Application of Box–Behnken design in the optimization of a magnetic nanoparticle procedure for zinc determination in analytical samples by inductively coupled plasma optical emission spectrometry

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ABSTRACT

This article describes the development of a procedure for zinc determination in water and biological samples after extraction by magnetic nanoparticles by inductively coupled plasma optical emission spectrometry (ICP-OES). The optimization strategy was carried out by using two level full factorial designs. Results of the two level full factorial design (2^4) based on an analysis of variance demonstrated that only the pH, amount of extractant and amount of nanoparticles were statistically significant. Optimal conditions for three variables: pH of solution, amount of extractant (E), and amount of nanoparticles (N) for the extraction of zinc samples were obtained by using Box–Behnken design. These values were 3.8, 3.1 and 3.3 mg, for pH of solution, amount of nanoparticles and amount of extractant, respectively. Under the optimized experimental conditions, the detection limit of the proposed method followed by ICP-OES was found to be $0.8\,\mu g\,L^{-1}$. The method was applied to the determination of zinc in water and biological samples.

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1. Introduction

Environmental pollution has given rise to concern about the accumulation of heavy metal species in humans. In order to access exposure to, and absorption of trace elements, a biological sample such as blood is often analyzed. A deficiency in trace elements such as the deviation of zinc from its normal level in blood is used for early diagnosis of certain illnesses [1]. The development of new methods for quantifying trace element is required and challenged. In some common procedures for the determination of low concentration of heavy metals a preconcentration step is needed before performing determination. The traditional separation and preconcentration methods for metallic ions include liquid–liquid extraction, coprecipitation, and ion exchange, etc. These methods often require large amount of high-purity organic solvents, some of which are harmful to health and cause environmental problems [2].

Magnetically assisted chemical separation (MACS) method, which uses micro-and-nanoparticles, is suitable for this purpose. It combines the selectivity and efficient separation offered by chemical extraction with magnetic recovery of extractant for selective separation of metal ions [3].

The use of multivariate experimental design techniques is becoming increasingly widespread in analytical chemistry. Multivariate experimental design techniques, which permit the simultaneous optimization of several control variables, are faster to implement and more cost-effective than traditional univariate (one at a time) approaches [4]. One of the most popular multivariate design techniques is two level full/fractional factorial, in which every factor is experimentally studied at only two levels. Due to their simplicity and relatively low cost, full factorial design techniques are very useful for preliminary studies or in the initial steps of an optimization, while fractional factorial designs are almost mandatory when the problem involves a large number of factors [5–7]. On the other hand, since only two levels are used, the models that may be fit to these designs are somewhat restricted. If a more sophisticated model is needed, as for the location of an optimum set of experimental conditions, then one must resort to augment response surface designs, which employ more than two factor levels. Among these, Box–Behnken is a second-order multivariate design technique based on three-level incomplete factorial designs that received widespread application for evaluation of critical experimental conditions, that is, maximum or minimum of response functions. The number of experiments (N) required for the development of this design is defined as $N = 2k(k-1) + C_0$, where k is the factor number and C_0 is the replicate number of the central point [8–16].

This work proposes a preconcentration procedure using magnetic nanoparticles for determination of zinc in water and biological

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Table 1
ICP-OES instrumental conditions.

Plasma	Argon
RF generator power (kW)	1.5
Plasma gas flow rate (L min^{-1})	15.0
Auxiliary gas flow rate (L min^{-1})	1.5
Frequency of RF generator (MHz)	40.0
Observation high (mm)	8.0
Nebulizer pressure (kPa)	240

samples by ICP-OES. The morin was used as extractant (ligand). The optimization of the preconcentration procedure is performed exploiting the Box–Behnken design.

2. Experimental

2.1. Apparatus

The measurements were performed with a simultaneous inductively coupled plasma optical emission spectrometry (ICP-OES, Varian Vista-Pro, Springvale, Australia) coupled to a V-groove nebulizer and equipped with a charge coupled device (CCD). The ICP conditions are shown in Table 1. The pH was determined with a model 630 Metrohm pH meter with combined glass-calomel electrode.

2.2. Materials

Morin (3,5,7,2′,4′-pentahydroxyflavone), acetic acid and acetonitrile were obtained from Merck (Darmstadt, Germany). All acids used were of the highest purity available from Merck and used as received. Reagent grade $FeCl_2$, $FeCl_3$ and nitrate or chloride salts of other cations (all from Merck) were of the highest purity available and were used without any further purification. A stock solution of Zn was prepared by dissolving the proper amount of standard solution of Zn (1000 mg L^{-1}) in doubly distilled water in a 10 mL flask. Dilute solutions were prepared by an appropriate dilution of the stock solution in doubly distilled water.

2.3. Preparation of morin-bound iron oxide magnetic nanoparticles

Iron oxide nanoparticle were prepared by coprecipitation Fe^{2+} and Fe^{3+} ions by ammonia solution and treating under hydrothermal conditions. The ferric and ferrous chlorides (mole ratio 2:1) were dissolved in water at a concentration of 0.3 mol L^{-1} iron ions. Chemical precipitation was achieved at 25 °C under vigorous stirring by adding NH_4OH solution (29.6%). During the reaction process, the pH of the solution was maintained at about 10.5. The precipitates were heated at 80 °C for 30 min, and then were washed several times with deionized water and ethanol. Finally, they were dried in a vacuum oven at 80 °C.

For the binding of morin, appropriate amount of dried nanoparticles was poured into a teflon beaker and then morin–ethanol solution was added. The mixture was gently stirred by a teflon rod and the solvent was slowly evaporated. The modified particles were then heated at 90 °C for 1 h to eliminate residual solvent completely.

2.4. Adsorption and desorption studies

Adsorption of zinc from aqueous solutions was investigated in batch experiments. Adsorptions were performed in test tubes containing 25 μg zinc ions in 50 mL deionized water. According to a preliminary experimental design, the pH was adjusted by drop-wise addition of 1 mol L^{-1} sodium hydroxide and 1 mol L^{-1} hydrochloric acid; and appropriate modified particles were added into solutions. After that, the mixture was shaken for an appropriate time to completely extract zinc ions from the solution. Finally, the test tubes were placed in a magnetic rack, where permanent magnet in the wall causes the particles to aggregate on one side of the test tube. The adsorption amounts of zinc were estimated from the concentration change of zinc in solution after adsorption by using an ICP-OES. The instrument response was periodically checked with known zinc standard solutions. Percent extraction of zinc was calculated from the following equation:

$$Extraction\% = \left(\frac{C_A - C_B}{C_A} \right) \times 100$$

where C_A and C_B are the concentration of zinc ions before and after extraction in the solution, respectively.

The phase distribution ratio (D) was calculated using the following equation:

$$D = \left(\frac{C_0 - C_f}{C_f} \right) \left(\frac{V}{W} \right) \tag{1}$$

where W is the weight of the nanoparticles in g and V is the volume of the aqueous phase in mL.

The adsorbed zinc was desorbed by putting the removed nanoparticles into 20 mL of CH_3COOH:CH_3CN:HNO_3 (5:5:10, v/v). After mixing for several minutes and removing the nanoparticles, the concentration of zinc in liquid solution was measured to estimate the amount of zinc desorbed.

2.5. Factorial design

A two level factorial 2^4 design with two replicates of center point was performed in order to determine the influence of these factors their interactions. The factorial design was evaluated using analytical response (A) of zinc. The experimental matrix and the A for each trial are shown in Table 2. An analysis of the variance (ANOVA) demonstrated that, within the experimental range, only pH, N and E were statistically significant and Pareto chart (Fig. 1) interpretation it.

2.6. Box–Behnken design

The significant variables like pH; amount of extractant; and amount of nanoparticles were chosen as the critical variables and designated as X_1, X_2 and X_3, respectively. The low, middle, and high levels of each variable were designated as −, 0, and + respectively, are given in Table 3. The actual design of experiments is given in Table 4.

Table 2
Experimental design and the results obtained in A.

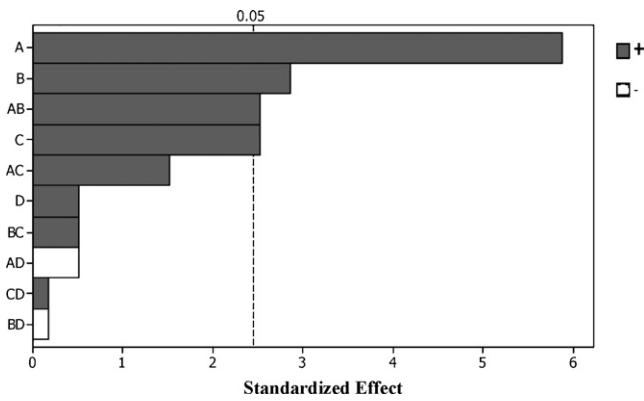| No.	pH	N	E	t	A
1	3.5	1	1	2	0.09
2	6.5	1	1	2	0.117
3	3.5	5	1	2	0.108
4	6.5	5	1	2	0.144
5	3.5	1	5	2	0.108
6	6.5	1	5	2	0.135
7	3.5	5	5	2	0.099
8	6.5	5	5	2	0.18
9	3.5	1	1	4	0.099
10	6.5	1	1	4	0.126
11	3.5	5	1	4	0.108
12	6.5	5	1	4	0.135
13	3.5	1	5	4	0.117
14	6.5	1	5	4	0.126
15	3.5	5	5	4	0.108
16	6.5	5	5	4	0.189
17	5	3	3	3	0.288
18	5	3	3	3	0.279

Fig. 1. Pareto chart of main effects obtained from 2^4 full factorial designs. The vertical line defines the 95% confidence interval (A = pH, B = N, C = E and D = t).

Table 3
The levels of variables chosen for the trials.

pH	Amount of nanoparticle (mg)	Amount of extractant (mg)
3.5 (−1)	1 (−1)	1 (−1)
5 (0)	3 (0)	3 (0)
6.5 (+1)	5 (+1)	5 (+1)

In a system involving three significant independent variables X_1, X_2, and X_3 the mathematical relationship of the response on these variables can be approximated by the quadratic (second degree) polynomial equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$
$$+ \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (2)$$

where Y = estimate response, β_0 = constant, β_1, β_2 and β_3 = linear coefficients, β_{12}, β_{13} and β_{23} = interaction coefficients between the three factors, β_{11}, β_{22} and β_{33} = quadratic coefficients.

A multiple regression analysis is done to obtain the coefficients and the equation can be used to predict the response. The degree of experiments chosen for this study was Box–Behnken, a fractional factorial design for three independent variables. It is applicable once the critical variables have been identified. In the model given in Eq. (2), interactions higher than second order have been neglected. A total of 15 experiments were necessary to estimate of the full model.

Table 4
The Box–Behnken design for the optimization of the magnetic nanoparticle extraction step.

Trial no.	pH	Amount of nanoparticle (mg)	Amount of extractant (mg)	A (Zn)
1	−1	−1	0	0.1140
2	+1	−1	0	0.2591
3	−1	+1	0	0.1120
4	+1	+1	0	0.2333
5	−1	0	−1	0.1088
6	+1	0	−1	0.2695
7	−1	0	+1	0.1132
8	+1	0	+1	0.2403
9	0	−1	−1	0.1429
10	0	+1	−1	0.1307
11	0	−1	+1	0.1263
12	0	+1	+1	0.1300
13	0	0	0	0.1500
14	0	0	0	0.1520
15	0	0	0	0.1470

3. Results and discussion

3.1. Optimization of the preconcentration procedure

The optimization step of the magnetic nanoparticle procedure was performed using a Box–Behnken design. Several variables that could potentially affect the extraction efficiency were chosen as: pH, amount of extractant, and amount of nanoparticles. Other parameters implicated in the extraction were kept constant, namely the adsorption time (30 min).

The number of experiments required to investigate the previously noted three parameters at three levels would be 27 (3^3). However, this was reduced to 15 using a Box–Behnken experimental design. The results from this limited number of experiments provided a statistical model, which was used to identify high yield trends for the extraction process. Table 4 shows the matrix with coded and the responses as analytical signals for zinc. The equations below illustrate the relationship of the three variables, that is, pH, amount of extractant (E), amount of nanoparticles (N) and analytical response.

$$A = 0.278675 - 0.118152(\text{pH}) + 0.0201(N) + 0.018769(E)$$
$$+ 0.017869(\text{pH})^2 - 0.002568(N)^2 - 0.0017302(E)^2$$
$$- 0.001983(\text{pH})(N) - 0.0028(\text{pH})(E) + 0.000994(N)(E)$$

The derivation of this general equation as (pH), (N) and (E) results in three new equations:

$$\frac{\delta(A)}{\delta(\text{pH})} = -0.118152 + 0.035738(\text{pH}) - 0.001983(N) - 0.0028(E)$$

$$\frac{\delta(A)}{\delta(N)} = 0.0201 - 0.001983(\text{pH}) - 0.005136(N) + 0.000994(E)$$

$$\frac{\delta(A)}{\delta(E)} = 0.018769 - 0.0028(\text{pH}) + 0.000994(N) - 0.00346(E)$$

The critical point in the surface response are found by solving these equation systems for the condition of $\delta(A)/\delta(\text{pH}) = 0$, $\delta(A)/\delta(N) = 0$ and $\delta(A)/\delta(E) = 0$. The way of calculating these critical points has been published in previous [16,17]. The summery of the analysis of variance (ANOVA) is shown in Table 5.

The calculated values for the critical point are as follows: pH 3.8, N = 3.1 mg and E = 3.3 mg.

The effect of pH on the extraction of zinc ions was studied. Results showed that the percent extraction was nearly constant and quantitative in the pH of 3.8. At the pH values below 3.8, however, the percent recovery was decreased.

The robustness of the analytical system (defined as the measure of its capacity to reproduce results when the procedure is performed under small changes in the nominal values established in the optimization step) was evaluated [18]. In the present work, the robustness was evaluated developing a full factorial design (2^3), involving, pH, N and E. The experiments were performed centered on the experimental conditions. The results considering the ANOVA

Table 5
ANOVA analysis for response Zn.

Source	Sum of squares	Degree of freedom	Mean square	F-value	P
Regression	0.046203	9	5.134E−03	207.84	<0.0001
Residual	1.24E−04	5	2.5E−05		
Lack-of-fit	1.11E−04	3	3.7E−05	5.83	0.15
Pure error	1.3E−05	2	6.0E−06		
R^2	0.997				

Table 6
Selectivity factors and distribution ratios of zinc.

Foreign ion	Distribution ratio (D)	Selectivity factor for nanoparticles (α)
Zn^{2+}	15	–
Cd^{2+}	4.1	3.6
Cu^{2+}	0.95	15.7
Ni^{2+}	3.6	4.1
Co^{2+}	5.4	2.8
Mn^{2+}	3.2	4.6

demonstrated that these factors are not significant in the levels studied. Then, it can be concluded that this procedure is robust for variation of (± 1 mg) of the variables N and E, and (± 0.5) units of pH value.

3.2. Analytical performance

Under the optimum conditions described, the calibration curve was linear over the concentration range of 10–200 $\mu g\,L^{-1}$. The least square equation at above dynamic linear range was as follows:

$$A = 0.0045C \ (\mu g\,L^{-1}) - 0.0076 \qquad (r^2 = 0.995)$$

The limit of detection (LOD) of the proposed method for the determination of zinc was studied under the optimal experimental conditions. The LOD obtained from $C_{LOD} = 3(S_d)_{blank}/m$ was 0.8 $\mu g\,L^{-1}$. The precision was also determined and expressed as relative standard deviation. The experimental enhancement factor calculated as the ratio of the slopes of the calibration graphs with and without preconcentration was 30.

All the statistical calculations are based on the average of triplicate readings for each standard solution in the given range.

The determination of zinc ion was quantitatively in the presence of large amounts of alkaline and alkaline-earth ions, which indicates method suitability for natural water analysis. Selecting coefficients of zinc ions over the other selected inorganic cations that coexisted with zinc ions in natural sources were studied by batch procedure. The nanoparticles were tested for separation of zinc ions from Cd^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+} and Ni^{2+} ions either individually or in their mixtures. The concentrations of transition metals were determined by ICP-OES. The selectivity of the zinc ions versus another cation was determined by the ratio of the two partition coefficients, D_{Zn}^{2+} and D_m^{n+} which is referred to the selectivity factor, α:

$$\alpha = \frac{D_{Zn}^{2+}}{D_m^{n+}} \tag{3}$$

The obtained results are summarized in Table 6. As it is clear from the results, quantitative separation of zinc from other cations is possible.

Table 7
Determination of zinc in water and biological samples.

Sample	Zn added ($\mu g\,mL^{-1}$)	Zinc found ($\mu g\,mL^{-1}$)	Recovery%	RSD%
Tap water	–	–	–	–
Tap water	0.2	0.1994	99.7	0.80
Chahnemah water	–	–	–	–
Chahnemah water	0.2	0.1989	99.5	0.92
Serum blood	–	1.96	–	4.3
Serum blood	0.2	2.155	97.5	5.0
Zinc sulfate tablet[a]	–	50.1	–	4.5
Zinc sulfate tablet	0.2	50.297	98.5	4.7

[a] The zinc sulfate tablet was provided from Darou Pakhsh pharmaceutical Mfg. Co.

3.3. Analytical application

In order to assess the applicability of the method to real samples with different matrices, it was applied to the extraction and separation of zinc ions from 100 mL of three different samples. Table 7 shows the extraction of 20.0 μg of added zinc ions from 100 mL of different samples. As seen, the results of three analysis of each sample show that the zinc recovery was almost quantitative.

4. Conclusion

In this work, magnetic nanoparticles were syntheses. The modification of surface of the magnetic nanoparticle through the use of morin was achieved easily. When the diameter of the particle is reduced to the nanometer scale, large external surface area available for the surface modifications, thereby is raising the rate of reaction. Magnetic nanoparticles may be quickly and easily recovered in the presence of external magnetic fields for employs again. Besides, the internal diffusion limitations may be avoided, because all of the available surface area of magnetic nanoparticles is external.

The response surface methodology (RSM) has several advantages compared to the traditional experimental procedures in which "one variable at a time" technique is used. Firstly, the RSM offers a large amount of information from a small number of experiments. Indeed, classical methods are time consuming and a large number of experiments are needed to explain the behavior of a system. Secondly, in the RSM, it is possible to observe the interaction effect of the independent parameters on the response. We can say that the RSM is a useful tool for the optimization. Finally, the RSM also represents a more economical approach, as the number of experiments may be significantly reduced. The Box–Behnken design technique has proved to be an extremely valuable tool, permitting accurate optimum values of experimental parameters to be determined as well as the possibility to evaluate the interaction between variables with a reduced number of experiments [19].

The application of a Box–Behnken matrix became possible, rapid, economical and efficient way of an optimization strategy of the proposed procedure. The magnetic nanoparticle procedure developed for the determination of zinc in water and biological samples needs pH 3.8, $N = 3.1$ mg and $E = 3.3$ mg.

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