



Effect of Citric Acid Addition on the Characteristics of Silver Nanoparticles Synthesized via Green Synthesis Using Spent Coffee Grounds Extract

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ABSTRACT

Spent coffee grounds (SCG) are an abundant coffee-processing waste containing bioactive compounds with potential as natural reducing and stabilizing agents in the green synthesis of silver nanoparticles (AgNPs). This study aimed to optimize the reaction time for AgNP synthesis using methanolic SCG extract and evaluate the effect of citric acid as an additional stabilizing agent. AgNPs were synthesized using methanolic SCG extract and silver nitrate at reaction times of 0, 30, 60, 90, and 120 min. The formation of AgNPs was monitored using UV-Visible spectrophotometry, while particle-size characteristics were evaluated using Particle Size Analyzer (PSA). The characteristic surface plasmon resonance (SPR) band appeared at approximately 424 nm, with the highest absorbance of 1.30 obtained at 60 min. Prolonging the reaction to 90 and 120 min resulted in decreased absorbance, indicating that longer reaction times did not further improve the optical response of the AgNPs. Therefore, 60 min was selected as the optimum reaction time. At the optimum reaction time, 0.1 mM citric acid was added as an additional stabilizing agent. PSA analysis showed that unstabilized AgNPs had D10, D50, and D90 values of 34.48, 65.83, and 125.64 nm, respectively, with a PI of 0.289. After citric acid addition, these values decreased to 30.33, 55.96, and 103.11 nm, respectively, with a PI of 0.255. These results indicate that citric acid improved particle dispersion and reduced the contribution of larger particle populations. The study demonstrates the potential of methanolic SCG extract for green AgNP synthesis and citric acid as an additional stabilizing agent.

Keyword: Spent Coffee Grounds, Silver Nanoparticles, Green Synthesis, Citric Acid, UV-Visible Spectroscopy, Particle Size Analyzer

ABSTRAK

Spent coffee grounds (SCG) merupakan limbah pengolahan kopi yang melimpah dan mengandung senyawa bioaktif yang berpotensi sebagai agen pereduksi dan penstabil alami dalam sintesis hijau nanopartikel perak (AgNPs). Penelitian ini bertujuan mengoptimalkan waktu reaksi sintesis AgNPs menggunakan ekstrak metanol SCG serta mengevaluasi pengaruh asam sitrat sebagai agen penstabil tambahan. AgNPs disintesis menggunakan ekstrak metanol SCG dan perak nitrat pada waktu reaksi 0, 30, 60, 90, dan 120 menit. Pembentukan AgNPs dipantau menggunakan spektrofotometri UV-Visible, sedangkan karakteristik ukuran partikel dievaluasi menggunakan *Particle Size Analyzer* (PSA). Pita *surface plasmon resonance* (SPR) karakteristik muncul pada sekitar 424 nm, dengan absorbansi tertinggi sebesar 1,30 pada waktu reaksi 60 menit. Perpanjangan waktu reaksi hingga 90 dan 120 menit menyebabkan penurunan absorbansi, menunjukkan bahwa waktu reaksi yang lebih lama tidak meningkatkan respons optik AgNPs. Oleh karena itu, waktu 60 menit dipilih sebagai kondisi optimum. Pada kondisi optimum sebanyak 0,1 mM asam sitrat ditambahkan sebagai agen penstabil. Hasil PSA menunjukkan bahwa AgNPs tanpa stabilizer memiliki nilai D10, D50, dan D90 masing-masing sebesar 34,48; 65,83; dan 125,64 nm dengan PI 0,289. Setelah penambahan asam sitrat, nilai tersebut menurun menjadi 30,33; 55,96; dan 103,11 nm dengan PI 0,255. Hasil ini menunjukkan bahwa asam sitrat meningkatkan dispersi partikel dan mengurangi kontribusi populasi partikel berukuran besar. Penelitian ini menunjukkan potensi ekstrak metanol SCG untuk sintesis hijau AgNPs serta asam sitrat sebagai agen penstabil tambahan.



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Kata kunci: *Spent Coffee Grounds*; Nanopartikel Perak; Sintesis Hijau; Asam Sitrat; Spektrofotometri UV-Visible; *Particle Size Analyzer*

1. Introduction

Silver nanoparticles (AgNPs) have attracted considerable attention due to their distinctive physicochemical properties and wide-ranging applications, particularly in antimicrobial materials, catalysis, sensing, and biomedical fields [1,2]. However, conventional synthesis methods commonly employ chemical reducing and stabilizing agents that may pose environmental concerns. Consequently, green synthesis has emerged as a more sustainable alternative by utilizing naturally derived compounds as reducing and stabilizing agents for nanoparticle production [2,3].

Green synthesis utilizes naturally occurring compounds from biological materials as reducing and stabilizing agents in nanoparticle formation. Spent coffee grounds (SCGs) are a promising material for this approach because they retain phenolic and other bioactive compounds capable of participating in the reduction and stabilization of silver nanoparticles [4,5]. Zuurro et al. demonstrated that SCG extracts could effectively reduce Ag^+ ions and produce stable AgNPs with an average particle size of approximately 10 nm under optimized conditions [4]. More recently, Nallusamy et al. further demonstrated the successful synthesis of AgNPs using spent coffee grounds, supporting their potential as a renewable source of reducing and stabilizing compounds for green nanoparticle synthesis [6].

Beyond its role as a reducing and stabilizing agent, the utilization of spent coffee grounds in AgNP synthesis also provides an opportunity to convert coffee waste into value-added functional materials [7,8]. However, the characteristics of SCG-mediated AgNPs are strongly influenced by synthesis conditions. Zuurro et al. [4] demonstrated that pH, temperature, solvent composition, and the silver-to-phenolic ratio significantly affected AgNP formation and particle characteristics. More recently, Kusumahastuti and Gintu [7] reported that variations in synthesis pH influenced the particle size, morphology, distribution, and stability of AgNPs synthesized using brewed coffee grounds extract. These findings highlight the importance of controlling synthesis conditions to obtain AgNPs with desirable characteristics.

One of the major challenges in AgNP synthesis is maintaining colloidal stability, as nanoparticles tend to aggregate due to their high surface energy. Citrate is commonly used as a capping agent because its negatively charged carboxylate groups can contribute to the electrostatic stabilization of AgNPs. However, the stability of citrate-capped AgNPs is strongly influenced by environmental conditions, particularly pH and ionic strength, which can alter surface charge and promote particle aggregation. Boughbina-Portolés et al. [9] demonstrated that changes in the surrounding medium affected the aggregation behavior and stability of citrate-capped AgNPs, while Marciniak et al. [10] showed that pH significantly influenced the size of AgNPs synthesized using citric acid. These findings indicate that the role of citrate in AgNP systems is closely associated with the physicochemical conditions of the synthesis medium.

In addition to its stabilizing function, citrate can also influence AgNP formation by affecting complexation, nucleation, and particle growth [11,12]. Citrate-derived species have also been shown to interact with AgNP surfaces through carboxylate-containing groups, supporting their role in surface capping and nanoparticle stabilization [13]. These functions may complement the reducing and stabilizing activity of bioactive compounds naturally present in SCG extracts [4,5], providing additional control over AgNP formation and stability. However, although SCG extracts have been successfully used for AgNP synthesis [4–8], the functional effect of citric acid addition in SCG-mediated systems remains insufficiently explored. Therefore, this study investigates whether citric acid addition influences the resulting characteristics of SCG-mediated AgNPs.

Based on these considerations, this study aimed to investigate the effect of citric acid addition on the characteristics of silver nanoparticles synthesized through a green synthesis approach using spent coffee grounds extract. Nanoparticles synthesized without citric acid were compared with those synthesized in the presence of citric acid to evaluate differences in their resulting characteristics. The findings of this study are expected to provide further insight into the role of citric acid in SCG-mediated AgNP synthesis and contribute

to the development of a simple and environmentally friendly approach for converting organic waste into value-added nanomaterials.

2. Materials and Method

2.1. Materials and Equipment

The materials used in this study included spent coffee grounds, methanol p.a, silver nitrate (AgNO_3), citric acid (0.1 mM), and distilled water. Spent coffee grounds were used as the source of natural bioactive compounds for the green synthesis of silver nanoparticles. Silver nitrate was used as the silver ion precursor, while citric acid was employed as a stabilizing agent in the treatment group.

The equipment used in this study included an analytical balance, glassware, magnetic stirrer, hot plate, filtration apparatus, rotary evaporator, UV-Visible spectrophotometer (Thermogenesis 150), and Particle Size Analyzer (PSA) (NKT-N9 Nano Particle Sizer). The UV-Visible spectrophotometer was used to monitor the formation of silver nanoparticles based on their characteristic surface plasmon resonance absorption, while the PSA was used to determine particle size and particle size distribution.

2.2. Preparation of Methanolic Extract of Spent Coffee Grounds

Spent coffee grounds were collected from coffee shops, and the coffee used for brewing was Arabica. The collected coffee grounds were first separated from foreign materials and sieved to remove coarse impurities and other unwanted residues. The samples were then dried at room temperature until a constant weight was obtained. The dried coffee grounds were subsequently processed into a fine, homogeneous powder and stored in a dry, sealed container at room temperature until further use. A total of 50 g of SCG powder was macerated with 500 mL of methanol, corresponding to a solid-to-solvent ratio of 1:10 (g/mL) for four days at room temperature. The four-day maceration period was not independently optimized in this study but was adopted from a previously reported methanolic maceration procedure for Arabica coffee by-products Maulana et al. [13], with modifications for spent coffee grounds. During the maceration process, the mixture was stirred every 24 h to facilitate the extraction of bioactive compounds. The use of methanol was adapted from the solvent extraction procedure reported by Mussatto et al., with modifications to the extraction time and maceration conditions [14].

After four days, the mixture was filtered to separate the extract from the solid residue. The resulting filtrate was concentrated using a rotary evaporator to remove the methanol and obtain a concentrated spent coffee grounds extract. The concentrated extract was subsequently used for the synthesis of silver nanoparticles.

2.3. Green Synthesis of Silver Nanoparticles

Silver nanoparticles (AgNPs) were synthesized using methanolic extract of spent coffee grounds as a natural reducing agent following a modified procedure reported by Srikhao [15]. A total of 20 mL of spent coffee grounds extract was added to 180 mL of 3 mM silver nitrate (AgNO_3) solution. The mixture was heated at 70 °C under continuous stirring throughout the reaction. The stirring condition was maintained consistently; however, the stirring speed was not recorded in rpm [16].

The synthesis process was initially evaluated at different reaction times of 0, 30, 60, 90, and 120 min to determine the optimum synthesis time for AgNP formation. At each reaction time, the formation of silver nanoparticles was monitored based on the change in color of the reaction mixture and confirmed by UV-Visible spectrophotometric analysis. The reaction time that produced the most favorable characteristics of AgNP formation was selected as the optimum synthesis time for the subsequent stabilization study.

Using the selected optimum reaction time, AgNPs were then synthesized under two different conditions to evaluate the effect of citric acid addition. The first formulation consisted of 20 mL of spent coffee grounds extract and 180 mL of 3 mM AgNO_3 solution without citric acid, resulting in a total reaction volume of 200 mL. For the second formulation, 20 mL of 0.1 mM citric acid solution was added to the reaction mixture containing 20 mL of spent coffee grounds extract and 180 mL of 3 mM AgNO_3 solution, resulting in a final reaction volume of 220 mL. The volumes of spent coffee grounds extract and AgNO_3 solution, as well as the reaction temperature, stirring conditions, and reaction time, were maintained unchanged between the two formulations [17].

All other synthesis parameters, including AgNO_3 concentration, extract volume, reaction temperature, stirring conditions, and reaction time, were maintained constant. The AgNPs synthesized with and without citric acid were subsequently compared to evaluate the effect of citric acid addition on the stability and characteristics of the resulting silver nanoparticles.

2.4. Characterization of Silver Nanoparticles

The formation of silver nanoparticles was confirmed using UV-Visible spectrophotometry. The synthesized AgNP suspensions were analyzed within the wavelength range associated with the surface plasmon resonance of silver nanoparticles. The appearance of an absorption peak within approximately 395–510 nm was used as an indication of AgNP formation.

Particle size and particle size distribution were determined using a Particle Size Analyzer (PSA). PSA measurement was performed once for each sample; therefore, the obtained particle-size data were considered preliminary comparative data. The results were compared to evaluate the effect of citric acid addition on the particle characteristics of the synthesized AgNPs.

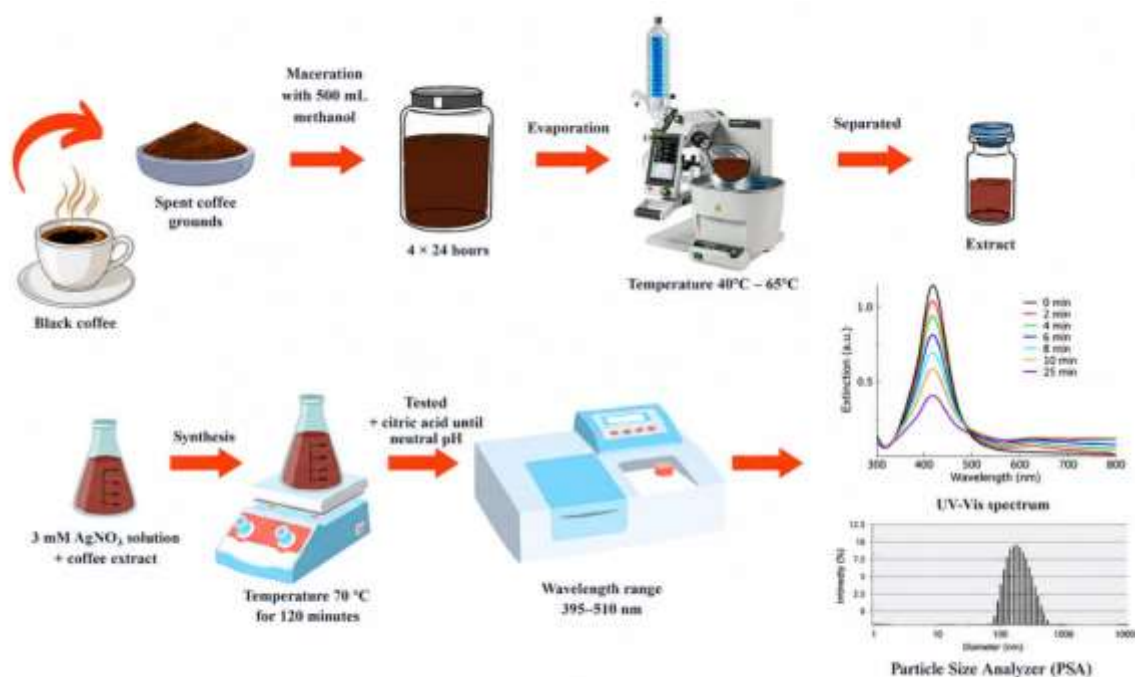


Figure 1. Schematic illustration of the preparation of methanolic spent coffee grounds extract, green synthesis of silver nanoparticles (AgNPs), and their characterization using UV-Visible spectrophotometry and particle size analysis.

3. Results and Discussion

3.1. Optimization of Silver Nanoparticle Synthesis Time

The formation of AgNPs at different reaction times was monitored using UV-Visible spectrophotometry in the wavelength range of 300–700 nm. UV-Visible spectroscopy is widely used as an initial characterization technique for AgNPs because silver nanoparticles exhibit a characteristic surface plasmon resonance (SPR) absorption band in the visible region. The SPR phenomenon originates from the collective oscillation of conduction electrons at the nanoparticle surface upon interaction with electromagnetic radiation. The intensity and position of the SPR band can be influenced by nanoparticle concentration, size, morphology, surface chemistry, aggregation state, and interparticle interactions. Therefore, changes in the UV-Visible spectra can be used to monitor the progress of AgNP formation and changes in their optical characteristics [18].

As shown in Figure 2, the spectrum at 0 min did not exhibit a distinct absorption maximum characteristic of AgNPs, suggesting that substantial formation of AgNPs had not yet occurred. After 30 min, an absorption band began to appear in the visible region, with an absorbance of approximately 0.79 at around 424 nm. The appearance of this characteristic SPR band provides evidence that the reduction of Ag^+ to Ag^0 and subsequent formation of AgNPs had begun. Similar observations have been reported for green synthesis systems, in which

the development of an SPR band accompanies AgNP formation following the interaction of a silver precursor with a biological extract[15,19].

The SPR intensity increased from 30 to 60 min, suggesting that the synthesis process continued to progress with reaction time. At 60 min, the spectrum exhibited the strongest SPR response, with an absorbance of approximately 1.30 and a λ_{max} of approximately 424 nm. The pronounced SPR band is consistent with substantial AgNP formation under the investigated reaction conditions. However, the higher absorbance should not be interpreted solely as a direct increase in the amount or concentration of AgNPs, because SPR intensity can also be influenced by particle size, morphology, aggregation state, surface characteristics, and dispersion conditions. Therefore, the spectrum obtained at 60 min is more appropriately interpreted as representing the most favorable optical response among the reaction times investigated.

The formation of AgNPs can be attributed to the reducing capacity of compounds present in the methanolic SCG extract. Phenolic compounds and other antioxidant constituents can donate electrons to Ag^+ ions, resulting in the formation of Ag^0 atoms. These atoms subsequently undergo nucleation and growth to form silver nanoparticles. Yust et al. [5] demonstrated that SCG extracts possess sufficient reducing activity to synthesize silver and gold nanoparticles and associated this capability with the antioxidant and caffeoylquinic acid content of the extracts. Similarly, Chien et al. [20] reported that SCG contains phenolic and other bioactive compounds that can participate in the reduction and surface interaction processes involved in AgNP synthesis.

The strongest SPR response observed at 60 min suggests that this reaction time provided favorable conditions for AgNP formation under the experimental conditions employed. At shorter reaction times, the reduction and nucleation processes may not have progressed to the same extent, resulting in a weaker SPR response. As the reaction proceeded, Ag^+ reduction, nucleation, particle growth, and changes in the dispersion state of the nanoparticles could collectively contribute to the observed changes in SPR intensity. A similar time-dependent increase in SPR intensity was reported by Liaqat et al. [21] who observed progressive changes in the SPR response with increasing reaction time and identified 60 min as the optimum reaction time. More recently, Ünüvar et al. [22] reported that the nucleation and growth of AgNPs were completed within approximately 60 min, as indicated by a gradual increase in SPR intensity up to 60 min followed by no substantial change between 60 and 90 min. These findings support the selection of 60 min in the present study as the reaction time that produced the most favorable SPR response.

After 60 min, the SPR intensity decreased slightly at 90 min, reaching an absorbance of approximately 1.25, while the λ_{max} remained around 424 nm. The relatively small change in λ_{max} suggests that the general optical characteristics of the AgNP system remained similar. However, the decrease in absorbance indicates that extending the reaction time did not further improve the SPR response. This change should not necessarily be interpreted as a decrease in the amount of AgNPs, since variations in SPR intensity may also result from changes in particle size, aggregation state, surface properties, or dispersion characteristics.

A more pronounced decrease in absorbance was observed at 120 min, with an absorbance of approximately 1.02 in the SPR region. Prolonging the reaction beyond the selected reaction time may alter particle growth and particle-particle interactions, potentially affecting the optical characteristics of the suspension [15]. Once substantial nucleation has occurred, further reaction may favor changes in existing particles rather than the formation of new nuclei. Consequently, changes in particle size, aggregation state, or dispersion may contribute to the decrease in SPR intensity observed at longer reaction times. Thus, the lower absorbance at 120 min is more appropriately interpreted as a change in the optical response of the AgNP dispersion rather than direct evidence of reduced nanoparticle formation.

The role of SCG extract in the surface characteristics of the synthesized nanoparticles should also be considered. In addition to reducing Ag^+ ions, bioactive compounds present in coffee waste extracts may interact with the surface of newly formed AgNPs and potentially contribute to surface capping. Several compounds, including phenolic compounds, tannins, flavonoids, and other organic constituents, have been reported to participate in the reduction and surface interaction processes during green synthesis of AgNPs [23]. For SCG specifically, Zuorro et al. [4] demonstrated that SCG extracts can function as reducing and capping sources during AgNP synthesis. The use of spent coffee grounds as a reducing source and surface-associated matrix has also been reported in the preparation of AgNP-SCG materials [20]. However, the extent of colloidal stabilization provided by SCG extract cannot be established from UV-Visible measurements alone.

Overall, the UV–Visible spectra demonstrate that reaction time influenced the SPR characteristics and optical response of AgNPs synthesized using methanolic SCG extract. The characteristic SPR response was absent at 0 min, became evident at 30 min, reached its highest intensity at 60 min, and subsequently decreased at 90 and 120 min. The strongest SPR response, with an absorbance of approximately 1.30 and a λ_{max} of approximately 424 nm, was observed at 60 min. Therefore, 60 min was selected as the optimum reaction time based on the most favorable SPR response under the experimental conditions investigated, rather than solely on the assumption of a higher AgNP concentration. This reaction time was subsequently used for the comparative preparation of AgNPs with and without citric acid to evaluate the effect of citric acid addition on the particle-size distribution and dispersion characteristics of the synthesized AgNPs.

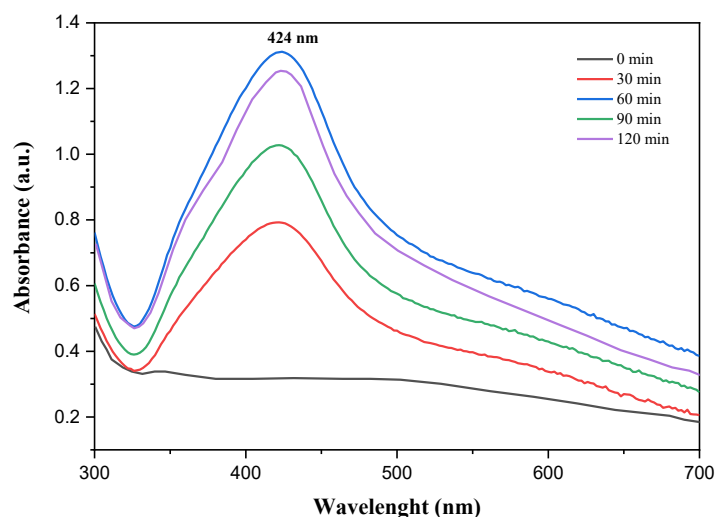
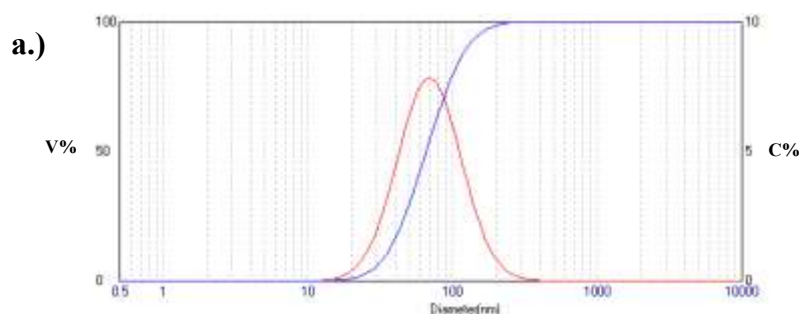


Figure 2. UV-Vis absorption spectra of AgNPs synthesized using methanolic spent coffee grounds extract at different reaction times

3.2 Particle Size Analyzer (PSA) Analysis

Particle size distribution of the synthesized AgNPs was evaluated using a Particle Size Analyzer (PSA) to investigate the effect of citric acid addition on the particle-size distribution. As shown in Figure 3, both AgNPs without citric acid and those containing citric acid exhibited relatively similar unimodal distribution profiles, although the latter showed a shift toward smaller particle sizes. AgNPs without citric acid exhibited D10, D50, and D90 values of 34.48, 65.83, and 125.64 nm, respectively, with a peak particle size of approximately 65.50 nm and a PI of 0.289. Following citric acid addition, the D10, D50, and D90 values decreased to 30.33, 55.96, and 103.11 nm, respectively, while the peak particle size decreased to 55.69 nm and the PI to 0.255. These results indicate a shift in the measured particle-size distribution toward smaller sizes and a reduced contribution of larger particle populations.



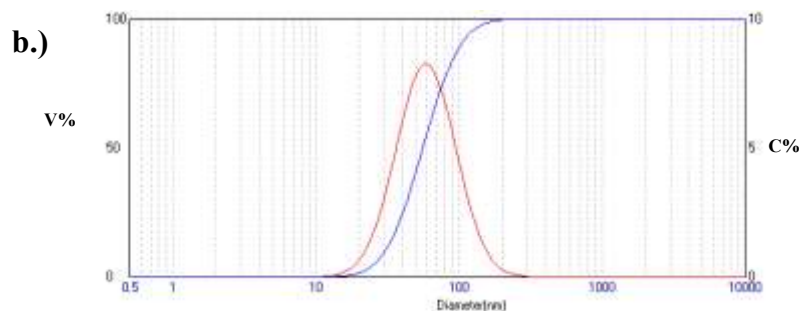


Figure 3. Particle size distribution of (a) AgNPs and (b) citric acid-stabilized AgNPs.

The observed changes may be associated with the interaction of citric acid with the AgNP surface. Citric acid contains carboxyl groups that can interact with the nanoparticle surface, while negatively charged carboxylate groups may promote electrostatic repulsion between particles and thereby limit close particle-particle interactions [24]. Accordingly, the lower D50 and D90 values and the decrease in PI observed after citric acid addition suggest a narrower particle-size distribution and fewer larger dispersed entities. These observations are consistent with a potential capping role of citric acid; however, they do not by themselves establish long-term colloidal stability.

The methanolic SCG extract may also contribute to the surface characteristics of the synthesized AgNPs because organic and bioactive compounds present in the extract can participate in Ag^+ reduction and may interact with the nanoparticle surface [4]. The additional presence of citric acid may therefore modify the dispersed state of the nanoparticles, as reflected by the observed changes in the PSA profile. This finding suggests that combining SCG extract with citric acid influences the particle-size distribution of the resulting AgNP suspension.

It should be noted that PSA measures the size of particles or dispersed entities in the measurement medium and can be influenced by aggregation, agglomeration, surface coating, and dispersion conditions. Therefore, the decrease in PSA-derived particle sizes should not be interpreted as direct evidence of a reduction in the primary crystalline size of the AgNPs. Rather, the results indicate a reduction in the apparent particle or aggregate size and a narrower particle-size distribution following citric acid addition. Further evaluation using zeta-potential measurements and time-dependent colloidal stability studies would be required to confirm the stabilizing effect of citric acid on the synthesized AgNPs.

4. Conclusion

Silver nanoparticles were successfully synthesized using methanolic spent coffee grounds (SCG) extract as a natural reducing source. Reaction time significantly influenced the optical characteristics of the synthesized AgNPs. A characteristic SPR band was observed at approximately 424 nm, with the strongest SPR response (absorbance ≈ 1.30) obtained after 60 min of reaction. The subsequent decrease in absorbance at 90 and 120 min suggests changes in the optical and dispersion characteristics of the nanoparticles; therefore, 60 min was selected as the optimum reaction time under the investigated conditions. The addition of citric acid influenced the particle-size distribution of the synthesized AgNPs. The AgNPs without citric acid exhibited D10, D50, and D90 values of 34.48, 65.83, and 125.64 nm, respectively, with a PI of 0.289, whereas the addition of citric acid decreased these values to 30.33, 55.96, and 103.11 nm, respectively, with a PI of 0.255. These changes indicate a narrower particle-size distribution and a reduced contribution of larger particle populations or aggregates in the citric acid-containing system. Thus, under the investigated conditions, citric acid showed a beneficial effect on the particle-size distribution and dispersion characteristics of SCG-mediated AgNPs. However, further studies using zeta-potential measurements and time-dependent colloidal stability evaluation are required to confirm its long-term stabilizing effect. Overall, the findings demonstrate the potential of combining SCG extract with citric acid as a promising approach for producing AgNPs with improved particle-size distribution.

5. Conflicts of Interest

Authors declare no conflicts of interest.

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