Jurnal Akta Kimia Indonesia
INDONESIA CHIMICA ACTA

PAPER

View Article Online
View Journal View Issue

Cite this: *Indo. Chim. Acta.*, 2026, **19**, **1**.

Received Date:
1th July, 2025
Accepted Date:
3th July, 2026

Keywords:
Citrate;
EDTA;
Gold Nanoparticle;

DOI:
http://dx.doi.org/10.70561/ica.v19i1.45327

Effect of Reducing Agent Type on the Morphology, Size, and Stability of Gold Nanoparticles (AuNPs): A Comparative Study of Sodium Citrate and Ethylenediaminetetraacetic Acid (EDTA)

Eduwin Saputra[1]*

Abstract. Gold nanoparticles (AuNPs) were successfully synthesized using two types of reducing agents (sodium citrate and EDTA) to investigate the effect of reducing agent type on the physicochemical properties of the resulting nanoparticles. The citrate-mediated synthesis yielded optimal results at pH 4 with the addition of 0.25 mL of 0.25% citrate solution, whereas the EDTA-mediated synthesis was optimal under the same pH conditions with 0.50 mL of 0.25% EDTA solution. Transmission Electron Microscopy (TEM) analysis revealed that citrate-AuNPs were spherical with an average size of 56 nm, while EDTA-AuNPs exhibited a hexagonal morphology with an average size of 27 nm. Furthermore, EDTA-AuNPs demonstrated superior stability during long-term storage compared to citrate-AuNPs. These results suggest that the type of reducing agent significantly affects the morphology, size, and stability of synthesized AuNPs.

Introduction

Nanoparticles are particles with nanometer dimensions (1-100 nm) that exhibit significantly different physical, chemical and biological properties compared to materials in macroscopic (bulk) form. There are many materials that are often used as nanoparticles such as gold (Au)(Dong et al., 2025), silver (Ag)(Tareq et al., 2025) and copper (Cu) (Serdar et al., 2025)metals. Gold nanoparticles (AuNPs) are one of the research topics that are experiencing rapid development due to their vast potential applications in various fields, including biomedicine(Samad et al., 2025), electronic(Wang et al., 2025), health(Saputra et al., 2025), and environment(Coelho et al., 2024). Gold nanoparticles have characteristics such as their large surface to volume ratio. This gives AuNPs the property of surface plasmon resonance, which gives them a color change that can be observed with the naked eye(Austin et al., 2014) This character arises due to the collective oscillation of electrons in the conduction band after the interaction between AuNPs and electromagnetic radiation. This character is very useful to be applied as a colorimetric sensor (Dong et al., 2025; Laghari & Khuhawar, 2020; Saputra, 2024).

The synthesis of AuNPs has been carried out by various methods such as reduction(Jingyue Zhao, 2015), thermal decomposition(Odularu, 2018), sonochemical(Fuentes-García et al., 2021), photochemical(Dong et al., 2025), and electrochemistry(Lewa et al., 2023). Among these methods, chemical reduction is the most popular way of synthesizing gold nanoparticles due to its simplicity, low-cost, and the possibility of being produced on a large scale(Hammami et al., 2021). The reduction method only requires reductants and precursors in the process of synthesizing gold nanoparticles. The sensitivity and selectivity of the AuNPs-based colorimetric sensor to detect analytes is influenced by the optimum conditions of the colorimetric sensor.

The optimum conditions for synthesis of AuNPs are influenced by several factors, including precursor pH, reductant concentration, incubation time, and the type of reductant used. Highly acidic solutions result in an excess of H^+ ions, which can reduce the availability of electrons needed to convert Au^{3+} ions into neutral Au^0 atoms. An increase in the amount of reductant added to $HAuCl_4$ is generally proportional to the amount of AuNPs formed.

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, University, Bengkulu, 38371;* ***Email: esaputra@unib.ac.id***

Determining the optimum incubation time is essential to identify the duration required for complete reduction of Au^{3+} to Au^0. Additionally, the type of reductant used plays a critical role in determining the physicochemical characteristics of the resulting AuNPs(Yoo et al., 2022). Therefore, further investigation into the effects of various reducing agents is essential for optimizing the synthesis of AuNPs.

The Turkevich method is one of the most widely employed approaches for the synthesis of gold nanoparticles (AuNPs), wherein colloidal AuNPs are produced via the chemical reduction of chloroauric acid ($HAuCl_4$) using an appropriate reducing agent(Zhao et al., 2013). Trisodium citrate is commonly utilized in this method due to its dual role as both a reducing and stabilizing agent, attributed to the presence of three carboxylate groups ($-COO^-$) and one hydroxyl group (-OH) in its molecular structure. Ethylenediaminetetraacetic acid (EDTA), an organic chelating agent with four carboxylate groups, serves as an alternative reducing agent(Mohammadi et al., 2013). In the present study, AuNPs were synthesized using EDTA to evaluate its effect on nanoparticle formation. The distinct structural features of trisodium citrate and EDTA are anticipated to significantly influence the physicochemical characteristics of the resulting AuNPs under optimized synthesis conditions.

Experimental

Material and Methods

The materials used in this study included 1.0 g of 99.9% pure gold supplied by Antam, aqua regia prepared from hydrochloric acid (HCl, 37%) and nitric acid (HNO_3) in a 3:1 ratio (12 mL:4 mL), and distilled water. Additional reagents obtained from Merck (Germany) included trisodium citrate ($Na_3C_6H_5O_7$), ethylenediaminetetraacetic acid (EDTA), and sodium hydroxide (NaOH). The instruments employed were a UV-Vis spectrophotometer (Shimadzu UV-Vis Thermo Scientific Genesys 10S), a Transmission Electron Microscope (TEM; JEOL JEM-1400), a Particle Size Analyzer (PSA; HORIBA Scientific SZ-100), a mini photo studio, and a Galaxy A33 smartphone camera.

Procedures

Synthesis of gold nanoparticles (AuNPs)

Preparation of $HAuCl_4$ solution. Amount of 1.0 g of gold was dissolved into aqua regia (a mixture of 37% HCl and 65% HNO_3 solutions in a ratio of 3:1 (12 mL: 4 mL)). The mixing was carried out in a fume hood and the dissolution process was carried out on an electric heater at 50°C and stirred at 60 rpm until all the gold metal was completely dissolved. The solution was then diluted with distilled water to a volume of 1000 mL.

pH Optimization of $HAuCl_4$

The pH of the $HAuCl_4$ precursor in this study was adjusted to 2, 3, 4, and 5. The pH adjustment was performed by adding several drops (approximately 0.1-0.3 mL) of 1.4 M NaOH to the precursor solution, followed by pH measurement using a pH meter. A 1.50 mL aliquot of the $HAuCl_4$ solution at each pH level was heated on a hotplate with stirring at 100 °C until boiling, then 0.50 mL of 0.5% (w/v) sodium citrate or EDTA was added. The synthesis continued until a visible color change occurred, ranging from yellow to gray, purple, reddish-purple, and red. After the formation of colloidal AuNPs, the absorbance of each solution was measured using a UV-Vis spectrophotometer.

Precursor concentration optimization

Amount of 0.50 mL of 0.5% (w/v) reductant solution was added to 1.50 mL of $HAuCl_4$ solution at optimum pH and varied in concentration from 50, 100, 150, and 200 mg/L. Synthesis of AuNPs was carried out at 100°C with stirring. After the formation of colloidal AuNPs, each solution was measured for absorbance using a UV-Vis spectrophotometer.

Optimization of reductant concentration and volume

The $HAuCl_4$ solution at the optimum concentration and volume was adjusted to pH 5 and then mixed separately with sodium citrate and EDTA at varying concentrations of 0.10%, 0.25%, 0.50%, 0.75%, and 1.00% (w/v). The synthesis of AuNPs was conducted at 100 °C under stirring. Subsequently, the volume of the reducing agents was varied at 0.10, 0.25, 0.50, 0.75, and 1.00 mL. After the formation of AuNPs, the absorbance of each solution was measured using a UV-Vis spectrophotometer.

Stability of AuNPs

The stability test was conducted by storing the colloidal AuNPs for specific time intervals, followed by absorbance measurements. AuNPs synthesized using trisodium citrate and EDTA under optimum conditions were analyzed on days 1, 2, 3, 7, 14, 21, and 28.

Characterization of AuNPs

Characterization of the AuNPs was conducted using UV-Vis spectrophotometry, transmission electron microscopy (TEM), and particle size analysis (PSA). TEM and PSA were performed on 5 mL aliquots of Cit-AuNPs and EDTA-AuNPs.

Result and Discussion

Synthesis of gold nanoparticles (AuNPs)

Gold nanoparticles (AuNPs) can be synthesized using reducing agents such as sodium citrate or ethylenediaminetetraacetic acid (EDTA), each demonstrating distinct reduction mechanisms and efficiencies in producing nanoscale particles. In citrate-mediated synthesis, gold ions (Au^{3+}) derived from the $HAuCl_4$ precursor are reduced to elemental gold (Au^0) under elevated temperature conditions. Sodium citrate functions both as a reducing and stabilizing agent; the resulting Au^0 atoms initiate nucleation, followed by particle growth through the successive addition of Au^0 atoms to the growing nuclei. Citrate ions subsequently adsorb onto the nanoparticle surface, imparting negative charges that induce electrostatic repulsion, thus preventing aggregation and enhancing colloidal stability(Monti et al., 2017).

Meanwhile, in the EDTA-based system, the reduction of Au^{3+} ions to Au^0 is facilitated by the reductive carboxylate and amine groups present in the EDTA molecule(Dozol et al., 2013). This process also occurs under heating conditions and proceeds through similar nucleation and particle growth stages. EDTA further acts as a stabilizing agent via chelation and adsorption mechanisms, providing steric hindrance and imparting a negative surface charge that enhances particle stability. Due to its strong metal-chelating ability, EDTA yields AuNPs with smaller particle sizes and greater colloidal stability compared to those synthesized using citrate. The reaction mechanism between the precursor and the reducing agent is illustrated in figure 1.

The synthesis of AuNPs was conducted by optimizing several parameters, including precursor pH, reductant concentration and volume, and reaction time, based on the resulting UV-Vis spectra.

pH Optimization of $HAuCl_4$

The formation of AuNPs is highly influenced by the pH of the reaction medium(Malassis et al., 2016). pH optimization is essential to establish the ideal conditions for reducing Au^{3+} to Au^0, particularly when using sodium citrate, which is highly sensitive to pH changes. In this study, the pH of the $HAuCl_4$ solution was varied from 2 to 5. Optimization was performed by adjusting the pH of the precursor solution prior to synthesis. Since the initial pH of $HAuCl_4$ was 1.7, it was adjusted with 1.4 M NaOH until the desired pH was reached, as confirmed by a pH meter. The NaOH solution was added only in small volumes; therefore, it did not significantly alter the concentration of $HAuCl_4$ in the reaction system. The effect of pH variations on AuNPs synthesis using citrate and EDTA as reductants is illustrated in the figure 2:

The optimum pH condition for the synthesis of citrate-AuNPs and EDTA-AuNPs was determined to be pH 4. Based on the absorbance data, the formation of gold nanoparticles was observed even at a precursor pH of 2, as indicated by absorbance peaks in the wavelength range of 500–550 nm and the appearance of a reddish-purple colloidal color(Shang et al., 2022). This color change is attributed to the phenomenon of localized surface plasmon resonance (LSPR), where incident light interacts with the free electrons on the nanoparticle surface, causing collective oscillations of electrons in the conduction band(Alebrahim et al., 2024). Under highly acidic conditions, the excess of H^+ ions reduce the availability of electrons for the reduction of Au^{3+} to Au^0, thereby inhibiting efficient nanoparticle formation. The formation of AuNPs is strongly influenced by the acidity of the solution, in accordance with Le Chatelier's principle. As the concentration of H^+ ions increase, the reducing ability of citrate diminishes, thereby decreasing the availability of electrons required to reduce Au^{3+} ions to elemental gold (Au^0) and form AuNPs(Tyagi et al., 2011).

As the pH of the precursor solution increases, the color of the resulting colloid shifts to burgundy, accompanied by a rise in maximum absorbance, which reaches its optimum at pH 4. Elevating the pH above 3 reduces the concentration of H^+ ions, allowing more carboxylate groups to ionize into their anionic form ($-COO^-$). These anionic groups can interact with Au(III) ions to form complexes, thereby generating a negatively charged layer on the surface of gold nanoparticles (AuNPs) during the reduction process. An increased number of anionic carboxylate groups enhances the negative surface charge, promoting greater electrostatic stabilization of the nanoparticles. Furthermore, pH measurement conducted after AuNPs synthesis showed that the final pH of the colloidal solution increased to approximately 6. This result supports the proposed mechanism, indicating the consumption of H^+ ions and the involvement of citrate and EDTA ionization during the reduction of Au^{3+} to Au^0. However, at pH 5, the UV-Vis spectra exhibit a decrease in maximum absorbance, indicating a reduced number of particles formed. The absorbance intensity observed in the UV-Vis spectra correlates with the concentration of nanoparticles in the solution(Notriawan et al., 2021). Thus, nanoparticle formation increases from pH 2 to 4, but declines at pH 5.

Based on the absorbance data of Citrate-AuNPs and EDTA-AuNPs, it was observed that increasing the precursor pH from 4 to 5 resulted in a decrease in absorbance. This reduction is attributed to nanoparticle aggregation under more alkaline conditions. These findings

are consistent with the study by Rizki et al., (2020), which reported that increasing the pH from 6 to 10 during synthesis led to AuNPs aggregation, indicated by a color change of the solution to blue or gray. This phenomenon is caused by the high concentration of OH^- ions in alkaline media, which enhances hydrogen bonding between reducing molecules on the surface of adjacent AuNPs, thereby reducing the interparticle distance and promoting aggregation.

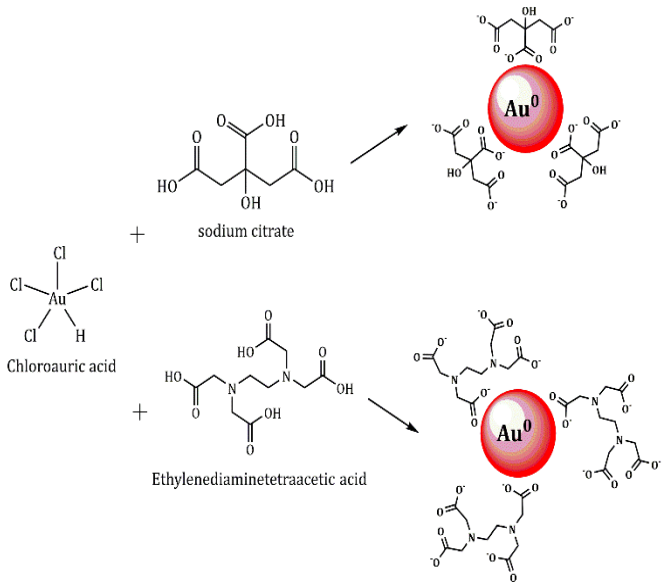

Figure 1. Mechanism of AuNPs Formation by Citrate and EDTA Reducing Agents.

The stability and formation of AuNPs are strongly influenced by the protonation state of citrate, which changes according to the pH of the solution. Citrate has three dissociation constants, namely pKa_1 = 3.14, pKa_2 = 4.67, and pKa_3 = 6.40, indicating the transition of citrate species at different pH ranges. In the pH range of 3.14–4.67, citrate predominantly exists in the monodeprotonated form, which provides favorable electrostatic interactions with gold ions. Under these conditions, citrate effectively acts as both a reducing stabilizer and capping agent, thereby preventing excessive particle growth and maintaining the nanoparticles within the nanoscale dimension(Krishnamurthy & Yun, 2013).

At lower pH conditions, the proportion of monodeprotonated citrate decreases, reducing its ability to stabilize the AuNPs surface. Therefore, the particles tend to aggregate, forming larger particles that may eventually precipitate. When the pH increases to the range of 4.67–6.40, the dominant citrate species shifts to the dideprotonated form, while above pH 6.40 the trideprotonated species becomes predominant. These highly deprotonated citrate species are considered less effective in maintaining nanoparticle stability compared to the monodeprotonated form, which can also lead to increased aggregation of AuNPs at higher pH values (Amir Tabrizi, Fatma Ayhan, 2009).

EDTA acts as both a reducing and stabilizing agent in the synthesis of gold nanoparticles (AuNPs). During the reaction, EDTA initially forms complexes with Au(III) ions and subsequently undergoes oxidative decarboxylation, producing intermediates such as ED3A, S-EDDA, glyoxal, and formaldehyde. In this process, Au(III) is reduced to Au(I) and finally to metallic Au(0), leading to the formation of gold nanoparticles. The formaldehyde generated during EDTA oxidation also contributes to the reduction and nucleation processes. Furthermore, the reaction mechanism is strongly influenced by pH; acidic conditions promote faster reduction and the formation of larger particles, whereas alkaline conditions result in slower reduction and the formation of smaller, more stable nanoparticles due to enhanced stabilization by EDTA (Dozol et al., 2013).

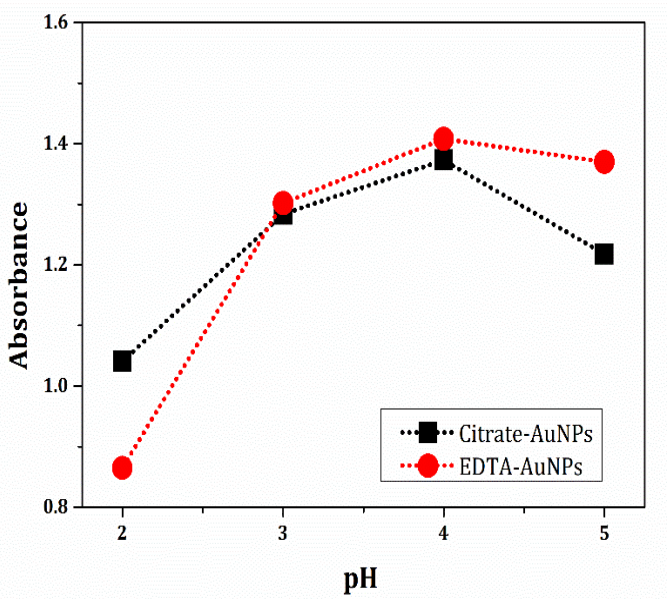

Figure 2. Absorbance data of synthesized Citrate-AuNPs and EDTA-AuNPs at various precursor pH.

Precursor concentration optimization

Optimization of $HAuCl_4$ precursor concentration was conducted to determine the optimal amount of $HAuCl_4$ required to produce AuNPs under ideal synthesis conditions. The data from the precursor concentration optimization experiment are presented in the following figure.

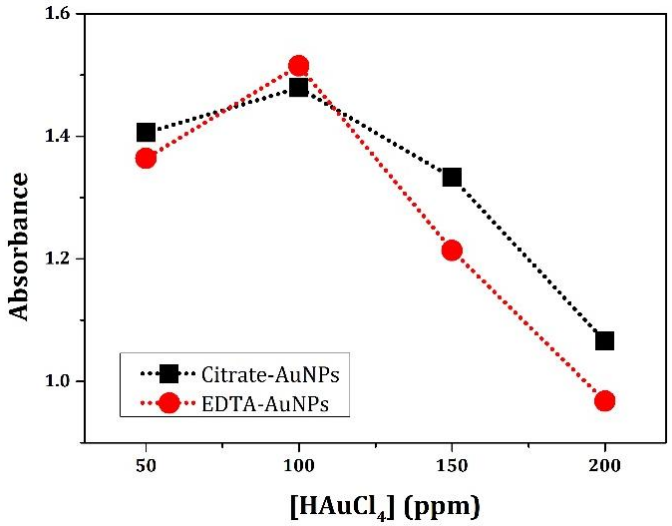

Figure 3. Absorbance data of AuNPs synthesis at various precursor concentrations.

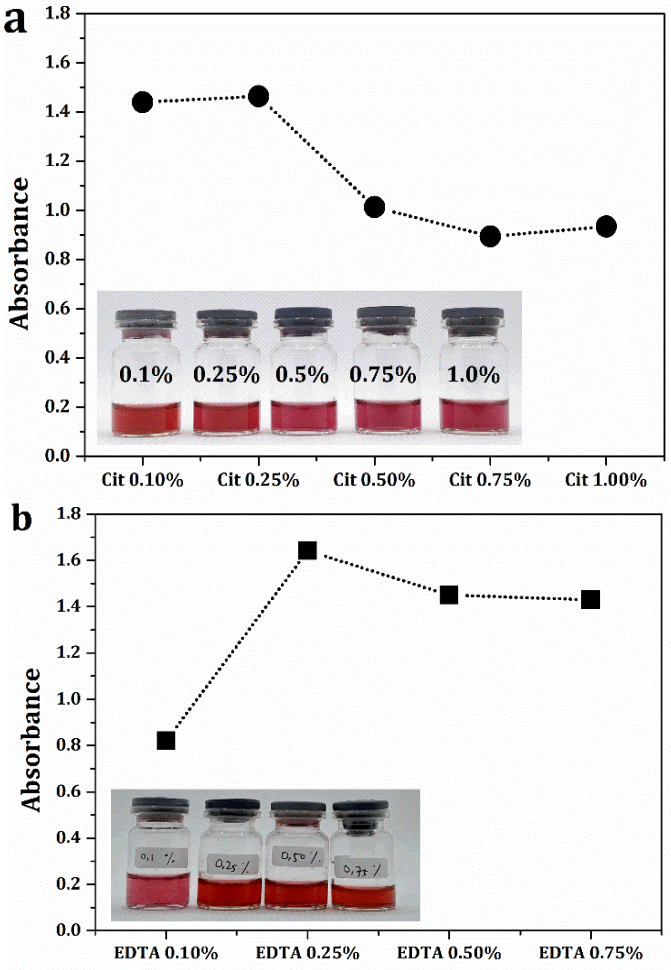

Figure 4. Absorbance data of synthesized (a) Citrate-AuNPs and (b) EDTA-AuNPs at varying reductant concentration.

Figure 3 shows a graph illustrating the relationship between the concentration of the $HAuCl_4$ precursor at pH 5 (in ppm) and the absorbance value of AuNPs synthesized using two types of reducing agents: citrate and EDTA. Higher absorbance values indicate a greater concentration of nanoparticles or a more efficient AuNPs formation process. It can be observed that for both Citrate-AuNPs and EDTA-AuNPs, the absorbance increases with increasing $HAuCl_4$ concentration, reaching a maximum at 100 ppm. This suggests that 100 ppm is the optimum concentration for AuNPs formation with both reducing agents. Beyond this point, further increases in precursor concentration lead to a decrease in absorbance, indicating the formation of larger particles or an imbalance in the precursor-to-reductant ratio.

Reductant concentration optimization

The effect of sodium citrate concentration was studied by reacting 1.5 mL of 100 mg/L HAuCl4 with 0.75 mL of sodium citrate at various concentrations of 0.10; 0.25; 0.50; 0.75 and 1.00% (b/v). Addition of EDTA as much as 0.5 mL at various concentrations namely 0.1%; 0.25%; 0.50% and 0.75%. Figure 4 shows the absorbance value and color of colloidal AuNPs.

From Figure 4a, it is observed that the optimum conditions for AuNPs synthesis are achieved at concentrations of 0.25% (b/v) trisodium citrate and 0.1% (b/v) EDTA. At 0.10% (b/v) trisodium citrate and 0.1% (b/v) EDTA, the presence of the reducing agents in sufficient amounts led to a change in colloidal color from yellow to red. The red coloration indicates that the reduction capability of both citrate and EDTA at these concentrations is optimal for AuNPs formation. Increasing the trisodium citrate concentration to 0.25% (b/v) did not result in a significant color change, but the absorbance increased slightly. The addition of 0.25% (b/v) EDTA produced a more intense red color in the colloidal AuNPs and a higher absorbance value, indicating the formation of more nanoparticles. Further increases in trisodium citrate concentration to 0.50%, 0.75%, and 1.00% (b/v) resulted in a color shift of the colloid from red to reddish purple. Similarly, increasing EDTA concentrations to 0.50% and 0.75% (b/v) yielded colloidal AuNPs with color and absorbance values comparable to those at 0.25% EDTA.

Electrosteric stabilization may occur at high concentrations of reductants due to their dense arrangement around the formed Au nanoparticles. However, excessively high reductant concentrations can also lead to a decrease in absorbance. At elevated levels of citrate and EDTA, the absorbance values tend to decline, indicating the formation of fewer Citrate-AuNPs and EDTA-AuNPs. Since absorbance correlates with nanoparticle concentration, it is possible that overly high concentrations

of citrate and EDTA reduce the effectiveness of the reduction process. This may be attributed to less efficient interactions between the reducing functional groups and Au(III), potentially due to increased solution viscosity. Nevertheless, given the abundance of carboxylate and hydroxyl groups capable of reducing Au(III), this explanation may be less likely. An alternative hypothesis is that stronger interactions between the citrate and EDTA molecules and the AuNPs surfaces dominate, causing the absorbance of the reductants to overshadow the characteristic surface plasmon resonance of the nanoparticles.

The results of this study prove that the formation of AuNPs is influenced by the concentration of reductant. The more trinatrium citrate and EDTA added to HAuCl4, the more functional groups available to reduce Au(III), so that nucleation is intensified. The addition of reductant at a certain concentration will not increase the absorbance intensity at the maximum wavelength. In this study, the optimum concentration of citrate and EDTA reductant is 0.25% (b/v).

Reductant volume optimization

After it is known that the synthesis of AuNPs is optimum at the concentration of citrate and EDTA 0.25% (b/v), then the effect of citrate and EDTA 0.25% (b/v) at various volumes is investigated. The optimum conditions for the synthesis of AuNPs at various volumes of reductant can be seen in figure 5.

Figure 5 shows that the optimum conditions for the synthesis of citrate-AuNPs at a concentration of 0.25% (b/v) citrate as much as 0.25 mL and 0.25% (b/v) EDTA as much as 0.5 mL. At a volume of 0.1 mL citrate and 0.25 mL EDTA has been successful to synthesize AuNPs, marked by a change in color to red. When the volume of reductant was increased to 0.25 mL, the color of the colloid formed still remained red, but the UV-Vis spectrum produced was higher and slender. This means that the particles formed are more numerous and have a more homogeneous size. When the volume of citrate reductant was increased to 1.00 mL, the color of the colloid formed was still red but the resulting UV-Vis spectrum decreased. The addition of EDTA up to 1 mL causes the AuNPs formed to be reddish purple in color and accompanied by a decrease in absorbance value. It can be concluded that the citrate-AuNPs synthesis is optimum at the addition of 0.25% (b/v) citrate volume of 0.25 mL and EDTA-AuNPs at 0.25% EDTA volume of 0.5 mL.

Based on the optimum conditions for the synthesis of citrate-AuNPs and EDTA-AuNPs, the UV-Vis spectra can be seen in figure 6.

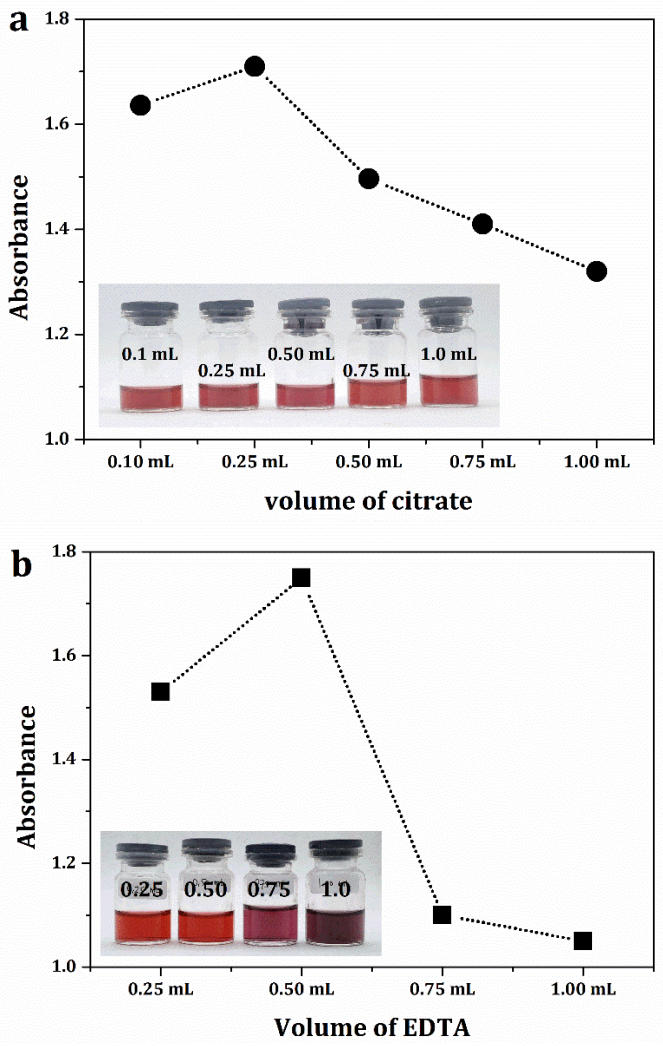

Figure 5. Absorbance data of synthesized (a) Citrate-AuNPs and (b) EDTA-AuNPs at varying reductant volume.

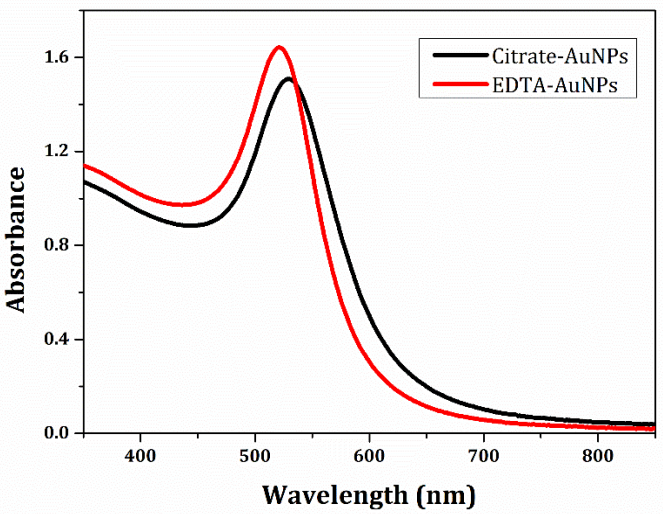

Figure 6. UV-Vis spectra of citrate-AuNPs and EDTA-AuNPs at optimum conditions.

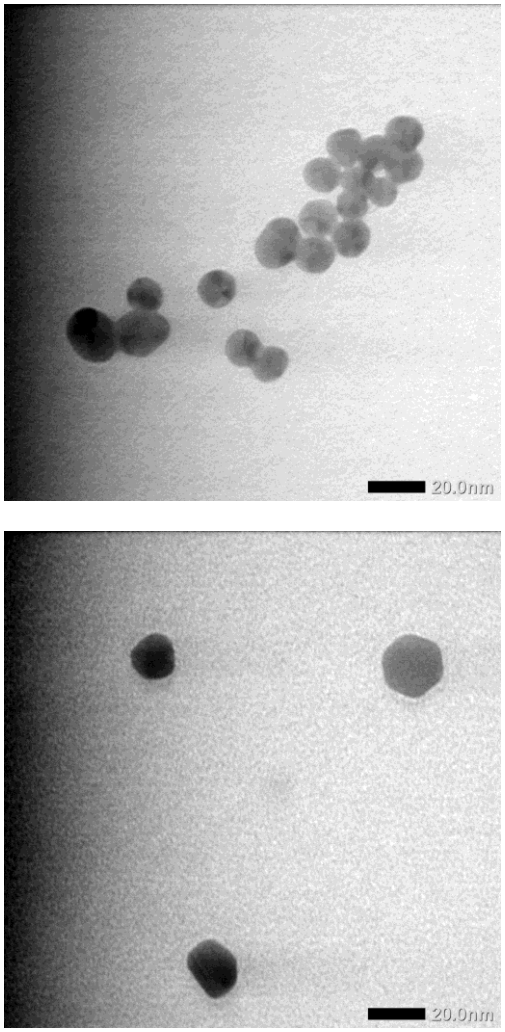

Figure 7. TEM analysis of Citrate-AuNPs(up) and EDTA-AuNPs(down).

Based on the spectra results, it can be seen that citrate-AuNPs have UV-Vis spectra at a wavelength of 526 nm, while EDTA-AuNPs have UV-Vis spectra at a wavelength of 521 nm. The appearance of the peak in the UV-Vis spectra of AuNPs is caused by the Surface Plasmon Resonance (SPR) phenomenon. SPR is a collective oscillation of free electrons on the surface of metal nanoparticles triggered by interaction with light at a certain wavelength. In addition, the slimmer shape of the spectra on EDTA-AuNPs indicates that the particles formed have a more homogeneous size than citrate-AuNPs. The position of the absorption maximum band depends on the average size of the nanoparticles and shifts to larger wavelengths with increasing particle diameter(Apyari et al., 2014).

TEM analysis of AuNPs

TEM analysis aims to determine the shape, size, and morphology of the synthesized nanoparticles. TEM image results represent differences in morphology and size distribution of nanoparticles as a function of synthesis parameters, such as pH, concentration of reductant, concentration of masking agent, concentration of metal precursor, temperature, etc. The results of the analysis using TEM can be seen in figure 7.

The synthesized nanoparticles at optimum conditions were analyzed for shape and size distribution using TEM. Figure 7 (a) shows that the successfully synthesized citrate-AuNPs have a spherical shape. While EDTA-AuNPs have an almost round shape and some are even hexagon-shaped. This particle shape is also the same as the research produced by Diamai & Negi (2016). The difference in particle shape between citrate-AuNPs and EDTA-AuNPs can be caused by the type of reductant used to synthesize AuNPs (Iqbal et al., 2016). The citrate structure has three carboxyl groups and one hydroxyl group. According to Frost et al., (2017), only one carboxylic group on citrate is bound to the surface of AuNPs, and the other two carboxylic groups are not bound to the surface of AuNPs. While EDTA has four carboxyl groups, with one carboxyl group attached to the surface of AuNPs and leaving three carboxyl groups free on the surface of AuNPs. The difference in the number of carboxyl groups on the surface of AuNPs is what causes the difference in particle shape on AuNPs.

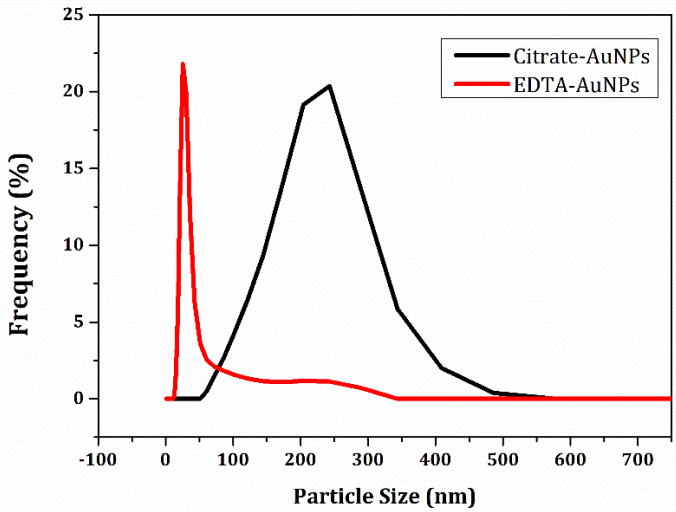

Figure 8. Particle size distribution of citrate-AuNPs and EDTA-AuNPs.

PSA analysis of AuNPs

PSA analysis aims to determine the size and size distribution of nanoparticles that have been synthesized. The measurement results using PSA can be seen in figure 8. Figure 8 shows the average size distribution of citrate-

AuNPs and EDTA-AuNPs which are 56 nm and 27 nm, respectively. This size difference can be caused by several factors such as the strength of the reducing agent. EDTA is a stronger reducing agent than citrate. Faster reduction results in more nuclei so that the particle growth is divided into more particles and therefore the particle size becomes smaller. In addition, EDTA-AuNPs have greater repulsion compared to citrate-AuNPs. This repulsion is related to the stability of the AuNPs generated by the charge on the surface of the AuNPs. The surface of EDTA-AuNPs has more carboxyl groups. These groups are negatively charged, so the more negative the surface, the greater the repulsion between surfaces. This repulsion will prevent the AuNPs particles from forming larger particles (aggregation).

Stability test of AuNPs

In this study, absorbance measurements were taken to evaluate the stability of AuNPs over a storage period lasting some time. The results of the AuNPs stability test can be seen in the following figure.

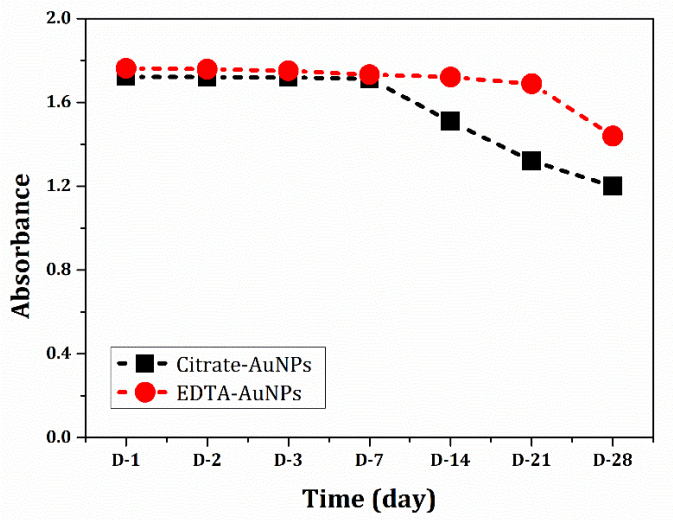

Figure 9. stability data of citrate-AuNPs and EDTA-AuNPs.

Based on the absorbance data of AuNPs at various times, citrate-AuNPs have storage stability until the seventh day while EDTA-AuNPs have stability until the twenty-first day. This can be seen from the relatively stable absorbance value. Whereas after longer storage, there is a significant decrease in absorbance value. This difference can be attributed to EDTA's ability as a strong and multifunctional chelating agent, which not only reduces gold ions but also forms strong bonds with the nanoparticle surface through carboxylate and amine groups. These bonds provide dual stabilization, namely electrostatic and steric, which effectively prevents particle aggregation and growth during storage. In addition, EDTA is also able to bind to residual metal ions that may trigger oxidation or changes in particle size, and is more resistant to changes in pH, temperature, and environmental ionic strength, making it a more effective protectant than citrate, which only provides relatively weak electrostatic stabilization.

Conclusion

Gold nanoparticles (AuNPs) can be synthesized using reducing agents such as sodium citrate and EDTA. The different types of reducing agents cause different optimum conditions for the synthesis of AuNPs. Synthesis using citrate showed optimum results at pH 4 with the addition of 0.25 mL of 0.25% citrate solution, while synthesis using EDTA reached optimum conditions at pH 4 with the addition of 0.50 mL of 0.25% EDTA solution. Based on the TEM image, citrate-AuNPs are spherical with an average size of 56 nm, while EDTA-AuNPs are hexagonal with an average size of 27 nm. In addition, EDTA-AuNPs showed higher stability during storage compared to citrate-AuNPs. These results indicate that the type of reducing agent has a significant effect on the physicochemical characteristics of the gold nanoparticles produced.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Alebrahim, M. A., Ahmad, A. A., Migdadi, A. B., & Al-Bataineh, Q. M. (2024). Localize surface plasmon resonance of gold nanoparticles and their effect on the polyethylene oxide nanocomposite films. *Physica B: Condensed Matter*, *679*, 415805. https://doi.org/10.1016/j.physb.2024.415805

Amir Tabrizi, Fatma Ayhan, H. A. (2009). Gold Nanoparticle Synthesis and Characterisation. *Hacettepe J. Biol. & Chem.*, *37*(3), 217–226. http://www.hjbc.hacettepe.edu.tr/site/assets/files/2941/37_3_217-226.pdf

Apyari, V. V., Arkhipova, V. V., Dmitrienko, S. G., & Zolotov, Y. A. (2014). Using gold nanoparticles in spectrophotometry. *Journal of Analytical Chemistry*, *69*(1), 1–11. https://doi.org/10.1134/S1061934814010031

Austin, L. A., MacKey, M. A., Dreaden, E. C., & El-Sayed, M. A. (2014). The optical, photothermal, and facile surface chemical properties of gold and silver nanoparticles in biodiagnostics, therapy, and drug delivery. *Archives of Toxicology*, *88*(7), 1391–1417. https://doi.org/10.1007/s00204-014-1245-3

Coelho, E., Xavier, D., Almeida, A., & Colherinhas, G. (2024). Dynamic interactions of negatively charged gold nanoparticles (AuNPs) in aqueous environments with different ionic compositions. *Journal of Molecular*

Liquids, *413*, 126021. https://doi.org/10.1016/j.molliq.2024.126021

Diamai, S., & Negi, D. P. S. (2016). EDTA-capped gold nanoparticles as a colorimetric probe for aluminium. *Materials Research Express*, *3*(11). https://doi.org/10.1088/2053-1591/3/11/115002

Dong, S., Zhang, Y., Shi, Q., Zhu, Z., Wang, Y., Luo, R., & Feng, J. (2025). Development of colorimetric immunochromatographic assay based on different morphologies of gold nanoparticles for the detection of triadimefon. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *343*, 126544. https://doi.org/10.1016/j.saa.2025.126544

Dozol, H., Mériguet, G., Ancian, B., Cabuil, V., Xu, H., Wang, D., & Abou-Hassan, A. (2013). On the Synthesis of Au Nanoparticles Using EDTA as a Reducing Agent. *The Journal of Physical Chemistry C*, *117*(40), 20958–20966. https://doi.org/10.1021/jp4067789

Frost, M. S., Dempsey, M. J., & Whitehead, D. E. (2017). The response of citrate functionalised gold and silver nanoparticles to the addition of heavy metal ions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *518*, 15–24. https://doi.org/10.1016/j.colsurfa.2016.12.036

Fuentes-García, J. A., Santoyo-Salzar, J., Rangel-Cortes, E., Goya, G. F., Cardozo-Mata, V., & Pescador-Rojas, J. A. (2021). Effect of ultrasonic irradiation power on sonochemical synthesis of gold nanoparticles. *Ultrasonics Sonochemistry*, *70*, 105274. https://doi.org/10.1016/j.ultsonch.2020.105274

Hammami, I., Alabdallah, N. M., Jomaa, A. Al, & Kamoun, M. (2021). Gold nanoparticles: Synthesis properties and applications. *Journal of King Saud University - Science*, *33*(7), 101560. https://doi.org/10.1016/j.jksus.2021.101560

Jingyue Zhao, B. F. (2015). Synthesis of Gold Nanoparticles via Chemical Reduction Methods. *Nanocon*, 18.

Krishnamurthy, S., & Yun, Y.-S. (2013). Recovery of microbially synthesized gold nanoparticles using sodium citrate and detergents. *Chemical Engineering Journal*, *214*, 253–261. https://doi.org/10.1016/j.cej.2012.10.028

Laghari, S., & Khuhawar, M. Y. (2020). Colorimetric detection of fluoxetine using citrate-capped silver nanoparticles. *SN Applied Sciences*, *2*(4), 1–10. https://doi.org/10.1007/s42452-020-2425-9

Lewa, I. W. L., Meifina, & Isnaeni. (2023). Electrochemically Synthesized Gold Nanoparticles Using Gold and Copper Electrodes. *Jurnal Sains Materi Indonesia*, *24*(2), 97–104. https://doi.org/10.55981/jsmi.2023.762

Malassis, L., Dreyfus, R., Murphy, R. J., Hough, L. A., Donnio, B., & Murray, C. B. (2016). One-step green synthesis of gold and silver nanoparticles with ascorbic acid and their versatile surface post-functionalization. *RSC Advances*, *6*(39), 33092–33100. https://doi.org/10.1039/c6ra00194g

Mohammadi, Z., Shalavi, S., & Jafarzadeh, H. (2013). Ethylenediaminetetraacetic acid in endodontics. *European Journal of Dentistry*, *07*(S 01), S135–S142. https://doi.org/10.4103/1305-7456.119091

Monti, S., Barcaro, G., Sementa, L., Carravetta, V., & Ågren, H. (2017). Characterization of the adsorption dynamics of trisodium citrate on gold in water solution. *RSC Adv.*, *7*(78), 49655–49663. https://doi.org/10.1039/C7RA10759E

Notriawan, D., Nesbah, N., Ernis, G., Fadhila, M. A., Wibowo, R. H., Pertiwi, R., & Ilfanisari, V. (2021). Aktivitas Antibakteri Membran Nanokomposit Kitosan/Nanopartikel Perak. *Alchemy*, *9*(1), 26–31. https://doi.org/10.18860/al.v9i1.11146

Odularu, A. T. (2018). Metal Nanoparticles: Thermal Decomposition, Biomedicinal Applications to Cancer Treatment, and Future Perspectives. *Bioinorganic Chemistry and Applications*, *2018*, 1–6. https://doi.org/10.1155/2018/9354708

Rizki, F. E., Santosa, J. S., & Suyanta. (2020). *Pembuatan Nanopartikel Emas Menggunakan Agen Penudung Natrium Sitrat dan Aplikasinya sebagai Sensor Hipoklorit secara Kolorimetri*.

Samad, A., Gnanasekaran, A., & Kim, Y. J. (2025). Hierarchical network modulation of cell death regulators and inflammatory genes by biogenically engineered gold nanoconstructs with CK in gastric tumorigenesis. *Cancer Nanotechnology*, *16*(1), 15. https://doi.org/10.1186/s12645-025-00310-9

Saputra, E. (2024). A colorimetric detection of creatinine based-on EDTA capped-gold nanoparticles (EDTA-AuNPs): Digital Image Colorimetry. *Sensors International*, *5*, 100286. https://doi.org/10.1016/j.sintl.2024.100286

Saputra, E., Kase, Z. R., Timur, I. A., Frisky, A. Z. K., Wicaksono, N. D. A., Jatmiko, W. A., Munawwaroh, Z., Alimin, Pambudi, F. I., & Santosa, S. J. (2025). A novel method utilizing a portable digital image-based colorimeter detector and a glutathione-functionalized gold nanoparticles (GSH-AuNPs) sensor for the highly selective and sensitive detection of creatinine in urine sample. *Sensors International*, *6*, 100327. https://doi.org/10.1016/j.sintl.2025.100327

Serdar, O., Aydın, A. N., Ölçülü, A., Çimen, I. C. Ç., Ak, T. P., Derman, T., Pala, A., & Yıldırım, N. C. (2025). Determination of the Effect of Nanoparticle Copper on Navicula cryptocephala var. veneta by Biomarkers and Bioaccumulation Amounts. *Turkish Journal of Fisheries and Aquatic Sciences*, *25*(12). https://doi.org/10.4194/TRJFAS25931

Shang, K., Wang, S., Chen, S., & Wang, X. (2022). Sensitivity Detection of Uric Acid and Creatinine in Human Urine Based on Nanoporous Gold. *Biosensors*, *12*(8), 1–14. https://doi.org/10.3390/bios12080588

Tareq, M. S., Hameed, A. K., Saleem, A. H., & Rasheed, B. G. (2025). In vitro antimicrobial activity of silver nanoparticles synthesis by lasers against teeth caries bacteria (S-mutans). *Lasers in Medical Science*, *40*(1), 271. https://doi.org/10.1007/s10103-025-04530-8

Tyagi, H., Kushwaha, A., & Kumar, A. (2011). *pH-Dependent Synthesis Of Stabilized Gold Nanoparticles Using Ascorbic Acid*. *10*, 4–7. https://doi.org/10.1142/S0219581X11009301

Wang, L., Wei, Y., Liu, X., Chen, J., Mao, C., & Jin, B. (2025). Electrochemiluminescence biosensor based on gold nanoparticles modulated cathode AIE-activated metal-organic frameworks for the ultrasensitive detection of CA15-3. *Biosensors and Bioelectronics*, *282*, 117465. https://doi.org/10.1016/j.bios.2025.117465

Yoo, S., Nam, D. H., Singh, T. I., Leem, G., & Lee, S. (2022). Effect of reducing agents on the synthesis of anisotropic gold nanoparticles. *Nano Convergence*, *9*(1), 5. https://doi.org/10.1186/s40580-021-00296-1

Zhao, P., Li, N., & Astruc, D. (2013). State of the art in gold nanoparticle synthesis. *Coordination Chemistry Reviews*, *257*(3–4), 638–665. https://doi.org/10.1016/j.ccr.2012.09.002