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Green Synthesis of Iron Oxide Nanoparticles Using Full Factorial Experimental Design: Technical and Analytical Impact of $FeCl_3.6H_2O$ Precursor on Yield

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Abstract: *Green synthesis of iron oxide nanoparticles (IONPs) has emerged as an eco-friendly alternative to conventional synthesis methods. This method utilizes natural ingredients, such as green tea extract, as a reducing agent that supports the green chemistry process. This research aims to analyze the effects of precursor concentration, reducing agent volume, and reaction temperature on the synthesis results using technical and pro-analytical $FeCl_3.6H_2O$ precursors. A full factorial design was employed to assess the effect of synthesis conditions on the IONPs yield. The yield was statistically analyzed using analysis of variance (ANOVA). The analysis results reveal that the predicted values are within reasonable consistency based on the experimental data of both IONPs from the technical and pro-analytical precursors. The ANOVA values for both precursor sources show that the reaction temperature factor is negatively correlated, while the precursor concentration and reducing agent volume positively correlate with the IONPs yield. Regression model variance (ANOVA) can be explained 98.15% for the technical precursor model and 99.83% for the analytical precursor model from data variations. This research can determine the optimal combination of synthesis factors that provide a basis for selecting a synthesis strategy tailored to specific performance requirements. These findings can support the wider application and reproducibility of green synthesis methods in various research contexts and implications for industry.*

Keyword*: ANOVA, Full Factorial, IONPs, Pro-analytical, Technical*

INTRODUCTION

Environmentally friendly techniques have attracted much attention for producing metal oxide nanoparticles (Priya et al., 2021). This environmentally friendly approach uses green synthesis, which has several advantages such as process simplification, economic feasibility, and little waste residue (Karade et al., 2019). Green synthesis utilizes natural resources and environmentally friendly procedures as a sustainable alternative to conventional chemical synthesis methods that often involve hazardous chemicals, higher-energy procedures, raise environmental issues, and contain toxic by-products (Osman et al., 2024).

Green synthesis for the production of metal oxide nanoparticles includes the use of green tea leaf extract. Green tea leaves have the highest antioxidant activity, followed by white, black, red, and other teas (Kolodziej et al., 2020). High antioxidant activity indicates superior reducing ability, essential for synthesizing metal oxide nanoparticles. Green tea mainly comprises catechins, while black tea comprises tannins (Prasanth et al., 2019). Catechins that consist of 80–90% of total polyphenols, epigallocatechin gallate (EGCG) is a catechin content that is more than 50% of total catechins (Venkata et al., 2018). One of these metal oxide nanoparticles is iron oxide nanoparticles (IONPs). Exploring green synthesis methods for IONPs offers an opportunity to investigate the intersection between nanotechnology, sustainability, and analytical precision.

IONPs have different forms, such as FeO (wustite), γ-Fe_2O_3 (maghemite), and Fe3O4 (magnetite). IONPs have various applications, including in magnetic storage media, battery electrodes, magnetic hyperthermia, drug delivery, antibacterial, solar cells, food packaging, nanofertilizers, and dead and poor spermatozoa separation (Ajinkya et al., 2020). These nanoparticles' size, shape, and surface features significantly affect their performance in nanoparticle applications (Altammar, 2023). Among the various methods used, the choice of precursor materials and the synthesis approach play an important role. IONPs produced from various iron chloride hexahydrate ($FeCl_3.6H_2O$) precursors are of technical and pro-analytical grade. Technical grade materials are generally used in industry with lower purity compared to pro-analytical materials, which have high purity and are often used in laboratory environments

A Full factorial experimental design will be applied to investigate the green synthesis process of IONPs systematically. Factors that influence the yield of green synthesis IONPs include precursor concentration, reducing agent volume, and reaction temperature (Kheshtzar et al., 2019; Arsalani et al., 2018). One element's influence on other factors' level is extremely well determined by design (Badr et al., 2020). Analysis of variance (ANOVA) of data is performed after identifying factor significance to assess the correlation between factors (Herlekar & Barve, 2015). Therefore, research was carried out to analyze the correlation between synthesis factors such as precursor concentration, reducing agent volume, and reaction temperature on the synthesis results using technical and pro-analytical $FeCl_3.6H_2O$ precursors.

RESEARCH METHODS

Materials and Tools

The materials used in this research include green tea leaf powder obtained from an online marketplace, $FeCl_3.6H_2O$ technical, $FeCl_3.6H_2O$ pro-analysis (MERCK), ethanol 96%, distilled water, and filter paper (Whattman).

The tools used in this research include an Acer Aspire S3 computer with 4 GB RAM, which has a Windows 10 operating system and Intel Core i5-3337U CPU @ 1.80GHz processor specifications, software in the form of Design Expert 13.0.5.0 Trial Version (https://www.statease.com/software/design-expert/), beaker glass (Pyrex), volumetric pipette (IWAKI), suction bulb, hotplate (IKA C-MAG HS 4), glass funnel (IWAKI), analytical balance (Quattro FH 300), volumetric flask (IWAKI), wire netting, tongs, desiccator (Duran desiccator 250 mm), oven (Memmert UN 55 531), aluminum dish, centrifuge (Gemmy PLC-05), centrifuge tubes (Sarstedt), spatula, and magnetic stirrer.

Methods

Experimental design

The experimental design used in this study was a full factorial to analyze the effect of independent variables in the form of reaction temperature (A), precursor concentration (B), and reduction extract volume (C) on technical and pro-analytical $FeCl_3.6H_2O$ precursor types on the dependent variable in the form of yield. The precursor concentrations were 0.1M and 0.3M (Arsalani et al., 2018; Mohamed et al., 2023). The reaction temperatures were 50°C and 70°C, and the reduction extract volumes used were 20 mL and 40 mL (Kheshtzar et al., 2019) with a synthesis time of 45 minutes (Akhbari et al., 2019). Determination of the value of the independent variables was based on the results of previous studies with different synthesis conditions. The factors and levels of the experiment were selected by considering their influence on the results of the synthesis of iron oxide nanoparticles (IONPs), where the variables used were two levels, namely low and high, which are presented in Table 1.

Table 1. Experimental factors code and levels

Factors	Factors code	Levels	
		Low	High
Temperature (°C)	A	50	70
Concentration (M)	B	0.1	0.3
Volume (mL)	C	20	40

The running number of experiments carried out is determined by factors and levels. The run of experiments between 3 factors and 2 levels used is 2^3 full factorial experimental designs, which are listed in Table 2 for the synthesis of IONPs. A factorial linear regression equation was used to analyze experimental data. This model represents the impact of process variables (A, B, and C) and interactions on the response variable (the yield of IONPs). The general structure of the chosen model can be shown as follows.

$$Y= b_0 +b_1A + b_2B + b_3C \qquad \text{(Eq. 1)}$$

The results obtained were subjected to an analysis of variance (ANOVA) to determine the correlation between significant factors using the Design Expert 13.0.5.0 software program.

Table 2. Design layout of full factorial experimental design

Run Number	Factors		
	Temperature (°C)	Concentration (M)	Volume (mL)
1	50	0.1	20
2	50	0.3	20
3	50	0.1	40
4	50	0.3	40
5	70	0.1	20
6	70	0.3	20
7	70	0.1	40
8	70	0.3	40

Preparation of plant extract

Green tea extract was prepared using commercial green tea leaf powder. A total of 12 grams of green tea leaf powder was weighed and then added with 200 mL of 96% ethanol. The solution was homogenized using a magnetic stirrer at room temperature for 35 minutes to ensure extraction occurred thoroughly. Following the stirring process, the solution is allowed to sit until the remaining green tea settles to the bottom. The supernatant solution was filtered through filter paper to remove solid residue after sedimentation was complete. The filtrate was then centrifuged at 786 RCF for 10 minutes to further purify the extract. The centrifuged green tea extract is poured into dark bottles for further use (Safa & Koohestani, 2024).

Green synthesis of iron oxide nanoparticles (IONPs)

Iron oxide nanoparticles (IONPs) green synthesis using technical and pro-analytical grade $FeCl_3.6H_2O$ precursors with varying reaction temperature, precursor concentration, and reducing extract volume as shown in Table 2. The first step involves using a technical $FeCl_3.6H_2O$ grade precursor to heat 20 mL of extract (C) pipetted using a volumetric pipette until it reaches 50°C (A) on a hotplate, thermometer placed in the extract solution is used to control the temperature then adding 5 mL of 0.1M $FeCl_3.6H_2O$ (B) drop by drop using a burette and continuing the synthesis for 45 minutes. The crucibles were heated in an oven until a constant weight was attained, after which the IONPs were placed into the crucibles. The crucibles containing IONPs were oven dried at 100°C until a constant weight was obtained (Ghafarzadegan et al., 2022). The same procedure was followed for runs 2 to 8, with different factors and repetitions also carried out using pro-analytical grade $FeCl_3.6H_2O$ precursor. The yield of IONPs percentage can be calculated using the following equation.

$$\text{Yield of IONPs } (\%\frac{w}{v}) = \frac{\text{Constant mass of IONPs}}{\text{5 mL precursor}} \times 100\% \qquad \text{(eq.2)}$$

RESULTS AND DISCUSSION

Green synthesis iron oxide nanoparticles (IONPs)

Green synthesis produces nanoparticles utilizing bioactive compounds from plant extracts as capping agents (Jamzad & Bidkorpeh, 2020). Gottimukkala et al (2017) state that IONPs can be synthesized using green tea extract. The OH-groups of flavonoid compounds in green tea leaves are used as capping agents in synthesizing precursors. Green tea is a reducing agent in synthesizing various morphologies of IONPs due to its high polyphenol and other organic group content. There are around 4000 species, with polyphenols accounting for one-third of them, that help reduce metal ion precursors to nanoparticles (Gottimukkala et al., 2017). Flavonoids and catechins are types of polyphenols. Catechins, specifically epigallocatechin gallate (EGCG), have a content of more than 50% total catechins (Venkata et al., 2018). Catechins are bioactive compounds that assist in reducing Fe^{3+} to Fe^0. The reaction involving EGCG proceeds according to the following equation.

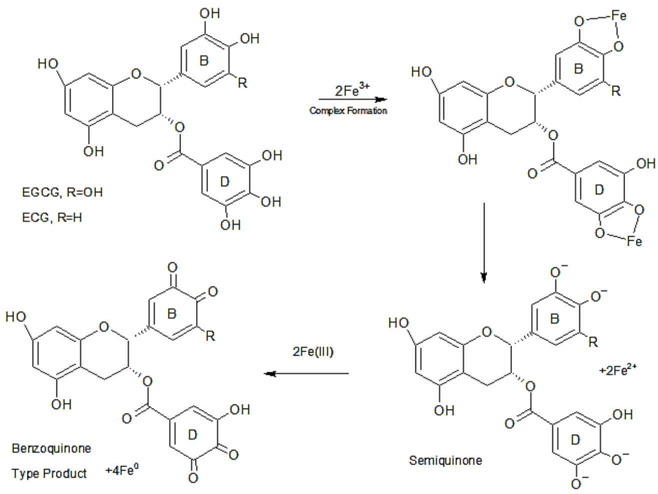

Figure 1. Reaction mechanism for the formation of IONPs by green tea extract (Gottimukkala et al., 2017)

According to the reaction in Figure 1, the reduction process occurs in two steps, with the first step involving the addition of the precursor to build a complex by splitting the OH bond and generating a partial bond with the Fe^{3+} ion. The second step involves partial bond cleavage and electron transfer, which reduces Fe^{3+} ions to Fe^0 and oxidizes EGCG to ortho quinone. IONPs are compounds consisting of oxygen and iron atoms linked in the form of a molecule. Iron is commonly found as hematite (Fe_2O_3), magnetite (Fe_3O_4), goethite ($FeO(OH)$), siderite ($FeCO_3$), or limonite ($FeO(OH)\cdot n\ (H_2O)$). IONPs are synthesized at a temperature of 100ºC. Different calcination temperatures will result in various phases of IONPs, namely Fe_3O_4 (at room temperature up to 100ºC), γ-Fe_2O_3 (calcination at 200–300ºC), and α-Fe_2O_3 (calcination at 300–600ºC) (Dewanto et al., 2023).

Analysis of model equation and statistical evaluation

The experimental research included eight experiments for each type of precursor. Actual data from the experiment is utilized to create empirical models that represent the results of synthesis with technical and pro-analytical precursors. The results of the experiment are shown in Table 3.

Table 3. Yield of IONPs

Run Number	Yield of IONPs from precursor ($\%\frac{w}{v}$)	
	Technical	Pro-analytical
1	10.60	9.70
2	19.60	13.00
3	18.20	17.60
4	25.70	20.90
5	9.80	8.50
6	15.00	12.50
7	14.70	16.40
8	22.40	19.70

The actual model equations for the yield of IONPs from technical precursors (Y_1) and pro-analytical precursors (Y_2) are given in Equations (3) and (4), respectively.

$$Y_1 = 9.05000 - 0.152500A + 36.75000B + 0.325000C \quad \text{(Eq. 3)}$$
$$Y_2 = 2.80000 - 0.051250A + 17.37500B + 0.386250C \quad \text{(Eq. 4)}$$

The technical grade precursor produces a higher yield value compared to the pro analytical grade precursor, this difference can be attributed to the addition of chemical agents or impurities in the technical grade material that have the potential to increase the yield of IONPs.

The research uses a linear regression calculation model with a factorial analysis type. Chicco et al (2021) state that good modeling has high accuracy because the predicted and actual values show relatively small systematic errors. When evaluated using linear regression modeling and factorial analysis, the pro analytical grade precursor showed superior predictive performance. This is evidenced by the coefficient of determination values of 0.9815 for technical grade and 0.9983 for pro analytical grade precursor based on Figure 2. A coefficient of determination close to 1 indicates that the modeling has relatively small errors and is not biased (Adamu et al., 2020). Therefore, although the yields obtained with technical grade precursors are slightly higher, the model based on pro analytical grade precursors shows greater reliability and lower bias.

The actual vs. predicted plots are a parameter in regression analysis and predictive modeling, helping to ensure that the model used is highly accurate and reliable in predicting experimental outcomes. Plots of actual vs. predicted yield results of IONPs from technical and pro-analytical precursors are presented in Figure 2.

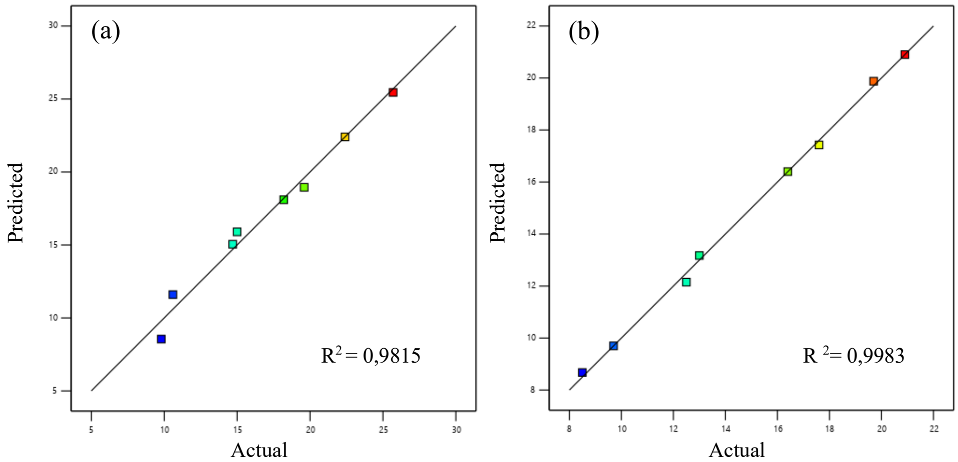

Figure 2. Relationship between actual and predicted values ((a) Technical (b) Pro-analytical precursor)

According to Figure 2, it can be seen that the points on the plots for technical and pro-analysis precursors are evenly distributed across the lines. Teja & Damodharan (2018) state that an even distribution shows that the model has strong predictive ability, characterized by the coefficient of determination (R^2), which describes how much the predicted value matches the actual value. The technical and pro-analytical precursors had R^2 values of 0.9815 and 0.9983, respectively. The coefficient of determination indicates which portion of the response variability is explained by the model. For a model to be considered suitable, the R^2 value must be close to 1 (Ahmad et al., 2016).

The residual vs. predicted plots are a visualization for analyzing prediction errors or residuals in statistical models. residual refers to a difference between the actual predicted values produced by the model (Rocha et al., 2023). The residuals can be used to graphically evaluate the significance of the established model (Rufina et al., 2023). Plots of the residual vs. predicted yield results of IONPs from technical and pro-analytical precursors are presented in Figure 3.

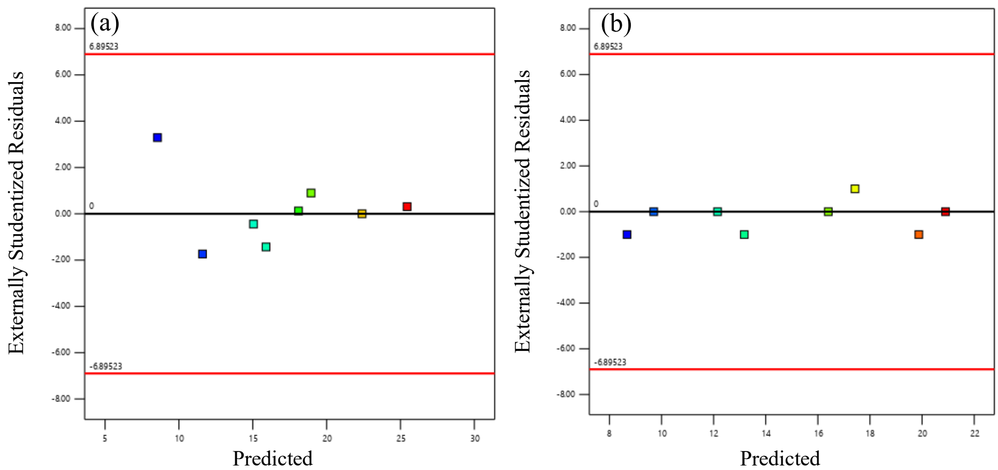

Figure 3. Residual plots for predicted values ((a) Technical (b) Pro-analytical precursor)

According to Figure 3, data distribution using technical precursors is more spread out around the zero-line compared to pro-analytic precursors, whose distribution is concentrated near the zero-line but both do not cross the lower and upper bounds. This indicates that model inconsistency occurs with the technical precursor data, while consistency is observed with the pro-analytical precursor data. The purity level of the materials used can cause inconsistencies and increase systematic errors based on the ANOVA test reviewed from its statistical parameters. The results of the ANOVA test are presented in Table 4.

Table 4. ANOVA of the yield of IONPs

Statistics	Source of precursor	
	Technical	Pro-analytical
F-value	70.56	792.40
P-value	0.0006	< 0.0001
CV %	5.87	1.67
Adjusted R^2	0.9675	0.9983
Predicted R^2	0.9258	0.9933
R^2	0.9815	0.9983

The F-value is obtained by dividing the variable's mean square by the error's mean square. The area under the relevant null sampling distribution of the F-value that exceeds the observed F statistic indicates the P-value. Factors with a P-value of less than 0.05 according to the 95% confidence interval have a statistically significant impact on the response (Enis et al., 2018). The results show that the model is significant because the F values in the technical and pro-analytical precursor models are very high, 70.56 and 792.40, respectively. The P-value > F in both models indicates a significant model because it is less than 0.05, especially 0.0006 and <0.0001.

CV% is used in some industries to judge the capability of a process. A smaller CV value indicates high consistency, precision, and reliability in the data or measurement results (Llerena et al., 2019). According to Table 4, the CV% values of the model for technical precursor and analytical precursor are 5.87 and 1.67, respectively. This shows that technical precursors have consistent and reliable data; however, technical precursors have a higher variation or distribution of data than pro-analytical precursors. The predicted R2 model values for technical precursor and analytical precursor are 0.9258 and 0.9933, while the adjusted R2 values are 0.9675 and 0.9983. This indicates that the predicted R2 value is reasonably consistent with the adjusted R2 value because the difference between these values is smaller than 0.2 for both models (Sen, 2016). A regression model variance study (ANOVA) detected that the R2 values of the two models were 0.9815 and 0.9983. This shows that the model can explain 98.15% of the technical precursor model and 99.83% of the analytical precursor model from data variations.

Table 5. Estimate coefficient value for the factor interaction effects.

Factors	Technical precursor			Pro-analytical precursor		
	Coef. estimate	F-value	P-value	Coef. estimate	F-Value	P-value
A-Temperature (°C)	-1.53	18.65	0.0125	-0.5125	34.31	0.0042
B-Concentration (M)	3.68	108.04	0.0005	1.74	394.31	<0.0001
C-Volume (mL)	3.25	84.50	0.0008	3.86	1948.59	<0.0001

The significance levels of the parameter estimated coefficients and parameter interactions are shown in Table 5. The P-value for the technical and pro-analytical precursor models is less than 0.05, so these two models are declared significant. The coefficient estimate indicates the estimated change in response for every unit change in factor value, while all other variables remain constant (Sen, 2016).

Estimated coefficient values calculated for the primary influence variables from both models show that reaction temperature (A) has a negative influence, precursor concentration (B) has a positive influence, and reducing extract volume has a positive influence on the IONPs yield results. Temperature has an adverse effect due to high temperatures that can cause the degradation or inactivation of active phytochemicals in green tea leaf extract. Increasing precursor concentration and reducing extract volume has a positive effect due to precursors acting as the primary source of metal ions, where optimal concentrations facilitate more efficient synthesis. In addition, green tea leaf extract containing various phytochemicals with adequate standard reduction potentials enhances the reduction of metal ions to suitable nanoparticles, thereby increasing the overall yield (Anchan et al., 2019; Bindes et al., 2019; Groiss et al., 2017).

Analysis of Influencing Factors

Green synthesis iron oxide nanoparticles (IONPs) use 3 types of factors to analyze the effect of reaction temperature (A), precursor concentration (B), and reducing extract volume (C). Factors and interactions were evaluated using the normal probability plots presented in Figure 4.

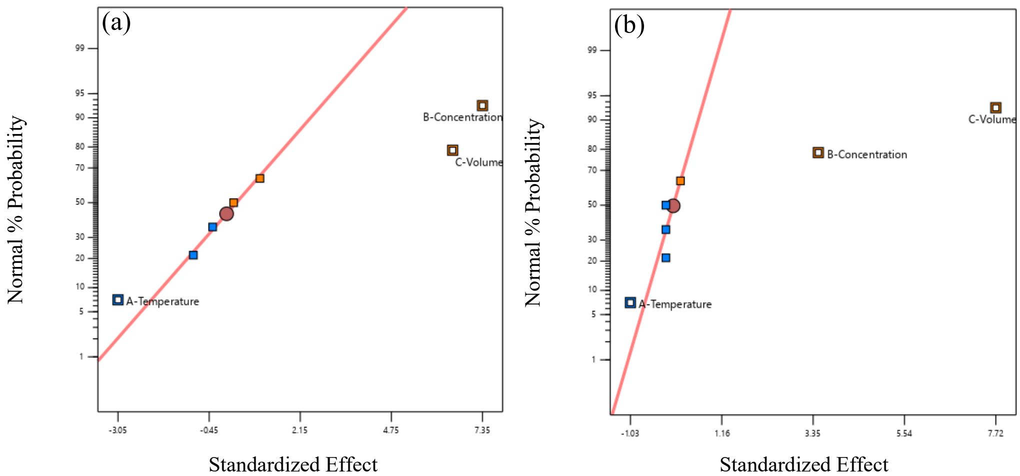

Figure 4. Normal plots of factor effects (**(a)** technical **(b)** pro-analytical precursor)

The normal plots are divided into two regions with a percentage <50% (negative effect) and another region with >50% (positive effect) (Badr et al., 2020). According to Figure 4, the normal plots using both technical and pro-analytical precursors have a positive effect on the concentration of precursor (B) and volume of reducing extract (C) while the reaction temperature (A) has a negative effect. The perturbation plots in Figure 5 further explain how the main effects and independent interaction variables affect the yield of IONPs.

Perturbation plots can be used to compare the independent influences of factors on a response variable (Zuorro et al., 2022). Perturbation plots in Figure 5 show the effect of reaction temperature (A), precursor concentration (B), and reducing extract volume (C) on the yield of IONPs using technical and pro-analytical precursors. Figure 5 (a) and (b) both show that factor C has little influence on the yield of IONPs. Figure 5 (a) shows that the influence of factor B is greater than factor C, while in Figure 5 (b) factor C has the main influence and factor B has a moderate factor on the yield of IONPs. This can be caused by technical precursors having unknown purity, which can cause systematic errors.

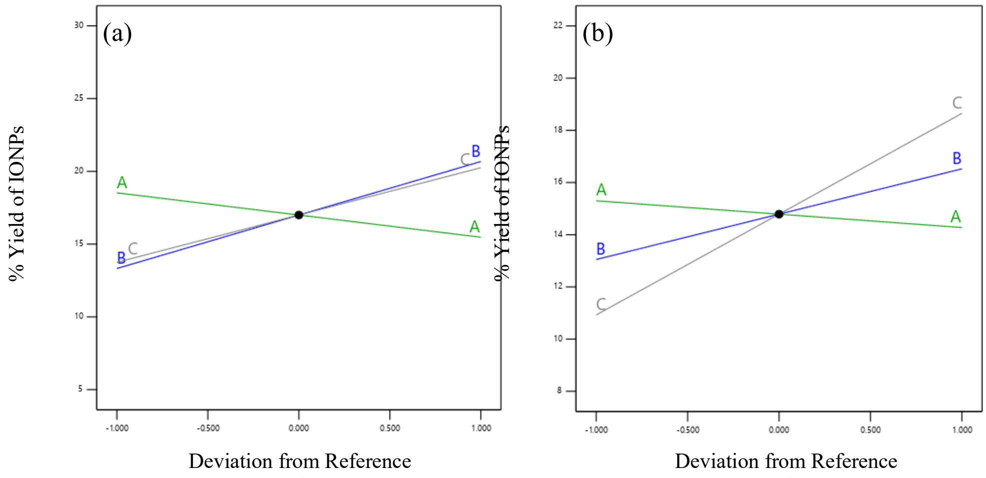

Figure 5. Perturbation plots of factor effects (**(a)** technical **(b)** pro-analytical precursor)

CONCLUSIONS

In this research, reaction temperature, precursor concentration, and reducing agent volume affect the green synthesis of IONPs using technical and pro-analytical precursors. The predicted values from the two precursors were within reasonable consistency for both IONPs. The ANOVA results of the two precursor sources show that the reaction temperature factor has a negative correlation. In contrast, the precursor concentration and reducing agent volume positively correlate with the yield of IONPs. Regression model variance can be explained 98.15% for the technical precursor model and 99.83% for the analytical precursor model from data variations. This research can determine the optimal combination of synthesis factors that support green synthesis methods' wider application and reproducibility in various research contexts and implications for industry. Further research is recommended to confirm the suggested model's results through model prediction optimization.

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