

Adsorption and degradation of enrofloxacin, a veterinary antibiotic on natural zeolite

Havva Merih Ötker, Işıl Akmeht-Balcıoğlu*

Bogazici University Institute of Environmental Sciences, Bebek, Istanbul, Turkey

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Abstract

The adsorption of enrofloxacin, a veterinary antibiotic onto natural zeolite and further decontamination of zeolite was investigated in the present study. In the first part of the study, the effects of pH, temperature, and presence of ammonium ion on the adsorption process were examined and evaluated on the basis of Langmuir and Freundlich isotherms. Adsorption of enrofloxacin on natural zeolite was found to be highly pH dependent, exhibiting increases correspondent to decreases in pH. The positive value of enthalpy change showed the endothermic nature of adsorption processes. The presence of ammonium ion enhanced the adsorption of enrofloxacin. In the second part of the study, infrared (IR) spectroscopy, and scanning electron microscopy (SEM) were used for the determination of the modifications on the zeolite surfaces resulting from adsorption and ozone treatment. It was found that ozone at sufficient concentrations over specified time periods was able to decompose the enrofloxacin adsorbed on zeolite.

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1. Introduction

The presence of antibacterial agents in terrestrial and aquatic environments is of growing interest worldwide due to the emergence and development of antibiotic-resistant bacteria. Antibiotics are used extensively in veterinary medicine for therapeutic purposes and as a growth promoter. The primary elimination mechanisms of antibiotics from the bodies of animals are via urine and feces, so essentially all antibiotics administered are eventually excreted, whether unchanged or in metabolite form. Accordingly, residues from several antibiotic classes are found in animal manure up to mg/kg range [1]. One of the reasons for the contamination of terrestrial environments by antibiotic residues and their biologically active metabolites could be the application of animal manure to agricultural fields as a fertilizer. Additionally, these substances may threaten nearby streams, rivers and groundwater depending on their mobility in the soil system, affecting aquatic organisms as a result of leaching from agricultural fields [2].

Enrofloxacin is a fluoroquinolone group antibiotic, which is widely used in poultry production in order to treat respiratory and enteric bacterial infections [3]. After administration, most of the fluoroquinolone group antibiotics are excreted in urine and feces as a parent compound [4]. Fluoroquinolones are strongly sorbing amphoteric antibiotics tightly bound by organic matter in feces, soil and clay minerals [5]. Consequently, their degradation by means of biotic and abiotic processes in manure is retarded leading to contamination of agricultural soils fertilized with manure. Therefore, removal of antibacterial agents in manure prior to land application is necessary to protect the environment.

Because of their unique adsorption and ion-exchange properties, natural zeolites, especially clinoptilolite, have a wide variety of applications in the treatment of animal wastes, including odour control to create healthier environment for confined livestock, wastewater cleanup, and control the viscosity and nutrient retention of manure. By considering the high adsorptivity of natural zeolites and the sorption tendencies of fluoroquinolone antibiotics [5,6], an interaction between natural zeolite and antibiotic substances excreted via urine and feces is logically inevitable. Consequently, it is important to promote the degradation of antibiotics that are

* Corresponding author. Tel.: +90 212 359 70 36; fax: +90 212 257 50 33.
E-mail address: balciogl@boun.edu.tr (I. Akmeht-Balcıoğlu).

strongly bound to manure, organic matter or zeolite minerals, which are further used as fertilizer.

Ozone is known to be an efficient oxidant for the degradation and removal of antibacterial agents in an aqueous state [7–9], as well as in the treatment of soils contaminated with conventional organic pollutants such as PAHs and PCBs [10–12]. Ozone has already been used for the deodorization of manure slurry [13]. By considering the above mentioned facts, the goals of the present study are, to investigate the adsorption capacity of enrofloxacin antibiotic by natural zeolite, elucidate the mechanism of its uptake, and evaluate the efficiency of ozone treatment in the decontamination and regeneration of contaminated zeolite. Adsorption isotherms of enrofloxacin at different pHs and temperatures were determined and modeled according to Langmuir and Freundlich isotherms. The enthalpy change (ΔH°) of the adsorption process was also calculated. Infrared (IR) spectroscopy and scanning electron microscopy (SEM) were used to detect antibiotic substances on zeolite materials and determine the efficiency of ozonation in zeolite decontamination.

2. Materials and methods

2.1. Materials

Adsorbent, natural zeolite was obtained from Manisa, Turkey. A powder form (<150 μm) of zeolite was used for all adsorption experiments. The pretreatment of zeolite was performed at 300 $^\circ\text{C}$, for 48 h in order to remove any organic contaminants or impurities and was stored in a dessiccator until it was used. According to X-ray powder diffraction analysis (Rigaku D/Max-Ultima+/PC XRD instrument), clinoptilolite was found to be a predominant material in the mineralogical composition of natural zeolite. The chemical composition of natural zeolite is given in Table 1.

Enrofloxacin (99.8% purity) (Fig. 1) was obtained from Bayer. Aqueous enrofloxacin solutions were prepared at different concentrations using distilled deionized water. NaOH and HCl were used for pH adjustment.

2.2. Methods

Enrofloxacin concentrations in the aqueous solutions were determined spectrophotometrically (UV–vis Shimadzu) at

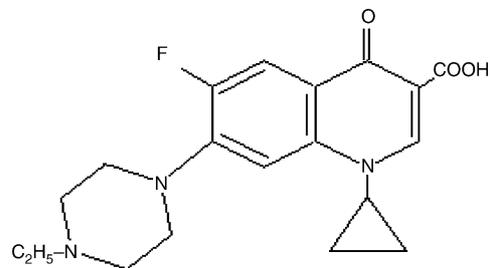


Fig. 1. Molecular structure of enrofloxacin.

maximum absorbance wavelength of 275 nm at pH 5, 272 nm at pH 7, and 271 nm at pH 10. The steady shift in absorption maximums correspondent to pH values can be attributed to the extent of ionization within the carboxylic group as a consequence of the removal of a proton [14].

To determine the adsorption isotherms at varying pHs and temperatures, batch adsorption experiments were performed with 75 ml of enrofloxacin solution (50–200 mg/l). In these experiments, 0.75 g of precisely weighed amounts of pre-treated zeolite were sealed in 100 mL flasks. All the flasks were agitated in a temperature (28–50 $^\circ\text{C}$) controlled shaker (Julabo Eco Temp TW12) at 200 rpm for 24 h. These samples were then centrifuged and filtered prior to spectrophotometric measurement.

In order to examine the influence of temperature on the uptake rate of enrofloxacin, kinetic experiments were performed. Two hundred and fifty milliliters of flasks containing 200 mL of enrofloxacin solution (200 mg/L) and 2 g of zeolite were agitated and 3 mL aliquots were taken periodically for spectrophotometric measurement.

In order to decontaminate the zeolite, ozonation experiments after the adsorption preparations were performed in 100 mL glass reactor. Ozone (1430 mg/h) was produced from pure oxygen using a Fisher OZ 500 ozone generator and was fed into a reactor containing 75 mL slurry of zeolite, on which enrofloxacin was adsorbed, at a flow rate of 50 L/h. Expired ozone gas was trapped in two gas absorption bottles containing 20% KI solution and ozone concentrations in that gas was determined by the iodometric method [15].

Prior to infrared and SEM analysis, the zeolite was filtered and dried both before and after ozone treatment. Infrared spectra (Perkin-Elmer 1600 series FTIR) of zeolite samples were measured in a wavenumber range between 400 and 4000 cm^{-1} via the KBr method. SEM images of the zeolite samples were taken by an ESEM-FEG/GSED-Philips instrument.

3. Results and discussion

3.1. Adsorption of enrofloxacin on natural zeolite

In the first part of the present study, the adsorption characteristics of enrofloxacin on natural zeolite were examined at different pHs and temperatures.

Table 1

Chemical composition of natural zeolite

Element	wt.%
O	41.18
Na	1.48
Mg	1.12
Al	8.86
Si	42.26
K	4.08
Ca	1.01

3.1.1. Effect of pH

Kinetic experiments for zeolite were performed with 200 mg/L enrofloxacin solutions at pH values of 5, 7, and 10 (Fig. 2). The results obtained at three different pH values show that equilibrium was almost attained in about 3 h. There was no significant change in equilibrium concentrations after 3 h. Although the major uptake of enrofloxacin by natural zeolite occurred in the first 3 h and equilibration was almost completed, the flasks were shaken for 24 h to ensure complete equilibration.

Because of the carboxylic acid and several basic amine functional groups in its structure (Fig. 1), enrofloxacin is amphoteric and considered zwitterionic with the pK_a values of approximately 6 and 8 [16]. Enrofloxacin is positively charged in acidic solutions and may undergo cation exchange reactions, while at high pH values it is found in its anionic form. On the other hand, the zeolite possesses a net negative structural charge in the pH range of 2–12, resulting from isomorphous substitutions of cations in the crystal lattice [17]. From the results presented in Fig. 2, it is obvious that, in acidic solutions, high ionic interactions occur between the cationic enrofloxacin and the negatively charged zeolite surface. At pH 7, where the dominant solution phase species was the zwitterion, a cationic state could still be accepted as a large contributor to enrofloxacin sorption since a considerable amount of enrofloxacin was removed from the solution via adsorption (Fig. 2). However, at a pH of 10, a significant decrease was observed in the sorption capacity of zeolite since an anionic state of enrofloxacin is the dominant species at an alkaline pH. Interaction between anionic enrofloxacin and exchangeable cations, which are bound to the negatively charged mineral surfaces (cation bridging) was proposed as a binding model for the sorption of anionic enrofloxacin [5] and could be an explanation for the lesser affinity of natural zeolite for enrofloxacin at alkaline pH medium (pH 10). Similar effects of the medium pH on the adsorption of amphoteric organic substances onto clay minerals were reported in the literature [18]. In the view of the obtained results, considerably high adsorption of enrofloxacin on zeolite is expected in slightly alkaline manures such as poultry, where pH varies between 7.5 and 8.5 [19].

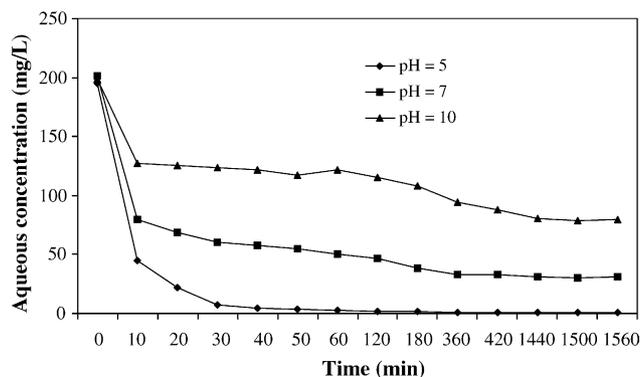


Fig. 2. Effect of pH on the adsorption of enrofloxacin.

The analysis of equilibrium data for the adsorption of enrofloxacin on zeolite was conducted using the Freundlich (Eq. (1)) and Langmuir (Eq. (2)) isotherms;

$$q_e = K_f C_e^n \quad (1)$$

where q_e is the amount of adsorbed enrofloxacin at equilibrium, K_f a Freundlich adsorption coefficient and n is a Freundlich constant, which indicates how dramatically the binding strength changes as the adsorption coefficient changes, C_e is the equilibrium concentration of enrofloxacin.

$$q_e = \left[\frac{K_L C_e}{1 + K_L C_e} \right] q_m \quad (2)$$

where q_m and K_L are the Langmuir constants related to the monolayer adsorption capacity and energy of adsorption, respectively.

Enrofloxacin adsorption equilibrium experiments for natural zeolite were performed at initial pHs of 7 and 10, in the concentration ranges of 50–200 mg/L for 24 h, in order to examine the effect of pH on adsorption capacity and adsorption isotherms are represented in Fig. 3.

The average percentage errors between the experimental and predicted values of Langmuir and Freundlich parameters were calculated using Eq. (3) [20].

$$E (\%) = \frac{\sum_{i=1}^N |(q_{e(i,exp)} - q_{e(i,cal)})/q_{e(i,exp)}|}{N} \times 100 \quad (3)$$

where $q_{e(i,exp)}$ and $q_{e(i,cal)}$ are experimental and calculated values of q_e , respectively and N is the number of the measurements. The adsorption constants at different pH values are listed in Table 2 with the average percent errors.

Both Langmuir and Freundlich models were found to generate a satisfactory fit to the experimental data as indicated by correlation coefficient values in Table 2. However, higher values of correlation coefficients as well as lower percentage errors for the Langmuir model compared to the Freundlich indicated that sorbed enrofloxacin forms monolayer coverage on the zeolite surface. As expected through the kinetic studies, a higher monolayer adsorption capacity q_m was obtained

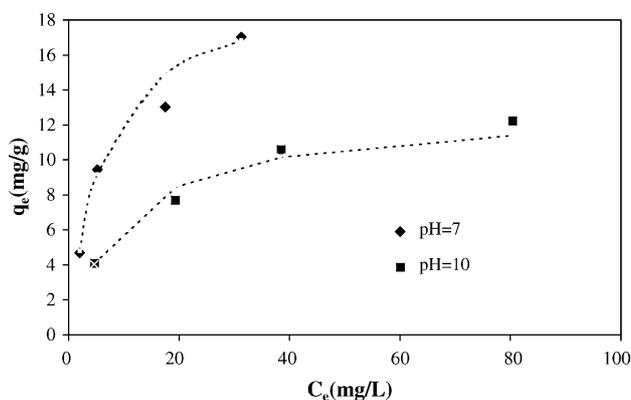


Fig. 3. Adsorption isotherms of enrofloxacin on natural zeolite at different pH values and 28 °C.

Table 2
Langmuir and Freundlich parameters of enrofloxacin adsorbed on zeolite at different pH values

pH	Langmuir				Freundlich			
	q_m (mg/g)	K_L (L/mg)	R^2	E (%)	K_f (mg/g)(L/mg) ⁿ	n	R^2	E (%)
7	19.34	0.17	0.99	5.28	3.87	0.44	0.96	8.27
10	12.8	0.10	0.99	5.22	2.31	0.39	0.98	5.42

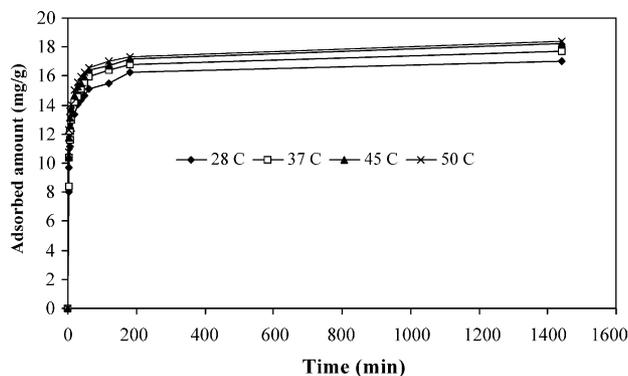


Fig. 4. Effect of temperature on the adsorption of enrofloxacin on natural zeolite at pH 7.

at pH 7 than that of pH 10. The equilibrium isotherm could not be established at pH 5, since enrofloxacin in the aqueous phase was almost completely adsorbed on the zeolite surface for all adsorbate concentrations.

3.1.2. Effect of temperature

To investigate the effect of temperature on the adsorption of enrofloxacin onto natural zeolite experiments were performed at 28, 37, 45, and 50 °C for 24 h (Fig. 4). While the results of adsorption isotherms at different temperatures are presented in Fig. 5, the Langmuir and Freundlich parameters with the percentage errors are listed in Table 3.

The change of the sorption capacity of natural zeolite due to temperature indicated that higher temperatures favored enrofloxacin removal from aqueous solutions. Similar to the pH effects, the application of the Langmuir model to data seemed to be more appropriate than that of the Freundlich model due to the higher correlation coefficients and considerably lower percentage errors, which vary between 2.1 and 5.2%. As obtained from the results of kinetic experiments, a slight increase was observed in the maximum adsorption density and in the affinity of the zeolite for enrofloxacin as the temperature was raised from 28 to 50 °C due to the fact that enrofloxacin adsorption by natural zeolite is an endothermic

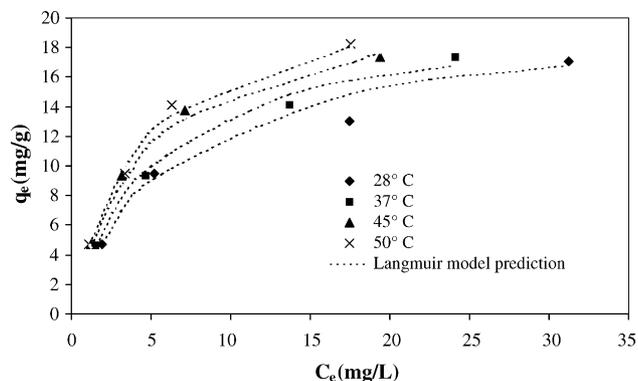


Fig. 5. Adsorption isotherms of enrofloxacin on natural zeolite at different temperatures.

process. In order to confirm this result, the enthalpy change ΔH° of the adsorption process was calculated using the van't Hoff equation;

$$\frac{d \ln K}{dT} = \frac{\Delta H^\circ}{RT^2} \quad (4)$$

where K is Langmuir constant, R the universal gas constant (8.314 J/mol K) and T is temperature in K. The enthalpy change was found to be 11.7 kJ/mol by plotting $\ln K$ versus $1/T$, which gives a straight line with a correlation coefficient of 0.98. The positive value of enthalpy change confirmed the endothermic nature of the adsorption process. Additionally, on the basis of relatively small enthalpy change, it can be concluded that the nature of the sorption process is physical, either ionic interactions or hydrogen bonding with the low exchange enthalpies (below 40 kJ/mol) [21]. These results are in agreement with the microcalorimetric measurements from Nowara et al. [5], who reported similar exchange enthalpies for the ionic binding of enrofloxacin on several clay minerals.

3.1.3. Effect of ammonia nitrogen

Natural zeolite is widely used in poultry production for the purpose of odour control. One of the major contributors

Table 3
Langmuir and Freundlich parameters of enrofloxacin adsorbed on zeolite at different temperatures and at pH 7

T (°C)	Langmuir				Freundlich			
	q_m (mg/g)	K_L (L/mg)	R^2	E (%)	K_f (mg/g)(L/mg) ⁿ	n	R^2	E (%)
28	19.3	0.17	0.99	5.28	3.87	0.44	0.96	8.27
37	19.8	0.20	0.99	2.64	4.03	0.47	0.98	5.76
45	22.1	0.22	0.99	2.11	4.84	0.47	0.94	11.73
50	22.6	0.23	0.99	2.80	4.85	0.50	0.96	10.16

Table 4

Langmuir and Freundlich parameters of enrofloxacin adsorbed zeolite in the presence of ammonium nitrogen at pH 7

NH ₄ -N (mg/L)	Langmuir			Freundlich		
	q_m (mg/g)	K_L (L/mg)	R^2	K_f (mg/g)(L/mg) ⁿ	n	R^2
0	19.3	0.17	0.99	3.87	0.44	0.96
50	33.5	0.25	0.97	7.24	0.49	0.87
200	65.7	0.24	0.94	11.20	0.58	0.80

of odour in animal production is the generation of ammonium from urea. Zeolite is used to capture ammonium ions from urine and faeces to prevent the release of odour causing ammonia. In order to evaluate the effects of ammonium ions on the adsorption efficiency of enrofloxacin onto zeolite, enrofloxacin adsorption experiments were carried out in the presence of 50 and 200 mg/L NH₄-N. The resulting Langmuir and Freundlich parameters are presented at Table 4.

As can be seen from the table, the maximum adsorption capacity of natural zeolite for enrofloxacin was greatly increased by the addition of ammonium nitrogen. By the increment of the initial ammonium nitrogen concentration from 50 to 200 mg/L, adsorbed ammonium nitrogen concentration showed an increase (data not shown) along with a more than two-fold increase in the monolayer adsorption capacity of natural zeolite for enrofloxacin. This might be due to the enhancement of ionic interaction between the deprotonated carboxylic group of enrofloxacin and the positively charged ammonium ion on the ammoniated zeolite surface.

3.2. Ozonation experiments

In order to evaluate the effect of ozonation on the adsorption capacity and regeneration of natural zeolite, zeolite (10 g/L) equilibrated with enrofloxacin (200 mg/L) at pH 7 for 24 h was subjected to ozonation for 10, 20, and 30 min with an applied ozone dose of 1430 mg/h. Adsorption experiments were conducted to use the same batch of zeolite repeatedly (up to three times). After each regeneration, the adsorption capacity of the zeolite was determined for each treatment period (Fig. 6) to see whether the capacity has changed after regeneration.

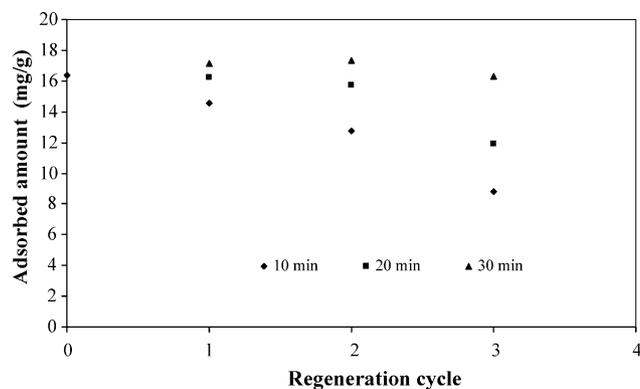


Fig. 6. Changes in the adsorption capacity of zeolite with ozone treatment for different treatment periods.

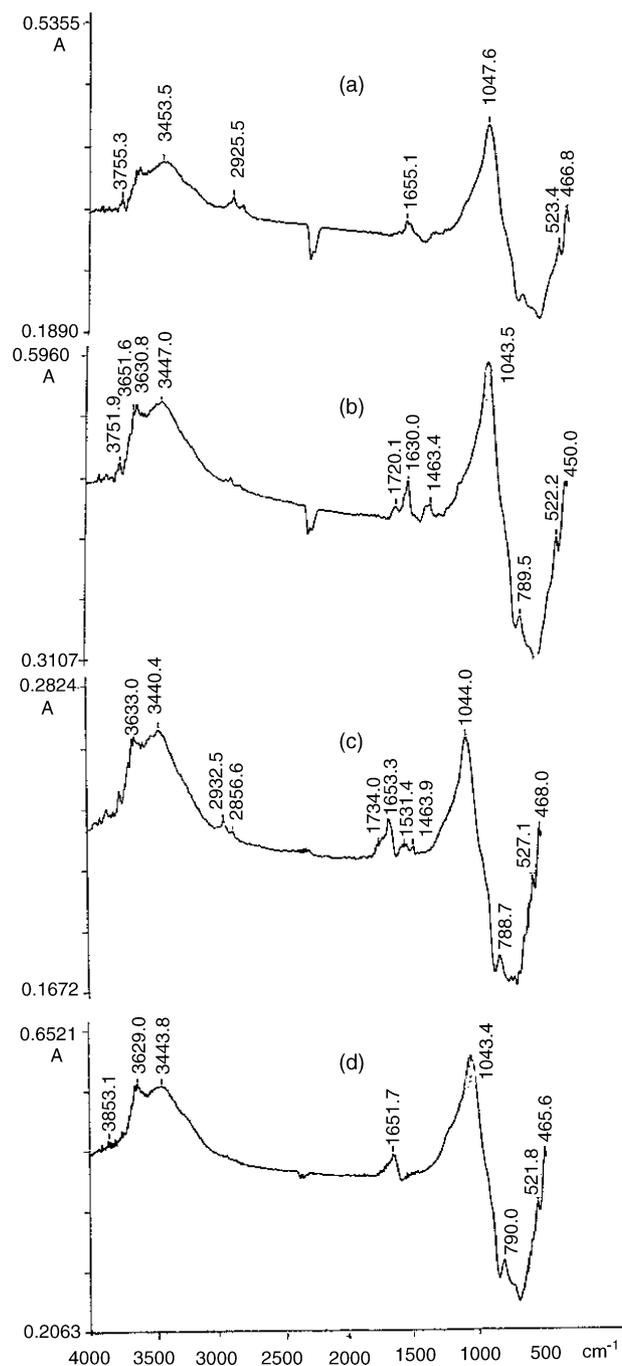


Fig. 7. IR spectra of natural zeolite (a), after (b) enrofloxacin uptake at pH 7, after treatment with ozone for 30 min (c) and 2 h (d).

It was observed that after 30 min ozonation (consumed ozone dose = 100 mg) period, there was no change in the adsorption capacity of zeolite. However, regeneration of 10 (consumed ozone dose = 40 mg) and 20 min (consumed ozone dose = 70 mg) ozone treatments exhibited 48% and 30% respective decreases in the adsorption capacity of natural zeolite.

Although 30 min of ozonation seemed to be sufficient for the reuse of zeolite without any decrease in the adsorption capacity, it is also important to examine the efficiency of ozonation on degradation of enrofloxacin and decontamination of natural zeolite. Our previous study indicated that enrofloxacin in aqueous solution easily reacted with ozone and produced biodegradable products [7]. In this study, in order to evaluate the efficiency of ozone treatment on enrofloxacin removal from the surface of natural zeolite, infrared spectroscopy and scanning electron microscopy were used. Fig. 7 presents the IR spectrums of natural zeolite, before and after uptake of enrofloxacin at pH 7, and after ozonation (30 min and 2 h).

As can be seen from the figure, by the adsorption of enrofloxacin on zeolite at pH 7, new peaks appears at 1463 and 1630 cm^{-1} , and they can be attributed to the asymmetric and symmetric stretch of the COO^- group, which interact with cations (cation bridging) bound to negative zeolite surfaces. In other studies, [22,23] binding of fluoroquinolone group antibiotics to several metal ions was investigated and

it was found that bidentate ligands were formed through the carbonyl oxygen atom and one of the oxygen atoms of the carboxylate group. Another peak in IR spectra (Fig. 7b) of zeolite equilibrated with enrofloxacin at 1720 cm^{-1} exhibited the presence of unionized carboxylic acid group on the zeolite surface completely vanished in the IR spectra of zeolite after adsorption at pH 10 (data not shown), as expected. The IR spectrum of zeolite ozonated for 30 min showed a decrease in the intensity of the above mentioned peaks, indicating failure in the complete removal of enrofloxacin from zeolite surfaces (Fig. 7c). On the other hand, the spectrum of zeolite ozonated for 2 h (Fig. 7d) in the $400\text{--}4000\text{ cm}^{-1}$ is similar to that of the original zeolite indicating the complete decontamination of natural zeolite. However, in real applications, for the determination of a required ozone dose, biodegradability and the toxicity of the oxidation products should also be considered, as well as the complete removal of parent compounds.

Fig. 8 shows the SEM images of natural zeolite, before and after enrofloxacin uptake, and after 30 min of ozone treatment. Comparing the images of virgin (Fig. 8a) and contaminated zeolite (Fig. 8b), the surface coverage of zeolite by enrofloxacin after 24 h adsorption process can be observed. On the other hand, SEM image of the ozonated zeolite obviously exhibited that complete decontamination could not be achieved within 30 min treatment period (Fig. 8c) confirming the results obtained from the IR analysis.

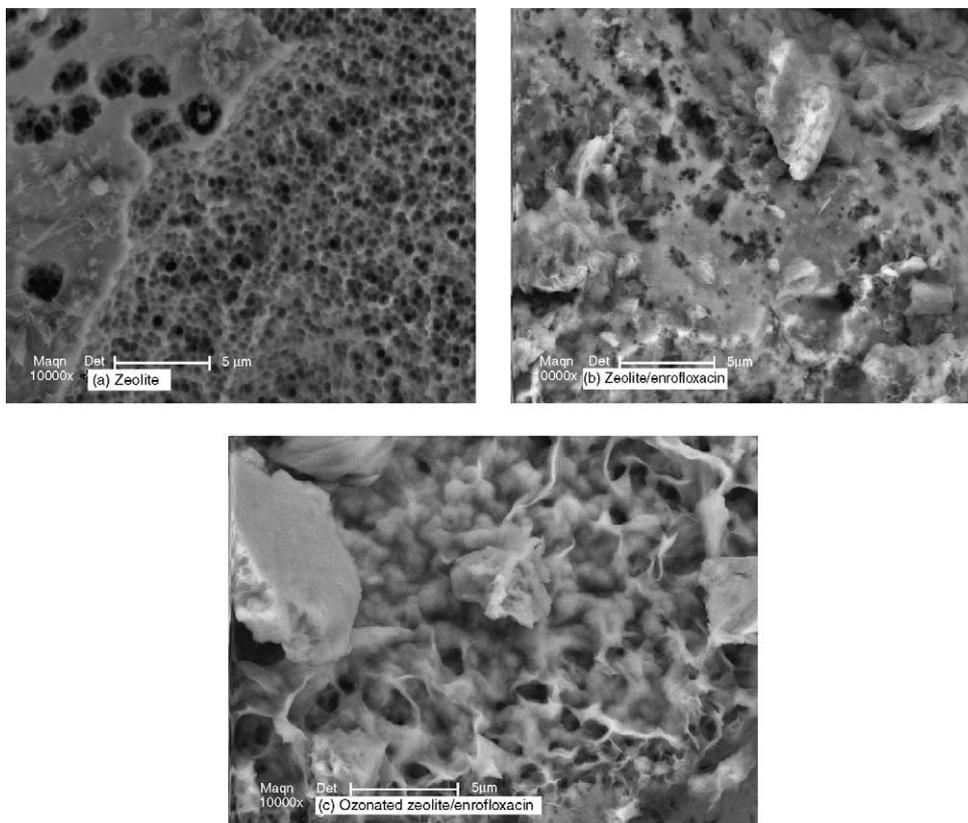


Fig. 8. (a–c) SEM images of natural zeolite before and after enrofloxacin uptake, and after 30 min ozone treatment.

SEM study also indicated that the original pore structure of zeolite changed with adsorption and ozonation. After the 24 h equilibration all pores greater than $0.1\ \mu\text{m}$ filled with enrofloxacin in contaminated zeolite. The volume of small pores ($<0.05\ \mu\text{m}$) constitutes 87% of total pore volume of contaminated zeolite and the remaining portion of the pores is in $0.09\text{--}0.05\ \mu\text{m}$ size range. While 30 min ozonation was able to restore 80% of virgin great pore volume after first regeneration cycle an increase was observed in the volume of pores $<0.05\ \mu\text{m}$. Furthermore maximum pore size of virgin zeolite decreased from $0.25\ \mu\text{m}$ to $0.17\ \mu\text{m}$ by ozonation.

4. Conclusion

The presence of antibiotics in terrestrial and aquatic environments indicates that degradation of strongly sorbing antibacterial agents in manure is essential to protect the environment from contamination. In the present study, decontamination of zeolite by ozone treatment was examined, as well as sorption characteristics of enrofloxacin on natural zeolite, which is widely used in animal treatment for different purposes. Following conclusions were drawn from this study:

- Increases in the uptake of enrofloxacin onto natural zeolite were obtained by decreasing pH and increasing temperature. On the basis of low enthalpy changes and strong pH effects, it can be concluded that, enrofloxacin sorption interactions with natural zeolite is controlled by ionic functional groups resulting in ionic binding between antibiotic and zeolite. Langmuir isotherm model gave a higher correlation coefficient value to describe adsorption equilibrium data for enrofloxacin on zeolite compare to Freundlich isotherm. The presence of ammonium ion in the medium enhanced the adsorption of enrofloxacin on zeolite. Increasing the ammonia concentration from $50\ \text{mg/L}$ to $200\ \text{mg/L}$ resulted in 50% increase in the adsorption of enrofloxacin.
- Ozone at sufficient concentration was able to decompose enrofloxacin adsorbed on zeolite. Use of low ozone dose for the treatment of contaminated adsorbent led to decrease of adsorption capacity of zeolite due to incomplete removal of enrofloxacin. Ozonation caused the change of zeolite pore structure by producing smaller pores.

As result of this study ozonation can be proposed as promising method for the treatment of agricultural waste. However, further experiments on the effect of manure components on the adsorption and degradation of enrofloxacin in manure, by ozonation as well as biodegradability and the toxicity of degradation byproducts are necessary. Furthermore the interaction between natural zeolite and enrofloxacin can also be considered as a detoxification method to remove this antibiotic from manure, if pH and ammonia concentration are opportunely

adjusted although adsorption is not a destructive method for contaminants.

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