OPTIMIZATION PROCESS FOR THE REMOVAL OF HEAVY METALS FROM AQUEOUS SOLUTION USING GRAPHENE OXIDE NANOSHEETS AND RESPONSE SURFACE METHODOLOGY

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Abstract. Water pollution due to heavy metals has become a critical problem worldwide. In this study, the removal of chromium(III), cadmium and lead by graphene oxide adsorbent was examined. Graphene oxide nanosheets were synthesized through Hummer's method, and their characteristics were examined using FTIR, XRD, and SEM. The effect of independent variables, pH, contact time and initial concentration of solution on the removal efficiency of Cr(III), Cd^{2+} and Pb^{2+} was evaluated according to the experimental Box-Behnken Design using response surface methodology (RSM). Applying quadratic model, the adsorption rate of Cd^{2+} and Pb^{2+} was obtained as 99% and the adsorption rate of Cr(III) was obtained as 98%. ANOVA was applied as statistical analysis of responses. According to FESEM images, the average size of graphene oxide sheets was 1 to 3 μm. After optimization by RSM, adsorption capacities of Cr(III), Pb^{2+} and Cd^{2+} were found to be 38 mg/g, 136 mg/g and 68 mg/g, respectively. Examination of isotherms suggested that Cd^{2+} and Cr(III) adsorptions follow Langmuir, and Pb^{2+} adsorption follows Freundlich isotherm. The results showed that graphene oxide has a good effect on removing Cr(III), Cd^{2+} and Pb^{2+} ions from aqueous solutions. pH of the solution and initial concentration of the contaminant had the highest effect on adsorption of the mentioned heavy metals. The results of RSM analysis showed that the obtained data were in agreement with the predicted model.
Keywords: *adsorption, water pollution, heavy metals, Box-Behnken Design, Langmuir, Freundlich*

Abbreviations: *GO:* Geraphene oxid; *RSM:* Response surface methodology; *FTIR:* Fourier transform infrared; *XRD:* X-ray diffraction; *SEM:* scanning electron microscope; *FESEM:* Field Emission Scanning Electron Microscope; *BBD:* Box-Behnken Design; *EPA:* Environmental Protection Agency ;*Cr:* Chromium; *Cd:* Cadmium; *Pb:* Lead; *pH:* potential hydrogen; *Conc:* Concentration; *PZC:* point of zero charge

Introduction

Growth of urbanization, rapid industrialization of cities and emission of heavy metals in the ecosystem - even at extremely low concentrations - have brought up concerns worldwide. Removal of heavy metals from water and wastewater has been at the center of attention owing to strict regulations on water pollution control in many countries (Amarasinghe and Williams, 2007). Lead, chromium, cadmium, zinc, and nickel are among the most water polluting heavy metals (Yang et al., 2014). Conventional methods for the removal of heavy metals from water include chemical oxidation, chemical precipitation, ion exchange, adsorption, filtration, reverse osmosis, solvent extraction and coagulation-flocculation (Rajasulochana

and Preethy, 2016). Adsorption method is known to be better than other techniques due to high efficiency, flexibility, simple design, low cost, easy operation and minimum sludge production (Fu and Wang, 2011). The most important characteristics for an adsorbent are high adsorption capacity and rapid adsorption. These can be achieved when the adsorbent possesses a large specific surface area and abundant adsorption sites (Wang et al., 2012). Majority of adsorbents rarely have both of the mentioned characteristics. Therefore, an investigation on new adsorbents which can properly adsorb heavy metals is required (Pan et al., 2009). Among the studied adsorbents, graphene oxide nanoparticles, owing to adsorption capacity and large specific surface area, are applied either independently or along with other adsorbents in order to remove heavy metals from aqueous solutions (Liu et al., 2011; McGrail et al., 2016). Considerable physical properties of graphene include heat superconductivity, extremely high electron mobility (Jiang et al., 2010), high mechanical strength (Yu et al., 2008) and adsorption of some metal ions (Hao et al., 2012). Sitko et al. (2013) examined the adsorption of heavy metal ions from aqueous solutions using graphene oxide, and observed that maximum adsorption occurred at pH ranges of 4-8 for cadmium and 3-7 for lead and chromium. Fan et al. (2013) examined a mixture of graphene oxide and magnetic chitosan (MCGO) as an adsorbent and reached a maximum adsorption capacity of 96% for lead ions. Kumar et al. (2014) studied the removal of lead and arsenic using graphene oxide nanosheets and concluded that graphene oxide is highly efficient in removing heavy metals from aqueous solutions. Among the contaminants, chromium(III), cadmium and lead are the most environment-polluting elements since they are widely used in industry. The efficiency of adsorption processes depends on various parameters such as pH, temperature, type of adsorbent, adsorbent dosage and organic or inorganic contaminants (Liu et al., 2011). The water containing high concentration of chromium may cause serious environmental problems and also skin sensitivity, cancer and DNA damage to humans (Tchounwou et al., 2012). Cadmium is categorized as a carcinogenic element, which leads to lung cancer, kidney diseases, digestive and immune system disorders in human bodies (Jaishankar et al., 2014). According to EPA, lead, after entering the body, does not leave the body and precipitates in blood, skin, organs and tissues including muscles, bones and joints (Huang et al., 2014; Li et al., 2007) and causes environmental pollution with severe toxic effects on human health and other creatures (Uluozlu et al., 2008).

The conducted studies show that graphene oxide nanosheets are the most effective adsorbents used for the removal of heavy metals from aqueous environments and soil. The key reason for using graphene oxide nanosheets in this study is their simultaneous possession of several properties.

In this study, the efficiency of graphene oxide nanosheets in removing chromium(III), cadmium and lead from aqueous solutions was examined. The aim of this study was to evaluate the effect of pH, initial concentration and contact time on graphene oxide adsorption capacity. The variables analyzed by response surface method (RSM) were applied according to Box-Behnken design in order to optimize heavy metals removal from aqueous solutions. Also, absorption isotherm was investigated using Langmuir and Freundlich adsorption isotherms.

Materials and methods

Materials and instruments

Chemicals applied in the experiments were lab grade. All the chemicals including nitric acid 65% (HNO_3), sulfuric acid 97% (H_2SO_4), hydrogen peroxide 30% (H_2O_2), hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium nitrate ($NaNO_3$), sodium chloride (NaCl), chromium(III) chloride ($CrCl_3$), potassium dichromate ($K_2Cr_2O_7$), potassium permanganate ($KMnO_4$), lead nitrate ($PbNO_3$) and graphite powder were provided by Merck, Germany. Deionized water was used in all the experiments. Synthesis of graphene oxide nanosheets was done using Hettich centrifuge (Model Universal 320 R), an ultrasonic bath (Elmasonic, E 60H Model, Germany) and a heater (IKA 2000 Model, Germany). Moreover, pH was determined by a pH-meter (pH-Meter 765, Germany), and agitation of the samples was carried out using an IKA-werke-KS260C shaker, Germany. The samples containing Cr(III), Cd^{2+} and Pb^{2+} ions were analyzed through ICP (Inductively Coupled Plasma) Varian-OES-710.

Synthesis of graphene oxide

Using the modified Hummer's method (which is suitable to synthesize graphene oxide), the synthesis of graphene oxide was carried out as follows. Initially, 200 mL concentrated sulfuric acid was poured into a 500 mL glass container and its temperature decreased after stirring in an ice-water bath for 30 min. Then, 4 g of sodium nitrate was added to the solution and its temperature decreased after stirring in an ice-water bath for 1 h. Afterward, 4 g of graphite (500 mesh) was added and mixed for 2 h in this condition. 4 g of graphite (500 mesh) was added to the solution and then mixed for 2 h. During 2 h, 24 g of potassium permanganate was added and stirred. The glass container containing the sample was fixed in an oil bath at 38 to 40 °C for 24 h. After removing the oil bath, 640 mL double distilled water was added to reaction container and stirred for 30 min. The reaction stopped by adding 140 mL hydrogen peroxide 30%. The sample was totally settled after three days. After removing the supernatant, the produced graphene oxide was washed four times using HCl (5%) and then filtered through a buchner funnel. The obtained suspension was washed four times using double distilled water. Then the suspension was treated by ultrasound for 30 min. Finally, the obtained brown solid substance was dried in a vacuum oven at 40 °C (Chaiyakun et al., 2012).

Determination of PZC

In order to examine the heavy metals removal more closely, pH of the zero charge point, where the adsorbent surface is electrically neutral and unladen, and also the adsorbent PZC (pH_{PZC}) were measured (Zhao et al., 2011, 2012). In order to measure PZC, 10 flasks containing 0.1 mol/L NaCl solution were used. The initial pH of the solution was adjusted in the range of 1 to 10 using NaOH and HCl 0.1 mol/L. Then, 0.1 g adsorbent was added to each solution, and the electrolyte solution was stirred with the adsorbent for 24 h. After reaching equilibrium, pH of the solutions was measured, ΔpH graph was drawn according to the initial pH, and pH_{PZC} was estimated.

Characterization of graphene oxide nanosheets

Morphology, size and shape of the synthesized adsorbent were examined using a field emission scanning electron microscope (FESEM model MIRA3, TESCAN).

Characteristics of graphene oxide nanoparticles were determined using an x-ray diffraction model: X'Pert PRO MPD PANalytical. Fourier transform infrared (FTIR) of graphene oxide in the range of 400-4000 cm^{-1} was analyzed using Bruker, Tensor 27, Germany.

Adsorption experiments

All the experiments were conducted in a batch mode. Initially, the solutions containing Cr(III), Cd^{2+} and Pb^{2+} were prepared by adding a certain amount of potassium dichromate, cadmium nitrate and lead nitrate to 1 L of DI water. In order to study the effect of environmental pH on adsorbing metals within the range of 2-6, 0.05 g/L and 0.02 g/L of the synthesized graphene oxide were added to the lead and cadmium-containing solutions and chromium-containing solution, respectively (the initial concentration was within the ranges of 10-100 mg/L for Cd^{2+} and Cr(III) and 50-150 mg/L for lead, and contact time of 5-60 min was allotted to each metal). The solutions were studied by the presented model and stirred on a magnetic stirrer with a constant speed of 200 rpm for a specified time. Finally, the solutions were stabilized as the mentioned time passed. At the next step, the suspension was filtered by centrifugal method and passed through 0.2 μm filters. The heavy metals concentration after adsorption was measured using ICP. Adsorption efficiencies of Cr(III), Pb^{2+} and Cd^{2+} were calculated by *Equation 1* (Belay et al., 2010).

$$R = \frac{(C_0 - C_e)}{C_0} * 100 \qquad \text{(Eq.1)}$$

Moreover, adsorption capacity of the synthesized graphene oxide at various heavy metals concentrations was determined using *Equation 2* (Li et al., 2011).

$$Qe = \frac{(C_0 - C_e)V}{m} \qquad \text{(Eq.2)}$$

where, R is adsorption removal efficiency, Qe is the amount of the heavy metal adsorbed onto the adsorbent in equilibrium conditions (mg/g), C_0 is the initial concentration of the heavy metal in solution (mg/L), C_e indicates equilibrium concentration of the heavy metal after equilibrium time elapsing (mg/L), V is volume of the solution in liter, and m is mass of the adsorbent in gram.

Experimental design and data analysis

RSM along with experimental techniques was used to assess the relationship among the effective variables in the experiment (Ghafari et al., 2009). ANOVA was applied as a statistical method for analysis of responses. Response surface method was applied according to Box-Behnken design in order to optimize heavy metals removal from aqueous solutions. This method has several advantages such as reducing the number of test steps, time and cost, and saving the consumption materials. This method is used in this study because it can 1) test several levels of testing with different factors, 2) identify how removal efficiencies are affected by changes in the level of pH, initial concentration and contact time (as factors), and 3) specify the optimized combination of adsorbent pH, initial concentration and contact time for the efficient removal of heavy metals.

In this study, pH of the solution, concentration of contaminants and contact time were chosen as the independent variables affecting the heavy metals removal efficiency and labeled as pH (pH), concentration (conc) and contact time (Time), respectively (*Tables 1A, 2B,* and *3A*). Version 10 of Design-Expert software was used to investigate the effect of independent variables on response surface methodology (adsorption capacity of adsorbent and adsorption efficiency of heavy metals). Designing was carried out using the Box-Behnken design. The number of steps in the experiment was determined using *Equation 3:*

$$N = 2K\left(K-1\right) + C_o \qquad (Eq.3)$$

where, K represents the number of factors investigated and C_o indicates the number of replication for the experiment (Khajeh, 2011). 17 experiments with five replicates at center points were done for each metal. Thus, totally 51 experiments were designed and conducted (Yetilmezsoy et al., 2009). A second-order polynomial regression model equation, which relates the removal efficiency to the investigated factors, can be presented as *Equation 4:*

$$Y = C_{k0} + \sum_{i=1}^{4} C_{ki}x_i + \sum_{i=1}^{4} C_{kii}x_i^2 + \sum_{i<j=2}^{4} C_{kij}x_ix_j \qquad (Eq.4)$$

where, Y is predicted response; C_{k0}, C_{ki}, C_{kii} and C_{kij} are regression coefficients for intercept, linear effects, quadratic effects and double interactions, respectively; and x_i and x_j are coded values of the independent variables (Wu et al., 2012).

In order to evaluate the accuracy of the models, statistical analysis was carried out using ANOVA with p-value (Prob $\leq$ F $<$ 0.0001). Adequacy and predictability of the model were examined using lack of fit criterion, R^2 linear regression coefficient of determination, adjusted R^2, adequate precision and residual detection (Wu et al., 2012).

Results and discussion

Characterization of graphene oxide nanosheets

The FESEM images illustrate the morphology of graphene oxide nanosheets (*Fig. 1*). Morphology of the sheet structure of graphene oxide is clear in the images. The size of graphene oxide sheets is approximately 1-3 µm.

As can be seen in *Figure 1,* graphene oxide is semitransparent with some particles and lateral dimensions of several micrometer size and small holes, indicating the production accuracy of the synthesized graphene oxide sheets. FTIR spectra can be categorized into three classes: 2000-4000, 1300-2000 and 800-1300 cm^{-1}. The first class is usually concerned with hydrogen bonded O-H; absorbed H_2O; mostly free O-H; symmetric, dehydration and aliphatic units; and asymmetric stretching vibrations in CH_3, CH_2 or C-H, bonds. The second class includes the most significant oxygen functionalities characterized by N-O and C-O-containing structures. The third class is normally related to various C-O bonds such as those in phenols, hydroxyl groups and ethers (Omar and Moloukhia, 2008). FTIR spectra of graphene oxide nanosheets are indicated in *Figure 2*. For graphene oxide, IR band in the range of 1032 cm^{-1} is assigned to alkoxy C-O groups situated at the edges of the GO nanosheets. In the ranges of 1384

and 1623 cm^{-1}, it is concerned with carboxyl C-O groups and C=C in stretching vibrations of aromatic structures, respectively. In the ranges of 1694, 2921, and 3422 cm^{-1}, IR band is assigned to stretching CH^2, SP of GO aromatic sheets and OH groups of GO, respectively.
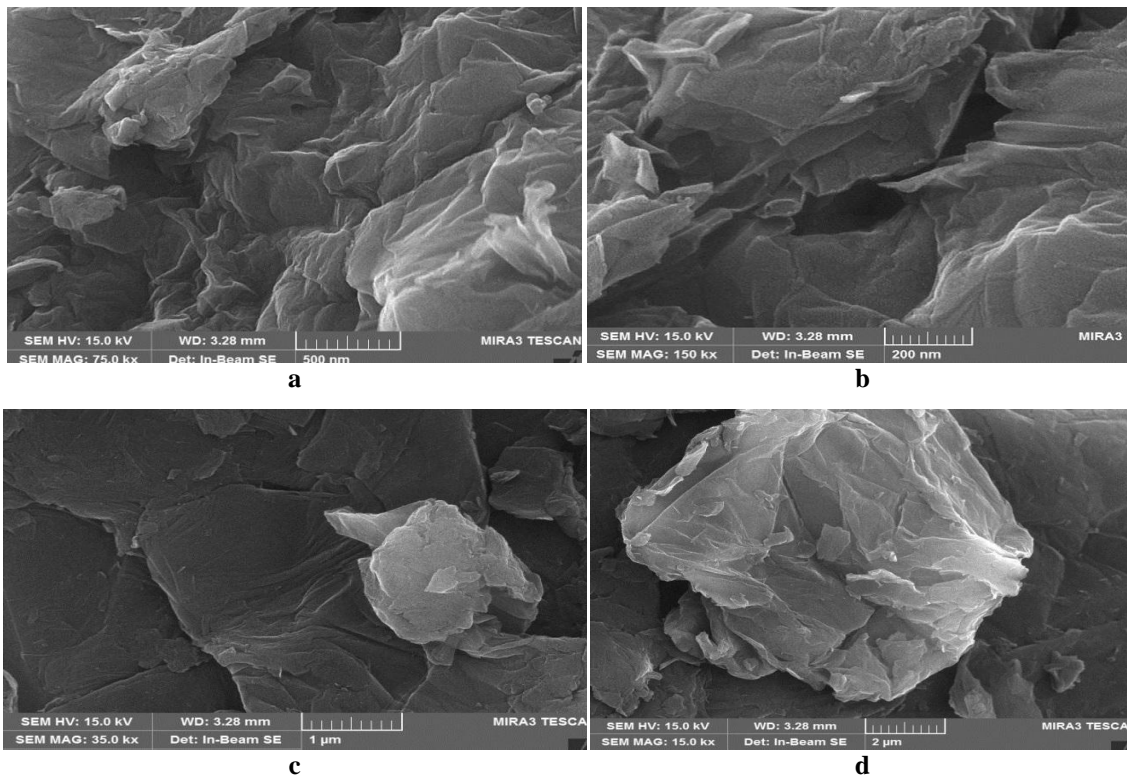

Figure 1. *FESEM image of the graphene oxide: a) 200 nm, b) 500 nm, c)1 µm, and d) 2 µm*
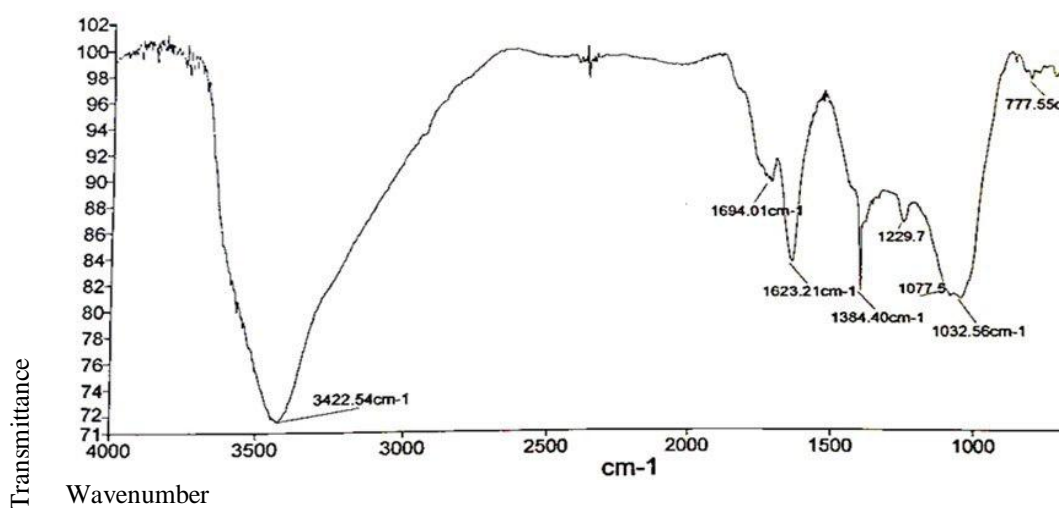

Figure 2. *FTIR spectra of the synthesized graphene oxide*

Figure 3 shows the XRD spectra of GO nanosheets. It is obvious that the synthesized adsorbent has only one intense peak centered at 11.3°.
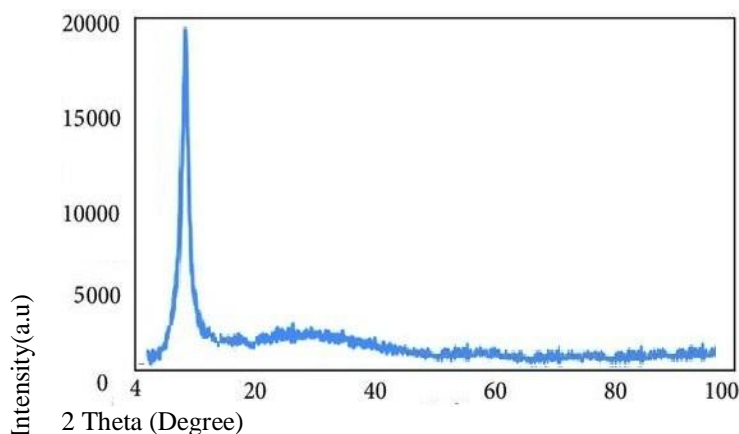
Figure 3. *XRD spectra of the synthesized GO*

Statistical analysis

The model and the quadratic equation were obtained using the Box-Behnken design. The maximum adsorption capacity of each experiment was finally recorded after adding the specified amounts of graphene oxide; adjusting the pH, concentration and time; and conducting the experiments. In order to examine the heavy metals removal, it is necessary to analyze the input data, output parameters, and optimized values.

The statistical significance of the variables was evaluated by analysis of variance (ANOVA) (*Tables 1C, 2C* and *3C*). The F-value implies that the terms in the model have a significant effect on the response. The p-value (<0.0001) indicates that the terms are significant at the probability level of 95% (*Tables 1A, 2A* and *3A*). The p-value of lack of fit (LOF) was not significant compared to the pure error, suggesting that the model fitted well with the experimental data. Adequate precision gives the ratio of signal to noise. The ratios greater than 4 are desirable. The predicted R^2 was in reasonable agreement with the adjusted R^2, which confirm the accuracy of the model (Hu et al., 2013; Tripathi et al., 2009). Software package of Design-Expert 10 version (Minneapolis, USA) was employed to design the experiments and analyze the obtained data.

Cadmium (Cd^{2+})

The following quadratic model was obtained using BBD and indicates the mathematical relation among the variables involved in Cd^{2+} (*Eq. 5*):

$$Qe = -(16.83) + (2.28 \times pH) + (0.36 \times Conc) + (0.35 \times Time) - (0.022 \times pH \times Conc) \quad \text{(Eq.5)}$$
$$+ (7.153 \times pH \times Time) + (0.709 \times pH^2 Conc^2) - (5.85 \times Time^2)$$

Plus signs preceding the parameters represent the synergistic effect of the variable, and minus signs indicate a reduction or reverse effect. When the value of one variable increases due to the rise in that of another variable, a positive correlation coefficient is formed, which represents the synergistic effect. On the contrary, when the value of a variable increases due to the reduction in that of another variable, the correlation coefficient is negative (Talib et al., 2017). Considering the parameters and statistical analysis of the model, the R^2 linear regression coefficient value is high ($R^2 = 0.9999$)

and it reasonably agrees with the adjusted R^2 value of 0.9999. Consequently, the quadratic model fits the experimental data within the range of operating factors. As the value of R^2 is closer to 1, power of the model will be greater in describing the response variations as a function of independent variables (Zarei et al., 2015). The significance of the predicted model is represented by the F value which equals 16712 for adsorption of Cd^{2+} by the synthesized graphene oxide. The F-value of the model was found to be significant. The value of adequate precision represents the difference between the value of the predicted response and the average value of the prediction error. If this proportion reaches > 4, it will be indicative of adequate signal in the model. According to the results, the mentioned value is 485 for Cd^{2+} adsorption.

Table 1. *Initial factors and values obtained for Cd^{2+}*

A) Independent variables and their levels					
Variables	**Factor**	**Unit**	**Levels**		
			−1	**0**	**+1**
pH	pH	-	2	4	6
Conc.	Cd Conc.	mg/L	10	55	100
Time	Time	min.	5	32.5	60

B) RSM design and the observed values				
Run	**Factor**			**Qe**
	pH	**Cd Conc.**	**Time**	
1	6	55	5	41.7895
2	4	55	32.5	29.8021
3	4	10	60	8.40417
4	6	10	32.5	31.653
5	6	100	32.5	68.349
6	4	55	32.5	29.8015
7	4	55	32.5	30.839
8	2	55	5	14.5829
9	4	55	32.5	30.1014
10	2	10	32.5	14.4269
11	4	100	60	49.4414
12	4	55	32.5	29.7955
13	2	55	60	14.0758
14	4	100	5	48.9891
15	4	10	5	8.31452
16	2	100	32.5	44.51
17	6	55	60	42.8255

Central point: pH = 4, Conc = 55, Time = 32.5 Average = 31.63

C) Analysis of variance for RSM					
Factor	**SS[a]**	**dF[b]**	**MS[c]**	**F-value**	**p-value**
Model	5021.51	8	627.69	16712.29	< 0.0001
pH	1544.50	1	1544.66	41122.58	< 0.0001
Conc.	3304.98	1	3304.98	87995.61	< 0.0001

Time	0.15	1	0.15	4.04	0.00794
pH* conc	16.52	1	16.52	439.76	< 0.0001
Conc* time	0.62	1	0.62	16.48	0.0036
pH ^{2}	33.83	1	33.83	900.86	< 0.0001
Conc. ^{2}	45.79	1	45.79	1219.44	< 0.0001
Time ^{2}	82.53	1	82.53	2197.44	< 0.0001
Residual	0.03	8	0.038		
Lack fit	0.02	4	0.049	1.91	0.2727
Pure error	0.01	4	0.026		

$R^2 = 0.9999$, Adj. $R^2 = 0.9999$, Adeq. precision = 485.3
[a]Sum of square
[b]Degree of freedom
[c]Mean square

Lead (Pb^{2+})

Equation 6 indicates the mathematical relations among the input parameters of lead.

$$Qe = -(10975639) + (37.36219 \times pH) + (0.90729 \times Conc) + (1.61406 \times Time) - (0.051692 \times pH \times Conc)$$
$$- (0.072090 \times pH \times Time) - (1.98777 \times (pH)^2) - (0.20492 \times (Time)^2) \quad \text{(Eq.6)}$$

The linear regression coefficient R^2 value (0.9999) and adjusted R^2 showed the effective removal of Pb^{2+} by graphene oxide and significance of the model for this metal with F = 8141. The obtained adequate precision is 313.

***Table 2.** Initial factors and values obtained Pb^{2+}*

A) Independent variables and their levels					
Variables	**Factor**	**Unit**	**Levels**		
			-1	**0**	**+1**
pH	pH	-	2	4	6
Conc.	Pb Conc.	mg/L	50	100	150
Time	Time	min	5	32.5	60

B) RSM design and the observed values				
Run	**Factor**			**Qe**
	pH	**Pb Conc.**	**Time**	
1	2	100	5	44.4753
2	4	100	32.5	99.8557
3	4	100	32.5	99.1589
4	4	100	32.5	99.5009
5	6	100	5	99.0929
6	4	150	60	118.551
7	6	150	32.5	149.553
8	4	100	32.5	98.8449
9	6	100	60	99.3135

10	4	50	5	48.7973
11	2	150	32.5	103.863
12	2	100	60	51.6843
13	6	50	32.5	49.5921
14	4	50	60	49.4307
15	4	100	32.5	99.2496
16	2	50	32.5	23.1052
17	4	150	5	112.356

Central point: pH = 4, Conc = 100, Time = 32.5 Average = 85.09

	C) Analysis of variance for RSM				
Factor	**SS[a]**	**dF[b]**	**MS[c]**	**F-value**	**p-value**
Model	16032.24	7	2290.32	8141.96	< 0.0001
pH	2075.12	1	2075.12	7376.94	< 0.0001
Conc.	4492.39	1	4492.39	15970.21	< 0.0001
Time	0.11	1	0.11	0.39	0.5545
pH* conc	45.86	1	45.86	163.02	< 0.0001
pH* time	26.98	1	26.98	95.91	< 0.0001
pH ²	120.86	1	120.86	429.66	< 0.0001
Time ²	589.09	1	589.09	2094.20	< 0.0001
Residual	1.69	6	0.28		
Lack fit	1.11	2	0.56	3.86	0.1146
Pure error	0.058	4	0.14		
Core total	16033.93	17			

R^2 = 0.9999, Adj. R^2 = 0.9998, Adeq. precision = 313.3
[a]Sum of square
[b]Degree of freedom
[c]Mean square

Chromium (Cr(III))

Equation 7 indicates the mathematical relations among the input parameters of chromium.

$$Qe = (4.65520) + (0.68819 \times pH) - (0.18288 \times Conc) - (0.098263 \times Time)$$
$$+ (0.11980 \times pH \times Conc) - (5.56740 \times (Conc)^2) + (1.52885 \times (Time)^2) \quad \text{(Eq.7)}$$

R^2 value (0.9980) and adjusted R^2 suggest good removal of Cr(III) by graphene oxide and significance of the model for this metal with F = 655. The obtained adequate precision is 90.

Table 3. *Initial factors and values obtained for Cr(III)*

A) Independent variables and their elevels					
Variable	**Factor**	**Unit**	**Levels**		
			-1	**0**	**+1**
pH	pH	-	2	3.25	4.5
Conc.	Cr Conc.	mg/L	10	55	100
Time	Time	Min	5	32.5	60

B) RSM design and the observed values				
Run	**Factor**			**Qe**
	pH	**Cr Conc.**	**Time**	
1	3.25	55	32.5	15.0606
2	3.25	55	32.5	15.0964
3	2	100	32.5	15.0061
4	4.5	100	32.5	36.4972
5	3.25	55	32.5	14.4681
6	4.5	10	32.5	9.52933
7	2	55	5	11.2105
8	3.25	100	60	21.8738
9	3.25	10	5	8.67247
10	3.25	100	5	21.0389
11	4.5	55	5	25.4581
12	3.25	55	32.5	14.9065
13	4.5	55	60	24.5924
14	2	55	60	7.1957
15	3.25	10	60	8.85581
16	2	10	32.5	4.54325
17	3.25	55	32.5	15.6882

Central point: pH = 3.25, Conc = 55, Time = 32.5

C) Analysis of variance for RSM					
Factor	**SS[a]**	**dF[b]**	**MS[c]**	**F-value**	**p-value**
Model	1000.19	6	166.70	655.0	< 0.0001
pH	386.27	1	386.27	1517.76	< 0.0001
Conc.	243.34	1	243.34	956.15	< 0.0001
Time	6.104e	1	6.104e	0.024	0.8808
pH* conc.	100.49	1	100.49	394.83	< 0.0001
Conc.^{2}	4.13	1	4.13	16.24	0.0038
Time^2	4.41	1	4.41	17.35	0.0031
Residual	2.04	8	0.25		
Lack fit	1.27	4	0.32	1.65	0.3199
Pure error	0.77	4	0.19		

$R^2 = 0.9980$, Adj. $R^2 = 0.9964$, Adeq. precision = 90.17
[a]Sum of square
[b]Degree of freedom
[c]Mean square

Comparative effects of the studied variables on removal efficiency

Figure 4 shows the effect of all the three variables on the removal rate at a point and the response level is visible and comparable in the designing method. Removal efficiency increased with the increase of pH (a) and concentration (b). However, increase in the contact time (c) did not have a significant effect on the value obtained for the heavy metals removal, and a different result was obtained for each metal.
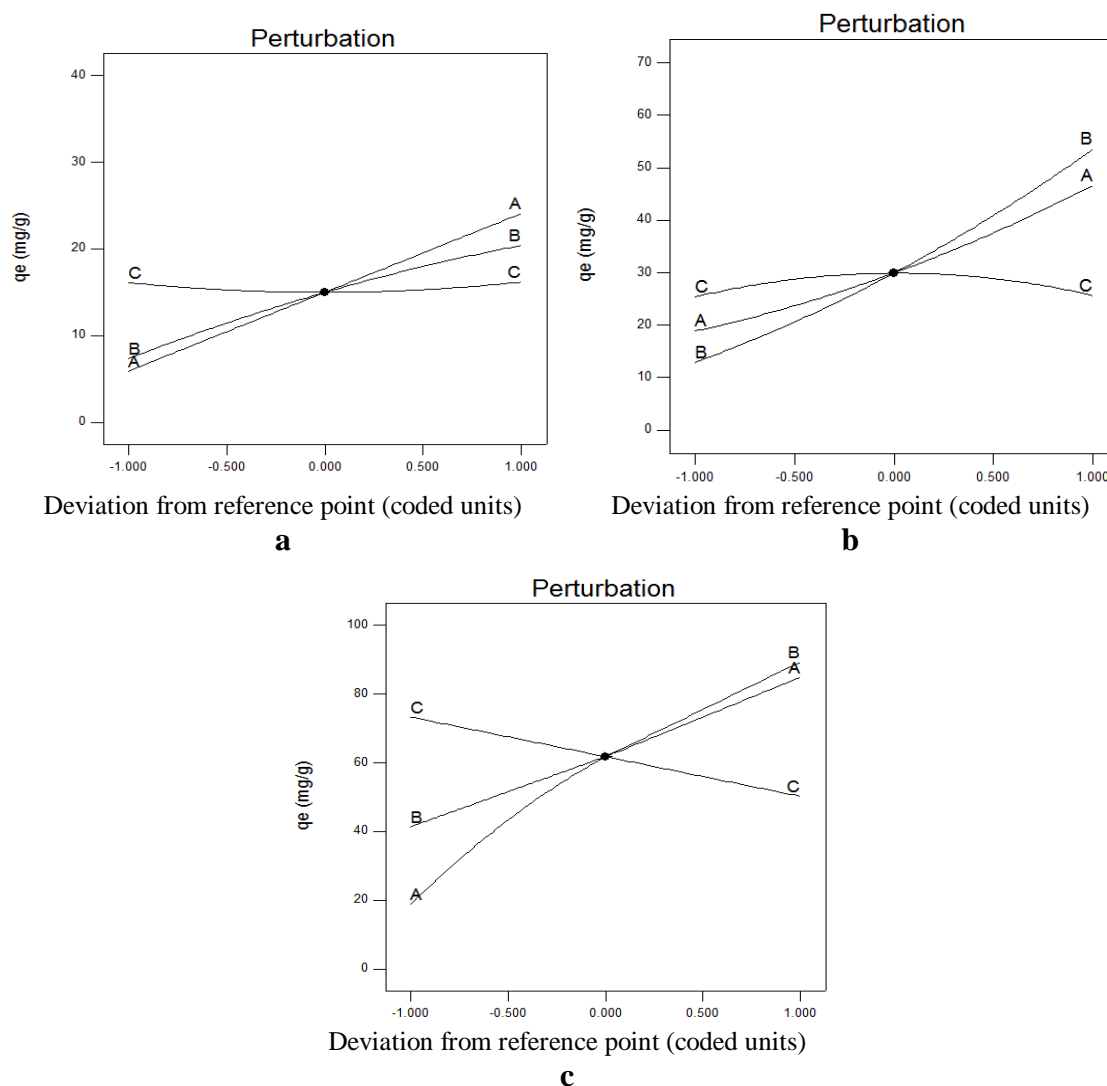

Figure 4. *Perturbation plots of removal by graphene oxide: a) chromium, b) cadmium, c) lead*

Effect of the variables on responses

Effect of pH

pH is a determinant factor in adsorption of Cr(III), Cd^{2+} and Pb^{2+} ions from aqueous solutions (Li and Wang, 2009; Ramkumar et al., 2008). To study the effect of pH on adsorption capacity of graphene oxide, pH of the solution was tested in the ranges of 2-6 for Cd^{2+} and Pb^{2+} and 2-4.5 for Cr(III). Adsorption capacity with contaminant concentration and also the contact time versus pH are illustrated in *Figure 5*. The value

of pH_{PZC} for synthesized graphene oxide was obtained as 3. When pH of the solution is below 3, the adsorbent surface is positively charged. Any increase in pH leads to change of surface charge from positive to negative. This demonstrates that heavy metals adsorption onto graphene oxide and thereby heavy metals removal are higher in pH $\geq$ 3 (Lingamdinne et al., 2008). Results showed that the removal of Cr(III), Cd^{2+} and Pb^2 by graphene nanoparticles was affected by pH of the solution. In the adsorption process, two ions of OH^- and H^+ are responsible for decomposition (Zarei et al., 2015). For chromium, by raising the initial pH of the solution from 2 to 4.5, the adsorption capacity of graphene oxide increases from 4.5 to 36.5 on a constant trend. As can be seen in *Figure 5a* and *b*, the maximum Cr(III) adsorption occurred at pH = 4.5. Analysis of *Figure 5* indicates that the negatively charged groups on the adsorbent surface and ionization of binding sites in functional groups in the cell wall increase with the increase of pH. Consequently, the removal of Cr(III) ion from aqueous solution and adsorption capacity were increased. Zhang et al. (2018) concluded that pH is the most effective variable in adsorbing chromium, and changes in pH may have a marked effect on kinetic characteristics and adsorption equilibrium. They studied the pH range of 2-11 and demonstrated that the highest adsorption capacity of chromium occurred at the pH range of 3-5 and subsequently the adsorption capacity considerably increased with the increase of pH. Another study was conducted by Jiang et al. (2017) on the effective synthesis of graphite compounds for the effective removal of chromium from aqueous solutions. The mentioned study was carried out in the pH range of 1-9, the initial concentrations of 10-100 mg/L and time duration of 5 to 300 min. The best result was achieved at pH range of 4-6 and concentration of 100 mg/L. By increasing the time from 5 to 180 min, initially the adsorption efficiency increased rapidly and then reached the saturation point (Jiang et al., 2017). According to *Figure 5c* and *d*, the effects of variables on Cd^{2+} adsorption indicate that with the increase of initial pH from 2 to 6, the graphene oxide adsorption capacity increases from 0.2 to 32.2 mg/g. At the first stage, adsorption capacity rapidly increases with the increase of pH from 2 to 4.5. Afterwards, with the increase of pH from 4.5 to 6, the slope of curve constantly increases and the maximum adsorption capacity for Cd^{2+} occurs at pH = 6. According to the tested conditions, cadmium, lead and chromium began to precipitate at pH values greater than 6 and 4.5. Similar results have been reported in other studies, confirming that cadmium and lead begin to precipitate at pH values greater than 6 (Zhao et al., 2011). A study conducted by Sitko et al. (2013) on adsorption of metal ions using graphene oxide revealed that when pH increase from 3 to 4, Cd^{2+} adsorption capacity rapidly increases and becomes constant at pH range of 5-8 (Depci et al., 2012; Zhao et al., 2011). The effects of variables on Pb^{2+} ion are shown in *Figure 5c*. Adsorption increases from 23 to 89.9 mg/g with the increase of initial pH from 2 to 6. At the first step, the adsorption capacity increases quickly with the increase of pH from 2 to 4. Afterwards the adsorption capacity increases with a slight slope with the increase of pH from 4 to 6. According to the results obtained in other studies, Cr(III) and Pb^{2+} adsorptions rapidly increase at the pH range of 2-3, and then the increasing rate slows down (Lingamdinne et al., 2016; Hao et al., 2012). Other studies have shown that most of the Cd^{2+} and Pb^{2+} adsorption processes take place at the pH range of 5.3 to 6, and Cr(III), Cd^{2+} and Pb^{2+} ions start to precipitate at the pH values higher than 6 (Fan et al., 2012; Jafari et al., 2016). As a result, the maximum rate of Pb^{2+} and Cd^{2+} adsorption occurs at pH = 5.5 and the maximum rate of Cr(III) adsorption occurs at pH = 4.5. *Figure 5* indicates the

effect of pH on time and concentration for Cr(III), Cd^{2+} and Pb^{2+} adsorptions in aqueous solutions.
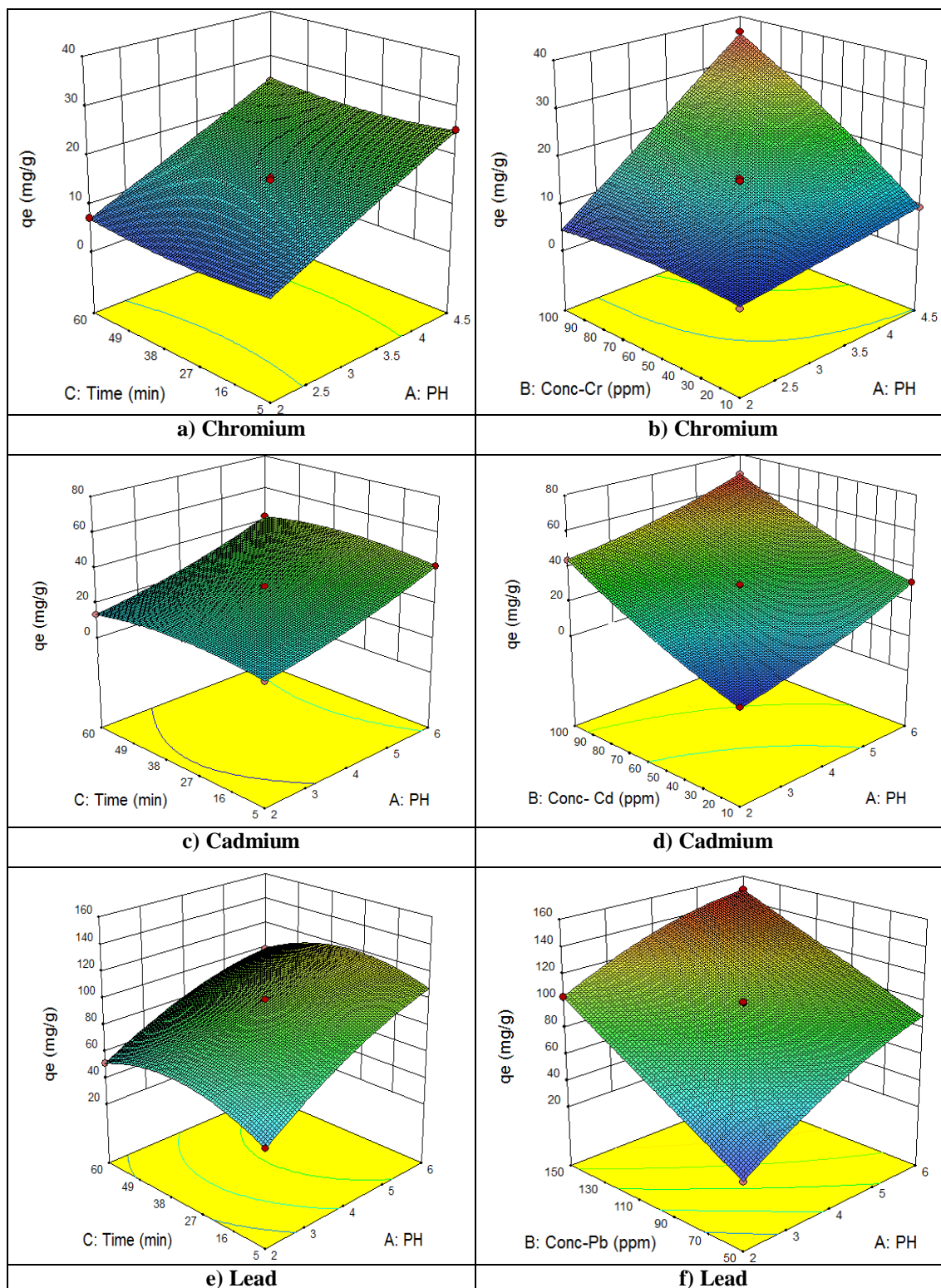

Figure 5. *The 3D response surface for Cr(III): a) pH and time, b) pH and concentration; for Cd^{2+}: c) pH and time, d) pH and concentration; and for Pb^{2+}: e) pH and time, f) pH and concentration*

Effects of initial concentration and contact time on adsorption capacity

The effect of concentration on adsorption capacity in optimum conditions (25 °C) shows that the adsorption capacity of graphene oxide increases smoothly from 63 to 135 mg/g for Pb^{2+} with the increase of initial concentration from 50 to 150 mg/L. Also, with the increase of initial concentration of the solution from 10 to 100 mg/L, graphene oxide adsorption capacity increases from 5 to 38 mg/g for Cr(III) and from 1.2 to 68 mg/g for Cd^{2+}. As can be seen in *Figure 5,* the maximum adsorption rate took place at high concentrations. This can be due to the fact that increase of adsorbent concentration increases the area of adsorbent and facilitates the access to more adsorbed sites, thus increasing the rate of heavy metals absorption. Due to the large number of sites available for absorption and absorption equilibrium in the initial stage of absorption, the adsorption process progresses steadily (Vasudevan et al., 2011). At different initial concentrations of heavy metals, the adsorption intensity was initially constant; however, over time, minor changes happened, and finally, it reached a constant value. Reaching the equilibrium time, the amount of adsorbed ions did not noticeably change over time. According to surface plots, increase of contact time did not lead to a considerable change in adsorption efficiency. Many studies yet have been done about the effect of initial concentration and contact time of adsorbent on the removal of metal ions. In some studies it has been suggested that the optimum contact time to reach the equilibrium condition for the removal of metal ions is less than 60 min (Barassi et al., 2009; Ramana et al., 2012; Sari and Tuzen, 2009). After optimization, the best values for heavy metals were obtained at pH = 6 for Pb^{2+}, and the maximum adsorption capacity of adsorbent reached 136 mg/g in initial concentration of 128 mg/L and contact time of 30 min. However, for Cd^{2+} ion at pH = 6, the maximum adsorption capacity was 68 mg/g in initial concentration of 100 mg/L and contact time of 33 min. Also, for Cr(III) at pH = 4.5, the maximum adsorption capacity was 37.63 mg/g initial concentration of 100 mg/L and contact time of 6 min. In a similar study, the maximum graphene adsorption capacity for lead was found to be 120 mg/g at pH = 5 and contact time of 30 min (Vasudevan and Lakshmi, 2012). Also, in another study, the maximum adsorption capacity for lead was obtained as 101 mg/g at pH = 6 (Awad et al., 2017). In a study conducted by Sitko et al. (2013) on cadmium, the maximum adsorption capacity was 49 mg/g at pH = 5 and contact time of 120 min. In another study, the maximum graphene oxide adsorption capacity for cadmium (88 mg/g) was obtained in initial concentration of 30 mg/L and a contact time of 30 min (Sheet et al., 2014). In this study, the values obtained as the maximum graphene adsorption capacity for Cr(III), Cd^{2+} and Pb^{2+} were consistent with the findings reported in other studies.

Examination of adsorption isotherms

Isotherm is the most important parameter in the design of adsorption systems. It represents the relation between the adsorption capacity of an adsorbent and the adsorbate concentration. The Langmuir and Freundlich adsorption isotherm models are used in this study. Langmuir isotherm is based on monolayer adsorption and homogeneity of the adsorbate with equal energy on the whole surface of the adsorbent. Contrary to Langmuir isotherm, Freundlich isotherm is on the basis of multilayer adsorption and heterogeneity of the adsorbate on the adsorbent (Jamali et al., 2015). The results of adsorption experiments with different pH values, contact times and initial concentrations of heavy metals have been analyzed using Langmuir and Freundlich

models. The values of adsorption isotherms are obtained using the equations presented in *Table 4*.

Table 4. Linear equations and isotherm parameters

Model		Parameters
Isotherm	Langmuir	$\dfrac{C_e}{q_e} = \dfrac{1}{Q^\circ b} + \dfrac{C_e}{Q^\circ}$
	Frendlich	$\log(q_e) = \log(K_f) + \dfrac{1}{n}\log(C_e)$

In Langmuir and Freundlich equations, C_e represents equilibrium concentration of heavy metals in mg/L; q_e indicates the amount of heavy metals adsorbed in equilibrium time in mg/g; Q° and b are Langmuir parameters related to the maximum absorption capacity and the energy of absorption correlation, respectively; and K_f and n are Freundlich constants which represent adsorption capacity and desirability of adsorption process, respectively. Values of K_f and n parameters are, respectively, determined through intercept and linear regression slope of $\log(C_e)$ versus $\log(q_e)$. In this model, n-values less than 1 indicate weak adsorption. The values between 1-2 and 2-10 represent difficult and desired adsorption, respectively (Jamali et al., 2015).

Referring to *Table 5*, "n" constant values of 1.9 for Cd^{2+} and Cr(III) and 7.4 for Pb^{2+} show the higher fitness of Freundlich model for Pb^{2+}. R^2 values for Cd^{2+} and Cr(III) obtained from Langmuir isotherm are greater than those obtained from Freundlich model, indicating the higher fitness of Langmuir isotherm for Cd^{2+} and Cr(III). However, Freundlich model has a higher fitness for Pb^{2+} ion (*Fig. 6*). Surface adsorption of Pb^{2+} ions takes place onto the monolayer of the adsorbent surface. Adsorption sites are equally and similarly present on the surface area of the adsorbent (Iram et al., 2010).

Table 5. Values of adsorption isotherm models

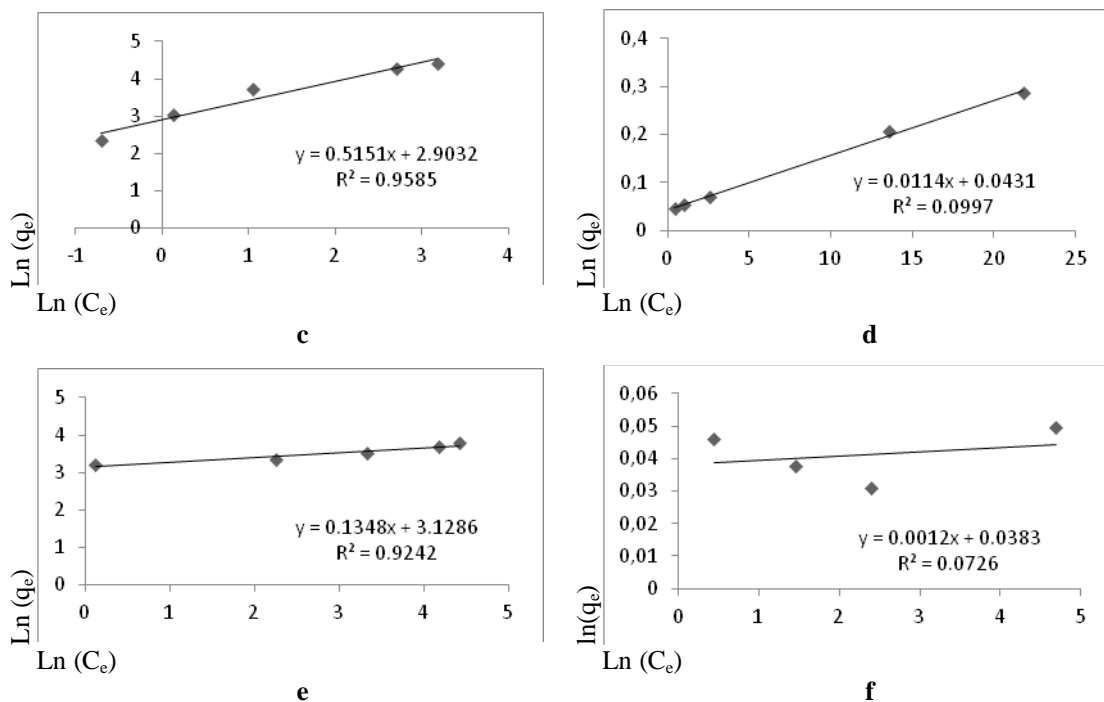| Heavy metals	Langmuir model			Freundlich model		
	R^2	b (L/mg)	q_{max} (mg/g)	R^2	N	K (mg/g) $(L/mg)^{1/n}$
Cr(III)	0.985	21.32	41.32	0.928	1.94	22.84
Pb^{2+}	0.072	0.031	833.33	0.958	7.42	24.46
Cd^{2+}	0.997	0.264	87.71	0.958	1.94	18.23
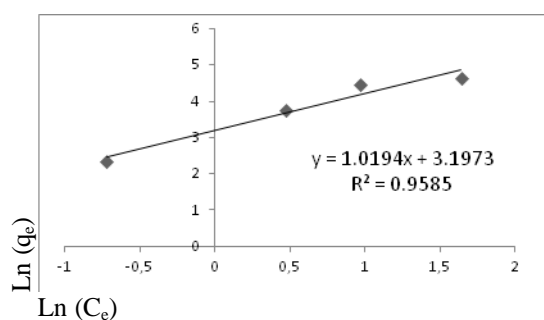
a
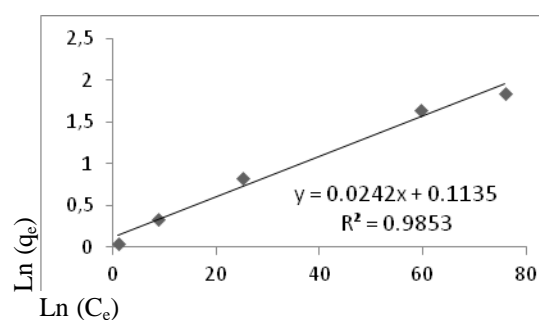
b

Figure 6. *Adsorption isotherm of graphene oxide: a) Freundlich Cr(III), b) Langmuir Cr(III), c) Freundlich Cd^{2+}, d) Langmuir Cd^{2+}, e) Freundlich Pb^{2+} ion, and f) Langmuir Pb^{2+}*

Conclusion

In the present study, graphene oxide nanoparticles were used to examine the removal of Cr(III), Cd^{2+} and Pb^{2+} which were synthesized by Hummer's method. RSM was used according to the Box-Behnken design to evaluate the effect of independent variables on response function. Properties of the synthesized adsorbent were studied using FESEM, FTIR, and XRD. In FESEM images, the size of graphene oxide sheets was 1 to 3 μm with wrinkled morphology. FTIR spectra were classified into three groups, and it was observed that ranges of alkoxy groups, carboxyl groups, stretching vibration of aromatic, stretching CH_2, aromatic sheets and OH groups of graphene oxide sheets were in agreement with the results of previous studies. XRD spectra exhibited an intense peak at $2\Theta = 11.3^o$ for graphene oxide. Some of the effective parameters for the removal of Cr(III), Cd^{2+} and Pb^{2+} were optimized. After optimization, the maximum adsorption capacity of adsorbent reached 136 mg/g for Pb^{2+} at pH = 6, initial concentration of 128 mg/L and contact time of 30 min. However, the maximum adsorption capacity was 68 mg/g for Cd^{2+} ion at pH = 6, initial concentration of 100 mg/L and contact time of 33 min. Also, the maximum adsorption capacity was 37.63 mg/g for Cr(III) at pH = 4.5, initial concentration of 100 mg/L and contact time of 6 min. The high value of R^2 linear regression coefficient was desirable. Values of R^2, adjusted coefficient (Adj. R^2) and predicted coefficient (Pred. R^2) were acceptable. The high value of R^2 linear regression coefficient guarantees the desirability of the presented quadratic equation and is representative of good fitness of the data with the predicted model. According to the results, all linear, quadratic and interaction effects of the variables were significant with a safety factor of 95%, indicating the effect of the variables on Cr(III), Cd^{2+} and Pb^{2+} removal using graphene oxide adsorbents. Adequate precision indicated the noise ratio, and the values greater than 4 represented the desirability of the quadratic equation

model for prediction. Replicability of the model, with C.V value of less than 10%, indicated a good condition. Values obtained from Langmuir isotherm equation for Cr(III) and Cd^{2+} exhibit the maximum fitness of this model with Cr(III) and Cd^{2+}. However, the values obtained from Freundlich isotherm for Pb^{2+} ions showed the maximum fitness of this isotherm with Pb^{2+} ions. The results showed that graphene oxide was effective in removing heavy metals from aqueous solutions. It was concluded that the adsorption process is a function of pH value, initial concentration of the solution and contact time, and graphene oxide can be effectively used to remove heavy metals from polluted waters. Finfings in this study can be applied in the treatment of industrial wastewater. Further studies should be carried out to investigate the efficiency of the proposed method for the removal of heavy metals at lower concentrations.

Conflict of interests. The authors state that there is no conflict of interests regarding publication of this article.

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