Optimization of oxytetracycline sorption onto iron modified montmorillonite

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ABSTRACT:
Aims: Iraqi montmorillonite was saturated with iron (III) to investigate the improvement in the adsorption efficiency towards the antibiotics represented by oxytetracycline

Methods
Design of Experiments was carried out to determine the effect of experimental conditions: initial oxytetracycline molarity concentration (0.1–1.0 mM), montmorillonite content (0.5 – 10 g/L) and the pH value of the solution (3-10) on the adsorption efficiency. Batch equilibration runs were also conducted to study the adsorption of OTC in aqueous solutions onto the modified clay mineral powder for 24 hours. The UV absorption of the residual material at 360 nm was employed to determine the remaining oxytetracycline after equilibration for 24 hours.

The iron introduction into montmorillonite resulted in enhancement of adsorption efficiency towards oxytetracycline which may be related to the possibility of stable complex with Fe(III) to make a 1:3 complex with it.

Results: On using the optimum conditions, a mathematical model simulating the operation for the treatment was obtained as follows:

\[
\text{Eff} \% = -0.467 + 0.160 \text{m} + 0.839C_{o} + 0.126 \text{pH} - 0.046 \text{m}C_{o} + 0.041 C_{o}^2 - 0.071 C_{o}\text{pH}
\]

Experimentally the OTC concentration could be decreased from 0.5 down to 0.03 mM in synthetic wastewater at a pH value of 5.5 by sorption onto 6.85g/L clay (montmorillonite)/L.

Conclusion: The treatment with iron allows the use of lower clay material to perform the OTC removal.

Key Words: Oxytetracycline removal; adsorption; Iron modified clay; montmorillonite, optimization, Experimental design.
Introduction
Antibiotics are among the widely used pharmaceutical compounds for human and veterinary applications. The usage of such materials widely in modern life led their emission to the water environment. Considerable amounts of antibiotics are excreted out of the living body not been metabolized. Antibiotics showed considerable persistence in the aqueous environment such as river and sea (Hernando et al., 2006). Large interest was directed in the last decade on the occurrence and fate of antibiotics in surface waters and towards their removal (Kim et al., 2005; and Košutić et al., 2007). Much concern was given for the environmental consequences of antibiotics where they lead to the appearance of drug resistant bacteria and the activation of sludge (Schwartz et al., 2003; Volkmann et al., 2004). Many authors reported the removal of the antibiotics from water, soils and living stocks. Oleszczuk et al, 2010, studied the application of multiwalled carbon nanotubes. Sediments as a major receptor play an important role in the removal of antibiotics from water (Pouliquen and Le Bris, 1996). Oxytetracycline, OTC, was successfully removed from water solution by adsorption on clay minerals (Barbooti, et al, 2014). The disposal of wastewater to the rivers, contribute to the overall retention of OTC onto the river sediments and significantly affected by the organic and iron content of the sediments (Barbooti, 2017). Harja and Ciobanu, 2017, used hydroxyapatite nano powders as adsorbent materials for OTC. They proved that at a pH value of 8 and ambient temperature, high OTC removal rates of about 97.58% with maximum adsorption capacities of 291.32 mg/g onto the uncalcined material was attained.

A number of drugs including OTC may be inhibited by the presence of iron and, thus, decreasing the bioavailability of them (Campbell and Hasinoff, 1991). The OTC interacts with Fe ions. Such behavior was utilized to describe a precise method to estimate the drug in various matrices (Alwarthan, et al., 1991). Modified sorption of OTC was reported by Mackay and Canterbury, 2005 when ion-exchange resins were loaded with metals like copper and calcium. The ability of organic matter of soil to sorb the OTC can also be improved by the addition of iron as a result of the strong complexing of OTC to iron. When the clay was saturated with Fe there was an increase in the rate of adsorption of azimsulfuron as reported by Vittorio et al, 2004. Mesoporous silicates impregnated with iron gave improve tetracycline adsorption from water (Vu et al, 2010). The adsorption of OTC onto activated sludge was highly pH-dependent on pH of the solution and enhanced by Cu²⁺ suggesting a surface complexation (Xiancai, et al., 2017). There was a competence between Chlortetracycline with tetracycline, TC, more intensely than OTC in soil (Fernández-Calviño, et al., 2015). The nano-carbon proved efficient adsorbent for TC and a capacity in the range of 125 mg/g was obtained and the adsorption nearly reached equilibrium within 2 h, with a removal rate of over 75%. (Wu, et al., 2016). The adsorption kinetics of OTC onto plant residues follows the pseudo-first-order model and the process is mostly physical in nature (Wang, et. al. 2018). They also noticed that the acid modified willow residues result in increase in adsorption efficiency.

The purpose of the present work is to investigate the potentials of using iron saturated Iraqi montmorillonite in the adsorption of OTC from water and to establish the optimum working conditions for the adsorption employing the experimental design method.

Materials and Methods
Materials and Reagents:
The OTC was a pure reagent from Sigma Aldrich. Standard OTC stock solution was freshly prepared using NanoPure water (Barnstead NanoPureII, Dubuque, IA). The working solutions were made by dilution to generate calibration curves. For the preparation of buffer solutions, the procedure described by Barbooti, et al., 2014 was used. The Montmorillonite claystone was supplied from Iraqi Geological Survey and with the characteristics given in Table 1 and Fig. 1.
Figure 1: The X – Ray Diffraction Pattern of the Montmorillonite.

Table 1: the Characteristics of the Iraqi montmorillonite

| Component   | Wt%  | Mineral composition                      |
|-------------|------|------------------------------------------|
| SiO₂        | 54.07| Monmorillonite (77.0%)                   |
| Fe₂O₃       | 5.59 | Palygorskite (Attapulgite)               |
| Al₂O₃       | 15.05| Quartz                                   |
| TiO₂        | 0.79 | Calcite                                  |
| CaO         | 5.65 | Gypsum                                   |
| MgO         | 3.20 | Apatite                                  |
| SO₃         | 1.1  | Feldspar                                 |
| Loss on Ignition | 11.0 |                                           |
| Na₂O        | 0.37 |                                         |
| K₂O         | 0.5  |                                         |
| Cl          | 0.92 |                                         |
| P₂O₅        | 0.42 |                                         |
| Organic Matter | 0.47 |                                           |
| CEC (meq/100 g) | 76.59 |                                          |

The procedure reported by was employed for the reparation of monmorillonite inserted with iron modified (Gerstl and Banin, 1980).

Instrumental 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). The operating conditions of HPLC analysis were as given in a previous paper (Barbooti, et al., 2014).
Design of Experiments
The use of Central Composite Design (Lawson, 2009) in this work allowed a comprehensive investigation of the effects of three main parameters: initial OTC concentration, clay content and the pH on the adsorption. Five levels of each parameter were used and the coded and real values of the variables are listed in Table 2.

| No. | Coded variables | Real variables | Adsorption Efficiency, % |
|-----|-----------------|----------------|-------------------------|
|     | $X_1/X_2/X_3$   | Clay content, g/L | Initial concentration, mM | pH |            |
| 1   | -1/1/-1         | 2.5            | 0.29                    | 4.48 | 47.6  |
| 2   | +1/-1/-1        | 8              | 0.29                    | 4.48 | 83.8  |
| 3   | -1/+1/-1        | 2.5            | 0.81                    | 4.48 | 80.86 |
| 4   | +1/+1/-1        | 8              | 0.81                    | 8.5  | 94.07 |
| 5   | -1/-1/+1        | 2.5            | 0.29                    | 8.5  | 72.41 |
| 6   | +1/-1/+1        | 8              | 0.29                    | 8.5  | 81.37 |
| 7   | -1/1/1          | 2.5            | 0.81                    | 8.5  | 79.38 |
| 8   | 1/1/1           | 8              | 0.81                    | 4.48 | 92.22 |
| 9   | -1.732/0/0      | 0.5            | 0.55                    | 6.5  | 93.27 |
| 10  | +1.732/0/0      | 10             | 0.55                    | 6.5  | 93.81 |
| 11  | 0/-1.732/0      | 5.25           | 0.1                     | 6.5  | 79.0  |
| 12  | 0/1.732/0       | 5.25           | 1                       | 6.5  | 84.6  |
| 13  | 0/0/-1.732      | 5.25           | 0.55                    | 3    | 65.3  |
| 14  | 0/0/+1.732      | 5.25           | 0.55                    | 10   | 88.54 |
| 15  | 0/0/0           | 5.25           | 0.55                    | 6.5  | 72.54 |
| 16  | 0/0/0           | 5.25           | 0.55                    | 6.5  | 87.24 |
| 17  | 0/0/0           | 5.25           | 0.55                    | 6.5  | 81.61 |
| 18  | 0/0/0           | 5.25           | 0.55                    | 6.5  | 88.86 |

Results and Discussion
The OTC exhibits two distinct absorption bands with 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 ($R^2=0.9996$) up to a concentration of 200 ppm which corresponds to a molar concentration of about 0.45 mM. Such a range is adequate for the purpose of the sorption study. The overall absorbance–concentration relation can be expressed by equation 1.
Using a cuvette thickness, b of 1 mm, the absorptivity, a, was 24 L.g⁻¹.cm⁻¹ and the molar absorptivity, Ε was 10990 L.mol⁻¹.cm⁻¹. 

\[ \text{Abs}_{360 \text{ nm}} = 0.0024 C_{\text{oc}t} + 0.0044 \] …………………………………..(1)

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

**Figure 2: Correlation of spectral and high performance liquid chromatography results.**

**Comparison with untreated clay:**
A set of experiments were conducted at the optimum conditions to investigate the removal of 0.2 mM OTC in the presence of various modified clay contents along with another set for the regular clay. The plots of the adsorption efficiency shown in Fig. 3 reflect the modification in the adsorption properties of the clay. The improvement was clearer with low clay content. Thus, the iron introduction to the clay allows the use of lower clay material to perform the OTC removal. Such a behavior may be a consequence of the possibility of the formation of a complex between OTC and iron. This resulted in modification in the affinity of the clay towards the drug. For higher clay content the difference in performance was limited and almost identical adsorption profiles were obtained.
Figure 3: Effect of clay content on adsorption efficiency of 0.2 mM Oxyt. Dark, Fe modified clay and light, regular montmorillonite.

**Optimization:**
A second order polynomial equation was employed in the range of the three independent variables to yield the equation that relates the adsorption efficiency, %, to these variables. The regression analysis of central composite design can be applied to the approximating model to obtain the optimum conditions for the treatment process. Using Statistica software, the coefficients of the response equation (2) were determined:

\[
\text{Eff} \% = -0.467 + 0.160 \, m + 0.839 \, C_o + 0.126 \, \text{pH} + 0.006 \, m^2 + 0.046 \, m \, C_o + 0.041 \, C_o^2 - 0.004 \, m \, \text{pH} - 0.071 \, C_o \, \text{pH} - 0.003 \, (\text{pH})^2 
\]

The symbol m refers to the amount of the clay in the solution.
Variance Explained = 84.14% ........................................(3)

The final Loss was equal to the sum of the squares of (Observed – Predicted)^2 and attained a value of 0.062.

% Average absolute error = 15.64%
SSE = Sum(Y_{obs} - Y_{pred})^2 = 0.062

The calculated coefficients values of the terms of equation (2) reflect the significance of the term being very low for the 5th, 8th and 10th terms. Equation (2) could then be modified into:

\[
\text{Eff} \% = -0.467 + 0.160 \, m + 0.839 \, C_o + 0.126 \, \text{pH} + 0.006 \, m^2 + 0.041 \, C_o^2 - 0.071 \, C_o \, \text{pH} 
\]

The F- distribution test [28] was used as a measure of the significance of terms in equation (2), using the variance of each term in the model. Tables 3 and 4 show the analysis of variance for orthogonal variables. The observed response was matched to the predicted one together with the errors for OTC concentrations are exhibited in Fig. 4.

| Table 3: Analysis of variance for orthogonal variables for adsorption Efficiency. |
The optimum conditions were estimated from these equations:

\[ X_1 = \text{clay content 6.85 g/L; } X_2 = \text{drug concentration, C 1 mM; and } X_3 = \text{pH 5.5} \]

### Table 4: Analysis of variance for orthogonal variables for adsorption Efficiency.

| Parameter  | Degrees of Freedom | Estimate | Standard Error | t Value | Pr > |t| |
|------------|-------------------|----------|----------------|--------|------|---|
| Intercept  | 1                 | -0.467293| 0.375110       | -1.25  | 0.2413 |
| clay       | 1                 | 0.160398 | 0.048616       | 3.30   | 0.0080 |
| drug       | 1                 | 0.838992 | 0.523199       | 1.60   | 0.1399 |
| pH         | 1                 | 0.125590 | 0.075447       | 1.66   | 0.1270 |
| Clay*Clay  | 1                 | -0.006331| 0.002642       | -2.40  | 0.0376 |
| drug*clay  | 1                 | -0.045586| 0.039033       | -1.17  | 0.2699 |
| drug*drug  | 1                 | 0.040620 | 0.294625       | 0.14   | 0.8931 |
| pH*clay    | 1                 | -0.003825| 0.005049       | -0.76  | 0.4662 |
| pH*drug    | 1                 | -0.070758| 0.053403       | -1.32  | 0.2147 |
| pH*pH      | 1                 | -0.003296| 0.004880       | -0.68  | 0.5147 |

DF = Degrees of Freedom
Figure 4: The observed versus the predicated response errors for adsorption Efficiency values.

A contour plot is the projection of the response surface as a two-dimensional plane. The analysis presents the necessary understanding of the effects of experimental variables together with their interaction on the efficiency (Chandran, et al., 2002). The study of the interactive effect of two parameters on the OTC sorption, the contour plots and the three dimensional representation are shown in Fig. 5. The center values: pH = 6.49, OTC concentration = 0.55 mmol/L and clay content = 5.25 g/L were chosen as the hold values.

The combination of the effects of initial concentration of OTC and clay content revealed only slight increase of the efficiency with the increase of the OTC concentration. Meanwhile, there was a significant increase in efficiency with the clay content increase even at low OTC concentration. However, the optimal efficiency can be obtained at moderate OTC and moderate pH values with the pH combined effect with OTC initial concentration. Meanwhile, the highest adsorption efficiency could be obtained at moderate clay content when combined with the pH values of (5-7.5) and moderate OTC concentration (Fig. 6).
Figure 5: Response Surfaces profiles of adsorption efficiency for the design points considering the experimental conditions: initial OTC concentration, 0.55 mM; clay content 5.25 g/L and pH = 6.49.
Figure 6: Response Contour for adsorption efficiency for the design points considering the experimental conditions: initial OTC concentration, 0.55 mM; clay content 5.25 g/L and pH = 6.49.

Effects of Operating parameters:

Effect of Clay Content:

The adsorption efficiency of OTC on the iron modified clay was proved to be highly dependent on the clay content as shown in Fig. 7. For the various OTC initial concentrations there was an increase in the efficiency with the increased clay content. The rate of increase was lower as the clay content exceeded above 6.5 g/L. Thus, 7.0 g/L can be the optimum content for the various levels of OTC concentration values. The increasing OTC concentrations result in increased efficiency. With fixed OTC initial concentration there was a clear increase in the sorption efficiency as the montmorillonite content increases (Fig. 8) for pH values ranging from 3-10. The sorption efficiency increases as the clay content increases and attained a maximum values over the range of 6-8 g/L. Again the efficiency favors lower pH values

Figure 7: The adsorption efficiency values versus clay content on for studied for initial OTC concentrations as studied at fixed pH value of 5.5.
Figure 8: The effect of clay contents on the adsorption efficiency at various pH values at fixed OTC concentration of 1.0.

Effect of pH of the Solution:

The pH of the solution was effective on the OTC adsorption using various clay contents and fixed OTC concentration (Fig. 9). For pH above 8.0, there exists a slight decrease in the efficiency which may be related to the negatively charged montmorillonite particles in the alkaline medium [Bao, et al., 2016] for pH values less than 7. There is a clear increase in the efficiency as the clay content increases. The high clay contents, e.g. 9, are associated with a sharp decrease at higher pH values. Meanwhile, the efficiency shows a steady state over the entire range of the pH values when the clay content is in the range of 5-7 g/L.
The rate of decrease was significantly less than those reported by Doi and Stoskopf, 2000, and Kulshrestha et al. 2004. 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. 2008 attributed the slight increase in adsorption to the partial degradation of OTC at the high pH value. However, the maximum adsorption was obtained by Avisar et al, 2009, to occur at pH range of 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, 2004, 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.
Figure 10: The effect of pH value on the adsorption efficiency for various OTC concentrations at fixed clay contents of 6.85 g/L.

The effect of changing the pH on the process of OTC sorption on a fixed clay content of 6.5 g/L is shown in Fig. 10 using various OTC concentrations. Moderate to higher OTC concentrations are associated with less dependence of the efficiency on the pH of the solutions. Above a pH value of 8.0, the efficiency showed a clear decrease especially for high OTC concentrations. The expected negative charges acquired by the clay particles in the alkaline medium may account for such an effect. Thus, there is no clear trend in efficiency over the pH range studied. Only a slight decrease in the efficiency beyond a pH of 8.0 as a result of the possible degradation of OTC in alkaline medium (Doi and Stoskopf, 2000).
Figure 11: The effect of OTC concentration on the adsorption efficiency at various pH values at fixed clay contents of 6.85 g/L.

Effect of initial OTC concentration:

The effects of initial concentration of OTC on sorption efficiency are shown in Fig. 11 by fixing the the Fe-modified clay content at various pH values. There appears a significant increase of the adsorption efficiency with the increase of OTC concentration. The rate of increase decreases as the pH value approaches 7. The efficiency was almost constant at the slightly alkaline medium. Further increase of pH caused a slight decrease in efficiency as the OTC concentration increases.

At a slightly acidic medium (pH=5.5), the adsorption efficiency increases with the increase of initial OTC concentration and various clay contents. As the clay content increases, the increase of the efficiency slows down to reach almost identical values at the high clay content (7-9 g.L⁻¹).

Conclusion
The sorption efficiency of oxytetracycline on montmorillonite can be improved by saturation of the clay with iron ions. Experimental design is a helpful tool for the investigation of varying experimental conditions effects on adsorption efficiency.

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