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New Synthesis Method of AuNPs Using *Moringa oleifera* Extract as Bioreductor and Potential Study as Colorimetric Microplastic Detection

Muhammad Bakhru Thohir[1]*, Dyah Setyaningrum[1], Arya Ananda Saputra[1] and Marta Citra Nursaida[1]

Abstract. Nanoparticles are multipurpose materials that have been utilized in the medical, energy, and environmental monitoring fields. The advantage of nanoparticles is that they have unique physicochemical properties such as surface area, optical activity, and surface modifiability. One of the important uses of nanoparticles is for environmental monitoring. This is because the surface of nanoparticles could be modified, and with their small size, they could reach analytes in difficult matrices. However, the synthesis of nanoparticles has been an unsustainable process. Therefore, the synthesis of AuNPs using bioreductors finds its urgency. The aim of this research is to determine the optimum conditions for the synthesis of AuNPs-MO, the character of the synthesized AuNPs-MO, and to study the application of AuNPs-MO for monitoring microplastic pollutants. This research was conducted through the stages of extraction, determination of optimum conditions, characterization, and literature study of the potential of AuNPs-MO as a detector. Determination of optimum conditions was carried out by applying variations in pH and precursor-reducing agent ratio. Characterization of AuNPs-MO was done with FTIR and PSA. In addition, microplastic detection models with AuNPs-MO have been obtained directly and indirectly. From this series of experiments, satisfactory results have been obtained.

Introduction

Microplastics are one of the most dangerous pollutants and have not been handled optimally at this time (Aragaw, 2021). Microplastics have dangers that have not all been revealed, but some that have been established are fat clumping (Liu et al., 2023). This is not surprising because microplastics have properties that are not lost from their macro size, namely lipophilic (Yuan & Xu, 2023). In addition, with its micro size, it has a high cruising range in the ecosystem. This ability increases its potential danger (Shamskhany et al., 2021).

Microplastics have properties as metal adsorbents. Metals could be adsorbed on the surface of plastic, and ironically when there is metal on the surface of the plastic, it will be carried into the system (Chen et al., 2023). Microplastics also have the property of accumulating like metals whose dangers will only appear in the long term (Thohir et al., 2022; Zhu et al., 2024).

So far, microplastic analysis has only been carried out using instruments such as FTIR and RAMAN which require special handling, expensive, and require energy (B. N. V. Kumar et al., 2021). In fact, nanoparticles such as gold nanoparticles (AuNPs) have the potential to replace the role of instruments as microplastic detector media.

Nanoparticles are defined as materials with a size of 1-100 nm that have taken an important role in the development of science and technology in this century (Bayda et al., 2019; Thohir & Tiyas, 2024). AuNPs could be used as pollutant detector media because they have biocompatible properties, multifunctional potential, high stability, and ease of modification (Mikhailova, 2021).

One method of synthesizing AuNPs is by using the

[1]*Chemistry Program, Faculty of Science and Engineering, Universitas Bojonegoro, Bojonegoro, 62119, Indonesia;* ***Email: bakhru@unigoro.ac.id.***

reduction method. The basic reaction is to reduce Au^{3+} ions to Au^{0}, then continue with the nucleation stage and form nanoscale material (Sangwan & Seth, 2022). In the reduction process, the materials commonly used are citric acid and ascorbic acid (Poklepovich-Caride et al., 2022; Waragai et al., 2021). Both of these synthetic materials have been widely reported for their optimum synthesis conditions (Oliveira et al., 2023). Unfortunately, the use of synthetic materials means wasting non-renewable materials. Therefore, it is important to synthesize AuNPs with renewable materials whose optimal points are known (Ganesh et al., 2021). In order to obtain AuNPs that have utility and benefits, do not ignore environmental sustainability.

Moringa oleifera is one of the plants that contains very high ascorbic acid. *Moringa oleifera* has the potential to substitute for the use of synthetic ascorbic acid. In addition to containing high ascorbic acid, *Moringa oleifera* also contains many secondary metabolites that could act as stabilizers (Perumalsamy et al., 2024). In the synthesis of AuNPs with synthetic reductants, stabilizers are still needed, while using *Moringa oleifera* could perform two roles at once. This, in addition to utilizing renewable materials, also includes saving the use of materials (Vijayaram et al., 2024).

From the research that has been reported, AuNPs could be made by green synthesis methods such as using *Curcuma pseudomontana*, *Capsicum annum*, *Garcinia kola*, and *Zingiber officinale* extracts (Akintelu et al., 2021; Fouda et al., 2022; Muniyappan et al., 2021; Patil et al., 2023). The use of AuNPs so far has only been as antioxidants, antibacterials, and anticancer (El-Borady et al., 2020); no one has used green AuNPs as a microplastic detector. Thus, the purpose of this study was to determine the optimum conditions for the synthesis of AuNPs whose ascorbic acid was substituted with *Moringa oleifera* extract with variations in pH and ratio, to determine the characteristics of AuNPs-MP, and to determine the colorimetric detection study of microplastics with AuNPs-MP.

Experimental

Material and Methods

The equipment used in this study were a UV-Vis Spectrophotometer, Fourier Transform Infrared Spectroscopy (FTIR), Particle Size Analyzer (PSA), hot plate, thermometer, stopwatch, and a set of laboratory glassware. While the materials needed in this study were *Moringa oleifera* powder, distilled water, pure gold, 37% hydrochloric acid (HCl), and nitric acid (HNO_3).

Procedures

Moringa oleifera Extraction

100 mL of distilled water was put into a beaker and heated on a hot plate until boiling while stirring continuously. *Moringa oleifera* powder of 0.25 grams was put into the boiling distilled water and left for 15 minutes while stirring occasionally. Then cooled to room temperature, then filtered using filter paper to separate the filtrate and residue, obtaining a brownish yellow *Moringa oleifera* extract. The extract is only used for 2 weeks; more than that must be regenerated. The extract is also stored at a temperature of 4°C.

Preparation of $HAuCl_4$ Precursor

The preparation of 0.3 mM $HAuCl_4$ solution was carried out by soaking 0.1 grams of pure gold in a mixture of 37% HCl and 65% HNO_3 with a ratio of 3:1 for 48 hours. Then heated to obtain solid $HAuCl_4$. The $HAuCl_4$ solid obtained was dissolved in distilled water to the boundary mark in a 100 mL measuring flask to obtain a concentration of 3 mM.

Synthesis of AuNPs-MO: pH Optimization

The pH optimization process of the synthesis was carried out at pH 4, 6, 8, 10, and 12. It began by heating 10 mL of 0.3 mM $HAuCl_4$ solution until it reached the boiling point with continuous stirring. After that, 5 mL of *Moringa oleifera* extract with a concentration of 0.25%, which had been conditioned for pH, was quickly added. The synthesis was carried out for 10 minutes with continuous stirring. After 10 minutes, stirring was continued without heating until room temperature so that optimal AuNPs-MO were formed. The AuNPs-MO formed were stored at a temperature of 4°C.

AuNPs-MO Synthesis: Synthesis Ratio Optimization

Optimization of the AuNPs synthesis ratio by utilizing *Moringa oleifera* extract bioreductor was carried out through the same stages as pH optimization, using the optimum pH from the previous treatment. The ratio variations used between 0.3 mM $HAuCl_4$ and *Moringa oleifera* extract were 5:15, 5:10, 7.5:7.5, 10:5, and 15:5.

Characterization of AuNPs-MO

The resulting AuNPs-MO were analyzed using UV-Visible Spectrophotometry to find the maximum wavelength in the range of 400-800 nm. Characterization was also carried out using Fourier Transform Infrared Spectroscopy (FT-IR) to identify functional groups of the bioreductant and interactions between functional groups. Analysis of AuNPs-

MO with FTIR was carried out at wave numbers of 4000 to 800 cm^{-1}. The last characterization was with Particle Size Analyzer (PSA), which aims to determine the particle size of the synthesized AuNPs-MO.

Result and Discussion

AuNPs are multipurpose materials that are currently used in many sectors, such as medicine, technology, and Environmental monitoring. This is due to the physicochemical properties of AuNPs. AuNPs have magnetic properties, modifiable surfaces, sizes that affect optical properties, and many others (Altammar, 2023). AuNPs are used by utilizing their physicochemical properties, such as drug carrier materials that can be directed with external magnets (W. Li et al., 2019), surfaces that can be modified with various functional groups for adsorption (Mitomo & Ijiro, 2021), and changes in optical appearance according to size (X. Li et al., 2021).

AuNPs are easy to synthesize, and their size can be directed as needed. The inherent properties of AuNPs also depend on the size and shape of the AuNPs synthesized. To obtain the most optimum conditions, variations are needed to achieve the best conditions in carrying out the synthesis. Some of the most important variables influencing the synthesis results and that must be optimized first are the concentration of H^{+} ions and the ratio of precursors and reducing agents (Hammami & Alabdallah, 2021; Yazdani et al., 2021).

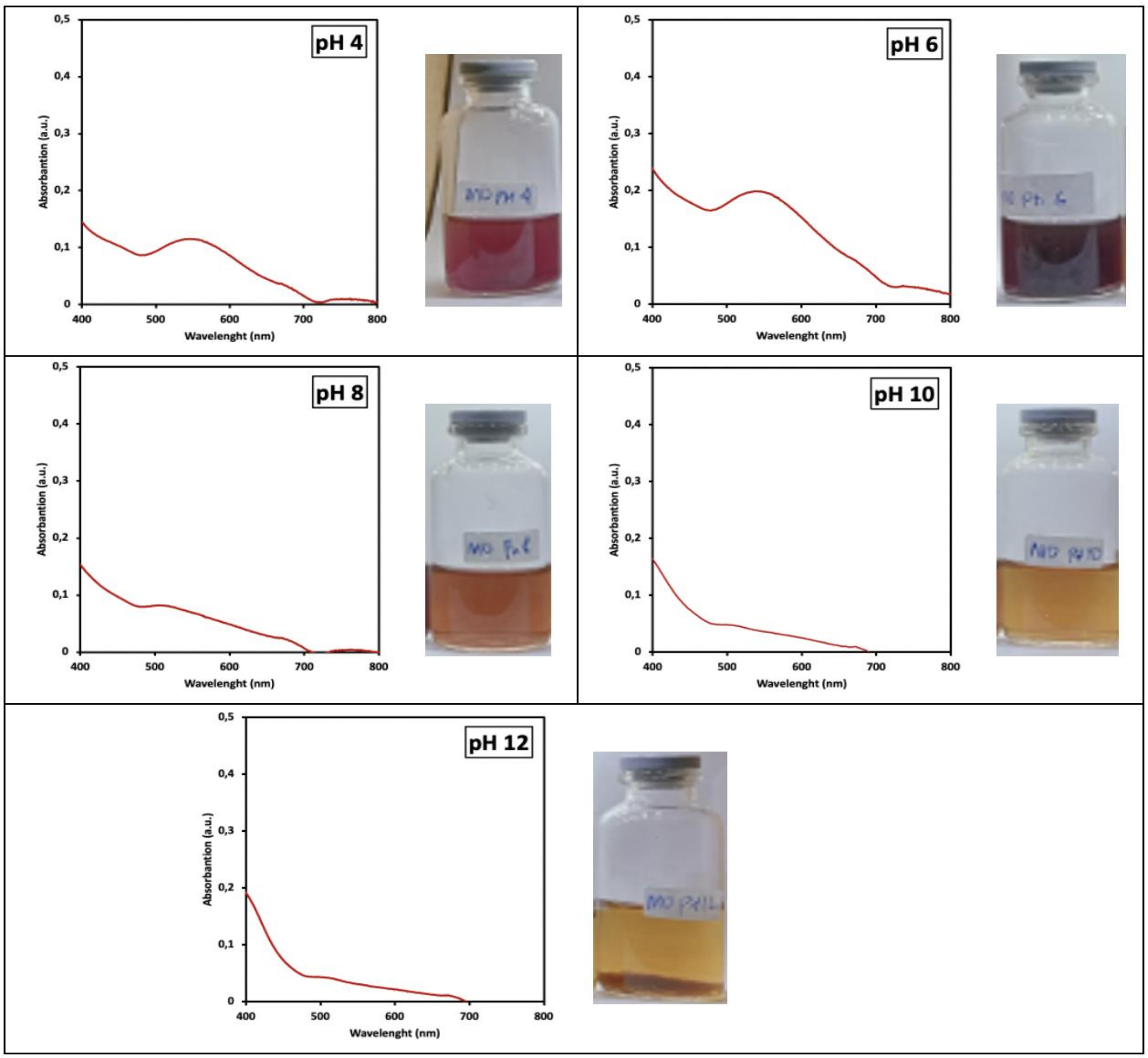

Figure 1. Synthesis Results of AuNPs-MO with Variations in pH of Reducing Agents. (left) UV-visible spectra of synthesis results and (right) visual appearance of AuNPs-MO.

Synthesis of AuNPs: determination of optimum pH

The pH applied in this study ranged from 4 to 12; this aims to determine the synthesis conditions from an environment with many H^+ ions (low pH) to a low H+ concentration (base).

The experiment began by heating the Au precursor to increase kinetic energy. This aims to ensure that the precursor has enough energy to react (Yang et al., 2021), because when no external energy is given, there is a risk that the reaction will not occur because it is unable to reach the activation energy or the reaction will run very slowly (Akintelu et al., 2021). In the heating process, the precursor is also always stirred so that the synthesis conditions can run faster because it increases the potential for growth between particles. Then when the precursor boils, the *Moringa oleifera* extract bioreductant, which has been conditioned at a pH ranging from 4 to 12, is quickly added and allowed to react for 10 minutes, then continued to cool at room temperature while still being stirred for 10 minutes to maximize the reaction (Patil et al., 2023).

The results obtained are shown in Figure 1. It could be seen that the AuNPs synthesized at pH 4 were successfully formed, marked by the appearance of a wine red color and having a lambda max at 550 nm (I. Kumar et al., 2019). From the UV-visible spectra curve, it can also be seen that the resulting AuNPs were formed quite a lot, and the homogeneity was not very good. This is indicated by the UV-Visible spectra, which show a rather high peak but with a slightly widened shape (Janah et al., 2022). Meanwhile, when it was increased to pH 6, AuNPs were formed a lot and with a homogeneous size. It can be seen from the very concentrated wine red AuNPs solution, the high and slimmer UV-Visible spectra peak. The size formed at pH 6 is also smaller than at pH 4 because in the spectra there is a blue shift to 540 nm.

Meanwhile, when the synthesis was carried out at pH 8, very few nanoparticles were formed, as indicated by the low lambda max peak. The results are not homogeneous, marked by a wide peak, and visually and physically no longer wine red. However, at pH 8, there are still a few AuNPs formed because there is a small peak at 500 nm. This means that there are AuNPs formed with very small sizes but in very small quantities (Ahmad et al., 2022).

Then when the conditions become more alkaline, namely at pH 10 and 12, it can be said that AuNPs were not successfully synthesized, because the physical color is already yellow-orange. Moreover, at pH 12 there is a precipitate as an indicator of coagulation, confirmed by the UV-visible spectra, which do not show a peak indicating AuNPs are formed.

These results indicate that AuNPs are optimally synthesized in acidic conditions, especially at pH 6. This is because in acidic conditions, Au is in the $AuCl_4^-$ species condition, which can be reduced. Meanwhile, when the pH increases, Au will begin to experience ligand substitution reactions. The Cl ligand will be substituted with the -OH group and form $AuCl_3(OH)$, $AuCl_2(OH)_2$, $AuCl(OH)_3$, and $Au(OH)_4$, which are less reactive to be reduced and even tend to precipitate (Pranata Putri et al., 2024).

In addition, ascorbic acid contained in *Moringa oleifera* extract becomes a reactive species for reduction at low pH. Ascorbic acid in acidic conditions exists in the form of $C_6H_8O_6$ and $C_6H_8O_6^-$. The $C_6H_8O_6$ species exists in greater quantities, but the most reactive one working as a bioreductant is $C_6H_8O_6^-$. Therefore, from here it can be seen that the acidic condition of the Au precursor is more ready to be reduced, and ascorbic acid is also more ready to reduce. While in basic conditions, ascorbic acid is in a more unstable and damaged $C_6H_8O_6^{2-}$ species condition so that it fails to reduce. In addition, the Au species that does not have a Cl ligand and has been replaced with OH will make electron transfer slow, because the one that acts as an electron bridge (bridge connection) is the Cl species (Pranata Putri et al., 2024).

Synthesis of AuNPs: Ratio of Precursor and Reducing Agent

The ratio comparison will affect the size, shape, and dispersion of the material and the resulting concentration, so a comparison experiment of precursors and reducing agents was carried out (Szczyglewska et al., 2023). At this stage, an experiment was carried out with the same flow as the previous stage, but the amount of precursor and reducing agent in the volume was different. The pH applied to the reducing agent was made at pH 6, according to the results of the optimum pH search stage in the previous stage. The ratios applied to precursors and reducing agents were 5:15, 5:10, 7.5:7.5, 10:5, and 15:5 (mL).

The results of this stage are shown in Figure 2. At a ratio of 5:15, when the amount of reducing agent is too much while the precursor is small, AuNPs will be obtained in very small amounts, as seen from the physical appearance of the color, which is red but bright. Meanwhile, from the UV-visible spectra, it has appeared at 530 nm, although with very small absorbance (Fouda et al., 2022). When the amount of gradual precursor is increased, the amount of AuNPs-MO formed will also increase. It can be seen that the higher the concentration of Au precursor, the more intense the wine red color produced, and the absorbance of the UV-Visible spectrophotometer graph is higher, and all are at 540 nm.

These results indicate that the reducing ability of *Moringa oleifera* extract is very high (Perumalsamy et al., 2024). It can be seen from the highest Au concentration that it has not shown constant results or has not clumped. Because when the precursor is more than the reducing agent, it will easily aggregate and form bridges between nano because there are Au precursors that are still in the form of $AuCl_4^-$ ions (El-Khawaga et al., 2023).

Another analysis of the results obtained: when the amount of reducing agent is too much and not in optimum conditions, it will act as a steric hindrance between precursors, and this is also likely what causes the ratio of 5:15 to produce very little AuNPs-MO synthesized. Too much reducing agent becomes a steric hindrance for the Au precursor and results in very few AuNPs being formed (Pasieczna-Patkowska et al., 2025). From these results, the most optimum ratio was taken as 15:5, because even though the synthesized size was larger (the lambda max peak was at a larger wavelength), the homogeneity and concentration obtained were greater.

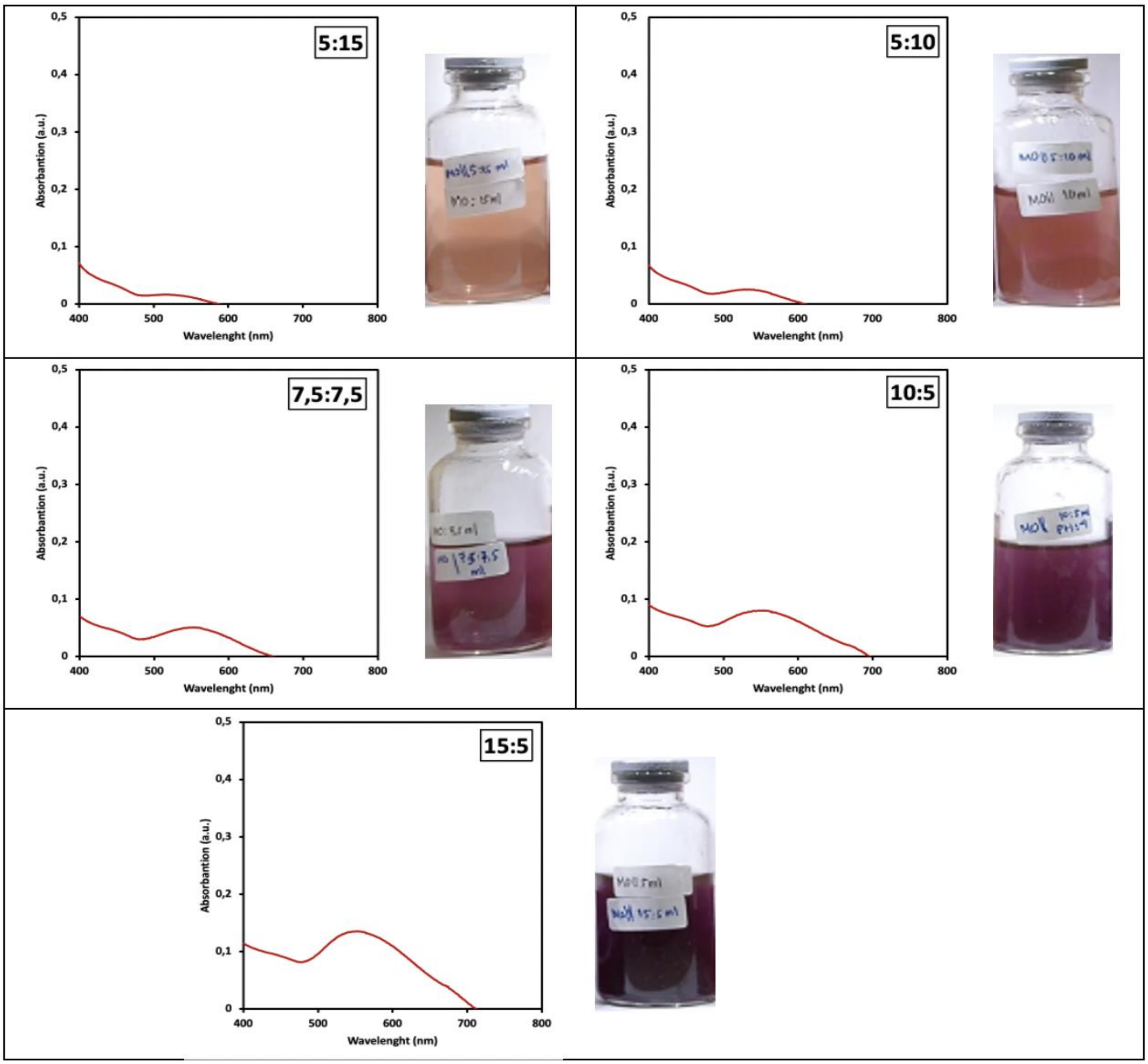

Figure 2. Synthesis Results of AuNPs-MO with Variations in the Ratio of Precursors to Reducing Agents. (left) UV-visible spectra of the synthesis results and (right) visual appearance of AuNPs-MO

Characterization of AuNPs-MO

AuNPs-MO synthesized at pH 6 and with a reducing agent precursor ratio of 15:5 were characterized using FTIR and PSA, shown in Figure 3 and Figure 4. Figure 3 shows the FTIR results where several absorptions appear, such as in the 3265.1 cm^{-1} area, which is the stretching vibration of the hydroxyl group (O-H) from the *Moringa* *oleifera* extract bioreductor (Fouda et al., 2022). In the 2124.6 cm^{-1} area is the stretching of the C≡C or C≡N group, and 1636.3 cm^{-1} shows the symmetric stretching of the carbonyl group (C=O), all of which are contributions from the *Moringa oleifera* bioreductor (Afrin & Vickram, 2025; Garg et al., 2025). The presence of functional groups such as O–H, C=O, and C≡N indicates the presence of biomolecules such as flavonoids, phenols, and organic acids, which are known to play a role in the reduction process of Au precursors (van de Langerijt et al., 2023).

Meanwhile, in the PSA characterization, the results of which are shown in Figure 4 and Table 1, the synthesized AuNPs-MO has an average size of 61.15 nm. This result is appropriate because it is still included in the size of nanomaterials, namely 1 to 100 nm. Moreover, the size of 61.15 nm is the dominant size, with a percentage reaching 92.64%, so it can be concluded that the reduction results of *Moringa oleifera* extract for the synthesis of AuNPs-MO have been successful (Fouda et al., 2022).

Table 1. PSA result data of AuNPs-MO synthesized under optimum conditions.

Data	Value (Average)
Peak 1, mean size (nm)	61.15
Peak 1, percentage size (%)	92.64
Peak 2, mean size (nm)	306.8
Peak 2, percentage size (%)	7.361

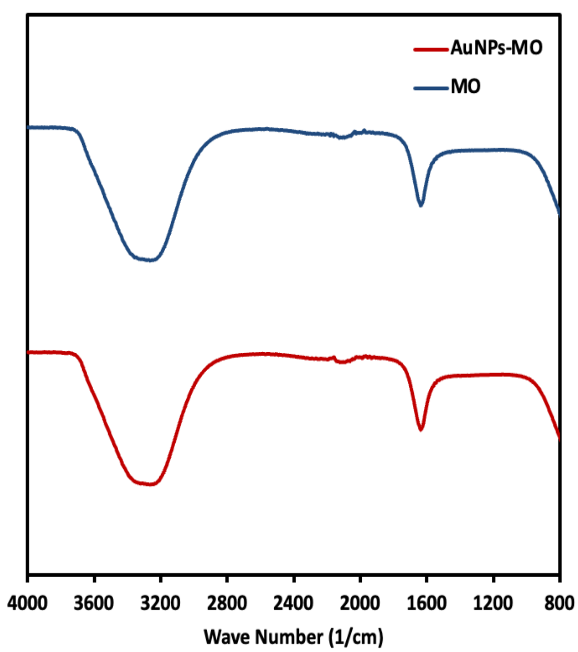

Figure 3. FTIR spectra (red) of AuNPs-MO synthesized under optimum conditions and (blue) *Moringa oleifera* extract.

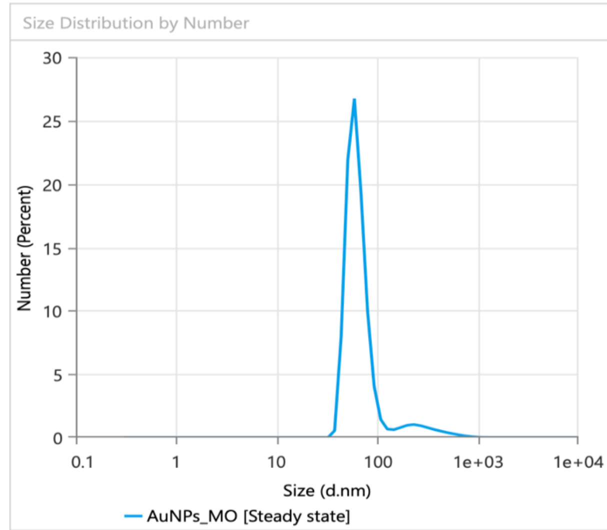

Figure 4. PSA spectra of AuNPs-MO synthesized under optimum conditions.

Colorimetric Study of AuNPs-MO for Microplastics

AuNPs have unique optical properties; they can change the color of their physical appearance simply by changing their size. In addition, the surface of AuNPs synthesized with bioreductors also has properties that can interact with microplastics because they are full of OH groups. However, the surface of AuNPs modified with peptides will give much better results. AuNPs thermodifferentiated with peptides will very easily interact electrostatically with microplastics. So when there are AuNPs-MO-Peptides in a vessel, initially they will be wine red, and when microplastics are added to the vessel, their size will enlarge, aggregate, and shift their wavelength to be larger. The color that was originally wine red will change to purple (Zhao et al., 2023).

Another way that the synthesized AuNPs-MO can be used for microplastic monitoring is to use acetone as an aggregator. AuNPs, when interacting with acetone, will aggregate and change their color from wine red to purple. Meanwhile, when AuNPs come into contact with a small amount of microplastic, the microplastic will become a steric hindrance and make acetone fail to aggregate AuNPs. In physical appearance, when AuNPs contain microplastic, they will be wine red, but when acetone is present, they will remain red and not turn purple, as the simulation shown in Figure 5 (Hong et al., 2022)

AuNPs-MO has great potential in the role of detecting new microplastic pollutants easily, quickly, and, of course, in an environmentally friendly way. However, the preparation process is still needed so that the results obtained are truly optimal, and it is possible that surface modifications are needed to make it more compatible when used as a detector.

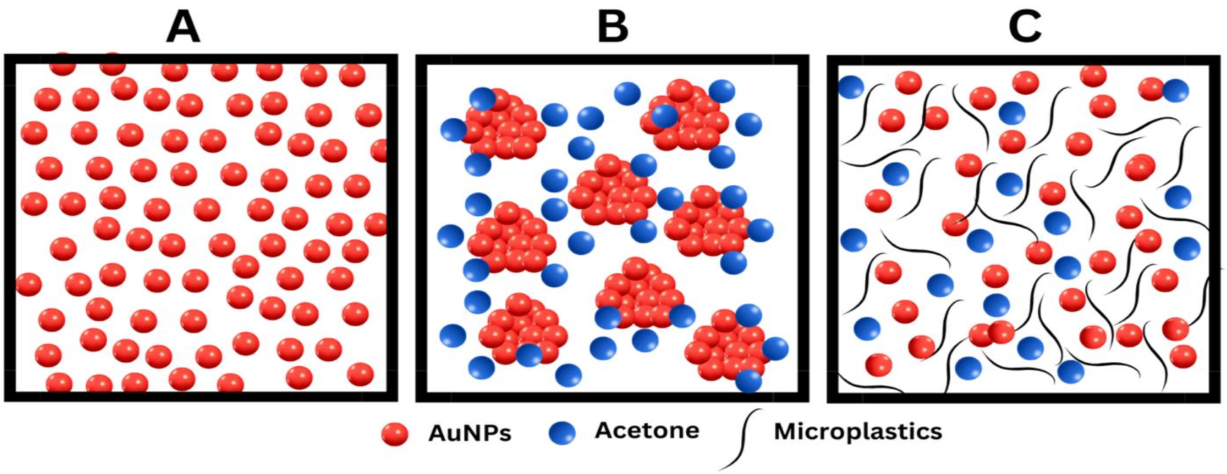

Figure 5. Simulation of the reaction of AuNPs with acetone and microplastics (A) AuNPs, (B) AuNPs aggregated due to being driven and directed by acetone, and (C) AuNPs not aggregated by acetone due to the presence of microplastics.

Conclusion

Satisfactory results have been obtained in the process of synthesizing AuNPs with *Moringa oleifera* bioreductors. The synthesis process was conditioned with pH and ratio, and the optimum AuNPs-MO results were obtained. The synthesis was carried out easily and simply. The pH was applied from acidic to basic, and the optimum pH of the synthesis was 6. While for the ratio applied, a high Au precursor was needed, and the best ratio was 15:5 (mL). The synthesized AuNPs-MO were characterized by FTIR and obtained absorption results at 3265.1 cm^{-1}, 2124.6 cm^{-1}, and 1636.3 cm^{-1} as a contribution from the existence of bioreductors containing flavonoids, phenols, and organic acids. Meanwhile, from the PSA test, the results of the AuNPs-MO size were 61.15 nm with an abundance of up to 92.64%. The synthesized AuNPs-MO have great potential to be a microplastic detector because they are able to interact electrostatically or detect with the help of an acceptor as an aggregator.

Conflict of Interest

The authors declare that there is no conflict of interest.

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