Removal of Metronidazole residues from aqueous solutions based on magnetic multiwalled carbon nanotubes by response surface methodology and isotherm study

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ABSTRACT
Antibiotics and pharmaceutical products cannot remove by conventional sewage treatment. In this work, an effective adsorbent magnetic multiwalled carbon nanotube (Fe₃O₄@MWCNTs; MMWCNTs) was synthesized by co-precipitation of MWCNTs with Fe₃O₄ and used for removal of Metronidazole from aqueous solutions. Response surface methodology on central composition design (CCD) was applied for designing of experiments and building of models for Metronidazole removal before determination by HPLC. Four factors including pH, the adsorbent dose, time, and temperature were studied and used for the quadratic equation model to the prediction of optimal points. By solvent the equation and considering the regression coefficient (R² = 0.9997), the optimal points obtained as follows: pH = 2.98; adsorbent dosage = 2.16 g; time = 22 min and temperature = 37.9 °C. The isotherm study of adsorption showed that the metronidazole adsorption on Fe₃O₄@MWCNTs follows the Langmuir model. The maximum adsorption capacity (AC) is 215 mg g⁻¹ obtained from Langmuir isotherm. The results showed that three factors including pH, amount of adsorbent, and temperature are significant on removal efficiency and an experimental point was found to agree satisfactorily with the predicted values. The proposed methods coupled to HPLC were used to analysis of metronidazole in six real samples. The results showed the best removal efficiency was obtained at optimal points. Moreover, the reusability of adsorbent showed that the Fe₃O₄@MWCNTs can be efficiently removed the Metronidazole from aqueous solutions as compared to other

ARTICLE INFO:
Received 11 Jun 2020
Revised form 5 Aug 2020
Accepted 28 Aug 2020
Available online 29 Sep 2020

Keywords: Magnetic multiwalled carbon Nanotubes, Metronidazole, Adsorption, Response surface methodology, Central composition design,

1. Introduction
Antibiotic residual in environmental ecosystems is a serious concern for human’s health. The conventional sewage treatment cannot remove antibiotics and pharmaceutical products and hence, a serious ecological risk that occurs by discharging of these effluent in environmental ecosystems and aquatic [1, 2]. Metronidazole (C₆H₉N₃O₃) is an antibiotic that used to treat a wide variety bacterial infections [3]. Recently, researchers reported that average concentration of metronidazole in river water and wastewater was approximately about 0.5
and 1.3 ng L\(^{-1}\), respectively [4]. In addition, many pharmaceutical industries discharge this antibiotic in environmental water by higher dosage [5]. Several methods such as fenton process, filtration and adsorption were used for removal of metronidazole from different matrix and determined with HPLC [6]. Due to cost and simplicity methods, the adsorption techniques are favorite method for removal of metronidazole in water and biological samples. Many techniques based on metal nanoparticles, the polymer structures, the nanosheets, MWCNTs modified with and neural network-genetic algorithm were used and developed for separation and determination metronidazole and other drug in different matrices [7]. Adsorption is simple, effective and economic way with high recovery, easy operation and low cost technique for removal of contaminants such as antibiotic within water or wastewater even at large concentration. The type and size of adsorbent is a key factor for adsorption process that influences on removal efficiency of pollutant [8]. Carbon nanotubes (SWCNTs and MWCNTs) and functionalized of CNTs are widely used as an adsorbent in the removal, extraction and preconcentration of many contaminants including medicinal compounds, pesticides, and other molecules [9]. High surface area, high permeability, good mechanical and thermal stability and repeatability are some of the unique properties of nanotubes. Also, the absorption capacity can be increased by modifying the surface of CNTs by \(\text{NH}_2\), \(\text{COO}\), \(\text{SH}\), \(\text{C}_6\text{H}_5\) groups and adsorption of contaminants would be more specific [10]. Response surface methodology (RSM) is a most applicable method used in many fields such as antibiotics and pharmaceutical products [11]. RSM is a technique that used for statistical analysis of complicated processes and can be utilized for investigating of relative significance of important factors even in the presence of complex interactions [12].

2. Experimental
2.1. Reagent and material
All reagent and solutions were analytical grade purchased from Merck (Darmstadt, Germany). Metronidazole (CASN: 443-48-1) was obtained from Aldrich chemical Co. (Germany). HPLC grade of acetonitrile (ACN) and DW purchased from Sharloa (Spain). Carbon nanotubes with outer diameter of 3–20 nm, length between 1–10 nm, number of walls 3–15 and surface area of around 350 m\(^2\) g\(^{-1}\) were prepared from Plasma Chem. GmbH (Berlin, Germany). Also, The pristine MWCNTs (308068, 98% carbon base, O.D= 10 nm, L=5-20 \(\mu\)m) was purchased from Sigma Aldrich. A standard solution of 1000 mg L\(^{-1}\) metronidazole prepared by dissolving of 1 gr metronidazole in 1 liter deionized water. All standard working solutions prepared daily by dilution of DW. The shaking and centrifuging of blood samples were used based on 300 rpm and 3500 rpm speeds by vortex mixer (Thermo, USA) and Falcon centrifuge (20 mL of polypropylene conical tubes, Thermo, USA), respectively. The pH was adjusted pH by 0.25 mol L\(^{-1}\) of sodium phosphate buffer solution (Merck, Germany) for pH of 5.5 to 8.2 (\(\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4\)).

2.2. Synthesis of MMWCNTs
Synthesis of magnetic carbon nanotube was
performed by co-precipitation methods that reported previously [13]. Briefly, 10 mg of pristine MWCNTs were added to 2 ml solution composed of 4.33 mmol Fe$^{2+}$ and 8.66 mmol Fe$^{3+}$ solution was stirred in ultrasonic bath for 10 min at 50°C while 10 ml concentrated ammonia (8 M) was added drop by drop to the solution. The pH of final solution should be alkaline in order to deposition of Fe$_3$O$_4$ on multi-walled carbon nanotubes. The adsorbent was washed for 7 times with distilled water and separated by a permanent magnet.

2.3. Metronidazole removal by MMWCNTs

Batch adsorption experiments were carried out as per the design developed with the central composite design methodology. Experiments were performed at a batch reactor in 500 ml beaker containing 50 ml of given concentration of metronidazole. Beakers were shaken during that shaked for the specified time period in a temperature controlled incubation shaker at 200 rpm. The pH was adjusted by addition of 0.1 M NaOH or HCl. After completion of experiments adsorbent was removed by an external magnet and remaining metronidazole was measured. The measurement of metronidazole was performed using Cecil HPLC (CECIL Corporation, England) equipped ACE C$_{18}$ column and UV-VIS detector at 230 nm. The mobile phase is ACN: WATER (60:40). The removal percentage of Metronidazole (%removal) was calculated as follow by equation 1:

\[
\%\text{Removal} = \frac{C_f - C_0}{C_0} \times 100
\]

(Eq. 1)

Where $C_f$ and $C_0$ are initial and final metronidazole concentration (mg L$^{-1}$) of solution, respectively.

2.4. Experimental design

CCD was applied in this work to investigation of variables for adsorption of metronidazole on to MMWCNTs. The CCD for four variables (pH, adsorbent dosage, time and the temperature), with two levels (minimum and maximum), was used for experimental design model. In the experimental design model, pH (2-10), adsorbent dosage (0.5-2.5 g), time (5-30 min) and temperature (20-60°C) were taken as input variables. Percentage removal of (30 mg L$^{-1}$) of metronidazole was selected as response of the system. The quadratic equation model for prediction of optimal point was expressed by Equation 2.

\[
Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_i x_i^2 + \sum_{i=0}^{k} \sum_{i+j=1}^{k} \beta_{ij} x_i x_j + \epsilon
\]

(Eq. 2)

Where $Y$ is the response of the system and $X_i$ and $X_j$ are the variables of action, $\beta_0$, $\beta_i$, $\beta_{ii}$, $\beta_{ij}$ are constant coefficient, linear effects, quadratic effects and interaction effects, respectively. The coefficient of determination, namely, $R^2$ and Adj-$R^2$ were used for the explanation of quality of the model. The statistical significance was expressed with adequate precision ratio and the F-test. Design expert (version 8) program was used for regression and graphical analysis. A total of 31 experiments were necessary to estimate of the full model (Table 1).

3. Result and discussion

In this work, removal of metronidazole by a nano-composite made of multi-walled carbon nanotubes and iron nanoparticles were studied. Design of Experiments were conducted using the RSM as well as factors affecting on absorption process of metronidazole such as pH, adsorbent dosage, time, and the temperature were optimized. Finally, the data obtained from experiments compared with model output to optimize and predict the results.

3.1. Regression model and statistical analysis

The CCD has been successfully used for optimizing conditions of Metronidazole removal. A second-order polynomial regression model equations relating the removal efficiency and process variables are given in Equation 3.
%Removal=+41.66308+2.39902 (pH)+9.57629
(adsorbent dose)+0.7686
(temperature)+1.00897 (time)-4.68750×
10⁻³ (pH×adsorbent dose)+0.011172 (pH×
temperature)+7.5×10⁻³(pH×time) -0.034687
(adsorbent×temperature)+0.077000 ( adsorbent
×time) -3.65×10⁻³ (temperature×time)-0.27659
(pH²)-1.99484(adsorbent²)-9.07506×
10⁻³(temperature²)-0.025495(time²)

(Eq.3)

Design-Expert 8 software was applied for
determination of the coefficients in Equation 3.
The optimal points were as follows: pH = 2.98;
adsorbent dosage = 2.16 g; time = 22.2 min and
temperature = 37.88 °C. The model prediction
of the metronidazole removal recovery is 82.8%
while the experimental amount of removal
efficiency is 83.4%. These results confirmed
that RSM effectively used for the investigation
of parameters in complex process could be
utilized to optimize the process parameters.
The mathematical expressions of relationship between the independent parameters and response of system are given in terms of encoded factors. The results of regression analysis on quadratic model are given in Table 3. The significance of each coefficient was expressed by F-values and p-values (Table 2). The larger of the F-values and the smaller of the p-values, indicated more significant of the corresponding coefficients. Values of “prob > F” less than 0.0500 also indicated high significant regression at 95 percent confidence level. According to the F- and p-values, temperature, time and adsorbent dose were found more effective on the adsorption process. The “Lack of Fit F-value” of 0.26 implies the Lack of Fit is not significant relative to the pure error. There is a 98.40% chance that a “Lack of Fit F-value” this large could occur due to noise. The fit of the model was checked by the determination coefficient (R^2). The “Pred R-squared” of 0.9660 is in satisfactory accordance with the “Adj R-Squared” of 0.9768.”Adeq Precision” measures the signal to noise ratio. A ratio greater than 4 is desirable. In this case “Adeq Precision” of 42.257 indicates an adequate signal. Thus, as a result of the statistical analysis, quadratic model was found satisfactory for describing the process and useful for developing empirical relation. Metronidazole removal showed to be very sensitive to changes in the adsorbent dosage and time of adsorption. Magnitude of F-value in Table 2 was expressed in comparison of these two factors adsorbent dosage was more effective on removal efficiency of metronidazole than time of experiments. Figure 1 showed 3D plots of interaction effects of all parameters on removal efficiency. As can be seen in Figure 1 when the pH increased from 2 to 6, the removal efficiency increased about 4%. Also (Figure 1), the results of the study showed that removal efficiency decreased in alkaline solutions. Adsorption time has more effect than pH on metronidazole

| Source         | Sum of square | Df | Square     | F-Value     | Prob > F |
|----------------|--------------|----|------------|-------------|----------|
| Model          | 3120.63835   | 14 | 222.902739 | 148.517383  | < 0.0001 |
| A-pH           | 84.4231475   | 1  | 84.4231475 | 56.2501161  | < 0.0001 |
| B-adsorbent    | 537.787036   | 1  | 537.787036 | 358.320959  | < 0.0001 |
| C-temperature  | 0.66785645   | 1  | 0.66785645 | 0.44498463  | 0.5091   |
| D-time         | 116.402257   | 1  | 116.402257 | 77.5574075  | < 0.0001 |
| AB             | 0.01125      | 1  | 0.01125    | 0.00749574  | 0.9315   |
| AC             | 25.56125     | 1  | 25.56125   | 17.0311499  | 0.0002   |
| AD             | 4.5          | 1  | 4.5        | 2.99829525  | 0.0922   |
| BC             | 15.40125     | 1  | 15.40125   | 10.2616655  | 0.0029   |
| BD             | 29.645       | 1  | 29.645     | 19.7521028  | < 0.0001 |
| CD             | 26.645       | 1  | 26.645     | 17.7532393  | 0.0002   |
| A^2            | 1107.54922   | 1  | 1107.54922 | 737.946571  | < 0.0001 |
| B^2            | 225.03502    | 1  | 225.03502  | 149.938096  | < 0.0001 |
| C^2            | 745.166816   | 1  | 745.166816 | 496.495584  | < 0.0001 |
| D^2            | 897.389678   | 1  | 897.389678 | 597.919825  | < 0.0001 |
| Residual       | 52.52985     | 35 | 1.50085286 |             |          |
| Lack of Fit    | 5.00385003   | 10 | 0.500385   | 0.26321645  | 0.9840   |
| Pure Error     | 47.526       | 25 | 1.90104    |             |          |
removal in this investigation. It was found that nearly 22.2 minute was enough to obtain highest yield. In addition, Figure 2 showed the parity plot of obtained results and predicted results that explains a satisfactory correlation between the observed results and fitted values. In this work, the plotted residuals indicate normal distribution; the data points form an approximately straight line. The data points farther from the line, expressed departure from normality [14, 15]. In this study, the residuals are approximately plotted along straight line for response, indicating no evidence of non-normality or unidentified variables.

Fig.1. Response surface modeling obtained by CCD.
3.2. Isotherm study

The adsorption isotherms for metronidazole adsorption on MMWCNTs were obtained with different metronidazole concentrations (1–30 mg L\(^{-1}\)). The Freundlich and the Langmuir adsorption isotherm models were used for evaluation experimental data. The Langmuir model and Freundlich model are given in Equations 4 and 5 as follows:

\[
\text{Langmuir: } \frac{C_f}{q_f} = \frac{1}{b q_m} + \frac{C_f}{q_m}
\]

(Eq. 4)

\[
\text{Freundlich: } \log q_f = \log K_f + \frac{1}{n} \log C_f
\]

(Eq. 5)

Where \(C_f\) (mg L\(^{-1}\)) is the equilibrium concentration of metronidazole, \(q_f\) (mg g\(^{-1}\)) is adsorption capacity at equilibrium, \(q_m\) (mg g\(^{-1}\)) is the maximum adsorption capacity, \(b\) (L mg\(^{-1}\)) is a constant related to the adsorption energy, \(K_f\) and \(n\) are Freundlich constants which characterize a particular adsorption isotherm. All the constants obtained according to the slope and intercept of the related lines and they are listed in Table 3. As shown in Table 3, the Langmuir isotherm plot fits better to the experimental adsorption data with higher correlation coefficient \((R^2 = 0.9994)\), which expressed that the adsorption of metronidazole ions onto MMWCNTs follows the Langmuir model (Fig. 3).

The \(q_m\) and \(b\) calculated from the slope and intercept of the regression line are 215.4 mg g\(^{-1}\) and 0.52 L mg\(^{-1}\), respectively (Table 3). Langmuir model depends on the acceptance of homogeneous distribution of metronidazole molecules on to surface of adsorbent.

| Table 3. Adsorption isotherm parameters of Langmuir and Freundlich models for adsorption of the metronidazole on the MMWCNTs |
|-----------------------------------------------|
| **Langmuir isotherm** | **Freundlich isotherm** |
| \(q_m\) (mg g\(^{-1}\)) | \(b\) (L mg\(^{-1}\)) | \(K_f\) (L g\(^{-1}\)) | \(n\) | \(R^2\) |
| 215.14 | 0.52 | 0.991 | 65.815 | 2.063 | 0.879 |

Fig. 2. The normal probability plot of the residuals and parity plot show the correlation between the observed and predicted values.
3.3. Desorption and reusability study
Desorption studies of metronidazole were carried out by using different volume of methanol containing NaOH 1% as eluent solvent. For desorption studies, when adsorption of metronidazole was completed, the adsorbent was magnetically separated and washed with deionized water. Then, (0.5-5) mL of the eluent was added to the adsorbent. After 30 min samples were collected to evaluate the metronidazole recoveries. Based on results, the best volume of eluent with high recovery of metronidazole was obtained by 2 mL. To assess the reusability and stability of the adsorbent, the adsorption–desorption experiment with eluent (methanol containing NaOH 1%) was repeated with 30 mg L\(^{-1}\) metronidazole several cycles. After 15 cycles, the adsorption capacity of the MMWCNTs decreases from 163 to 87 mg g\(^{-1}\). This result shows that the adsorbent can be applied effectively in a real process such as pharmaceutical industries wastewater treatment. Moreover, in order to investigate the inter-day validation of the results, the method was used for the determination of metronidazole (30.0 mg l\(^{-1}\)) in three consecutive days by HPLC, and the Relative Standard Deviation percent was found to be 4.1%. These results indicate that MMWCNTs are usable and stable for the extraction of metronidazole and the method has high reproducibility and repeatability for the determination and extraction of the antibiotics by HPLC.

3.4. Application of proposed methods to real sample
To verify the potential application of proposed method to real samples, the adsorption performance within real wastewater that spiked with different amount of metronidazole in optimum condition that obtained from model is also provided in Table 4. As expected, magnetic carbon nanotubes therefore show high removal efficiency (95-102 %) for the tested concentrations. It must be noted; the adsorbent cannot completely removed the metronidazole due to the competition between other substances which is also present in the wastewater.

| Sample  | Added | Initial concentration | *Found   | %Removal Efficiency |
|---------|-------|-----------------------|----------|---------------------|
| Sample A | ------ | 100                   | 3.7 ± 0.2 | 96.3               |
|         | 50     | 150                   | 5.2 ± 0.3 | 96.5               |
| Sample B | 200    |                        | 10.8 ± 0.5 | 94.6               |
|         | 100    | 300                   | 14.2 ± 0.7 | 95.3               |
| Sample C | 30     |                        | 0.5 ± 0.03 | 98.3               |
|         | 30     | 60                    | 1.9 ± 0.9 | 96.8               |

*Mean of three determinations of samples ± confidence interval (P = 0.95, n =8)
4. Conclusions
In this study, a central composition design (CCD) was used for evaluation of four variables of adsorption (time, temperature, initial ion concentration and amount of adsorbent) for Metronidazole by Fe₃O₄@MWCNTs. Quadratic model was developed to correlate the variables to the response. Through the analysis of response surfaces, adsorbent dose, pH and adsorption time were found to have significant effects on removal efficiency, whereas adsorbent dose showed that most significant. All removal analysis of Metronidazole were done based on Fe₃O₄@MWCNTs by HPLC in water and wastewater samples. Optimization was carried out and the experimental values were found to agree satisfactorily with the predicted values. Isotherm study of process showed maximum adsorption capacity of Fe₃O₄@MWCNTs for removal of metronidazole (215 mg g⁻¹). Also, application of proposed method for real wastewater sample showed high removal efficiency of proposed sorbent.

5. Acknowledgement
Authors hereby appreciate the staffs of health laboratory of Zabol University of medical sciences for their cooperation to perform this research. The research was funded by the Zabol University of medical sciences.

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