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 (Fe_3O_4@MWCNTs; MMWCNTs) was synthesized by co-precipitation of MWCNTs with Fe_3O_4 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 (R^2 =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 Fe_3O_4@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 Fe_3O_4@MWCNTs can be efficiently removed the Metronidazole from aqueous solutions as compared to other

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 ($C_6H_9N_3O_3$) 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

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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 matrixes [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 is could be increased by modifying the surface of CNTs by NH$_2$, COO, SH, C$_6$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]. High performance liquid chromatography (HPLC) is a suitable method for isolation, identification and measurement of many drugs. Combining liquid chromatography with mass spectrometer(LC-MS, HPLC-MS) is the most appropriate method for

identifying different species of organic compounds in complex matrixes. Examples of these compound are amino acids, proteins, nucleic acids, hydrocarbons, carbohydrates, drugs, terpenoids and pesticides, antibiotics, steroids, any organic or inorganic metal and a group of various materials.

Experimental data points were obtained during optimization based on Fe$_3$O$_4$@MWCNTs and a model for central composition design (CCD). It is good method for the consecutive experimentation and illustrate accurate information for testing several parameters while not involving an unusually large number of data points. The adsorption Metronidazole based on Fe$_3$O$_4$@MWCNTs was determination by HPLC instrument.

2. Experimental
2.1. Reagent and material
All reagent and solutions were analytical grade and 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 μm) was purchased from Sigma Aldrich. A standard solution of 1000 mg L^{-1} metronidazole prepared by dissolving of 1 gr metronidazole in 1liter deionized water. All standard working solutions prepared daily by dilution of DW.) The shacking 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 (Na$_2$HPO$_4$/ NaH$_2$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_3O_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 that 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**:

$$\%Removal = \frac{Cf - C0}{C0} * 100$$

(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**.

$$Y = \beta 0 + \sum_{i=1}^{k} \beta i x i + \sum_{i=1}^{k} \beta i i x i^2 + \sum_{i=0}^{k} \sum_{j=i+1}^{k} \beta x i x j + \epsilon$$

(Eq. 2)

Where Y is the response of the system and X_i and X_J are the variables of action, β_0, β_i, β_{ii}, β_{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.**

Table 1. Central composite design matrix with experimental and predicted values

Column1	Factor 1	Factor 2	Factor 3	Factor 4	Actual	Predicted
Run	A:pH	B:adsorbent dose	C:temperature	D:time	Actual Value	Predicted Value
1	2	2.5	60	5	65	65.79284266
2	6	1.5	40	10	59	60.50063264
3	6	1.5	40	10	74	72.34013804
4	10	0.5	60	5	66	66.97292803
5	2	0.5	60	15	67	68.94958105
6	2	0.5	60	5	68.7	66.96949568
7	2	2.5	20	15	70.8	70.74179107
8	10	2.5	20	15	70	68.94958105
9	2	2.5	60	15	69.1	68.22151495
10	6	1.5	40	10	65	64.42930494
11	2	0.5	20	5	73	74.75160032
12	10	2.5	60	15	63	65.53095796
13	2	0.5	20	15	74	73.37046337
14	2	0.5	60	5	73	73.07825335
15	10	2.5	60	5	67	65.74816798
16	10	2.5	20	15	74	73.07825335
17	6	1.5	40	15	63	63.19805057
18	6	1.5	40	15	80.5	79.9594382
19	10	0.5	60	15	81	82.86325088
20	10	2.5	60	15	83.4	82.86325088
21	2	2.5	60	5	63	62.62408038
22	2	2.5	20	5	62	62.0334084
23	10	0.5	60	15	56	56.42972396
24	6	1.5	40	10	65	64.22776481
25	10	2.5	60	5	83.5	82.86325088
26	2	2.5	60	5	83.2	82.86325088
27	10	0.5	20	5	82.9	82.86325088
28	10	0.5	20	15	82.4	82.86325088
29	2	0.5	60	15	83.1	82.86325088
30	2	2.5	20	15	83.2	82.86325088
31	6	1.5	40	10	83	82.86325088

%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 (pH^2)-1.99484(adsorbent^2)-9.07506× 10^{-3} (temperature^2)-0.025495(time^2)

(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 ℃. 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 dueto noise. The fit of the model was checked by the determination coefficient (R^2).The "Pred R-quared" 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

Table 2. ANOVA analysis for removal of metronidazole

Source	Sum of square	Df	Mean Square	F-Value	P-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 dose	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 22.2minute was enough to obtain highest yield.

In addition, **Figuer 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.
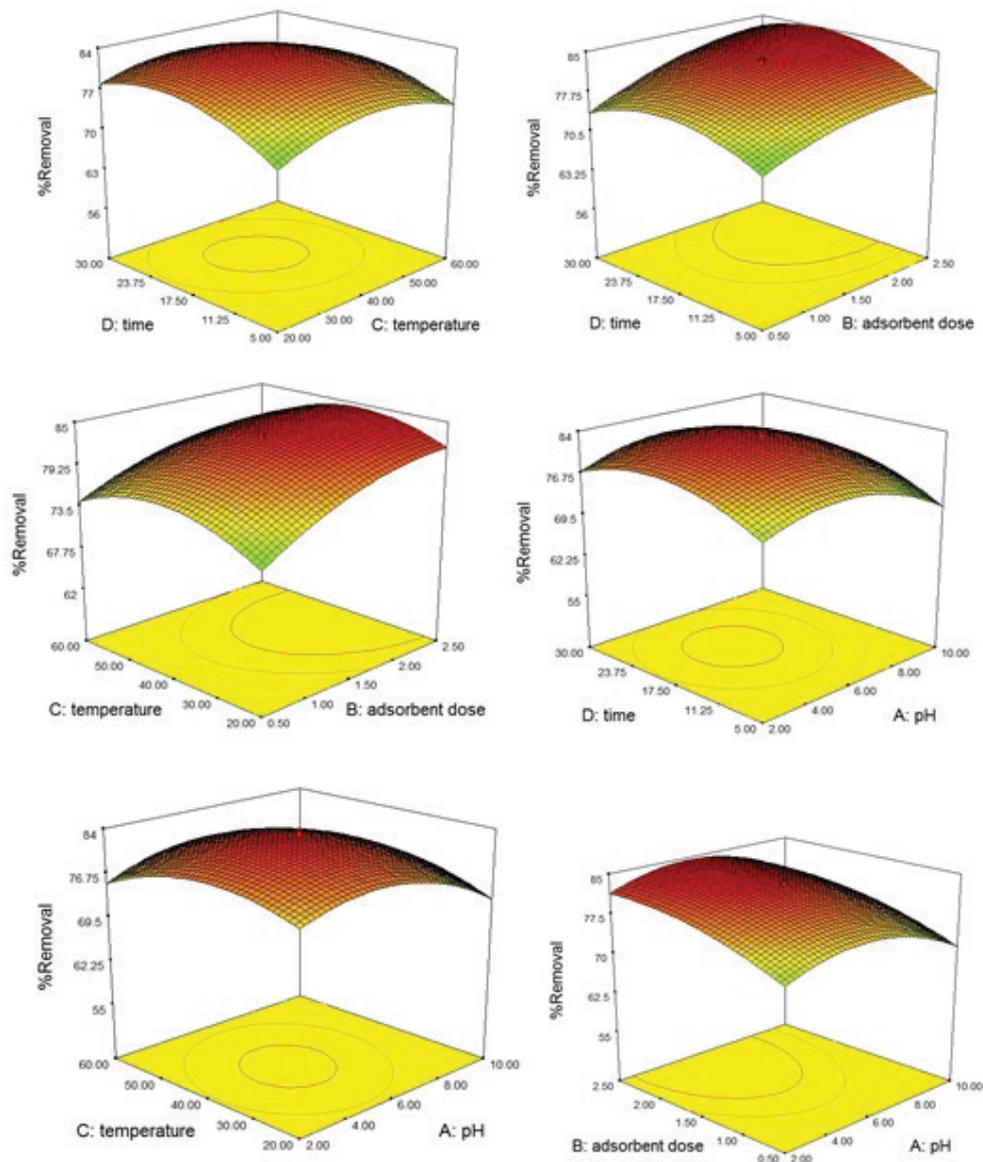

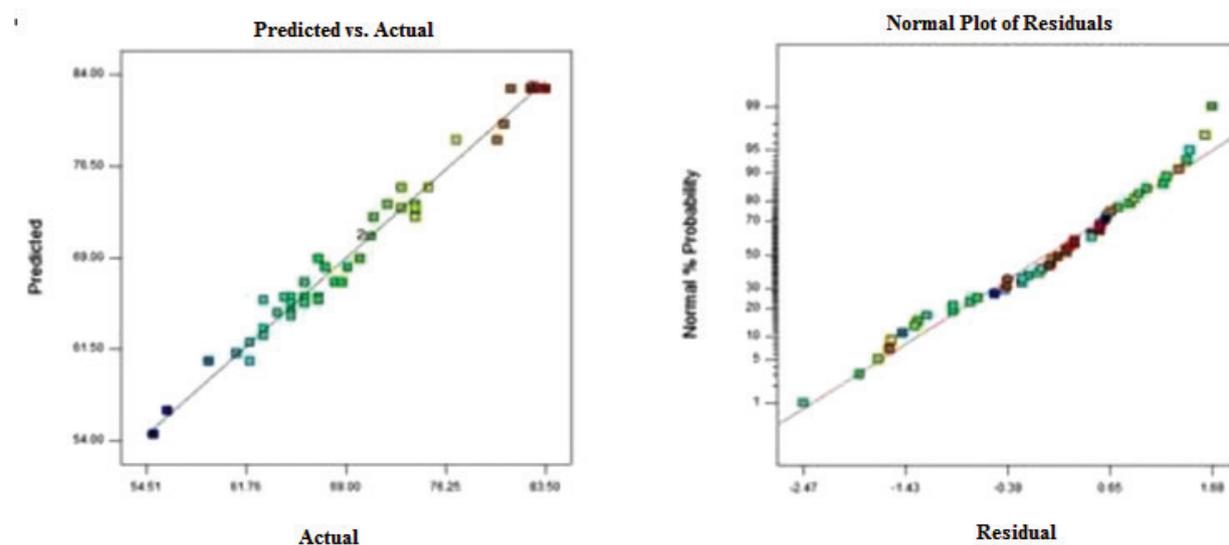**Fig.1.** Response surface modeling obtained by CCD.

Fig.2 The normal probability plot of the residuals and parity plot show the correlation between the observed and predicted values

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 **Equation 4 and 5** as follows:

$$\text{Langmuir:} \quad \frac{Cf}{qf} = \frac{1}{bqm} + \frac{Cf}{qm}$$

(Eq. 4)

$$\text{Freundlich:} \quad \log qf = \log Kf + \frac{1}{n}\log Cf$$

(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 acceptation 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

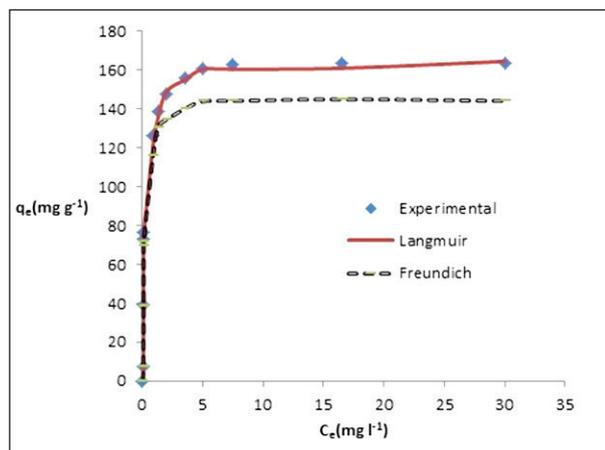| Langmuir isotherm			Ferundlich isotherm		
q_m(mg g^{-1})	b(L mg^{-1})	R^2	K_f(l g^{-1})	n	R^2
215.14	0.52	0.991	65.815	2.063	0.879

Fig.3. Fitting results of Langmuir and Ferundlich models with experimental data for adsorption of the metronidazole on the MMWCNTs

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.

Table 4. Adsorption of metronidazole in wastewater samples
by spiking of metronidazole at different concentration by HPLC (mg L^{-1})

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 $Fe_3O_4@$ 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 $Fe_3O_4@$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 $Fe_3O_4@$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

6. References

[1] W. Yan, Y. Xiao, W. Yan, The effect of bioelectrochemical systems on antibiotics removal and antibiotic resistance genes: a review, Chem. Eng. J. 358 (2019) 1421-1437.

[2] J. Xu, Z. Cao, Y. Wang, Y. Zhang, X. Gao, Distributing sulfidized nanoscale zerovalent iron onto phosphorus-functionalized biochar for enhanced removal of antibiotic florfenicol, Chem. Eng. J., 359 (2019) 713-722.

[3] T. D. Pham, T. Ngan Vu, H. L. Nguyen, Adsorptive removal of antibiotic ciprofloxacin from aqueous solution using protein-modified nanosilica, Polymers, 12 (2020) 57.

[4] N. Hanna, P. Sun, Q. Sun, Presence of antibiotic residues in various environmental compartments of Shandong province in eastern China: its potential for resistance development and ecological and human risk, Environ. Int., 114 (2018) 131-142.

[5] R. Pallares-Vega, Determinants of presence and removal of antibiotic resistance genes during WWTP treatment: a cross-sectional study, Water Res., 161 (2019) 319-328.

[6] T. Ariyanto, R. Aprillia G. Sarwendah, Y. Maulana, Modifying nanoporous carbon through hydrogen peroxide oxidation for removal of metronidazole antibiotics from simulated wastewater, Processes, 7 (2019) 835.

[7] M. L. Tran, C.-C. Fu, R.-S. Juang, Removal of metronidazole and amoxicillin mixtures by UV/TiO 2 photocatalysis: an insight into degradation pathways and performance improvement, Environ. Sci. Pollut. Res., 26 (2019) 11846-11855.

[8] Y. Zhou, T. Wang, D. Zhi, Applications of nanoscale zero-valent iron and its composites to the removal of antibiotics: a review, J. Mater. Sci., (2019) 1-18.

[9] Zhang, Shuai, et al. "A review of bioelectrochemical systems for antibiotic removal: Efficient antibiotic removal and dissemination of antibiotic resistance genes, J. Water Process Eng., 37 (2020) 101421.

[10] S. Talwar, A. K. Verma, V. K. Sangal, U. L. Štangar, Once through continuous flow removal of metronidazole by dual effect of photo-Fenton and photocatalysis in a compound parabolic concentrator at pilot plant scale, Chem. Eng. J. 388(2020) 124184.

[11] B. Davoud, A. Dokht Khatibi, K. Chandrika, Antibiotics removal from aqueous Solution and pharmaceutical wastewater by adsorption process: A review, Int. J. Pharm. Investigate., 10 (2020) 106-111.

[12] H. Cai, T. Zhao, Z. Ma, J. Liu, Efficient Removal of Metronidazole by the Photo-

Fenton Process with a Magnetic Fe3O4@ PBC Composite, J. Environ. Eng., 146 (2020) 04020056.

[13] K. Hasani, A. Peyghami, A. Moharrami, The efficacy of sono-electro-Fenton process for removal of Cefixime antibiotic from aqueous solutions by response surface methodology (RSM) and evaluation of toxicity of effluent by microorganisms, Arab. J. Chem. 13 (2020) 6122-6139.

[14] M. Galedari, M. Mehdipour Ghazi, S. R. Mirmasoomi, Photocatalytic process for the tetracycline removal under visible light: Presenting a degradation model and optimization using response surface methodology (RSM), Chem. Eng. Res. Design, 145 (2019) 323-333.

[15] S. Gholamiyan, M. Hamzehloo, F. Abdolhadi, RSM optimized adsorptive removal of erythromycin using magnetic activated carbon: Adsorption isotherm, kinetic modeling and thermodynamic studies, Sustain. Chem. Pharm., 17 (2020) 100309.