***Short Communication***

# Optimization of Iron Oxide Nanoparticle Preparation for Biomedical Applications by Using Box-Behenken Design

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***Abstract:***
*Magnetic nanoparticles can bind to different drug delivery systems and can be used for drug targeting to a specific organ by using an external magnetic field as well as used in hyperthermia by heating in alternating magnetic fields. The characteristics of iron oxide nanoparticles are significantly affected by particle size, shape and zeta potential, among which the particle size plays the most important role. In this study, a chemometric approach was applied for the optimization of iron oxide nanoparticle size. To optimize the size of nanoparticles, the effect of three experimental parameters on size was investigated by means of multivariant analysis for which $Fe^{2+}/Fe^{3+}$ ratio, pH and ionic strength of the media were considered. The experiments were performed on the basis of the Box-Behenken experimental design. The obtained regression model was characterized by both descriptive and predictive abilities ($R^2 = 1$). The method was optimized with respect to Z average diameter as a response. The Box-Behenken experimental design provides optimality in optimizing and testing the robustness of iron oxide nanoparticles preparation method.*
***Keywords:*** *Box-Behenken design, Iron oxide, Nanoparticles, Optimization.*

## 1. INTRODUCTION

Magnetic iron oxide nanoparticles with appropriate surface chemistry have been widely used experimentally for different biomedical applications, such as laboratory diagnostics, medical drug targeting and so on. For such biomedical applications, these nanoparticles must have high magnetization values and size smaller than 100 nm with overall narrow particle size distribution [1]. Different efforts have been carried out with regard to improving the quality of magnetic particles in terms of size distribution, shape and surface.

Magnetic nanoparticles can bind to the drug, proteins, enzymes, antibodies or nucleotides and can be directed to an organ, tissue or tumor by using an external magnetic field and used in hyperthermia by heating in alternating magnetic fields [2]. The properties of iron oxide nanoparticles are significantly affected by particle size, shape, zeta potential, and so on, and the particle size plays the main role. On intravenous administration of nanoparticles with size 30–100 nm, the liver eliminates the larger particles faster from the bloodstream compared with the smaller ones. Furthermore, large particles will be removed only by the cells that are capable of phagocytosis, whereas smaller particles can be removed by all types of cells through pinocytosis. For medical applications, the particles should be well dispersed in a liquid, usually in water, or as composites with organic or inorganic matrices. Liposomes that contain magnetic oxide particles in their cavities are called magnetosomes, whereas aggregates that contain organic compounds (dextran, starch, etc.) in combination with nanoscale iron oxide particles are called superparamagnetic beads or agglomerates [3]. Various methods such as microemulsion approaches, organometallic synthesis, and chemical reduction were used to prepare magnetic nanoparticles [4,5]. The magnetic hydrogels were fabricated by the chemical cross-linking of gelatin hydrogels and $Fe_3O_4$ nanoparticles by using genipin as the cross-linking agent [6]. Various thermal-sensitive ferrofluids for drug delivery application were synthesized [7, 8]. Iron oxide nanoparticles are widely used for various applications, such as drug release, drug targeting, imaging, and gene delivery [9, 10]. A magnetite polymer nanosphere loaded with indomethacin is used in anti-inflammatory therapy [11]. Cytokines are attached to magnetic nanoparticles and are examined as a system for controlled local drug release in cancer therapy [12].

The traditional experimentation is advantageous on considering efficiency and time, especially if complex processes are evaluated. Designed experiments enhance the value of research and minimize the process development time very efficient. Nowadays, experimental design is used to optimize different processes on the basis of mathematical modeling. Optimization is generally carried out on analyses such as capillary electrophoresis and liquid chromatography [13–15]. Optimization in the field of formulation, especially formulation of nanosystems, is often done according to traditional methods, which involve changing one variable at a time [16]. To optimize a formulation, various experimental design methods are used [17,18]. Optimization of nanoparticle production is difficult because of the wide array of parameters and variables that must be controlled to achieve a specific particle size. With central composite designs, Box-Behenken design is the response surface method used to examine the relationship between one or more response variables and a set of quantitative experimental parameters. This method has major advantages compared with other methods [19].

The objectives of this work are as follows: 1. optimize $Fe_3O_4$ nanoparticles by using chemical reduction preparation method; 2. test the use of the Box-Behenken design to optimize this process; and 3. create a model revealing the influence of various factors on the size of iron oxide nanoparticles.

## 2. EXPERIMENTAL PROCEDURE

$FeCl_3{\cdot}6H_2O$, $FeCl_2{\cdot}4H_2O$, NaCl, and NaOH were obtained from Merck company (Darmstadt, Germany).

### 2.1. Preparation of nanoparticles

Iron oxide nanoparticles were prepared by adding a base to an aqueous mixture of $Fe^{2+}$ and $Fe^{3+}$ chloride with various molar ratios. The precipitated magnetite was black in color. According to the thermodynamics of the reaction, a complete precipitation of $Fe_3O_4$ is under a nonoxidizing (oxygen-free) environment. Otherwise, $Fe_3O_4$ might also be oxidized to $Fe(OH)_3$, which is commonly called rust. The synthesis was done in an oxygen-free environment by passing $N_2$ gas:

$$Fe^{+2} + 2OH^- \longrightarrow Fe(OH)_2 \longrightarrow FeO{\cdot}xH_2O$$

$$Fe^{+3} + 3OH^- \longrightarrow Fe(OH)_3 \longrightarrow Fe_2O_3{\cdot}xH_2O$$

$$FeO + Fe_2O_3 \longrightarrow Fe_3O_4$$

The pH of the solution was adjusted 9–14 by adding NaOH (0.1M) and was measured using a pH meter, model HANNA.

### 2.2. Size and polydispersity determination

The size and polydispersity of particles were determined using a Zetasizer Nano Particle Analyzer with Zetasizer 3600 under 25°C at a scattering angle of 90° (Malvern instruments, UK). The analysis of the surface and shape characteristics of the nanoparticles was determined using scanning electron microscope, model 2360 (Leo Oxford England), after coating.

## 2.3. Experimental design

For finding the optimum condition of preparation of iron oxide nanoparticles, three experimental parameters were considered at three levels (Table 1). These parameters were chosen as they were considered to have the most significant effect on the size of nanoparticles. The levels were selected on the basis of the knowledge acquired from the initial experiment. All experiments were carried out in duplicate. A three-factor, at three levels, Box-Behenken experimental design was also used for testing the robustness of the method. The experiment in the central point provided a more precise estimate of pure experimental error and provided a measure for the adequacy of the model (lack of fit). All statistical analysis was performed on range-scaled factor values of [–1, +1] with SPSS software.

***Table 1.*** *The three factors and the corresponding three level settings*

| Level | pH | $Fe^{+2}/Fe^{+3}$ ratio | Ionic Strength (IS, mmol) |
|---|---|---|---|
| -1 | 9 | 0 | 1 |
| 0 | 11.5 | 0.5 | 25.5 |
| +1 | 14 | 1 | 50 |

## 3. RESULTS

The effect of three factors on the Z average diameter of nanoparticles was studied. The $Fe^{2+}/Fe^{3+}$ ratio, pH, and ionic strength (IS) of the media were considered. The media's ionic strength was adjusted by different NaCl concentrations as given Table 1. The experiments were performed in a random order on three levels for each factor by using the Box-Behenken design (Table 2) which provides enough information for calculation of the regression model containing linear interactions and curved factor effects. The values of response data obtained in the experiments are presented in Table 2. The graph of particle size distribution and the SEM photograph under experiment number 10 in Table 2 are shown in Figures 1 and 2.

The Z average diameters of nanoparticles for all 15 experiments were fitted to the polynomial model for each response factor. The coefficients of the model were calculated using backward multiple regression technique and validated using the

***Table 2.*** *Experimental conditions for Box-Behenken design and average response for particle size*

| Trial | pH | $Fe^{+2}/Fe^{+3}$ ratio | Ionic Strength (mmol) | Z average diameter (nm) |
|---|---|---|---|---|
| 1 | + | + | 0 | 186.00 |
| 2 | + | - | **0** | 33.49 |
| 3 | - | + | 0 | 7.51 |
| 4 | - | - | 0 | 10.57 |
| 5 | + | 0 | + | 176.40 |
| 6 | + | 0 | - | 155.90 |
| 7 | - | 0 | + | 5.57 |
| 8 | - | 0 | - | 162.80 |
| 9 | 0 | + | + | 14.43 |
| 10 | 0 | + | - | 17.18 |
| 11 | 0 | - | + | 35.74 |
| 12 | 0 | - | - | 183.80 |
| 13 | 0 | 0 | 0 | 0.70 |
| 14 | 0 | 0 | 0 | 0.64 |
| 15 | 0 | 0 | 0 | 0.64 |

analysis of variance (ANOVA). The criteria for the evaluation of descriptive capability of a polynomial were Fisher ratio value (*F*), adjusted $R^2$, and standard error of estimation (S.E.). The models that successfully described the size of nanoparticles were polynomials containing various numbers of terms and different combinations of factors. The related model and its statistics are presented in Table 3.

After developing the MLR model, the size of nanoparticles in 360 experimental conditions was predicted using the obtained equation. Then, the best obtained conditions were tested and the experimental results were compared with the

***Table 3.*** *Intercept, coefficients and mean effects for the predictive model obtained for three factors, pH, IS (Ion strength) and Fe ($Fe^{+2}/Fe^{+3}$ mole ratio)*

| Variable | Coefficients (p-value) | Mean effect |
|---|---|---|
| intercept | -0.462 | - |
| pH | 45.668 (0.000) | 0.436 |
| Fe | -42.173 (0.000) | -0.402 |
| IS | 35.943 (0.000) | 0.343 |
| $pH^2$ | 60.697 (0.000) | 0.396 |
| $IS^2$ | 64.092 (0.000) | 0.418 |
| pH×Fe | -38.893 (0.000) | -0.262 |
| pH×IS | -44.433 (0.000) | -0.300 |
| Fe×IS | -36.328 (0.000) | -0.245 |

[a]$R^2$=0.998, Standard error= 8.14 and F ratio=164.970; the number in parenthesis is the p-value

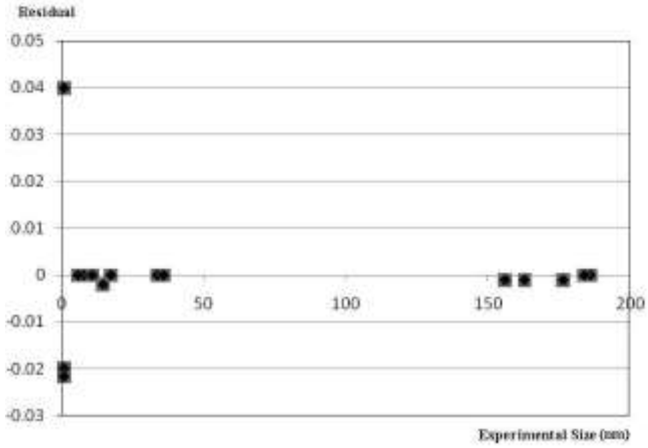


*Figure 1.* *The residual plot for the predicted size of iron oxide nanoparticles according to the regression model reported in Table 3*

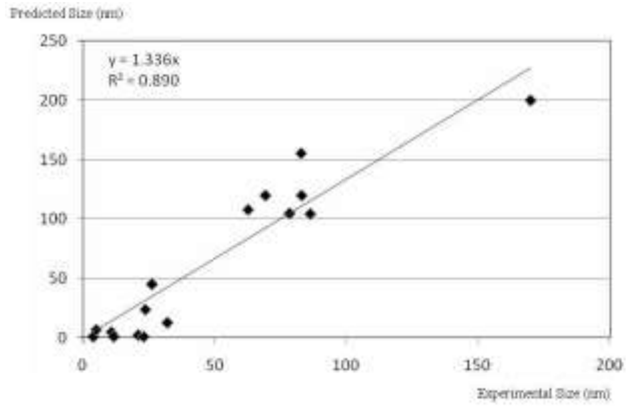


*Figure 2.* *Predicted vs. experimental iron oxide nanoparticle size for 17 experimental condition not entered in the modelling*

predicted ones. In addition, to assess the suitability of the obtained MLR model the residuals were analyzed statistically (Figure 2). Figure 2 also shows a good agreement between predicted and experimental results obtained under the optimized conditions. It can be observed that the fitting of this model and the model prediction ability are quite satisfactory.

## 4. DISCUSSION

The results of ANOVA demonstrated that the largest effects on nanoparticle size were due to the main effect of pH and Fe ratio. The main effect and quadratic term of IS were also significant in the size of nanoparticles. No significant effect was observed owing to the quadratic term of the $Fe^{2+}/Fe^{3+}$ ratio. The *p*-value in Table 3 shows that the coefficient is completely significant in the obtained MLR model. However, the interaction of these factors together shows significant effect on the *Z* average size of nanoparticles, that is, the respective response in the multidimensional factor space is curved in the sphere of the experimental design. The existence of interactions among the principal factors under the conditions of our experiments emphasizes the necessity to carry out active multifactor experiments for optimization of the preparation process of nanoparticles. This indicates that the use of simultaneous method is essential for optimizing the preparation process of iron oxide nanoparticles.

## 5. CONCLUSION

In this study, the effect of $Fe^{2+}/Fe^{3+}$ ratio, pH, and ionic strength of the media was investigated on the size of iron oxide nanoparticles. Box-Behenken experiments and multivariant analysis were used to optimize the size of iron oxide nanoparticles synthesized by applying a chemometric approach. The obtained regression model was characterized by both descriptive and predictive abilities ($R^2$ = 1). The method was optimized with respect to the *Z* average diameter as a response. It can be concluded that the Box-Behenken experimental design provides optimality in optimizing and testing the robustness of the preparation method of iron oxide nanoparticles.

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