RESEARCH ARTICLE

Comprehensive Evaluation of Process Parameters in the Green Synthesis of Iron Oxide Nanoparticles Using *Moringa olifera* extract

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Received: 12nd February, 2024; Revised: 28th April, 2024; Accepted: 10th June, 2024; Available Online: 31st August, 2024

ABSTRACT

This study investigates the green synthesis of iron oxide nanoparticles using *Moringa olifera* ethanolic extract and evaluates the impact of various process parameters on their physicochemical characteristics. The parameters examined include synthesis time, temperature, pH, stirring speed (RPM), and the ratio of extract to iron precursors. The findings reveal that a lower proportion of the extract increases the entrapment efficiency of the nanoparticles. Specifically, batch ME3 (3:1 ratio) produced the smallest nanoparticles at 117.8 nm, with a drug release of 75.54% and the highest entrapment efficiency at 60.28%. Extending the synthesis time to three hours in batch TM3 resulted in a drug release of 74.23% and a particle size of 116.3 nm. Batch TEM1, synthesized at 70°C, showed the highest entrapment efficiency and a favorable drug release profile of 71.09%, with the smallest particle size of 105.24 nm, suggesting that higher synthesis temperatures favor smaller particle sizes. Adjusting the pH to 10 in batch PM3 achieved a drug release efficiency of 76.02%. Batch RM5, synthesized with a stirring speed of 750 RPM, demonstrated a particle size of 95.7 nm and a drug release of 78.26%, indicating that higher stirring rates produce smaller nanoparticles and enhance drug release. These results highlight the significance of optimizing synthesis conditions to produce nanoparticles with desired properties. The use of *M. olifera* extract as a sustainable reducing and stabilizing agent presents an environmentally friendly approach to nanotechnology. This method has potential applications in drug delivery systems and various other industries, emphasizing the importance of green synthesis in creating sustainable and efficient nanomaterials.

Keywords: Green synthesis, Iron oxide nanoparticles, *Moringa olifera* extract, Nanoparticle characterization, Drug delivery systems.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.3.67

How to cite this article: Mahaparale S, Patil A, Chougule AD. Comprehensive Evaluation of Process Parameters in the Green Synthesis of Iron Oxide Nanoparticles Using *Moringa olifera* extract. International Journal of Pharmaceutical Quality Assurance. 2024;15(3):1536-1543.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Iron oxide nanoparticles (IONPs) have attracted considerable attention for their wide range of uses in biomedicine, environmental remediation, and magnetic storage devices. Their exceptional magnetic properties, along with their compatibility with biological systems and ease of customization, make them highly valuable in these fields. Nevertheless, conventional approaches to producing IONPs frequently depend on risky substances and resource-intensive procedures, prompting worries about the environment's long-term viability and overall safety. As a result, there has been a growing trend towards utilizing natural resources to produce nanoparticles in a more environmentally friendly way, known as green synthesis methods.[1]

Green synthesis utilizes biological entities like plant extracts, bacteria, fungi, and algae to facilitate the formation of nanoparticles. Among these options, plant extracts are highly appealing because of their abundant phytochemical content, which serves as effective reducing and stabilizing agents. This approach streamlines the synthesis process and improves the biocompatibility of the resulting nanoparticles. *Moringa olifera*, commonly known as the drumstick tree or miracle tree, is highly regarded for its suitability in this role. The leaves contain a rich concentration of antioxidants, flavonoids, and other bioactive compounds, which greatly enhances its efficacy in green synthesis applications.[2]

Previous research has shown that *M. olifera* material can be used to make different kinds of metal nanoparticles. However, there haven't been many in-depth studies on IONPs. For this study, the ethanolic extract of *M. olifera* will be used to systematically look at the effects of key process factors on the green production of IONPs. This study looked at a number of factors, such as the amount of extract compared to iron sources (ferric chloride and ferrous sulphate), the time it took to make

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the mixture, the temperature, the pH, and the number of turns per minute (RPM).[3]

By learning how these factors affect the process, we hope to find the best way to make IONPs with the right physical features. This in-depth review will not only help to improve green synthesis methods, but it will also make it easier to use IONPs in many different areas of life. Because of the natural potential of *M. olifera*, the results of this study could lead to a more sustainable and effective way to make iron oxide nanoparticles.[4]

MATERIAL AND METHOD

Materials

In this study, the synthesis of IONPs was carried out using the ethanolic extract from the dried powder of *M. olifera* leaves. The ethanol used for the extraction of *M. olifera* was procured from AnaLab Fine Chemicals, Mumbai. For the synthesis of magnetic nanoparticles, ferric chloride hexahydrate and ferrous sulfate were obtained from Research Lab Fine Chem Industries, and 25% ammonia solution was acquired from Pallav Chemicals & Solvents Pvt. Ltd. All chemicals used were of analytical reagent (AR) grade and were utilized without further purification to ensure consistency in the synthesis process.

Distilled water was used for all processes and dilutions. The reaction components were mixed using a magnetic stirrer (LMMS-300, Labman Scientific Instruments Pvt Ltd, Chennai) at controlled temperatures, RPM, and durations. The synthesized nanoparticles were separated using a centrifuge. Following synthesis, calcination was performed using a muffle furnace to enhance the properties of the nanoparticles. These materials and equipment were essential to ensure that the green synthesis method was reliable and reproducible.[5]

Preparation of Ethanolic Extract of *M. olifera*

Fresh *M. olifera* leaves were thoroughly washed with distilled water to remove any dirt or germs. The cleaned leaves were air-dried at room temperature until their weight remained constant. Once dried, the leaves were ground into a fine powder using a laboratory grinder. This powdered form of the leaves was then subjected to maceration with a 70% ethanol solution in a 1:40 (w/v) ratio at room temperature for 72 hours, with occasional shaking to ensure thorough extraction. After maceration, the mixture was filtered to separate the liquid extract from the solid residue (marc). The marc was then re-macerated using fresh dried *M. olifera* powder to ensure complete extraction, and this re-extraction process was repeated until saturation was achieved. The combined filtrates were collected and stored in a dark bottle at 4°C until further use. This method ensured a consistent and potent ethanolic extract of *M. olifera* for the subsequent synthesis of iron oxide nanoparticle.[6]

Synthesis of Iron Oxide Nanoparticles

The synthesis of iron oxide nanoparticles was carried out by mixing the ethanolic extract of *M. olifera* with a combination of 0.1M ferric chloride (FeCl2) and 0.1M ferrous sulfate (FeSO4) solutions, as previously studied. The coprecipitation method was employed using a 25% ammonia solution to precipitate the nanoparticles. Various process parameters, such as temperature, pH, and stirring speed, were systematically varied to observe their effects on the final product.

The mixture was stirred using a magnetic stirrer under controlled conditions to ensure thorough mixing and reaction. After the reaction, the mixture was centrifuged to separate the precipitate from the solution. The separated precipitate was then subjected to calcination at 400°C for 2 hours to improve the crystallinity and stability of the nanoparticles. Post-calcination, the nanoparticles were purified by washing with distilled water and ethanol to remove any residual impurities. The purified nanoparticles were then dried and subsequently evaluated for their physicochemical properties. This systematic approach ensured the production of high-quality iron oxide nanoparticles suitable for further applications.

Effect of Extract Proportion

The proportion of *M. olifera* extract added to the iron precursors was varied to create different batches of iron oxide nanoparticles. Specifically, the ratios of *M. olifera* extract to the combination of 0.1M FeCl2 and 0.1M FeSO4 were adjusted to 1:1, 2:1, 3:1, 1:2, and 1:3, labeled as ME1, ME2, ME3, ME4, and ME5, respectively. These variations were carried out under fixed conditions of temperature, stirring speed (RPM), and synthesis duration to isolate the effect of the extract proportion on the characteristics of the nanoparticles. This systematic variation allowed for the assessment of how different amounts of *M. olifera* extract influence the size, entrapment efficiency, and drug release properties of the synthesized iron oxide nanoparticles.[7]

Effect of different parameters on preparation of Iron oxide Nanoparticles

Effect of synthesis time

To look at how synthesis time affected the reaction, 10 mL of ethanolic extract, 10 mL of 0.1 M FeCl3, and 10 mL of 0.1 M FeSO4 were stirred for different amounts of time: batch TM1 stirred for 1hour, batch TM2 stirred for 2 hours, and batch TM3 stirred for 3 hours. At the conclusion of each time period, samples were taken for further study.[8]

Effect of temperature

We tested how temperature affects the creation of iron oxide nanoparticles by doing the process at various temperatures. About 10 mL of ethanolic extract were mixed with 10 mL of 0.1 M FeCl3 and 10 mL of 0.1 M FeSO4. The mixture was stirred for one hour at different temperatures for each batch: 40°C for batch TEM1, 50°C for batch TEM2, 60°C for batch TEM3, 70°C for batch TEM4, and 80°C for batch TEM5. The temperature was fixed and watched with a thermometer.[9]

Effect of pH

The pH of the reaction mixture was adjusted to specific values in order to assess its impact on nanoparticle synthesis. The ethanolic extract, 0.1 M FeCl3, and 0.1 M FeSO4 were

combined in equal amounts for each batch. The pH levels were adjusted accordingly: 8 for batch PM1, 9 for batch PM2, 10 for batch PM3, and 11 for batch PM4. The mixtures were stirred for 1 hour under identical conditions.[10]

Effect of revolutions per minute (RPM)

The properties of the synthesized nanoparticles were evaluated by varying the revolutions per minute (RPM) to determine the impact of stirring speed. The ethanolic extract was combined with FeCl3 and FeSO4 in equal amounts and stirred for 1 hour at various speeds for each batch. The stirring speeds were 150 RPM for batch RM1, 300 RPM for batch RM2, 450 RPM for batch RM3, 600 RPM for batch RM4, 750 RPM for batch RM5, and 900 RPM for batch RM6.[11]

Characterization of Iron Oxide Nanoparticles

Particle size and zeta potential

The measurement of particle size and zeta potential was conducted using dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). This technique offers valuable insights into the size distribution and surface charge of nanoparticles, which play a crucial role in maintaining stability and dispersion within biological systems.[12,13]

Thermogravimetric analysis (TGA)

The thermal stability of the nanoparticles was determined by performing thermogravimetric analysis (TGA) using a TGA Q500 (TA Instruments, USA). The nanoparticle sample was heated from room temperature to 800°C at a rate of 10°C per minute under a nitrogen atmosphere, and the weight loss was recorded as a function of temperature.[14]

Vibrating sample magnetometry (VSM)

The magnetic properties of the nanoparticles were evaluated using vibrating sample magnetometry (VSM). The measurements were conducted using a Microsense EZ. The samples underwent testing in an external magnetic field, allowing for the measurement of various parameters including coercivity, retentivity, and saturation magnetization.[15]

Entrapment efficiency (EE)

The entrapment efficiency (EE) was determined by quantifying the quantity of a model drug that was encapsulated within the nanoparticles. The drug-loaded nanoparticles were separated by centrifugation at 15,000 rpm for 30 minutes using an Eppendorf 5804R centrifuge (Eppendorf, Germany). The supernatant was analyzed using a UV-vis spectrophotometer from Shimadzu (model UV-1800, made in Japan) to quantify the free drug content.[16]

Drug release studies

Drug release studies were conducted to assess the release profile of the encapsulated drug from the nanoparticles. The drug-loaded nanoparticles were suspended in a phosphate buffer solution with a pH of 7.4 and incubated at 37°C in a shaking water bath from Julabo SW23 in Germany. Samples were withdrawn at specific time intervals and analyzed using the UV-vis spectrophotometer to determine the quantity of drug released.[17]

RESULT AND DISCUSSION

Effect of Extract Proportion with Ferric Chloride & Ferrous Sulphate on Iron Oxide Nanoparticles

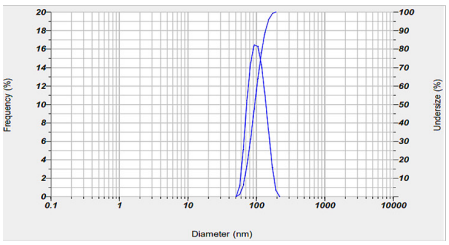
The properties of the synthesized iron oxide nanoparticles were significantly influenced by the proportion of *M. olifera* extract to ferric chloride and ferrous sulfate. The data in Table 1 and Figure 1 demonstrates a consistent trend: as the extract proportion increased, the particle size decreased and the entrapment efficiency improved. Batch ME2 had the smallest particle size of 98.7 nm and a significant drug release of 75.49% with a 2:1 proportion of extract to iron precursors. Batch ME3 had the highest efficiency of 60.28% and a drug release of 75.54% with a 3:1 proportion. The findings indicate that a greater proportion of the plant extract improves the bio-reduction and stabilization process, resulting in smaller and more effective nanoparticles. The thermal stability (TGA) and magnetic properties (VSM) of the nanoparticles vary across different batches, suggesting that the extract proportion plays a role in influencing their thermal and magnetic behavior.

Effect of Synthesis Time on Iron Oxide Nanoparticles

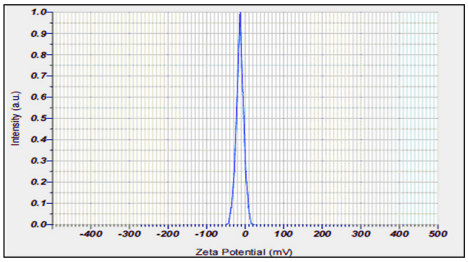
Various synthesis times were tested to examine the influence on the properties of the nanoparticles. Table 2 and Figure 2 show that longer synthesis times tend to result in smaller particle sizes and better drug release efficiency. As an illustration, the particle size of batch TM1, which was synthesized for 1 hour, measured 182.8 nm. On the other hand, batch TM2, synthesized for 2 hours, exhibited a smaller particle size of 108.3 nm. Batch TM3 had a synthesis time of 3 hours and yielded a particle size of 116.3 nm. Additionally, it demonstrated the highest drug release of 74.23%. The consistent zeta potential values observed across various synthesis times indicate the presence of stable surface charges, which are crucial for maintaining colloidal stability. The thermal stability (TGA) and magnetic

Table 1: Effect of extract proportion with ferric chloride & ferrous sulphate on iron oxide nanoparticles by using ethanolic extract of *M. olifera*

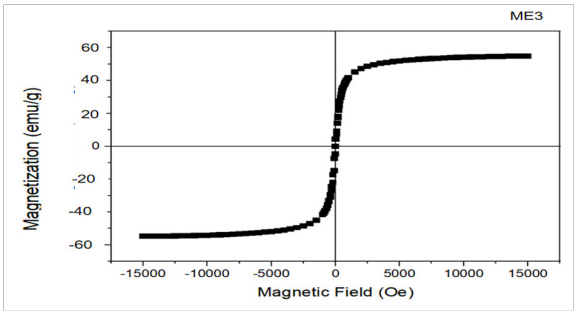
Batch No.	*Particle size*	*Zeta potential (mV)*	*TGA*	*VSM*	*EE (%)*	*Drug release (After 12 hours)*
ME1	127	- 5.2	312.25	0.35	48.12	70.99
ME2	98.7	-5.1	332.56	0.20	50.26	75.49
ME3	117.8	- 5.1	315.25	0.23	60.28	75.54
ME4	98.8	-5.4	315.78	6	58.96	75.49
ME5	146.4	-5.5	317.65	15	47.26	75.25



A. Particle Size of batch ME3

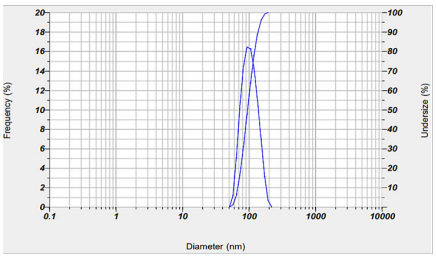


B. Zeta Potential of batch ME3

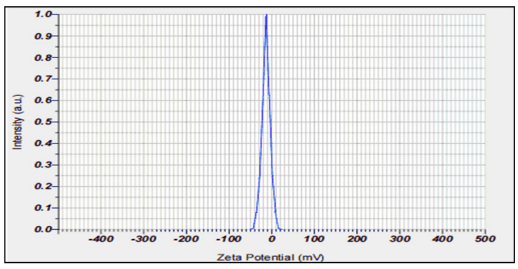


C. VSM of batch ME3

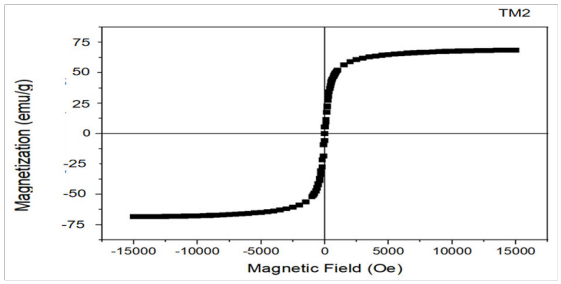
Figure 1: Particle size, zeta Potential and VSM of batch ME3



A. Particle Size of batch TM2



B. Zeta Potential of batch TM2



C. VSM of batch TM2

Figure 2: Particle size, zeta potential and VSM of batch TM2

Table 2: Effect of time on iron oxide nanoparticles by using ethanolic extract of *M. olifera*

Batch No.	*Particle size*	*Zeta potential*	*TGA*	*VSM (emu/g)*	*EE (%)*	*Drug release*
TM1	182.8 nm	-5.5 mv	512.26	0.0020	25.26	71.6
TM2	108.3 nm	-5.1 mv	550.32	12.5	31.70	72.56
TM3	116.3 nm	-5.1 mv	521.30	0.9	42.86	74.23

properties (VSM) also varied with synthesis time, suggesting that longer reaction times could result in increased thermal stability and magnetic activity of the nanoparticles.

Effect of Temperature on Iron Oxide Nanoparticles

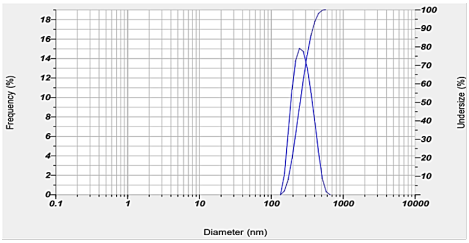
The impact of temperature on nanoparticle synthesis was assessed by conducting the reaction at various temperatures, as indicated in Table 3. Increasing the synthesis temperatures typically resulted in the formation of larger particles. As an example, the particle size of batch TEM1 synthesized at 40°C measured 182.6 nm, whereas batch TEM5 synthesized at 80°C exhibited a noticeably larger particle size of 265.2 nm. Batch TEM1 exhibited the highest drug release of 75.59%, indicating that lower temperatures, such as 40°C, could potentially be more advantageous in achieving optimal drug release profiles. Temperature is a key factor in determining the thermal and magnetic properties of the nanoparticles, as evidenced by the variations in TGA and VSM data.

Effect of pH on iron oxide nanoparticles

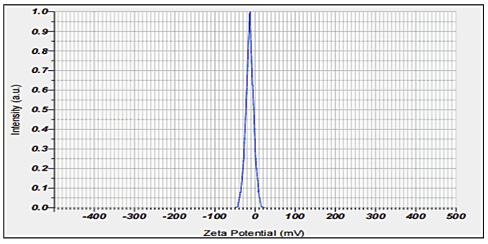
The pH of the reaction mixture was adjusted to investigate its impact on nanoparticle synthesis, as shown in Table 4 and Figure 4. The pH had a significant impact on the particle size, zeta potential, and drug release. The particle size of batch PM1 was 122.1 nm, and it had a drug release of 71.35%. On the other hand, batch PM3 with a pH of 10 showed the highest drug release of 76.02%. The results indicate that a higher pH level of 10 creates a more favorable environment for improving drug release efficiency. The thermal stability (TGA) and magnetic properties (VSM) also showed variations with pH, suggesting that pH adjustment plays a crucial role in optimizing the overall properties of the nanoparticles.

Effect of Revolutions Per Minute (RPM) on Iron Oxide Nanoparticles

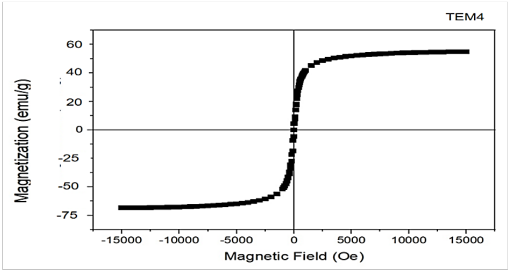
The stirring speed was adjusted to evaluate its influence on the characteristics of the synthesized nanoparticles, as indicated in Table 5 and Figure 5. Increased RPMs typically led to reduced particle sizes and improved drug release efficiencies. The particle size of batch RM2 with 300 RPM was the smallest at 73 nm, and it had a drug release of 75.28%. On the other hand, batch RM5 with 750 RPM exhibited the highest drug release of 78.26%. The findings indicate that increasing the stirring speeds can enhance the dispersion and formation of nanoparticles, resulting in a more uniform outcome. The TGA and VSM data show that the thermal and magnetic properties of the nanoparticles are affected by the stirring speed, as evidenced by the variations observed across different RPMs.



A. Particle Size of batch TEM4



B. Zeta Potential of batch TEM4



C. VSM of batch TEM4

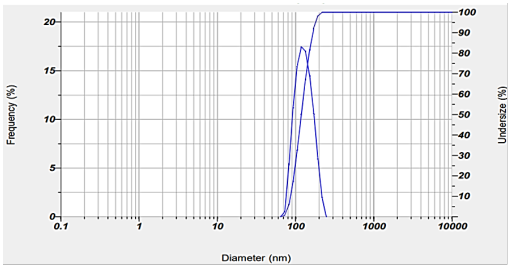
Figure 3: Particle size, zeta potential and VSM of batch TEM4

Table 3: Effect of temperature on iron oxide nanoparticles by using ethanolic extract of *M. olifera*

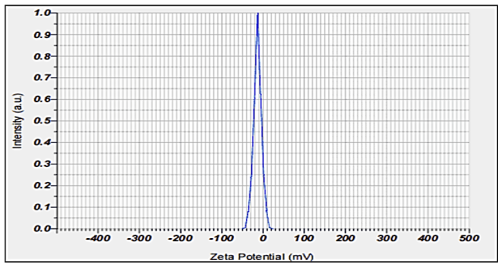
Batch No.	*Particle size (nm)*	*Zeta potential (mV)*	*TGA*	*VSM*	*EE (%)*	*Drug release*
TEM1	182.6	- 5.2	396.10	3.2	12.26	75.59
TEM2	122.5	- 5.1	418.27	17	17.25	68.99
TEM3	105.2	-4.9	475.62	03	15.98	72.54
TEM4	105.24	-4.5	418.56	19	18.28	71.09
TEM5	265.2	-4.2	412.58	8	17.52	67.11

Table 4: Effect of pH on iron oxide nanoparticles by using ethanolic extract of *M. olifera*

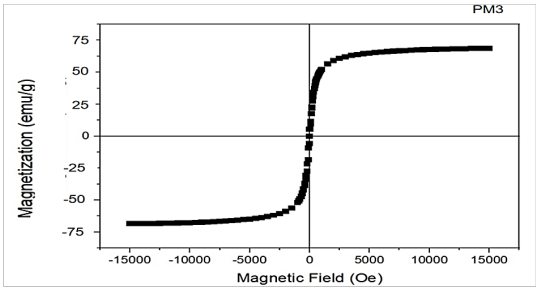
Batch No.	*Particle size (nm)*	*Zeta potential (mV)*	*TGA*	*VSM*	*EE (%)*	*Drug release*
PM1	122.1	-4.4	410.26	0.5	65.14	71.35
PM2	176.3	-5.4	510.78	0.68	68.95	73.12
PM3	123.1	-5.9	590.15	0.5	57.95	76.02
PM4	116.5	-6.1	512.69	1.5	55.18	72.33



A. Particle Size of batch PM3



B. Zeta Potential of batch PM3



C. VSM of batch PM3

Figure 4: Particle size, zeta potential and VSM of batch PM3

DISCUSSION

The synthesis of iron oxide nanoparticles using *M. olifera* extract showed that the ratio of extract to iron precursors had a notable impact on both particle size and entrapment efficiency. The smaller nanoparticles with enhanced entrapment efficiency were observed in batch ME2 (98.7 nm, 50.26% EE) and ME3 (117.8 nm, 60.28% EE), indicating a direct correlation between higher extract proportions and these improvements.

The duration of synthesis time played a crucial role, as longer durations led to smaller particle sizes and improved drug release profiles. The particle size of batch TM3, synthesized over a period of 3 hours, measured at 116.3 nm. Additionally, it exhibited the highest drug release of 74.23%. These results suggest that longer reaction times contribute to a more thorough reduction and stabilization process facilitated by the extract.

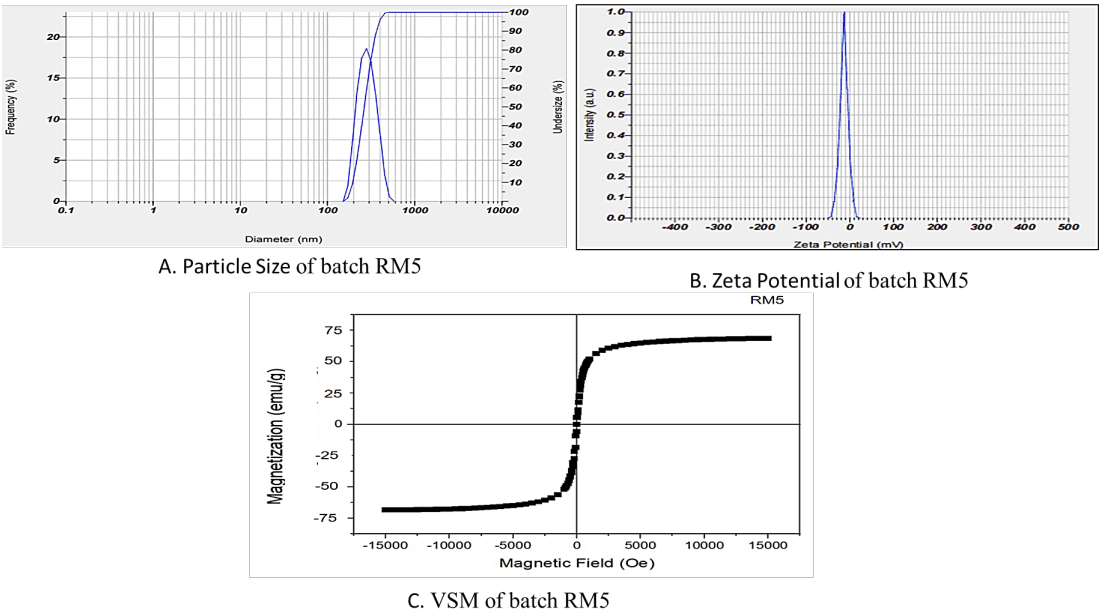
The study found that lower temperatures resulted in the production of smaller nanoparticles, which in turn led to improved drug release efficiencies. TEM1, synthesized at 40°C, exhibited a particle size of 182.6 nm and achieved the highest drug release of 75.59%. This indicates that lower temperatures are more conducive to achieving optimal nanoparticle characteristics.

The drug release efficiency was found to be enhanced in an alkaline environment with a pH of 10. The drug release of Batch PM3 at pH 10 was the highest at 76.02%, highlighting the significance of pH in optimizing nanoparticle properties.

The speed at which the mixture was stirred had a notable impact on the size of the particles and the efficiency of drug

Table 5: Effect of Revolutions Per Minute (RPM) on Iron oxide Nanoparticles by Using Ethanolic extract of *M. olifera*

Batch No.	*Particle size (nm)*	*Zeta potential (mV)*	*TGA*	*VSM*	*EE (%)*	*Drug release*
RM1	118.4	-3.8	376.25	19	48.12	77.72
RM2	73	-3.9	415.20	28	56.25	75.28
RM3	95.8	-4.0	470.26	0.23	61.29	78.70
RM4	107.4	-4.1		0.25	65.85	74.12
RM5	95.7	-4.1		0.28	66.89	78.26
RM6	129.2	-4.1		1.4	67.15	74.59



A. Particle Size of batch RM5

B. Zeta Potential of batch RM5

C. VSM of batch RM5

Figure 5: Particle size, Zeta Potential and VSM of batch RM5

release. Higher stirring speeds led to smaller particle sizes and improved drug release. Batch RM2, stirred at 300 RPM, achieved a particle size of 73 nm, which was the smallest among the batches. On the other hand, batch RM5, stirred at 750 RPM, exhibited the highest drug release of 78.26%. These results emphasize the significant influence of mechanical agitation on the formation of nanoparticles.

These findings highlight the significance of optimizing synthesis parameters to achieve desired nanoparticle characteristics. This study highlights the potential of using *M. olifera* extract as a reducing and stabilizing agent in nanoparticle synthesis, which aligns with the goal of promoting eco-friendly nanotechnology.

CONCLUSION

In conclusion, the ethanolic extract of *M. olifera* may be used to greenly synthesize iron oxide nanoparticles, underlining the importance of process factors on nanoparticle properties. Batch ME3, which had 117.8 nm nanoparticles and 60.28% entrapment effectiveness, showed that higher extract quantities produced smaller nanoparticles. Batch TM3 had 116.3 nm particle sizes and 74.23% drug release due to longer production durations.

Lower synthesis temperatures produced smaller nanoparticles and improved drug release, as evidenced in batch TEM1, which contained 182.6 nm nanoparticles and 75.59% drug release at 40°C. Drug release efficiency was optimized in an alkaline reaction mixture (pH 10), as shown by batch PM3, which released 76.02% of drug. For batch RM5, higher RPM improved nanoparticle dispersion and homogeneity, with 95.7 nm particles and 78.26% drug release. To attain desired nanoparticle properties, synthesis parameters must be optimized. Nanoparticle manufacturing using *M. olifera* extract as a reducing and stabilizing agent is sustainable and eco-friendly, with potential medicine delivery applications. This study enhances green nanotechnology and prepares for future research on the scalability and practical uses of environmental nanoparticles.

ACKNOWLEDGMENT

The authors would like to express their sincere gratitude to the Department of Pharmaceutical Chemistry, Dr. D. Y. Patil College of Pharmacy, Akurdi Pune for their invaluable support and resources provided throughout the course of this research and Infinite Biotech Institute of Research and Analytics, Sangli for their in completion of testing.

AUTHOR CREDIT STATEMENT

Sonali Mahaparale: Conceptualization, Methodology, Investigation, Formal Analysis, Writing - Original Draft, Writing - Review & Editing.

Amruta Patil: Supervision, Validation, Resources, Project Administration, Writing - Review & Editing.

Ashok Chougule: Conceptualization, Validation, Resources, Project Administration

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