



EVALUATION OF IRAQI MONTMORILLONITE AS ADSORBENT FOR THE REMOVAL OF OXYTETRACYCLINE FROM WATER

*Mahmood M. Barbooti , **Khaldoun S. Al-Bassam , *Bashar H. Qasim

bhc_chem@yahoo.com

*School of Applied Sciences, University of Technology. Baghdad, Iraq.

**State Company of Minerals and Geological Survey, Nidhal Street. Baghdad, Iraq

Abstract

This work is a contribution to the global interest of scientific community in the field of cleaning the environment from drug and antibiotic residues which lead to the development of new generations of bacteria with improved resistance to antibiotics. The sorption of oxytetracyclin, OTC, on an Iraqi certified clay mineral, the montmorillonite, is used as indicative for the success of the used treatments. Batch equilibration experiments were carried out to follow the sorption behavior of OTC on the clay. The concentration of OTC was determined by spectral absorption at 360 nm which presented excellent correlation with high performance liquid chromatography, HPLC, method over a wide range of concentration ($R^2 = 0.977$). After equilibration of synthetic OTC solutions with the clay for various intervals of time and various pH values, the concentration of OTC was employed to estimate the sorption efficiency of montmorillonite. The pH of the solution has only minor effect on the sorption efficiency. The kinetic treatment of the sorption results indicated two distinctive steps for the adsorption of OTC on the clay. Montmorillonite was proved as a candidate sorption material for OTC ensuring a removal efficiency of (94-96%) even at low clay contents. Experimentally the OTC concentration could be decreased from 0.5 to 0.03 mol/L at a pH value of 5.5 by sorption onto 2.0 g clay (montmorillonite)/L.

Key Words: Oxytetracycline removal; adsorption; montmorillonite, Optimization, Experimental design.

تقييم المونت موريلونائيت العراقي كعامل امتزاز في ازالة الاوكسي تتراسايكلين من الماء

*محمود مهدي بربوتي ، **خلدون صبحي البصام ، *بشار حسين قاسم

*فرع الكيمياء التطبيقية ، قسم العلوم التطبيقية ، الجامعة التكنولوجية . بغداد – العراق

** الشركة العامة للتعدين والمسح الجيولوجي، شارع النضال . بغداد – العراق

الخلاصة

يتضمن العمل مساهمة بسيطة تصب في مصلحة المجتمع العلمي العالمي في مجال تنظيف البيئة من مخلفات الادوية والمضادات الحيوية والتي يؤدي وجودها الى تطوير اجيال جديدة من البكتريا المقاومة للمضادات الحيوية. ان امتزاز المركب اوكسي تتراسايكلين على الطين العراقي المعدني (المونت موريلونائيت) كان مؤشرا ايجابيا على نجاح المعالجات المستخدمة. تم اجراء العديد من التجارب من اجل متابعة ومعرفة التصرف الامتزازي للاوكسي تتراسايكلين على الطين . تم تقدير تركيز الاوكسي تتراسايكلين بواسطة

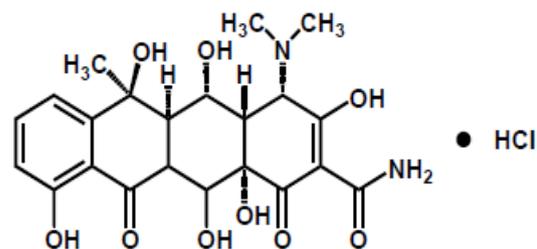
القياسات الطيفية وعند الطول الموجي ٣٦٠ نانومتر حيث اظهر معامل ارتباط جيد قيمته ($R^2 = 0.977$) عند اجراء التحليل بكميات عالية السائل العالي الاداء HPLC ولمدى واسع من التراكيز. كان الغرض من تحضير محاليل الاوكسي تتراسايكلين مع الطين وبفترات زمنية متغايرة وقيم أس هيدروجيني pH متغايرة ايضا هو توظيف تركيز الاوكسي تتراسايكلين لتقييم كفاءة الامتزاز للمونتموريلونايت. لقد وجد من خلال التجارب ان قيمة الاس الهيدروجيني pH للمحلول لها تأثيرا بسيطا جدا على كفاءة الامتزاز. عند دراسة حركية عملية الامتزاز، اظهرت النتائج ان عملية امتزاز الاوكسي تتراسايكلين تتم بخطوتين. لقد اثبت المونتموريلونايت بأنه مادة الامتزاز المناسبة جدا للاوكسي تتراسايكلين حيث ان كفاءة الامتزاز تراوحت بين (94 - 96%). عمليا، لقد وجد بأن تركيز الاوكسي تتراسايكلين قد انخفض من 0.5 الى 0.03 مول/ لتر عند قيمة اس هيدروجيني 5.5 عند امتزازها خلال ٢ غرام / لتر من الطين (المونتموريلونايت).

الكلمات المفتاحية:- ازالة الاوكسي تتراسايكلين ، الامتزاز الكيميائي، المونتموريلونايت

Introduction

Antibiotics are among the widely used pharmaceutical compounds for human and veterinary applications. The large amounts of antibiotic usage may result in their release to the environment, because up to 75-90% of the administered antibiotics can be excreted without undergoing metabolism. Antibiotics showed considerable persistence in the aquatic environment such as river and sea [1]. Large interest was directed in the last decade on the occurrence and fate of antibiotics in surface water and towards their removal. Much concern was given for the environmental consequences of antibiotics where they lead to the appearance and growth of drug resistant bacteria and the activation of sludges [2,3]. Many authors reported on the removal of the antibiotics from water, soils and living stocks. Oleszczuk et al.[4], studied the application of multiwalled carbon nanotubes. Sediments as a major receptor play an important role in the removal of antibiotics from water[5].

Tetracyclines, TC, antibiotic group is characterized by three different moieties of the molecule that can undergo protonation-deprotonation reactions and the possibility to adopt different ionic species and conformations depending on the pH of the aqueous solution in which TC is dissolved[6]. Hayes and Geiger[7], reported that 25-75% of tetracyclines administered to animals are excreted in the active form. The OTC, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a octahydro-3,5,6,10,12,12a-hexahydro-6-methyl-1,11-dioxo-2-naphthacene-carboxamide, (M.wt. 460 gm/mol), is a widely used antibiotic for human and veterinary purposes.



OTC, levels that exceed 1000 $\mu\text{g/g}$ could be detected in manure of beef cattle[8]. Considerable concern was directed for the fate of antibiotic residues in the manure of treated animals[9]. The consequences of antibiotic presence in water stimulated much effort towards their removal from wastewater and manure. Parolo et al.[6], described a Patagonian montmorillonite that appeared as a good adsorbent of TC. Choi et al.[10], used two types of granular active carbon for the removal of OTC from synthetic and real wastewaters and reported a removal efficiency of >90%. They found that the removal efficiencies slightly deteriorated (44~67%) in the river water due to organic interference. The purpose of the present work is to investigate the importance of Iraqi certified montmorillonite as a potential sorbent to remove OTC from aqueous solutions to aid remediation efforts. The purpose of the present investigation is to evaluate Iraqi certified montmorillonite for the adsorption of OTC from water. The study involves the evaluation of the kinetics and mechanism of the adsorption. The applicability of Langmuir model for the adsorption isotherm is also investigated.

Materials and Methods

Materials and Reagents:

The OTC standard was supplied by Sigma Aldrich (99%+). The KCl which was used to maintain certain ionic strength necessary for the adsorption was analytical grade reagent from

(Merck, AG). HPLC grade methanol and acetonitrile were purchased from Fisher Chemicals (Fairlawn, NJ). Standard stock solutions were freshly prepared by dilution of the OTC in 10 ml NanoPure water (Barnstead NanoPureII, Dubuque, IA). These solutions were diluted to generate a series of working standard solutions for preparing calibration curves and standard addition spikes that were prepared daily.

The clay mineral used was a high quality Montmorillonite claystone of Wadi Bashira certified clay obtained from the Iraqi Geological Survey and with the following characteristics: Montmorillonite (77.0%); Loss on Ignition = 11.0; Organic Matter = 0.47; CEC = 76.59 meq/100 g. The x-ray diffraction is shown in (Fig. 1).

Buffer solutions were prepared from Pipes which is the common name for piperazine-N,N'-bis(2-ethanesulfonic acid). A stock solution of 10M concentration was prepared from which the working pH buffers were prepared by simple adjustment of the main buffer with drops of 0.1 M HCl and 0.1 M NaOH.

Instrument Parameters

The spectral measurements were performed on a JASCO V-660 UV-Vis spectrophotometer operated at a band width of 2.0 nm for three cycles and absorption cuvette of 1 mm thickness. For comparison purposes, a group of the samples were analyzed by high performance liquid chromatography, HPLC on a Finnigan /Surveyor/ plus /HPLC system (Thermo Scientific) equipped with quadruple pumps coupled with Surveyor PDA plus detector (Photodiode Array) and a Surveyor/ Plus autosampler was used for all the analyses. A Hypersil Gold C18 column (150 x 4.6mm, 5 μ m) with a corresponding Hypersil Gold guard column (10 x 4 mm, 5 μ m) at room temperature was used for all separations. Samples were eluted isocratically with a mobile phase consisting of 0.010 M aqueous oxalic acid: acetonitrile: methanol (150:20:20 by volume)[11]. The mobile phase was mixed and sonicated for 5 min before use. The flow rate was maintained at 1.5 ml/min, with an injection volume of full loop (25 μ l), while the UV detector was set at 360 nm. Linear calibration was used for quantification based on the curves between the concentration and peak area of known standard of OTC.

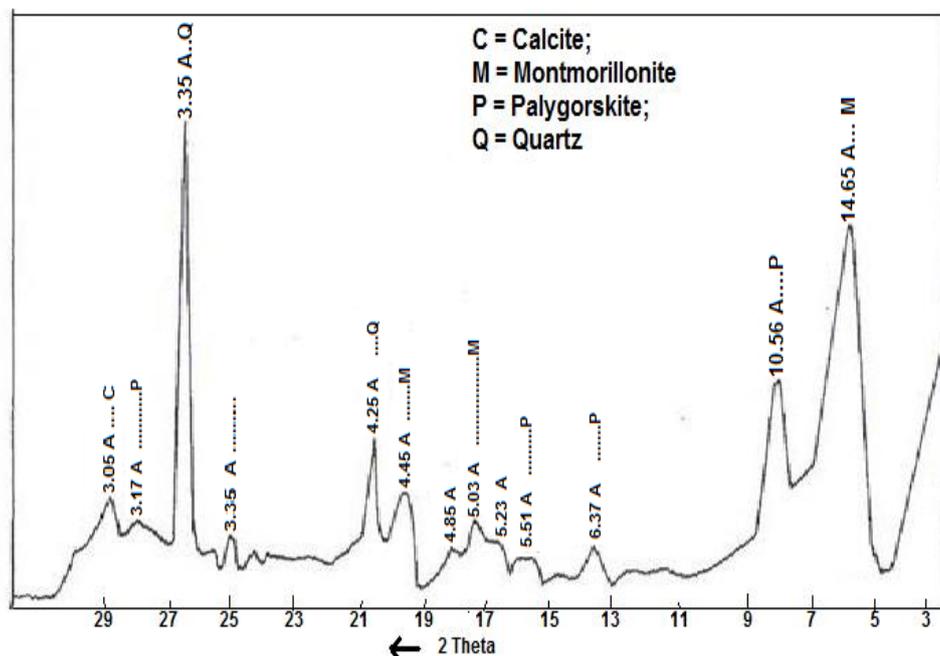


Figure 1: X – Ray Diffraction Pattern of the montmorillonite

Procedures:

Determination of point of zero charge (pH_{pzc}): The method of Orfao et al.[12], was adopted for the determination of pH_{pzc} of montmorillonite. The pH_{pzc} is defined as the

point where the curve pH_{final} vs $pH_{initial}$ crosses the line $pH_{final} = pH_{initial}$.

Adsorption Studies:

In 15-mL centrifuge tubes, a weighed amount of montmorillonite was placed together with 0.1

mL of 1.0 M KCl and the various volume of the OTC stock solution. The final dilution was made to 10 mL with the pre-prepared buffer solution. All the sample solutions were prepared in duplicate. The sampling tubes were covered with aluminum foil and placed on a rotary shaker for 24 hrs and run at a speed of 250 rpm at the lab temperature which ranged between 19-21°C. After equilibration, the samples were centrifuged at 3000 rpm for 10 mins to aid solid separation. The solutions were filtered through 0.22 µm syringe filters into amber glass vials to avoid photo-degradation and kept away from light at 4°C until the time of analysis.

For kinetic experiments the shaking of the sample solutions was done manually for the short time periods. The shaker was used for intervals exceeding 30 mins. Sample solutions were separated by centrifuging and syringe filters as above.

Data Presentation

The antibiotic sorption capacity, C_s (mmol/g), was calculated by Eq. 1:

$$C_s = (C_{cont} - C_{eq}) \times \frac{V}{m} \dots \dots (1)$$

where C_{cont} and C_{eq} (mmol/L) are the control and equilibrium aqueous antibiotic concentrations, respectively, V (L) is the volume of solution, and m (g) is the mass of the clay. However, the initial concentration of the OTC was not considered for the estimation of the amount adsorbed because of the slight degradation of the drug in aqueous solution with time[13].

The solid-water distribution coefficient (K_d , L/g) is represented by Eq. 2. The coefficient is defined as the ratio between the concentration of the sorbate

$$K_d = \frac{C_s}{C_{eq}} \dots \dots (2)$$

Results and Discussion

The OTC exhibits the spectral absorption displayed in (Fig 2). Two distinct absorption bands could be observed with peak maxima at 360 and 272 nm. The two wavelengths can be used for the quantitative evaluation of OTC. For this work we used the 360 nm absorption for the quantitation of OTC. The calibration graph was linear up to a concentration of 200 ppm which corresponds to a molar concentration of about 0.045 mM (Fig. 3). However such a range is adequate for the purpose of the sorption study.

For confirmation purposes the randomly selected samples were reanalyzed with HPLC and the results were plotted against those obtained by UV spectrophotometry as can be seen in (Fig. 4). It seems that over a relatively wide range of concentration a reasonable correlation coefficient, R^2 of 0.967 and a negligible intercept were obtained. Thus, spectrophotometry was employed for the rest of the work for the determination of the residual OTC in the studied solutions.

The point of zero charge

The point of zero charges of adsorbents (pH_{pzc}) is a point where an adsorbent have zero potential charge on its surface. The presence of H^+ or OH^- ions in solutions may change the potential surface charges of adsorbents. If the pH of solution is above its pH_{pzc} the surface functional groups on adsorbents will be protonated by the excess H^+ ions; on the contrary if it is below its pH_{pzc} , the surface functional groups will be deprotonated by the OH^- ions presence in the solution[14]. The pH of the various buffer solutions after equilibration with a fixed amount of the clay (0.1 g) were plotted against its original pH values as can be seen in (Fig. 5). The point of zero charge of Iraqi montmorillonite was found at a pH value of 8.78.

Preliminary experiments:

The analysis of the sample solutions equilibrated with various amounts of montmorillonite indicated a clear reduction in OTC concentration for all the samples. This accounted for a clear removal of antibiotic into the swelling clay particles. Samples with low clay contents showed a limited reduction in OTC concentration especially for high OTC levels. There was no clear and significant change in the reduction extent for the various pH ranges. The resultant water solutions were significantly low in OTC content and depending on the initial OTC concentration; the adsorption efficiency was 74-96% .

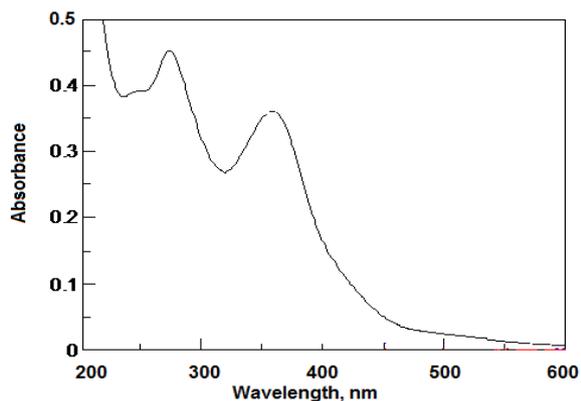


Figure 2: Absorption Spectrum of Oxytetracycline solution in water

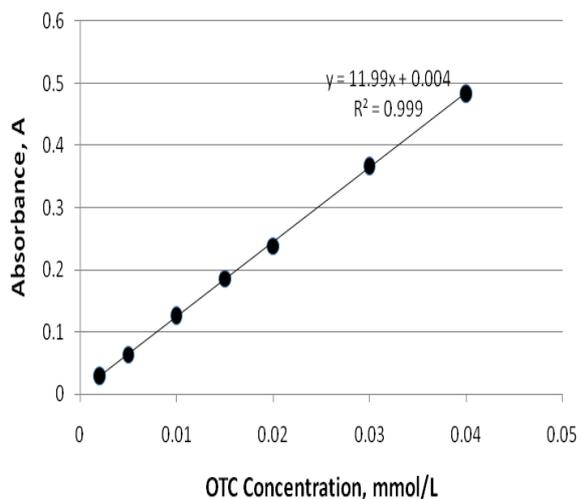


Figure 3: Calibration Graph of OTC Spectrophotometric Determination at 360 nm. Cuvette thickness, $b = 1$ mm, $a = 24$ L. g-1. cm-1. The molar absorptivity, $\epsilon = 10990$ L. mol-1. cm-1

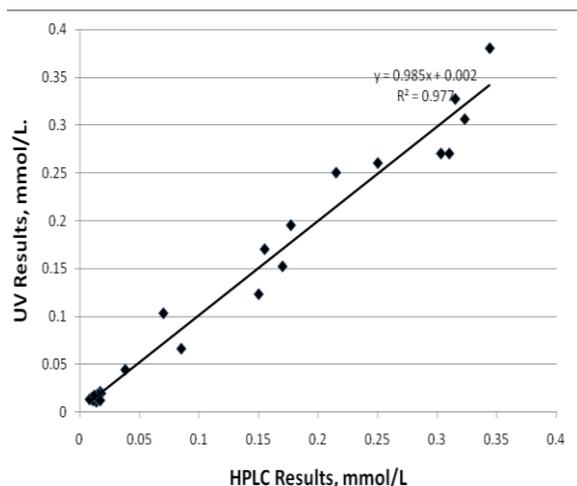


Figure 4: Correlation of spectral and HPLC. Spectral Conditions: $\lambda = 360$ nm, Cuvette 1 mm; HPLC Conditions: C18 column (150 x 4.6mm, 5 μ m), Hypersil Gold guard column (10x4 mm, 5 μ m), isocratic elution, mobile phase oxalic acid: acetonitrile: methanol (150:20:20 by volume)

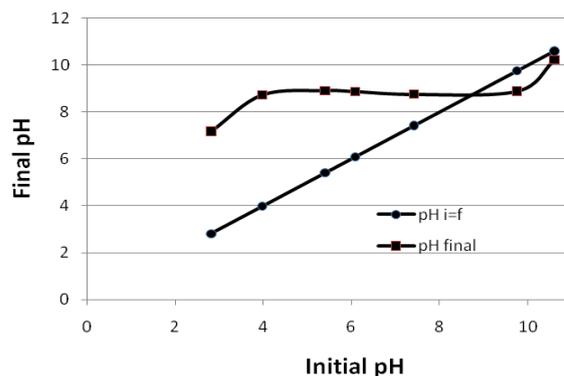


Fig. 5: Determination of point of zero charge for Iraqi montmorillonite

Effect of pH on the adsorption

(Fig. 6) shows the effect of the pH on the OTC adsorption. It is clear that the increase of pH resulted in a slight decrease in adsorption capacity up till pH value of 7.0. The rate of decrease was significantly less than those reported by Doi and Stoskopf [13], and Kulshrestha et al.[15]. Further, at a pH value of 10, the equilibrium concentration showed a slight decrease, indicating an improvement in the adsorption. However, at such a pH range, Parolo et al.[6], attributed the slight increase in adsorption to the partial degradation of OTC at the high pH value. Avisar et al.[16], detected maximum adsorption at pH 2–4 and concluded that the sorption capacity of the montmorillonite used by them at pH 5–7 was still described as rather high. Figueroa and MacKay[17], reported similar trend of the adsorption increase with iron rich soils and minerals. This correlation was attributed to the cationic exchange interactions that are dominant at lower pH values when OTC is positively charge.

Effect of adsorbent amount

A set of experiments were conducted to follow up the adsorption of 0.2 mM OTC in solution containing increasing amounts of the montmorillonite. The adsorption capacity, mmol/L was calculated from the concentration of the residual OTC after equilibration for 24 hr. (Fig. 7) shows the adsorption capacity of montmorillonite. The adsorption capacity decreases exponentially with the increase of the adsorbent content in the solution.

Kinetics of OTC adsorption on Montmorillonite

Several experiments were carried out to study the effect of time on the extent of adsorption of 0.2 mM OTC. Because the adsorption starts rapidly and extreme care must be undertaken to

isolate solutions after definite interval of times to ensure stopping the adsorption at the required time. Most of the change in OTC concentration occurred within the first 60 mins. The kinetic plots are shown in (Fig 8). Beyond two hours, the rate of reduction in residual OTC concentration was relatively slow. Doi and Stoskopf [13], reported that the presence of a substrate (bentonite clay) resulted in an approximate 17% decrease in OTC concentration within 5 min of contact. Thus, search was focused on the determination of the boundary between the rapid and the slow process.

The two steps could not be resolved from the normal adsorption extent plot versus time. However, kinetic plots can be employed for the resolution of rather overlapped processes related to an overall change[18]. The plot indicates two distinct segments of the adsorption process. The first is rapid and extends from the beginning of the process and proceeds upto 50 minutes and characterized by the equation:

$$\text{Log}C_s = -\frac{0.185}{t} - 0.964 \dots\dots (3)$$

The second step is slow and extends over 20 hours and follows a well defined linear relationship as in the equation:

$$\text{Log}C_s = -\frac{12.44}{t} - 0.735 \dots\dots (4)$$

Adsorption Isotherms:

The experimental OTC adsorption capacity values of OTC onto montmorillonite, C_s , were plotted versus the equilibrium OTC concentration, C_{eq} as in (Fig 7). The data fit well with a linear relationship with R^2 of 0.993:

$$C_s = 5.5149C_{eq} + 0.0182 \dots\dots (5)$$

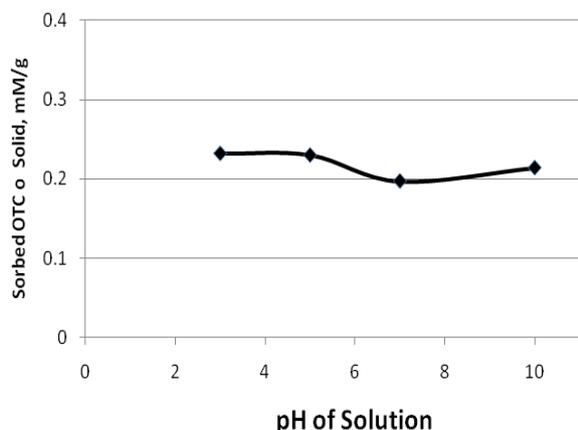


Figure 6: The pH dependence of OTC adsorption on Iraqi montmorillonite

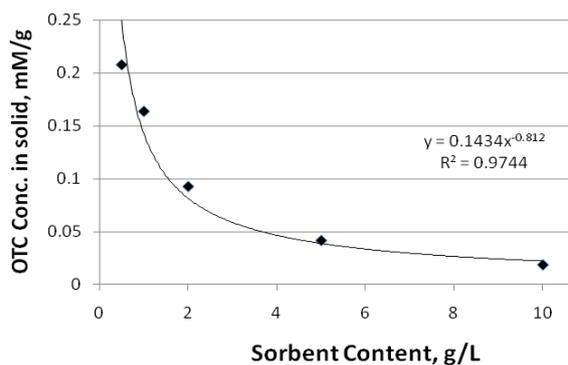


Figure 7: Effect of solid to solution content on adsorption capacity of montmorillonite

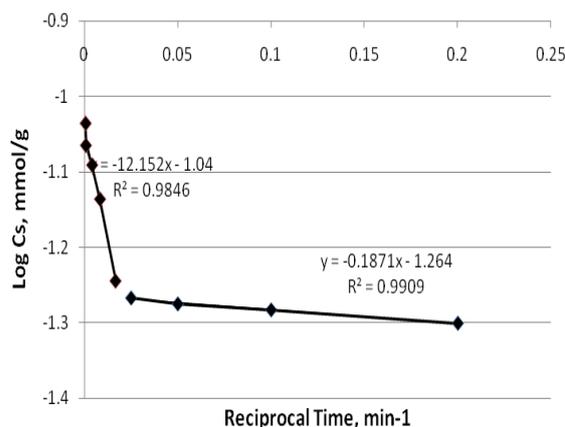


Figure 8: Kinetic plot for OTC adsorption on Iraqi Montmorillonite

The slope of the line was used to calculate the overall sorption coefficient, K_d , (L/equiv) using the formula:

$$K_d = \frac{C_s}{C_{eq}(CEC)} \dots\dots (6)$$

where the CEC (meq/g) is the cation exchange capacity of the clay, 76.59. The slope of the plot was divided by the CEC value of the clay mineral and the result was 0.072 l/meq. Sorption coefficients were normalized to the sorbent cation exchange capacity because interactions of the OTC with specific charge sites on the clay surface were anticipated (Fig.9). This K_d value was comparable to that obtained for sodium montmorillonite for relatively high iron concentration levels[17]. The data were also fitted to Langmuir model and the isotherm parameters were determined. The Langmuir adsorption isotherm ($1/C_s$ versus $1/C_{eq}$) for OTC on montmorillonite is shown in Fig.10. The plot indicates a linear relationship that controls the

adsorption with a correlation coefficient, R^2 , of 0.9178:

$$1/C_s = 0.1427 (1/C_{eq}) + 0.0809 \quad \dots(7)$$

The applicability of the isotherm models can be compared depending on the values of regression coefficients (R^2) that are regarded as a measure of the quality of fit of experimental data on the isotherm models[19]. The closer the R^2 value to unity, the better the fit to the isotherm equation. The OTC adsorption experimental data on montmorillonite fit well to both models, although the Langmuir isotherm model fits better.

Mechanism of Adsorption:

When the conditions are set properly, OTC adsorption capacities of <94% can be experienced on Iraqi Montmorillonite. The OTC molecule possesses some functional groups which facilitate the adsorption. However, the model proposed by Hayes and Geiger[7], for the environmental interfaces of tetracyclines with the soil particles provides acceptable concept for the interaction of oxytetracycline with the clay. Generally, ortho - substituted anionic and cationic groups on a zwitterionic molecule may result in undesirable repulsive interactions between the anionic moiety and the negatively charged surface of montmorillonite and disturbed sorption[20].

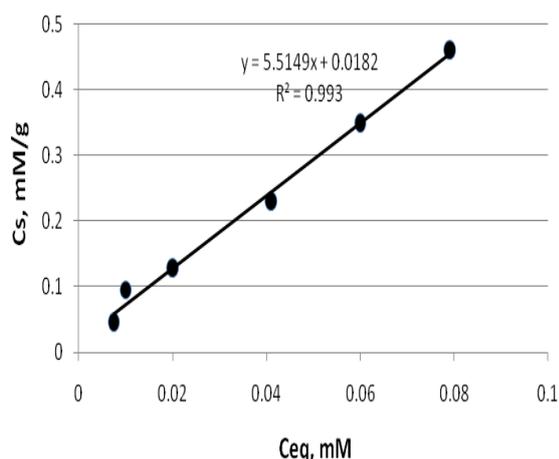


Figure 9: Adsorption Coefficient plot of OTC adsorption onto montmorillonite

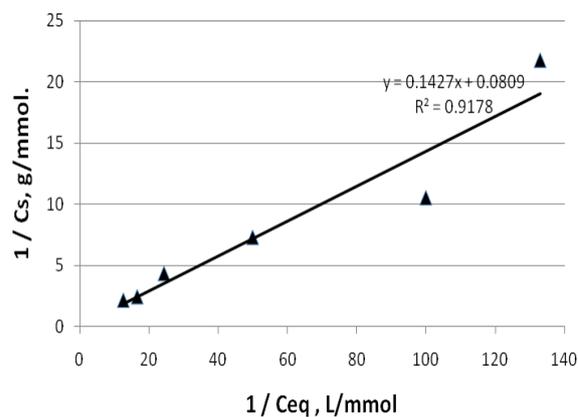


Figure 10: The plot of Langmuir adsorption isotherm for OTC onto montmorillonite

Conclusion

From the results of the present study it can be concluded that Iraqi montmorillonite is a potential sorbent for the removal of OTC from wastewaters. Kinetic treatment indicated two distinct adsorption steps: a fast and a slow process. The adsorption is well fitted with Langmuir model.

Acknowledgement

One of the authors (M. M. B.) is indebted to the Institute of International Education, IIE, for the financial support of the visit to the United States. The authors are grateful to Dr. D. Sarkar for the permission of using the laboratory facilities at MSU. The help of Mr. Pravin Punamiya in the practical work is greatly appreciated.

References:

1. Hernando, M.D., Mezcuca, M., Fernandez-Alba, A.R., Barcelo', D. **2006**. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. *Talanta*, **69** (2) : 334–342.
2. Schwartz, T., Kohnen, W., Jansen, B., Obst, U. **2003**. Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. *FEMS Microbiol. Ecol.* **43**(2), 325–335.
3. Volkmann, H., Schwartz, T., Bischoff, P., Kirchen, S., Obst, U., **2004**. Detection of clinically relevant antibiotic-resistance genes in municipal wastewater using real-time PCR (taqman). *J. Microbiol. Methods* **56**(3): 277–286.

4. Oleszczuk, P.; Pan, B. and Xing, B. **2009**. Adsorption and Desorption of Oxytetracycline and Carbamazepine by Multiwalled Carbon Nanotubes. *Environ. Sci. Technol.*, **43**(2): 9167-73.
5. Pouliquen, H. and Le Bris, H. **1996**. Sorption of oxolinic acid and oxytetracycline to marine sediments. *Chemosphere*, **33**(3): 801-815.
6. Parolo, M. E., Savini, M. C., Vallés, J. M., Baschini, M. T., and Avena, M. G., **2008**. Tetracycline adsorption on montmorillonite: pH and ionic strength effects. *Appl. Clay Sci.*, **40**(3): 179–186.
7. Hayes P L and Geiger F M. **2008**. Oxytetracycline at Environmental Interfaces Studied by Second Harmonic generation. WMRC Symposium on PPCPs in the Illinois Environment. pp.567-598.
8. Duff A, **2005**. Presence of Tetracycline Antibiotics In Surface Water - A Study of The Presence / Absence Of Tetracycline In The Raccoon River Watershed. Des Moines Water Works Laboratory. *Environ. Sci. Technol.*, **39** (1): 496-503.
9. Arikan O A; Sikora L J; Mulbry W; Khan S U; Rice C and Foster G D., **2006**. The fate and effect of oxytetracycline during the anaerobic digestion of manure from therapeutically treated calves. *Process Biochem.*, **41**(1):1637–1643.
10. Choi K J; Kim S G and Kim S H, **2008**. Removal of Antibiotics by Coagulation and Granular Activated Carbon Filtration. *J. Hazardous Materials*, **151**(1): 38-43.
11. Fritz J Wand Zuo Y, **2007**. Simultaneous determination of tetracycline, oxytetracycline, and 4-epitetracycline in milk by high-performance liquid chromatography. *Food Chem.*, **105**(3): 1297–1301.
12. Orfao J J M ; Silva A I M; Pereira J C V; Barata S A; Fonseca I M; Faria P C C and Pereira M F R, **2006**. Adsorption of reactive dye on chemically modified activated carbon – influence of pH. *J. Colloid Interface Sci.*, **296**(5): 480–489.
13. Doi, A. M. and Stoskopf, M. K., **2000**. The Kinetics of Oxytetracycline Degradation in Deionized Water under Varying Temperature, pH, Light, Substrate, and Organic Matter. *J. Aquatic Animal Health*, **12**(1): 246–253.
14. Kubilay S; Gurkan R; Savran A and Sahar T., **2007**. Removal of Cu(II), Zn(II) and Co(II) ions from aqueous solutions by adsorption onto natural bentonite. *Adsorption* **13**(1): 41–51.
15. Kulshrestha, P. Giese, Jr., R. F. and Aga, D. S., **2004**. Investigating the Molecular Interactions of Oxytetracycline in Clay and Organic Matter: Insights on Factors Affecting Its Mobility in Soil. *Environ. Sci. Technol.*, **38**(3):4097–4105.
16. Avisar, D.; Primor, O.; Gozlan I. and Mamane, H., **2009**. Sorption of Sulfonamides and Tetracyclines to Montmorillonite Clay. *Water Air Soil Pollut.*, DOI 10.1007/s11270-009-0212-8.
17. Figueroa, A. L. and Mackay, A., **2004**. Modeling Tetracycline Antibiotic Sorption to Clays. *Environ. Sci. Technol.*, **38** (2): 476-483.
18. Barbooti, M. M., **1983**. The possibility of resolving overlapping decomposition reactions by the kinetic plots of thermogravimetric curves. *Thermochim. Acta*, **68** (3): 363 – 370.
19. Al-Asheh S and Duvnjak Z, **1997**. Sorption of cadmium and other heavy metals by pine bark. *J. Hazard. Mater.*, **56** (2): 35–51.
20. Al-Asheh S and Duvnjak Z, **1997**. Sorption of cadmium and other heavy metals by pine bark. *J. Hazard. Mater.*, **56** (3): 35–51.