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Original Article

Optimizing Magnetite Synthesis for DNA Extraction: A Factorial Design Analysis of Temperature, pH, and TEOS Ratio Effects on Yield and Purity

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Abstract

Background: *Magnetite nanoparticles are widely used in DNA extraction due to their magnetic properties. Co-precipitation is a commonly used synthesis method, yet the influence of specific synthesis parameters on DNA extraction performance remains unexplored.*

Methods: *This study investigated a factorial design to evaluate the effects of temperature, pH, and tetraethyl orthosilicate (TEOS) ratio on DNA extraction performance, measured by yield and purity. Magnetite was synthesized under various conditions, and DNA was extracted and analyzed for quantity and quality, including PCR amplifiability using a 16S rRNA primer.*

Results: *Among the parameters tested, only the TEOS ratio significantly influenced DNA yield and purity, while temperature and pH showed no significant effects. The optimal conditions identified were 25 °C, pH 10, and a TEOS ratio of 0.25, yielding the highest DNA quality, which could be successfully amplified using PCR.*

Conclusion: *These findings suggest that while co-precipitation is robust, further optimization of the TEOS ratio could enhance DNA extraction efficiency. Future research should explore additional factors influencing magnetite's DNA extraction capabilities.*

Keywords

co-precipitation, DNA extraction, factorial design, magnetite, TEOS ratio, A260/280, silica coating, magnetic nanoparticles, DNA yield, simultaneous optimization

INTRODUCTION

Co-precipitation is a widely used method for preparing magnetite, with parameters such as temperature and pH frequently studied for their impact. Different synthesis temperatures can yield varying magnetite properties, which have been explored in applications like biosensors (Antarnusa et al., 2022) and medical fields (Gutierrez et al., 2024). While pH has been shown not to influence magnetite size (Ramadan et al., 2011), some studies continue to investigate its effects for specific applications, such as cancer drug delivery (Sirivat & Paradee, 2019) and dye waste remediation (Ba-Abbad et al, 2022; Hariani et al., 2013). However, the influence of these two parameters on DNA extraction applications remains unexplored, even though varying pH and temperature are commonly used in magnetite preparation protocols.

Magnetite used for DNA extraction is often modified to enhance its dispersibility, with TEOS (Tetraethyl orthosilicate) commonly used as a compound. Despite its frequent use, there has been no analysis of the effect of TEOS on the quality of extracted DNA. Notably, the amount of TEOS used to coat Fe_3O_4 varies widely across studies (Gai et al., 2010; Hieu et al., 2017; Nguyen et al., 2022).

The quality of DNA is commonly assessed by its yield and purity. Yield reflects the amount of DNA extracted, which is crucial for downstream applications that require a specific quantity of DNA. DNA purity is measured using the A260/280 ratio. A value close to 1.8 ± 0.2 indicates high purity (Lucena-Aguillar et al., 2016), while lower values suggest the presence of impurities such as proteins or organic compounds that absorb strongly at 280 nm. A higher A260/280 ratio indicates RNA contamination, which can interfere with DNA yield measurements, as the spectrophotometer cannot distinguish between DNA and RNA absorption.

Factorial design is an optimization method that enables researchers to simultaneously examine the effects of multiple factors, helping to understand the individual impact of each factor on the observed response and the interactions between factors. To the best of the authors' knowledge, no previous studies have applied factorial design to investigate the relationship between magnetite preparation parameters (such as temperature, pH, and TEOS ratio) and the quality of extracted DNA. This study fills this gap by employing a factorial design to optimize these factors for DNA extraction applications. While factorial design has been widely used in optimizing various processes (Akar Sen, 2016; Echeverría et al., 2021; Olasupo et al., 2020), its use to explore the relationship between magnetite preparation parameters and DNA extraction quality is novel. Studies such as those by Nguyen et al. (2022), Hieu et al. (2017), and Gai et al. (2010) have focused on DNA extraction using magnetic nanoparticles but have not employed factorial design in their optimization.

This research aims to apply factorial design to assess the influence of temperature, pH, and TEOS ratio and their potential interactions on the properties of magnetite used for DNA extraction. The responses evaluated include DNA yield and purity, based on the A260/280 ratio.

MATERIALS AND METHODS

Magnetite synthesis: Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$, ≥99%, Glentham Life Sciences), Ferric chloride hexahydrate ($FeCl_3.6H_2O$, ≥99%, Glentham Life Sciences), Tetraethyl orthosilicate (TEOS, for synthesis, ≥98%, Merck), Sodium hydroxide pellets (NaOH, EMPROVE, Merck), ammonia solution (NH_3, 25% w/w, Merck), Ethanol (96%, analytical grade, Bratachem), and Isopropanol (99.9%, analytical grade, Bratachem). **DNA extraction:** Triton X-100 (laboratory grade, Vivantis), Sodium Dodecyl Sulfate (SDS, SLS Fine, BASF), Polyethylene Glycol 6000 (PEG 6000, Clariant), Tris(hydroxymethyl)aminomethane (Tris base, molecular biology grade, Merck), Ethylenediaminetetraacetic acid (EDTA, EMPROVE, Merck), and Sodium chloride (NaCl, pharmaceutical grade, Dominion salt). **DNA evaluation:** Agarose (molecular biological grade, Himedia) for electrophoresis visualization and 2x PCR Master Mix (Bio-Rad) for DNA amplification. The bacterial culture was a mixed consortium of environmental bacteria, obtained through passive air sampling at the Konimex Diagnostic Center research laboratory. The culture was not taxonomically identified and was used as a natural microbial consortium. It was grown overnight in Tryptone Soy Broth medium (Himedia) at 37°C under static aerobic conditions.

Magnetite Preparation

A solution of $FeSO_4$ and $FeCl_3$ with a molar ratio of 1:2 was prepared by dissolving the salts in 80 mL of distilled water. While continuously stirring at 250 rpm and heating (25°C and 50°C, detailed in Table 1) for 30 minutes, a NaOH solution was added dropwise to the mixture until the pH reached 10 and 12, as specified in Table 1. The volume of the solution was adjusted to 100 mL with distilled water. The mixture was allowed to settle, and the supernatant was discarded. The precipitate was washed twice with distilled water and dried in an oven at 80 °C for two hours. The addition of TEOS followed the method described by Thangaraj et al. (2019). Magnetite and TEOS were mixed at weight-to-volume ratios of 0.25 and 4, respectively, as detailed in Table 1, and dissolved in 90% ethanol. The mixture was sonicated for 20 minutes, then stirred at 250 rpm. A 0.05% v/v ammonia solution (relative to the ethanol volume) was added dropwise under continuous stirring at 250 rpm. The suspension was incubated at room temperature for 6 hours. The resulting precipitate was washed once with 96% ethanol and twice with distilled water, then dried in an oven at 80 °C for 2 hours.

DNA Extraction Procedure

A 100 µL aliquot of bacterial culture was lysed using 200 µL of lysis solution containing 10 mM Tris base,

1 mM EDTA, 0.6% SDS, and 0.2% Triton X-100. The lysate was then mixed with magnetite, followed by the addition of 200 µL of binding buffer (2% PEG 6000, 2.5 M NaCl in isopropanol). The mixture was homogenized using a vortex (V-1 Plus, Biosan) at maximum speed for one minute and allowed to stand at room temperature for five minutes to facilitate DNA binding. Magnetite and the supernatant using a neodymium magnet for three minutes. The magnetite was then washed twice with 70% ethanol (room temperature), and DNA was eluted with nuclease-free water by magnetic separation.

Experimental Design

A full factorial design was employed to evaluate the effects of three factors on DNA extraction performance: temperature (25 °C and 50 °C), pH (10 and 12), and TEOS ratio (0.25 and 4 w/v). The complete treatment matrix is shown in Table 1. Synthesized magnetite from each treatment was applied in DNA extraction, and the resulting DNA yield and purity were assessed by measuring absorbance at 260 nm and 280 nm using a Nanophotometer N60 (Implen, Germany). All measurements were performed in duplicate with blank correction using nuclease-free water.

The extracted DNA from the optimal treatment was subjected to PCR amplification using primers 27F and 907R targeting the 16S rRNA gene fragment (Lane, 1991) to evaluate the presence of potential amplification inhibitors. Although no positive or negative controls were included, successful amplification was used to indicate the absence of major inhibitors. Amplification products were visualized by agarose gel electrophoresis.

Data Analysis

Data analysis was performed using Minitab 19 with the Design of Experiments (DOE) module. A full factorial design was applied, involving three factors—temperature, pH, and TEOS ratio—each at two levels. For each treatment combination, four replicates were conducted, defined as independent DNA extractions using the same batch of synthesized magnetite applied to different bacterial samples. Statistical significance was evaluated at an alpha level of $p < 0.05$. The analysis included a Pareto chart of standardized effects to identify significant factors, a main effects plot to examine individual factor contributions, and an interaction plot to evaluate the combined effects of factor interactions on DNA yield and purity.

RESULTS

This study conducted eight experiments combining three factors using a factorial design. The design matrix and the corresponding experimental results for each magnetite sample are presented in Table 1.

Table 1. *DNA yield concentration and A260/280 value for every experiment conducted*

No.	Temperature (°C)	TEOS ratio	pH	DNA yield concentration (ng/µl)	A260/280
1	25°C	0.25	10	87.1±16.49	1.84±0.02
2	25 °C	4	10	202.14±175.72	1.59±0.09
3	50 °C	0.25	10	71.33±34.37	1.75±0.11
4	50 °C	4	10	143.18±90.66	1.62±0.04
5	25 °C	0.25	12	64.63±26.96	1.89±0.07
6	25 °C	4	12	177.76±64.93	1.54±0.07
7	50 °C	0.25	12	56.73±18.21	1.86±0.06
8	50 °C	4	12	50.11±39.33	1.54±0.4

The DNA extraction results were subsequently analyzed using a Pareto chart, illustrating effects, as shown in Figure 1, where Figure 1 (A) represents the concentration response and Figure 1 (D) represents the A260/280 response. The analysis revealed that the TEOS ratio significantly affected both responses analyzed, with both effects crossing the reference line.

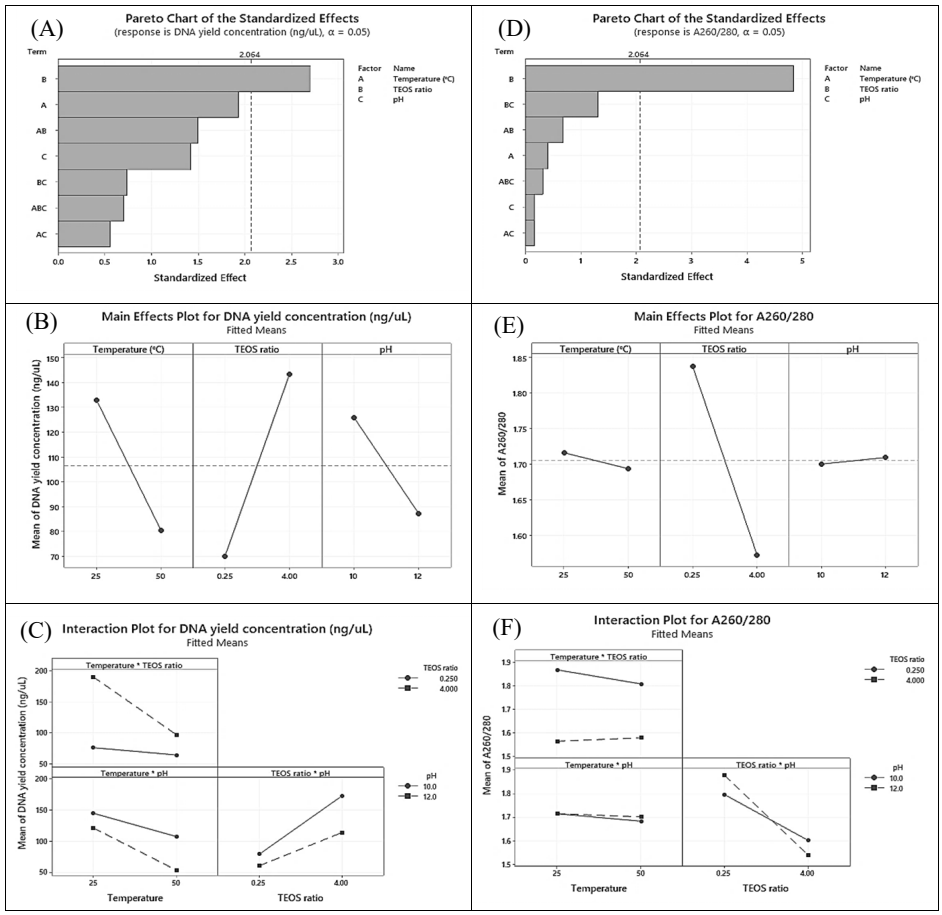

Figure 1. *(A) and (D) Pareto charts of the effects, (B) and (E) main effects plot, and (C) and (F) interaction plot for both DNA quality responses. (A), (B), (C) for DNA yield concentration (ng/µl). (D), (E), (F) for A260/280 response*

The statistical validation of the analysis was conducted by comparing the observed and predicted values using Formula (1) for concentration response and Formula (2) for A260/280 response. These equations were derived from a previous factorial design analysis. The analysis demonstrated a strong relationship between the observed and predicted results, with a coefficient of determination greater than 0.9 and a regression model p-value less than 0.05, indicating statistical significance for both responses (data not shown).

$$\text{DNA yield concentration} = 269 - 3.1 \times \text{Temperature (°C)} - 57 \times \text{TEOS ratio} - 17.7 \times \text{pH} + 3.62 \times \text{Temperature (°C)} \times \text{TEOS ratio} + 0.26 \times \text{Temperature (°C)} \times \text{pH} + 10.0 \times \text{TEOS ratio} \times \text{pH} - 0.408 \times \text{Temperature (°C)} \times \text{TEOS ratio} \times \text{pH} \quad (1)$$

$$\text{A260/280} = 1.93 - 0.0153 \times \text{Temperature (°C)} - 0.043 \times \text{TEOS ratio} + 0.002 \times \text{pH} + 0.0049 \times \text{Temperature (°C)} \times \text{TEOS ratio} + 0.00115 \times \text{Temperature (°C)} \times \text{pH} - 0.0052 \times \text{TEOS ratio} \times \text{pH} - 0.00037 \times \text{Temperature (°C)} \times \text{TEOS ratio} \times \text{pH} \quad (2)$$

Since the Pareto chart did not clearly show the effects of the factors on the response, the analysis was extended by using the main effects and interaction plots. The main effects and interaction plots for both DNA yield and A260/280 response are shown in Figure 1.

Figure 1(B) shows the effect of the three factors: temperature, TEOS ratio, and pH on DNA yield. Lower temperatures and pH values in the preparation procedure resulted in magnetite that extracted more DNA than those with higher temperatures and pH values. Conversely, a higher TEOS ratio produced magnetite that extracted more DNA than a lower TEOS ratio.

On the other hand, a higher TEOS ratio did not yield pure DNA. As shown in Figure 1(E), a TEOS ratio of 4 resulted in a DNA purity value below 1.6, indicating the presence of protein impurities in the DNA extract. In contrast, temperature and pH had no significant effect on DNA purity, with their values remaining around 1.7 across all conditions.

In the interaction plot (Figure 1(C)) for the DNA yield response, it can be observed that a temperature of 25°C consistently produced higher DNA concentrations across all levels of pH and TEOS ratio. Additionally, pH 10 resulted in higher DNA yields at all temperature and TEOS ratio levels than pH 12.

Figure 1(F) illustrates the pH, temperature, and TEOS ratio interaction. However, this interaction did not show a significant effect, as confirmed by the Pareto chart in Figure 1(D). Observations indicated that using a 0.25 TEOS ratio resulted in DNA purity within the range of 1.7 to 1.9. In contrast, with a 4 TEOS ratio, there was minimal change in purity across temperatures ranging from 25°C to 50°C. When considering the interaction between TEOS ratio and pH, pH 10 with a 4 TEOS ratio exhibited a relatively better purity level close to 1.6, compared to pH 12, which yielded an A260/280 value below 1.6. As for the interaction between pH and temperature, no significant differences were observed within the experimental range (temperature: 25°C–50°C; pH: 10–12).

Based on the main effects and interaction plots for DNA yield and A260/280 values, magnetite prepared at 25°C, pH 10, and a 0.25 TEOS ratio was selected for further testing in downstream processes. DNA extracted using magnetite prepared at 25°C, pH 10, and 0.25 TEOS ratio was successfully amplified using the primer pair 27F and 907R. Figure 2 shows amplicons of approximately 1500 bp, confirming that the extracted DNA was suitable for PCR amplification.

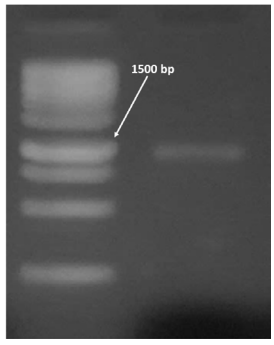

Figure 2. *PCR product electrophoresis visualization. The amplicon's size is about 1500 bp*

DISCUSSION

The co-precipitation method was employed to synthesize magnetite nanoparticles due to its simplicity, cost-effectiveness, and suitability for large-scale preparation. While synthesis conditions such as temperature and pH are traditionally known to influence particle size, morphology, and magnetic properties, our findings demonstrate that, within the tested ranges (25°C and 50°C for temperature; pH 10 and 12), these variables had limited impact on DNA extraction performance. This aligns with prior studies that reported stable particle characteristics, namely spherical morphology and a size distribution of approximately 6 to 13 nm, across 25°C to 80°C temperatures and 8 to 12.5 pH ranges (Mascolo et al., 2013; Nkurikiyimfura et al., 2020; Ramadan et

al., 2011; Saragi et al., 2018). The consistency in nanoparticle properties under these conditions likely explains the minimal variation observed in DNA yield and purity, reinforcing the robustness of the co-precipitation method.

In contrast, the TEOS ratio emerged as a critical factor influencing the yield and purity of extracted DNA. Increasing the TEOS ratio enhanced the silica coating around the magnetite core, consistent with reports that TEOS promotes silica shell formation, thereby increasing the surface area for biomolecular interaction (Hui et al., 2011). This larger surface area likely facilitated improved DNA adsorption, as reflected by the higher yield at the TEOS ratio of 4. This is particularly relevant for molecular applications requiring large quantities of DNA, such as next-generation sequencing.

However, the benefits of increased TEOS concentration came at the cost of DNA purity. At the highest tested ratio (TEOS = 4), A260/280 values dropped below 1.6, indicating potential protein contamination. One possible mechanism is the formation of positively charged salt bridges between proteins and the denser silica surface (Chen et al., 2020), leading to protein co-extraction even after washing. Such contamination is unfavorable for downstream applications, as it can inhibit enzymatic processes like PCR. These findings highlight the importance of balancing DNA yield and purity and emphasize the need to optimize TEOS concentration during nanoparticle synthesis.

The gel electrophoresis results further support the effectiveness of the optimized magnetite formulation. Clear and distinct amplification bands from the 16S rRNA PCR indicate that the extracted DNA was of sufficient quality for downstream amplification. This confirms not only the absence of significant PCR inhibitors but also the suitability of the extracted DNA for microbial community profiling. Despite the absence of formal positive or negative controls in the PCR setup, the successful amplification using universal bacterial primers (27F and 907R) provides practical validation of the extraction protocol.

Based on the factorial analysis, magnetite synthesized at 25°C, pH 10, and a TEOS ratio of 0.25 was selected as the optimal condition. This combination provides a practical balance of efficiency and simplicity. The lower synthesis temperature and moderate pH also reduce energy consumption and eliminate the need for strict pH adjustments, making the protocol more accessible for routine laboratory use. The selected TEOS ratio strikes a balance by enabling efficient DNA binding while maintaining high purity, an essential requirement for sensitive downstream applications such as PCR, where contaminants or inhibitors can disrupt amplification accuracy and lead to misleading or failed results (Acharya et al., 2017).

This study demonstrates the potential to tailor nanoparticle synthesis for specific bioanalytical applications, with TEOS concentration identified as a key factor influencing extraction performance. Using an environmental bacterial consortium enhances the method's relevance for metagenomics and microbial diversity studies. Future research should assess nanoparticle performance across diverse sample types, including clinical and environmental matrices, to support broader practical adoption.

CONCLUSION

The co-precipitation method proved to be a robust procedure, with parameters such as temperature and pH showing minimal impact on the DNA extraction capability of magnetite. However, the TEOS ratio significantly influenced the magnetite's DNA extraction performance, as evidenced by changes in DNA yield and purity (A260/280 ratio). Further optimization of the TEOS ratio in magnetite preparation serve as an intriguing avenue for future research.

Author Contributions

S. E. E. Tjoa: Conceptualization, Investigation, and writing original draft; **M. Mudasir:** Conceptualization, supervision, and writing – review; **E. Suharyadi:** Conceptualization, supervision, and writing – review; **B. S. Daryono:** Conceptualization, supervision, and writing - review.

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Ethical Approval
Not applicable.

Competing interest
The authors declare no conflicts of interest.

Data Availability
Data will be made available by the corresponding author on request.

Declaration of Artificial Intelligence Use
Not applicable.

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