



# Magnetic bentonite nanocomposite for removal of amoxicillin from wastewater samples using response surface methodology before determination by high performance liquid chromatography

Mohammad Reza Rezaei Kahkha<sup>a,\*</sup>, Ali Faghihi Zarandi<sup>b</sup>, Nahid Shafighi<sup>a</sup>, Saeedeh Kosari<sup>a</sup>  
and Batool Rezaei Kahkha<sup>a</sup>

<sup>a</sup> Department of Environmental Health Engineering, Faculty of Health, Zabol University of Medical Sciences, Zabol, Iran.

<sup>b</sup> Department of Occupational Health Engineering, Faculty of Health, Kerman University of Medical Sciences, Kerman, Iran.

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## ABSTRACT

In this study, feasibility of magnetic bentonite nanocomposite for removal of amoxicillin from wastewater samples was evaluated by high performance liquid chromatography (HPLC). Magnetic bentonite synthesized by co-precipitation of bentonite and  $\text{Fe}_3\text{O}_4$  and used for removal of amoxicillin from water samples. Response surface methodology on central composition design (CCD) was applied for designing of experiment and building of model. Three factors including pH, adsorbent dose and temperature were studied and used for quadratic equation model to prediction of optimal points. By solving the equation and considering regression coefficient ( $R^2 = 0.98$ ). The optimal points of main parameters were obtained as a pH of 4.68, the adsorbent dosage of 1.50 g and the temperature of 48.9<sup>o</sup> C. Results showed that three factor are significant on removal efficiency and experimental data are in good agreement with predicted data. Proposed methods were used to analysis of amoxicillin in three real samples.

## 1. Introduction

Nowadays, antibiotic residual is a serious concern for many of environmental researchers. Because of insufficient ability of conventional sewage treatment, some antibiotics such as ampicillin, erythromycin, tetracycline and penicillin are not even removed in sewage treatment processes and cause many environmental hazards [1]. Recent

studies have shown that some antibiotics have toxic effects on the life of microorganisms and, over the long term, have undesirable effects on ecological sustainability [2]. Amoxicillin ( $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S}_3$ ) is a  $\beta$ -lactam antibiotic with a molecular weight of 365 gram per mole is used to treat bacterial infections [3]. The concentration of this type of antibiotics groups in surface waters is 48 ng L<sup>-1</sup> and in the hospital sewage between 28 -82 mg L<sup>-1</sup> have been reported. In many pharmaceutical effluent output higher concentration of these drugs can also be found. Several methods such as; the ozonation [4],

\* Corresponding Author: Mohammad Reza Rezaei Kahkha

Email: [m.r.rezaei.k@gmail.com](mailto:m.r.rezaei.k@gmail.com)

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the Fenton process [5], electrochemical methods [6], the nano filtration [7] and the adsorption process [8] were applied for removal of antibiotics from aqueous environments samples. Absorption is one of the most effective methods to removal of antibiotics compounds from water and sewage even at low concentrations (less than 1 mg L<sup>-1</sup>). Adsorption is very simple and low cost method in comparison to other techniques that applied for removal of common pollutants from aqueous samples [9]. Natural clay compounds are one of the best adsorbents to removal of contaminants from air and water samples. This ability obtained from their high surface area, the porous structure, the chemical stability, and their layered structure. Bentonite is a natural clay that used as an adsorbent to removal of pollutants from water and wastewater samples. Response surface methodology (RSM) is appropriate technique that used in many fields [10]. The main objective of RSM is to determine optimum operating conditions for the system or designated area of the practical satisfaction [11]. Experimental data points were obtained during our optimization and used to build a model for CCD which was ideal for sequential testing and allows the right amount of information to test the lack of fit a large number of unusual design points. In this study, removal of amoxicillin by 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 amoxicillin such as pH, amount of adsorbent, and the temperature were optimized. Finally, the data obtained from experiments compared with model output to optimize and predict the results. The concentration of amoxicillin determined by high performance liquid chromatography. HPLC is simple, accurate and precise technique that used for separation, identification and analysis of drugs. It can be successfully and efficiently adopted for routine quality control analysis of drugs in bulk and pharmaceutical dosage form. It can also be used in combination with other analytical methods to further elucidate the components of mixtures.

## **2. Material and methods**

### **2.1. Apparatus and reagent**

The measurement of amoxicillin was performed using high performance liquid chromatography accessory (CECIL Corporation, HPLC, England) equipped ACE C<sub>18</sub> column and UV-VIS detector at 230 nm. The mobile phase is ACN: water (60:40). Analytical grade of different reagents such as; HCl and NaOH were purchased from Merck (Darmstadt, Germany). The amoxicillin was prepared from Aldrich chemical Co. HPLC grade of acetonitrile and water purchased from Sharloa (Spain). Bentonite clay was purchased and from Merck (Darmstadt, Germany) and used for further work. The bentonite samples were powdered and sieved by 80-mesh sieve and washed with double distilled water (DDW) for 4 times before using by procedure.

### **2.2. Synthesize of adsorbent**

Synthesize of magnetic bentonite are performed by co-precipitation methods by Hashem et al. First, 20 g of bentonite was added into 100 ml of distilled water containing FeCl<sub>2</sub> (0.02 mol L<sup>-1</sup>) and FeCl<sub>3</sub> (0.04 mol L<sup>-1</sup>). The pH of solution was set around 10 by adding NH<sub>4</sub>OH buffer solution (1 mol L<sup>-1</sup>) and stirred for 30 min at 300 rpm. Next, 40 ml of HNO<sub>3</sub> solution (2 M) was added with stirring for 5 minutes and then 60 ml of Fe(NO<sub>3</sub>)<sub>3</sub> solution (0.35M) was added to the previous solution and solution boiled for one hour. After settling suspension, the residual was filtered and solid of magnetic bentonite was separated by washing of DDW for 3-5 times. Finally, the product was heated in an oven at 80°C for 24 h [12].

### **2.3. Removal Procedure**

Experiments were performed with the central composite design (CCD) methodology. A standard solution of 1000 mg L<sup>-1</sup> amoxicillin was prepared by dissolving of 1 g of amoxicillin in 1 liter of deionized water. All standard working prepared from this solution. Experiments were performed at a batch reactor in 500 ml beaker that containing of 50 ml of amoxicillin concentration and the solution was shaken for the 30 minutes in incubation shaker at 200rpm by controlling of temperature. The pH

of the solution was adjusted by adding 0.1 M of NaOH and HCl. After completion of experiments, magnetic nanocomposite was removed by an external magnet and remaining amoxicillin was measured. The removal percentage of Amoxicillin (% removal) was calculated as Equation 1:

$$\% \text{Removal} = \frac{C_e - C_0}{C_0} * 100$$

**(Eq. 1)**

Where  $C_e$  and  $C_0$  are initial and final Amoxicillin concentration ( $\text{mg l}^{-1}$ ) in solution, respectively.

#### 2.4. Experimental design

A central composition design (CCD) was carried out in this research for evaluate the variables for adsorption of amoxicillin from aqueous solution using in a batch reactor. The CCD method for three variables (pH, amount of nanocomposite and temperature), with two levels (the minimum and maximum) was used as experimental design model. In the experimental design model, the pH between

2-9, the adsorbent dosage from 0.5 g to 1.5 g and the temperature between 20-60° C were employed as the input variables. Percentage removal of amoxicillin was the response of the system. Table. 1 showed the experimental design matrix that obtained from CCD procedure. The amoxicillin concentration determined by HPLC. The quadratic equation model for predicting the optimal point of adsorption processes was expressed by Equation 2.

$$Y = \beta_i + \sum_{i=1}^k \beta_{ixi} + \sum_{i=1}^k \beta_{iixi^2} + \sum_{i=0}^k \sum_{j=i+1}^k \beta_{xixj} + \epsilon$$

**(Eq.2)**

Where Y is the response of the system and  $X_i$  and  $X_j$  are the variables of action.  $R^2$  is coefficient of determination of polynomial model. The statistical significance was verified with adequate precision ratio and by the F-test. Design expert (version 8) program was used for regression and graphical analysis. A total of 19 experiments were necessary to estimate of the full model (**Table 1**)

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

Run	pH	T(°c)	adsorbent(g)	Actual Value (%)	Predicted Value(%)
1	5.5	45	1	98	92.20
2	11.39	45.00	1.00	20	19.20
3	5.50	45.00	1.00	96	92.20
4	9.00	65.00	.050	57	60.29
5	5.50	45.00	1.00	83	92.20
6	5.50	45.00	1.84	95	92.20
7	2.00	65.00	1.50	99	91.64
8	2.00	25.00	1.50	97	77.97
9	5.50	45.00	1.00	98	92.20
10	5.50	11.36	1.00	86	100
11	2.00	65.00	0.50	83	65.08
12	5.50	45.00	0.16	90	100
13	9.00	65	1.50	70	66.82
14	9.00	25.00	0.50	83	74.58
15	2.00	25.00	0.50	97	84.41
16	5.50	78.64	1.00	93	100
17	5.50	45.00	1.00	92	92.20
18	9.00	25.00	1.50	46	48.14
19	5.50	45.00	1.00	90	92.20

### 3. Result and discussion

#### 3.1. Regression model and statistical analysis

The CCD method has been successfully used for optimizing affecting factor that influenced on the percentage of amoxicillin removal. For the best response of system, the experimental results were analyzed through RSM to obtain an empirical model. The regression model equations (second-order polynomial) relating the removal efficiency of amoxicillin and related parameters were developed as Equation 3:

$$\begin{aligned} \% \text{Removal} = & +35.00926 + 7.71191 \times \text{pH} + \\ & 0.91252 \times T + 23.48479 \times \text{Dose} - 7.14286 \times \\ & 10^{-4} \times \text{pH} \times T + 0.38571 \times \text{pH} \times \text{Dose} + 5 \times 10^{-3} \times T \times \text{Dose} - \\ & 0.68596 \times \text{pH}^2 - 0.013048 \times T^2 - 8.99753 \times \text{Dose}^2 \end{aligned}$$

(Eq. 3)

The coefficients of Equation 3 are determined by using software Design-Expert 8. The optimal parameters are as follows: pH = 4.68, the adsorbent dosage = 1.5 g, and the temperature = 48.90° C.

The model prediction of the amoxicillin removal was obtained %99.48 while the experimental amount of removal efficiency achieved %99. These results confirmed that the RSM could be effectively applied to optimize the factors and parameters in complex processes. The term of encoded factor expressed relation between the independent variables and dependent response of system. Apart from the line are effects of the parameter for the amoxicillin removal, the RSM also gives an insight in to the quadratic and interaction effect of the parameters. Table 2 showed the results of regression analysis of obtained quadratic model. The F-values and p-values are related to significant of each coefficient (Table 2). High magnitude of the F-values and small magnitude of the p-values indicated that corresponding coefficients was more significant. Also, Values of “prob> F” less than 0.0500 implied high significant regression at 95 percent confidence level. According to the F-value and p-values, the amount of adsorbent was found more effective on the adsorption process of amoxicillin. The “Lack of Fit F-value” of 0.26

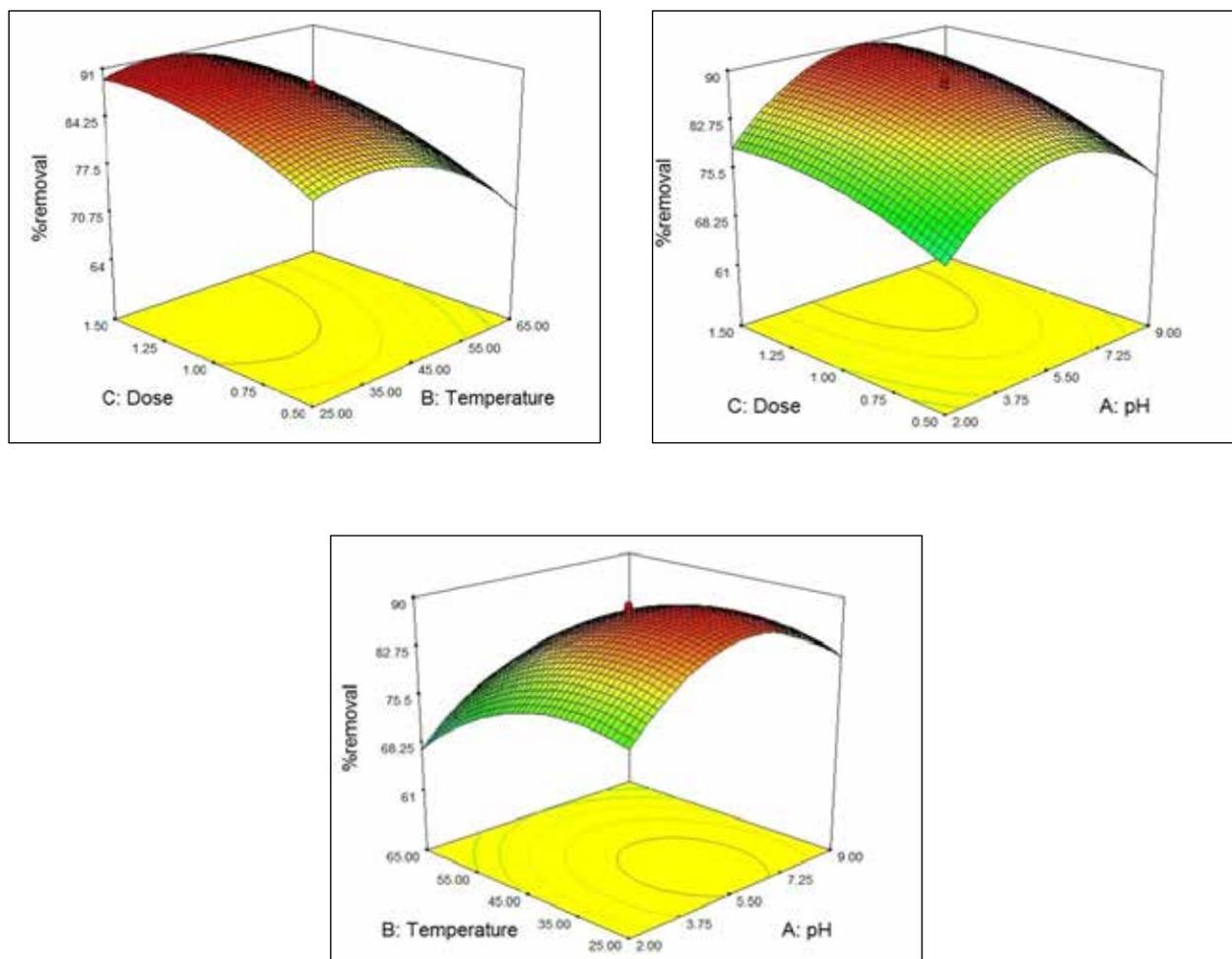
**Table 2.** ANOVA analysis for removal of amoxicillin

Source	sum of square	df	Mean		P-value
			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 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 <sup>2</sup>	1107.54922	1	1107.54922	737.946571	< 0.0001
B <sup>2</sup>	225.03502	1	225.03502	149.938096	< 0.0001
C <sup>2</sup>	745.166816	1	745.166816	496.495584	< 0.0001
D <sup>2</sup>	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 rror	47.526	25	1.90104		

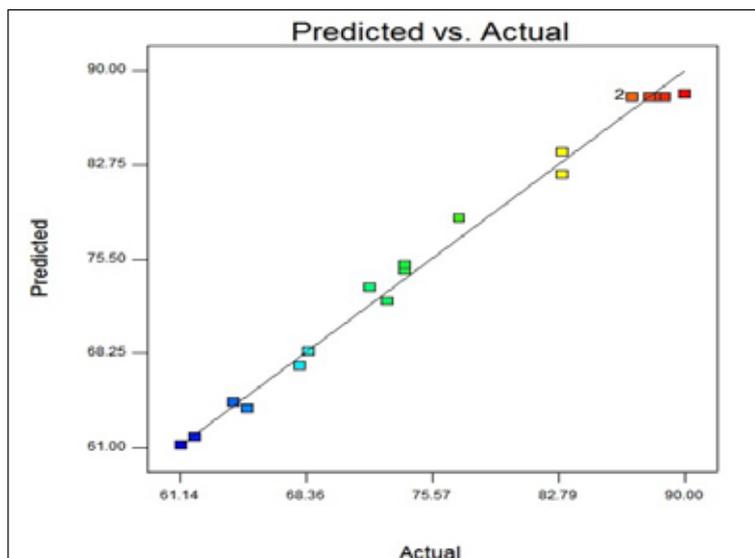
indicated that the Lack of Fit is not significant. The fitness of the model was expressed by the determination coefficient ( $R^2$ ). The “Pred R-squared” of 0.9848 is in agreement with the “Adj R-Squared” of 0.9768. “Adeq Precision” expressed the value of signal to noise ratio. A ratio greater than 4 is favorable. In this case “Adeq Precision” of 30.63 indicates an adequate signal. Thus, as a result of the statistical analysis, quadratic model was found suitable for demonstrate the adsorption process and useful for developing empirical relationships. Removal efficiency of amoxicillin was sensitively depended to the amount of adsorbent. The removal efficiency was sharply increased when the amount of adsorbent increased from 0.5 g to 1.5 g. The increase in yield due to increase in adsorbent dosage was more dominated

than other significant factors. The pH of the solution was another factor that influenced the response of system. As can be seen on **Figure 1**, when the pH increased from 2 to 5, the removal efficiency of amoxicillin was increased. Temperature is a key factor which effect on adsorption process. In this study, effect of temperature on removal efficiency of amoxicillin was investigated. Results showed that by increasing of temperature, the removal efficiency of amoxicillin decreased and in 48°C the highest removal efficiency was obtained.

The parity plot are presented in **Figure 2**. As shown in **Fig. 2**, there was a satisfactory correlation between the observed and fitted values. In addition, a normal distribution of residual were observed in **Figure 2** which indicated the data points can be formed approximately straight line.



**Fig.1.** Response of surface by CCD method



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

**Table 3.** Adsorption of amoxicillin in real samples based on magnetic bentonite nanocomposite using response surface methodology

Sample	Added (mg L <sup>-1</sup> )	Initial Amount (mg L <sup>-1</sup> )	Final Amount (mg L <sup>-1</sup> )	Recovery (%)
Sample 1	-----	-----	56.6 ± 2.1	-----
	50	-----	105.4 ± 4.7	97.6
Sample 2	-----	-----	66.3 ± 2.8	-----
	50	-----	117.1 ± 5.6	101.4
Sample 3	-----	-----	23.9 ± 0.9	-----
	20	-----	43.5 ± 1.7	98.0
CRM 1	-----	23.4	22.2 ± 1.1	94.8
	20	-----	41.6 ± 1.9	97.0
CRM 2	-----	58.9	56.8 ± 2.3	96.4
	50	-----	107.2 ± 5.1	100.8
CRM 3	-----	102.5	98.7 ± 4.8	98.9
	100	-----	96.9 ± 4.6	96.9
CRM 4	-----	47.1	48.2 ± 1.7	102.3
	50	-----	96.8 ± 1.7	97.2

Mean of three determinations ± standard deviation (P = 0.95, n = 5)

Sample 1: Wastewater Drug Factory

Sample 2: Wastewater Hospital

Sample 2: Wastewater Drug Razi

CRM 1, 2, 3 and 4 analysis with HPLC-MS with concentration of 23.4, 58.9, 102.5 and 47.1 respectively

### 3.2. Application of proposed methods to real sample

To verify the potential application of proposed method to real samples, three real wastewater samples were selected and used by proposed procedure. Different concentrations of amoxicillin include 100, 150 and 200 mg L<sup>-1</sup> were spiked to the samples and removal efficiency of amoxicillin was tested using proposed methods. Results are shown in **Table 3**. As expected, the magnetic bentonite nanocomposite has good recovery for adsorption of the spiked amoxicillin from all three real samples.

### 4. Conclusions

In this work, a magnetic bentonite nanocomposite was synthesized and applied for adsorption of amoxicillin from wastewater. A central composition design was applied for study of the effects of parameters such as; pH, temperature and amount of adsorbent which was influenced on the removal efficiency of amoxicillin. A quadratic model was solved and developed to correlate the independent variables and the response of system. Through the analysis of response surfaces, was found that amount of adsorbent was most significant variable on removal efficiency of amoxicillin by HPLC. Process optimization was performed and results showed that the experimental data were found to agree with the predicted values.

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