotics may cause a variety of adverse effects in the environment, such as antibiotics resistance of micro-organisms and chronic and acute toxicity for organisms.[5,6] Therefore removal of antibiotics from aqueous effluents has received much attention within environmental research.[7]

Antibiotics are among the most widely used pharmaceuticals and, according to the chemical structure of sub-categories, can be divided into beta-lactams, quinolones, tetracyclines, aminoglycosides, macrolides and sulfonamides.[8] Fluoroquinolones (FQs) were among the most used antibiotics applied to human therapy and veterinary treatment.[9] Due to the continuous increase of global needs and inappropriate discharge, they were frequently detected in a variety of natural environments.[10] Ciprofloxacin (CIP), one of the commonly used FQs, was found in the wastewater and surface water.[11]

Various techniques have been used for the removal of drugs and antibiotics from contaminated water which include chemical precipitation, ion exchange, solvent extraction, adsorption, membrane filtration, reverse osmosis, chemical oxidation and reduction and evaporation.[12,13] Most of these techniques are sometimes ineffective and involves high capital cost, which are not suitable for small scale industries.[14]

Adsorption is a very effective separation technique in terms of initial cost, simplicity of design and ease of operation and insensitive to toxic substances.[15,16] Activated carbon is the most efficient adsorbent used for antibiotics removal. But it is expensive to produce and regenerate.[17,18] During the last decade, a number of non-conventional, low-cost adsorbent such as rice hull ash, canola, *Lemna minor*, sawdust, azolla filiculoides and eucalyptus bark have been used for the removal of antibiotics from aqueous solution.[19,20]

Lemna minor, the common duckweed, is an aquatic freshwater plant of the genus *Lemna*. They belong to the family of *Lemnaceae*, which are monophyletic to the *Araceae* family.[21] As more leaves grow; the plants divide and become separate individuals. It is present wherever freshwater ponds and slow-moving streams occur.[22] Growth of colonies is rapid and the plants form a carpet covering still pools when conditions are suitable.[21] *Lemna minor* is used as animal fodder, wastewater nutrient recovery and other application.[22]

In this paper, the possibility of using LM to remove CIP was investigated using batch adsorption studies. The effects of various factors such as contact time, concentration and temperature on the removal efficiency of CIP onto LM were also studied. The kinetic and thermodynamic data were analyzed so that we can understand the adsorption mechanism and different models were applied to fit the experimental data.

MATERIALS AND METHODS

All the analytical grade reagents were used in this study. The ciprofloxacin (99%) (CAS Number=723-46-6; chemical formula: $C_{17}H_{18}FN_3O_3$; molecular weight=331.35 g/mol; maximum wavelength=277 nm) were obtained from Sigma-Aldrich Ltd., USA and used as received without any further purification. Stock solutions of 1000 mg/L were prepared by dissolving proper amounts of CIP in double distilled deionized water. To prepare the solutions of suitable concentration 10–100 mg/L of CIP, the

stock solution was diluted with double distilled water. The Regulation of pH was undertaken using 0.1 N HCl and 0.1 N NaOH.

Lemna minor was collected from a local source in the north of Iran. LM were washed several times with distilled water to remove surface impurities, dried at 100°C overnight, crushed by a hammer mill and sieved.

Batch adsorption experiments were carried out typically by stirring 0.25 g of LM with 100 mL of unbuffered CIP solution (pH 6.5) in 200 mL glass cells. Experiments were also carried out to determine the effects of varying both the contact time and CIP concentration of the solution. Working solutions of CIP were prepared from the stock solution (1000 mg/L) to the desired concentrations (10-50 mg/L) for each experimental run. All the experiments were carried out at a constant speed of 100 rpm with mechanical stirring. The adsorption study was conducted at three different temperatures (273, 283, 293, 303, 313 and 323 K) in a thermo-stated system, with an outer circulating-water bath. During each run, aliquots of 0.1 ml were withdrawn from the solutions at regular intervals of time, diluted and centrifuged for 10 min at 3000 rpm and the absorbance of the supernatant solution was measured. The CIP concentration was estimated spectrophotometrically by using HPLC at absorbance at λ_{max} = 277 nm. The mobile phase was 0.05 M phosphoric acid/acetonitrile with a volumetric ratio of 87/13 with an injection flow rate of 1 mL/min.

The amount of CIP adsorbed by the LM in each time interval t (q_t), was calculated by the following mass balance equation:[20]

$$q_t = (C_0 - Ct)V/m \quad (1)$$

Where q_t is the amount of CIP adsorbed per unit weight of LM at any time *t* (mg/g); C_0 and C_t are, respectively, the initial and liquid-phase concentrations of the CIP solution at any time t (mg/L); V is the volume of the CIP solution and m is the mass of the LM used.

RESULTS

Kinetic studies: The uptake of pollutants such as drugs from aqueous solutions by use of adsorbent is called adsorption. The prediction of batch adsorption kinetics provides the most important information for designing adsorption systems. Kinetics includes the search for the best model that well represents the experiment data as a function of environmental conditions. In the present study, pseudo-first and second-order kinetic and Intraparticle diffusion models were applied to describe the adsorption of CIP on LM as a function of initial CIP concentrations and contact time.

The pseudo first-order Lagergren equation is given as:[22]

$$\frac{dq}{dt} = k_1(q_e - q_t) \quad (2)$$

Where q_e and q_t (mg/g) are the amount of adsorbed CIP on the adsorbent at equilibrium and at time t. K_1 is the rate constant of pseudo first-order kinetic model. For applying boundary conditions t=0 to t and q=0 to qt the integrated form of Eq. (2) leads to:[23]

$$\log(q_e - q_t) = \log q_e - \frac{k_t}{2.303}t \quad (3)$$

The parameters and correlation coefficients of pseudo first order kinetic model for the adsorption of CIP were determined and summarized in Table 1. The values of K_1 and q_e, can also be determined from the plot of log (q_e-q_t) vs. t (Figure 1). It was suitable to describe the whole adsorption process of CIP on LM, but there was significant difference between experimental and predicted q_e values from first second-order.

The pseudo second-order model is related with the sorption capacity of adsorbent. The pseudo second-order kinetic model is represented as:[20]

$$\frac{dq_t}{dt} = K_2(q_e - q_t)^2 \quad (4)$$

Where K_2 is the pseudo second-order rate constant (g/mg.min). For boundary conditions t=0 to t and q=0 to qt the integrated form of Eq. (4) leads to:[24]

$$\frac{t}{qt} = \frac{1}{k_2 qe^2} + \frac{t}{q_e} \quad (5)$$

The parameters (K_2 and q_e) obtained from pseudo second-order kinetic model, correlation coefficient (R^2) are given in Table 2. The values of K_2 and q_e, can also be determined from slope and intercept of t/q_t vs. t plot (Figure 2). From Table 1, there was no significant difference between experimental and predicted q_e values from pseudo second-order, unlike the number obtained from the pseudo-first order model. The pseudo-second-order kinetic model could be regarded as sufficient to describe the adsorption of CIP on LM.

To predict the rate determining step in the adsorption process of CIP, Intraparticle diffusion equation (Eq 6) was used as follows:[25]

$$q_t = K_d t^{1/2} + C \quad (6)$$

Where K_d (mg/g $min^{0.5}$) is the intraparticle diffusion rate constant and C (mg/g) is proportional to the boundary layer thickness. The constants K_d and C are obtained from the slope and intercept of the straight line of q_t vs. $t^{1/2}$ (Figure 3) and listed in Table 1.

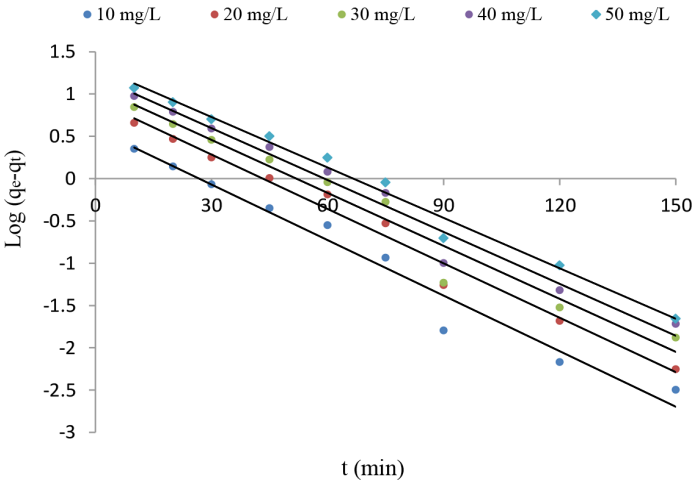

Figure 1: Pseudo-first-order of CIP adsorption onto LM at different concentration.

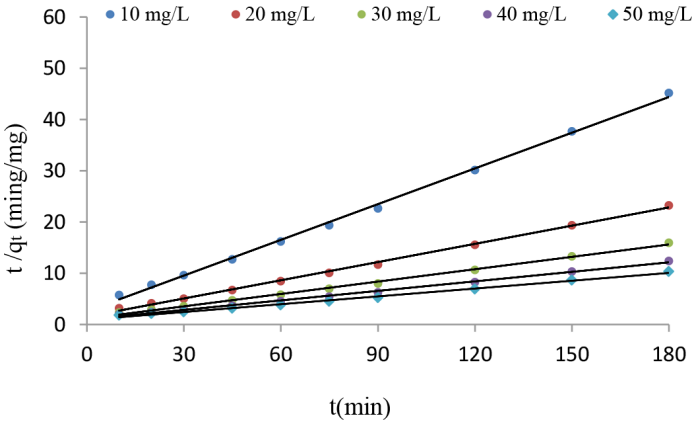

Figure 2: Pseudo-second-order of CIP adsorption onto LM at different concentration.

Table 1: The results of kinetic model studies related to the CIP adsorption onto LM.

CIP Concentration (mg/L)	$(q_e)_{exp}$ (mg/g)	Intraparticle diffusion Model			Pseudo-first order			Pseudo-second order		
		K_d	C	R^2	$(q_e)_{cal}$	K_1	R^2	$(q_e)_{cal}$	K_2	R^2
10	3.96	0.193	1.853	0.74	3.36	0.048	0.948	4.31	0.0208	0.998
20	8.22	0.398	3.33	0.759	6.47	0.041	0.954	8.474	0.0092	0.997
30	12.14	0.614	4.458	0.765	10.25	0.035	0.943	12.5	0.0055	0.997
40	15.41	0.839	5.196	0.769	16.21	0.024	0.953	16.39	0.0037	0.995
50	18.29	1.063	5.536	0.785	21.08	0.019	0.959	19.62	0.0027	0.994

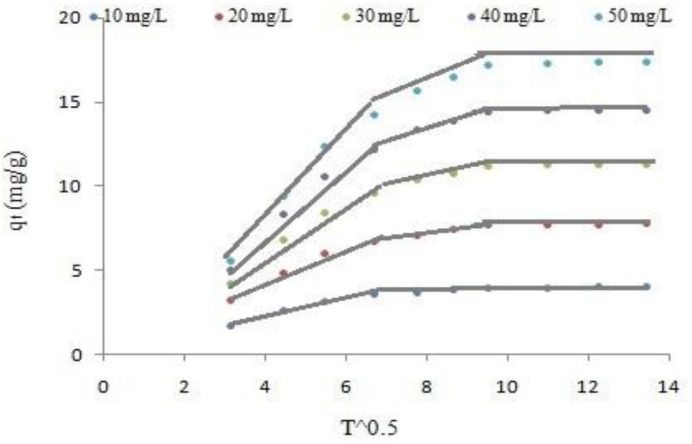

Figure 3: Intraparticle diffusion of CIP adsorption onto LM at different concentration.

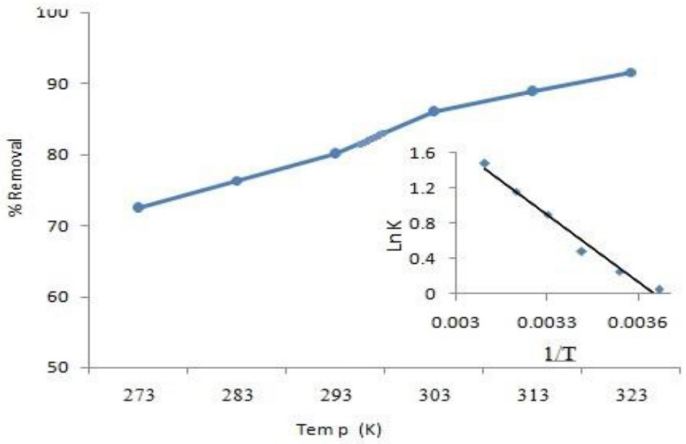

Figure 4: Effect of temperature and Van't Hoff plot for CIP adsorption on LM.

Thermodynamic studies: Figure 4 illustrates the effect of temperature on the adsorption of CIP, initially at 50 mg/L, onto LM as a function of time. The equilibrium adsorption capacity was clearly affected by temperature, with the amount of CIP adsorbed increasing from 14.82 (72.41% removal) to 18.32 mg/g (91.6% removal) when the temperature was raised from 273 to 323 K. This 19% increase in CIP removal for a 50°C rise in temperature indicates that temperature has a significant effect on adsorption.

The thermodynamic modeling study provides significant information about the change in entropy and enthalpy during the process. The thermodynamic parameters provided information about the spontaneous nature of CIP adsorption. Van't Hoff equation was used to evaluate the thermodynamic parameters such as change in enthalpy (ΔH^o), entropy (ΔS^0) and Gibb's free energy (ΔG^0) during the process.[25,26]

$$G^0 = -RT\ Ln\ K_d \quad (7)$$

$$Ln\ K_d = (\Delta S^0/R) - (\Delta H^0/RT) \quad (8)$$

Where R is gas constant = 8.314 J/mole.K, ΔG^0 is kJ/mole, T= temperature (K), ΔS^0 is kJ/mol K, ΔH^0 is the kJ/mole and K is the equilibrium constant (amount on biosorbent/amount in solution). On the basis of a plot of Ln K_L versus 1/T (Figure 4), ΔS^0 can be estimated from the intercept and ΔH^0 from the slope of what should be a straight line passing through the points. Figure 4 shows just such a plot with a correlation coefficient of 0.981.

The values of Thermodynamic parameters as ΔH^o, ΔS^0 and ΔG^0 are summarized in Table 2. The ΔS^0 and ΔH^o values are thus found to be 0.077 KJ/mol.K and 21.38 KJ/mol, respectively.

Table 2: Values of thermodynamic parameters for the adsorption of CIP onto LM.

Temperature (K)	G^0 (KJ/mol)	H^0 (KJ/mol)	S^0 (KJ/mol.K)
273	-0.11		
283	-0.58		
293	-1.08	21.38	0.077
303	-2.02		
313	-2.62		
323	-3.34		

DISCUSSION

The contact time between adsorbate and adsorbent is the most important design parameter that affects the performance of adsorption processes. The equilibrium was found to be nearly 90 min when the maximum CIP adsorption capacity was reached. The rapid adsorption at the initial contact time was due to the availability of more active surface of the adsorbents, which leads to fast adsorption of the CIP from the solution.[27] The later slow rate of CIP adsorption probably occurred due to the less availability of active site onto the surface of adsorbent as well as the slow pore diffusion of the solute into the adsorbent.[28] Similar results were observed by Mahvi who investigated the effect of contact time on removal of tetracycline from aqueous solution by azolla and indicated that adsorption increases with increasing contact time.[19]

There was an inverse relationship observed between the pseudo second-order rate constant K_2 and initial CIP concentrations values. These results also observed in previous adsorption studies.[29,30] A pseudo-second-order

kinetic model with high R^2 values exhibited well fitting to experimental data, in agreement with findings of Balarak *et al.*[27] and Zhang *et al.*[28]

Intraparticle diffusion is the rate limiting step, when the plot passes through the origin (C = 0). If the value of C is higher than zero, difference in the rate of mass transfer during initial and final stages occurred. It can be seen from Figure 3 that the plot do not path through the origin (the value of C>0), hence the mechanism of the adsorption process is probably a combination of boundary layer and pore diffusion which contribute to the rate determining step.[31,32] Therefore, the adsorption of CIP onto LM surface is a complex, involving more than one mechanism as shown in Figure 3.

Temperature has two main effects on the adsorption process. It is known that an increase in temperature increases the diffusion rate of the adsorbate molecule through the outer boundary layer and into the pores of the adsorbent particles due to the reduced viscosity of the solution.[33] The study of temperature dependence on the adsorption process provides valuable information about adsorption-related changes in enthalpy and entropy.

Effect of temperature results showed the adsorption of CIP onto the MC adsorbent surface is an endothermic process.[34] Before equilibrium was reached; increased temperature led to an increase in the rate of CIP adsorption, implying a kinetically controlling process, as found in many other systems.[35,36]

Also decrease of the temperature allows the CIP to desorb from the interface to the solution or the damage of active binding sites in the adsorbent which explain the decrease in the retention of CIP. So, the optimum temperature has been selected as 323 K for further adsorption experiments and results of this study agreement with findings of Balarak *et al.*[4] and Danalıoğlu *et al.*[1]

The positive value of H^o confirms the endothermic nature of the adsorption process, as has been found in most cases.[37,38] This feature may be an indication of the occurrence of monolayer adsorption.

The positive value of ΔS^0 indicates the increased uncertainty at the solid–solution edge throughout the fixation of the CIP on the active binding sites of the LM. Whatever the explanation for the positive entropy change, this is the factor leading overall to a negative free energy change. Adsorption processes with ΔG^0 values in the -20 to 0 KJ/mol range correspond to spontaneous physical processes, while those with values in the −80 to −400 KJ/mol range correspond to chemisorption.[39,40] As ΔG^0 changed from -0.11 to -3.34 KJ/mol when the temperature increased from 273-323 K, It can be concluded that the adsorption mechanism is dominated by physisorption, in keeping with the finding that the adsorption is rapid and more spontaneous at higher temperature.

CONCLUSION

In this study, LM shows promising adsorption capacity for CIP removal. The operating parameters for the maximum sorption were CIP solution concentration (10 mg/L) and temperature (323 K). The rate of sorption was found to obey pseudo-second order kinetics model with a good correlation coefficient. The negative G^0 values indicated that the sorption of CIP onto LM biomass was feasible and spontaneous. The positive H^0 value depicted endothermic nature of the sorption.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

LM: *Lemna minor*; **CIP:** Ciprofloxacin.

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