BIOSYNTHESIS OF IRON OXIDE NANOPARTICLES USING CENTRAL COMPOSITE DESIGN OF RESPONSE SURFACE METHODOLOGY

***Alaya-Ibrahim, S[1,2]., Kovo, A. S[1,2]., Abdulkareem A. S[1,2]., and Adeniyi, O. D[1,2].**

[1]Nanotechnology Group, Centre for Biotechnology and Genetic Engineering, Federal University of Technology, PMB 65, Minna, Niger State, Nigeria;

[2]Chemical Engineering Department, Federal University of Technology, PMB 65, Minna, Niger State, Nigeria.

*Corresponding author email: neekyai@yahoo.com 08032877199

ABSTRACT

Central Composite Design (CCD) of Response Surface Methodology has been applied in optimizing the size of iron oxide nanoparticles (FeONPs) synthesised by studying the effects of three (3) important elements which are reaction time, reaction temperature and extract volume. The quadratic model was selected and fitness of the model was studied using sequential model of sum of squares and model of summary statistics. The effects and interaction between the elements studied on the size of synthesised FeONPs were investigated using analysis of variance (ANOVA), 2D contour plots and 3D surface plot. ANOVA showed that the time of reaction has the greatest effect on the size of FeONPs synthesised and the 3D surface plots revealed that to obtain the smallest size of FeONPs, the reaction time and the reaction temperature has to be set at 15 mins & 25 °C respectively.

Keywords. *Iron oxide nanoparticles, Biosynthesis, CCD. RSM*

I. INTRODUCTION

Iron oxide nanoparticles (FeONPs) are considered as one of the most multipurpose and safe nanoparticles because of their biodegradability, biocompatibility, ease of surface modification and magnetic properties which enable them to be controlled by external magnetic field. Thus, they are useful in various biomedical applications such as targeted drug delivery, cell sorting, contrast agents for magnetic resonance imaging (MRI) and hyperthermia (Meyyappan *et al*., 2015; Al-Ruqeish *et al*.,2016). They also play important role in environmental remediation circle, as it is used in removal of both organic and inorganic heavy metal pollutants from polluted water (Balamurugan *et al*., 2014).

However, the antibacterial and the catalytic activities of FeONPs just like any other metal nanoparticles are relatively dependent on their sizes, structure, shape, size distribution & chemical-physical environment. Hence, it is of paramount importance to have control over the size distribution and this is often achieved by specifically varying the system methods, reducing agents and stabilizer (Abou El- Nour *et al*., 2010).

Response Surface Methodology (RSM) is a mathematical tool which aids in better understanding & optimizing the response of an experiment (Fisher, 1920) by basically feeding the software with information which in turn provide an accurate prediction of response. RSM was purposely designed to replace experimental response with predictive one by studying the various effects of parameters that will result to optimum response (Neda *et al*., 2002). Thus, it is widely applied in optimizing many processes which include; chemical & pharmaceutical, biological/biomedical, food sciences, production engineering, air quality analysis & toxicological research, and simulation studies (Ray, 2006; Montegometry, 2005; Carley *et al*., 2004; Neda *et al*., 2002; Allen and Yu, 2000). This is due to numerous advantages of RSM over conventional method as follows; (1) ability to efficiently predict values from numerical or practical experiments at discrete data points (2) it minimizes cost of analysis methods, the associated resources & high numerical data analysis (3) it also reduces the process development (Cira *et al*., 2016; Ray, 2006) and (4) it estimates the interactions between the process parameters (Asadi and Zilouei, 2017)

In this study, Central Composite Design (CCD) of Response Surface Methodology was employed in optimizing the particle size of iron oxide nanoparticles (FeONPs) through investigations of influence of the major synthesis parameters such as reaction time, reaction temperature and the extract volume.

2. MATERIALS AND METHODS

Materials

$FeCl_3$ and $FeCl_2$. $4H_2O$ salts used in this work are of analytical grade, manufactured by BDH, England. Deionized water was used throughout the study and the mango leaves was collected from Tunga area of Minna, Niger state, Nigeria.

Preparation of Mango Leaves Extract

The mango leaves collected were washed thoroughly with distilled water to remove dust from their surfaces before drying them under shade at room temperature for 18 days. After drying, they were crushed using porcelain mortar and pestle.

40 g of crushed mango leaves was boiled in 400 ml of deionized water for 15 mins. The mixture was allowed to cool & centrifuged at 6600 rpm for 30 mins, it was decanted, filtered using Whattman filter paper. The filtrate was collected as the MLE and stored in a bottle at a temperature of 4 °C for further experimental use.

Biosynthesis of FeONPs

Certain volume of MLE was reacted with 5 ml of 0.01 M of $FeCl_3$ and $FeCl_2$. $4H_2O$ (ratio 2:1) in a water bath shaker at specific temperature & reaction time based on the optimization parameters generated by Design Expert 10 using CCD of RSM. This procedure was carried out on all the samples and upon completion of their reaction time, samples were withdrawn from the shaker for UV-Visible Spectroscopy (UV) analysis (the UV result is in the main work) and the sizes were calculated from TEM analysis using Zeiss Auriga HRTEM

Table 1. Alpha ($\propto$) values of the factors studied and their coded form

Name	Coded factors	Units	Low	High	-alpha	+alpha
Time	A	Minute	5	15	1.59104	18.409
Temperature	B	Celsius	25	80	6.2507	98.7493
Extract volume	C	millilitre	5	14	1.93193	17.0681

Optimization Process

The rotatable CCD of RSM was employed because of its flexibility. Central Composite Design (CCD) is a combination of 2 level factorial design, centre points which is usually replicated and axial or star points. The distance of each axial points from the centre is called alpha ($\propto$). The value of $\propto$ is subject to the number of factors considered as well as some desired properties. Also, the star points are usually twice the number of factors in the design and it's a representation of extreme values for each factor in a design (minimum & maximum) (http://www.itl.nist.gov/div898/handbook/, 2017). In CCD, the centre points determine the orthogonality as well as the rotatability of a design.

- Orthogonality is a property of RSM that allows the model terms & block effects to be estimated independently by minimizing the variation in the regression coefficient.
- Rotatability is the measure of the variance of the predicted response at any point "x" which depends on the distance of x from the design center point. This property of RSM improves the precision of prediction (Asadi and Zilouei, 2017; Box and Draper, 1987).

Thus, for rotatable design, the alpha ($\propto$) = $[2^k]^{1/4} = 2^{3/4}$ = 1.682, for this design, where k is number of factors = 3. The ($\propto$) values of the factors studied and their coded form are shown in Table 1

The factors studied are reaction time, reaction temperature and the plant extract volume which are coded as A, B & C respectively. A total number of 20 runs was generated for the optimization of the size of FeONPs and after the experiments, the various sizes obtained are shown in Table 2.

Characterization Techniques

The FeONPs synthesized were characterized using UV-Visible Spectroscopy and Transmission Electron Microscopy (TEM)

Table 2. Experimental Design and Response Factor of Response Surface Analysis for FeONPs

Experimental Run	Time (min)	Temperature (Celcius)	Extract Volume (ml)	Size (nm)
1	5	25	14	0
2	10	52.5	9.5	8.11
3	5	80	5	0
4	15	25	14	0
5	15	80	5	10.11
6	15	80	14	0
7	15	25	5	9.37
8	5	25	5	0
9	5	80	14	0
10	10	52.5	9.5	8.11
11	10	52.5	9.5	8.11
12	10	52.5	9.5	8.11
13	18.41	52.5	9.5	12.21
14	1.51	52.5	9.5	0
15	10	52.5	9.5	8.11
16	10	98.75	9.5	0
17	10	52.5	1.93	18.11
18	10	6.25	9.5	0
19	10	52.5	9.5	8.11
20	10	52.5	17.07	6.25

UV-Visible Spectrum analysis: The syntheses of FeONPs were monitored using UV-Vis spectra by sampling 1 ml of FeONPs solution in 10 ml of distilled water using Shimadzu UV-visible spectrophotometer 1800 at the range of 190-800 nm.

Transmission Electrons Microscopy (TEM) analysis: The formation and sizes of the FeONPs synthesized were determined using Zeiss Auriga High Resolution Transmission Electrons Microscopy (HRTEM). 0.02 g of the FeONPs was suspended in 10 ml of methanol which was ultra- sonicated until there was complete dispersion of the FeONPs. A drop of the slurry was dropped on the holey carbon grid and photo light was used to dry the sample. The dried sample was loaded onto the sample holder and the electron microscope was operated for imaging

3.0. RESULTS AND DISCUSSIONS

UV-Vis spectra

Iron oxide nanoparticles was synthesised using different volume of plants extract with 5 ml of $FeCl_3$ & $FeCl_2$ (ratio 2:1) at different temperatures and reaction time as illustrated in Table 2 based on CCD of Response Surface Method of optimization. An immediate colour change was observed from light yellow to black colour, as soon as the Mango Leaves Extract (MLE) was added to the salt in all cases. This is an indication of formation of iron oxide nanoparticles, as the colour change is the easiest & commonest form of identification of nanoparticle formation (Balamurugan *et al.*, 2014). The change in colour is due to the excitation of the Surface Plasmon Vibrations in the Iron oxide nanoparticles (Song and Kim, 2009; Sneha Shah *et al.*, 2014). The wavelength obtained from the synthesis of FeONPs ranges from 199 nm–267 nm which is of typical spectra of FeONPs (Pattanayak and Nayak, 2013; Sranvanths et al., 2016) as shown in Figure 1

& predicted responses are well correlated; and adequate precision of 15.565 is an indication of strong signal that the model can be used for optimization as its value is higher than 4

Table 5. Analysis of Variance (ANOVA) of selected Response Model

Source	Sum of Squares	df	Mean Square	F- Value	p-value Prob>F
Model	507.87	9	56.43	25.52	<0.0001
A-Time	117.24	1	117.24	53.03	<0.0001
B-Temperature	0.04	1	0.04	0.018	0.8958
C-Extract volume	113.82	1	113.82	51.48	<0.0001
AB	0.068	1	0.068	0.031	0.8642
AC	47.43	1	47.43	21.45	0.0012
BC	0.068	1	0.068	0.031	0.8642
A	32.19	1	32.19	14.56	0.0041
B	192.21	1	192.21	86.94	<0.0001
C	6.14	1	6.14	2.78	0.1301
Std. Dev	1.49		R-Squared	0.9623	
Mean	5.24		Adjusted R	0.9246	
			Predicted R	0.7384	
			Adequate Precision	15.565	

The correlation between the actual and predictive size of FeONPs was also examined using predictive vs actual plot as shown in Figure 3. There is close agreement between the predicted and actual size of synthesised FeONPs as shown by points of the data, which are not too far apart.

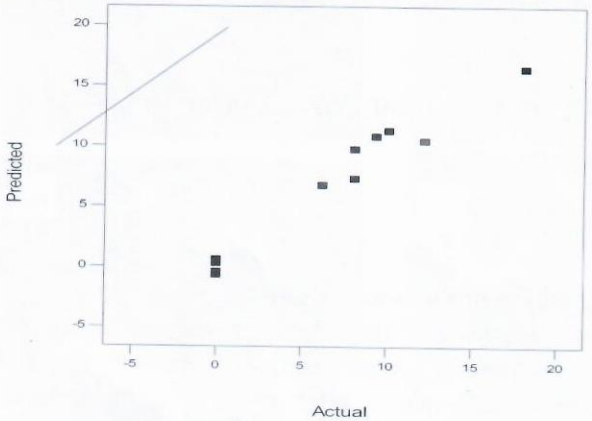

Figure 3. Predicted vs Actual values

Effects of Reaction Time, Reaction Temperature and the Extract volume on the Size of the Synthesised FeONPs

After the adequacy of the model has been established, the effects &interaction between the model terms on the response of the model was studied using ANOVA, 2D contour plot and 3-D surface plot.With reference to Table 5 (ANOVA) It can be deduced that time & extract volume are significant factors which influences the size formation of FeONPs with their p-value (Prob >F) of < 0.0001 & < 0.0001 respectively, while temperature has insignificant effect. Also, the interaction between time & extract volume has significant effect on the response of the model and its p-value (Prob >F) is < 0.0012. The F-Values of time, temperature & extract volume were found to be 53.03, 0.018 & 51.48 respectively. This is an indication that time has the highest influence on the size formation of FeONPs with highest F-value of 53.03. Thus, increasing the reaction time results to bigger size of FeONPs and vice versa.

Figure 4a and Figure 4b shows the 2D contour of temperature vs time & that of extract volume vs time respectively. Figure 4a examines the effect of temperature and time on the size formation of FeONPs synthesised. It could be deduced that keeping temperature constant and increasing the reaction time leads to increased size of the FeONPs. For instance, at temperature of 36 °C and reaction time of 6.03 min, a size of 3.98 nm was obtained while at the same temperature, with increased reaction time of 8.12 min, a size of 5.91nm was obtained. This is in support of ANOVA result identifying temperature as an insignificant.

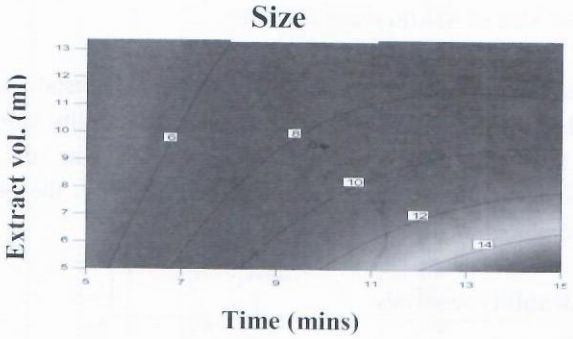

Figure 4a. 2D Contour Plot of Temperature vs Time

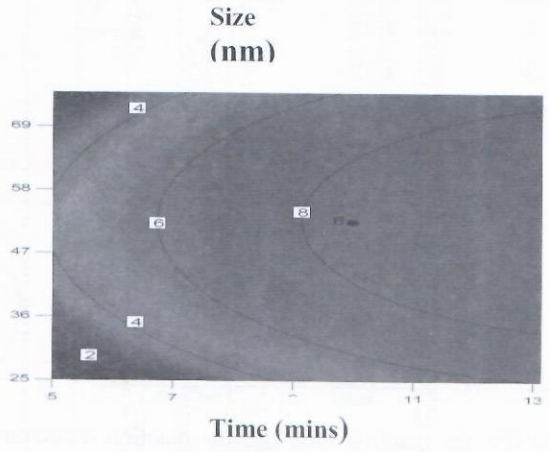

Figure 4b. 2D Contour Plot of Extract Volume vs Time

On the other hand, figure 4b relates the effect of extract volume and time as a function of size. The plot shows that lowering the volume of extract at constant reaction time produces a bigger size of FeONPs.

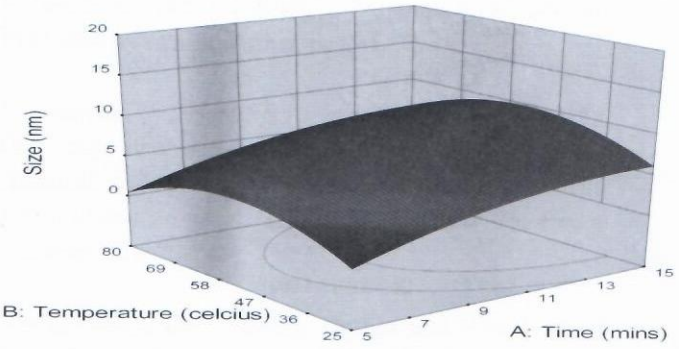

Figure 5. 3D Surface plot of Temperature & Time with Extract volume at the Central

The effects of reaction temperature and reaction time on the size of FeONPs with the extract volume at the central level is shown in Figure 5 The figure shows that in order to obtain the smallest size of FeONPs, the reaction time and the reaction temperature has to be set at 15 mins & 25 °C respectively

Numerical Optimization for Desirability of the Response

Numerical optimization was employed by the Design Expert software to determine the desirability of the size of FeONPs synthesised. The actual values of the operating variables (time, temperature & extract volume) as well as the response (size) were set, ranging from the minimum to maximum values. The software then proffers solution of their various optimal values and determine the desirability of the response (size). Desirability ranges from zero to one (1), desirability value of 1 is an ideal case and that of zero does not fit well. Thus, the desirability test was conducted on the FeONPs experimental variables & the obtained sizes as illustrated with Ramp view in Figure 6.

The figure shows that the optimal reaction time temperature and extract volume for obtaining FeONPs are,

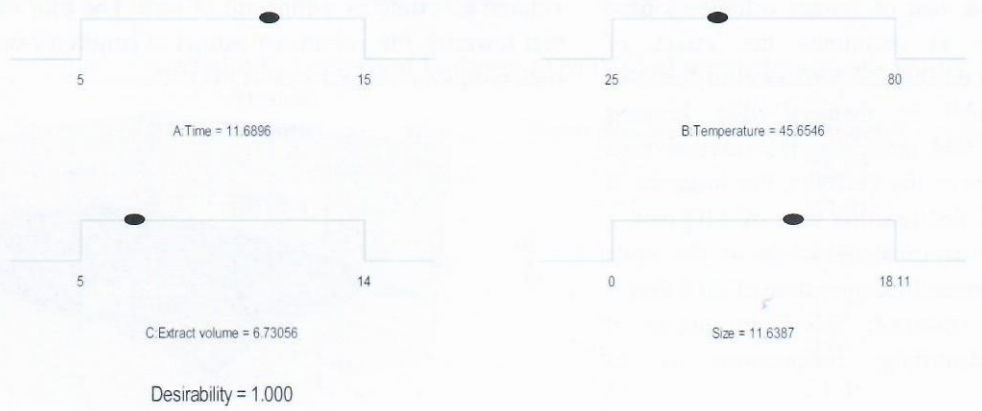

Figure 6. Ramp View of desirability of Size of Synthesized FeONPs

11.69 mins, 45.65 °C and 6.73 ml respectively, with their combined desirability of 1. The results of the desirability analysis as shown in Table 6, show different combinations of predicted synthesis parameters (time, temperature & extract volume) and their corresponding sizes of the FeONPs as well as their desirability value. They all fitted well with desirability value of 1, however, the Ramps result was confirmed as the best combinations.

Table 6. Results of Desirability Analysis

Number	Time (min)	Temperature (°C)	Extract volume (ml)	Size (nm)	Desirability
1	11.69	45.66	6.73	11.64	1
2	5	80	5	1.63	1
3	5	25	5	1.52	1
4	10	52.5	9.5	8.55	1
5	15	80	14	1.72	1
6	5	25	14	0.8	1
7	15	80	5	12.55	1
8	15	25	14	1.61	1
9	5	80	14	0.54	1
10	9.68	62.2	11.55	6.8	1

Hence the optimum experimental parameters for the synthesis of FeONPs by RSM are **11.69 mins 6.73 ml & 45.66 °C** for reaction time, extract volume and reaction temperature respectively

4.0. CONCLUSIONS

The CCD of RSM has been successfully used in optimizing the size of FeONPs and it was found to be an effective tool. Time and extract volume were found to have significant effect on the size formation of FeONPs, with time having the greater effect, the 3D surface plot of temperature & time with extract volume at the central level shows that in order to obtain the smallest size of FeONPs, the reaction time and the reaction temperature has to be set at 15 mins & 25 °C respectively.

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