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Silver Nanoparticles Capped with *p*-Hydroxybenzoic Acid for Colorimetric Determination of Cu(II)

Dewi Puspita, Gusrizal Gusrizal*, Endah Sayekti

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura, Pontianak 78124, Indonesia

*Corresponding author email: gusrizal@chemistry.untan.ac.id

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ABSTRACT. Silver nanoparticles (AgNPs) have attracted significant attention from researchers and are widely used in the development of chemical sensors due to their unique surface plasmon resonance properties. This study focuses on a colorimetric method for determining Cu(II) using AgNPs capped with *p*-hydroxybenzoic acid. The AgNPs were synthesized through a chemical reduction process, using *p*-hydroxybenzoic acid as both a reducing and capping agent. The resulting AgNPs exhibited a yellow color and displayed a characteristic absorption peak at 420 nm, with an average particle size of about 60 nm. These nanoparticles were then used for the colorimetric detection of Cu(II) in the presence of ethylenediaminetetraacetic acid (EDTA), which serves as a ligand. The addition of Cu(II) to the mixture of AgNPs and EDTA induced nanoparticle aggregation, leading to a decrease in absorption intensity at 420 nm and the emergence of a new peak at 680 nm. A linear correlation was established between the concentration of Cu(II) and the absorbance ratio at 680 nm to 420 nm. The limits of detection and quantitation for determining Cu(II) were found to be 0.4×10^{-3} M and 1.4×10^{-3} M, respectively, with a recovery rate of 104%. These results demonstrate that AgNPs capped with *p*-hydroxybenzoic acid, combined with EDTA, can provide a simple colorimetric determination of Cu(II) and offer insight into ligand-mediated aggregation behavior in AgNPs-based sensing systems.

Keywords: colorimetry; copper; hydroxybenzoic acid; nanoparticles; silver; water

INTRODUCTION

Copper is widely used in various industrial applications due to its excellent electrical and thermal conductivity. However, industrial activities may release waste containing copper into the environment, particularly into aquatic systems. Prolonged exposure to copper can adversely affect growth, reproduction, brain function, enzyme activity, blood chemistry, and metabolism in living organisms (Rehman et al., 2019).

Metal pollution in the environment, especially in aquatic systems, has become a significant global concern due to its harmful effects on human health and the ecosystem. Continuous monitoring of metal concentrations is crucial for detecting their presence in water bodies and for developing effective strategies to address metal pollution. Currently, the most common methods for detecting metals include atomic absorption spectrophotometry (AAS), X-ray fluorescence (XRF), and inductively coupled plasma-optical emission spectrometry (ICP-OES). However, these methods can be expensive due to the high costs of the instruments and the need for complex sample preparation and skilled technical expertise. Therefore, it is important to develop alternative analytical methods to overcome these challenges and reduce the costs associated with instrumentation.

Colorimetric methods that utilize nanoparticles have emerged as a promising alternative to expensive instrumentation. Silver nanoparticles (AgNPs) have garnered significant attention due to their potential as chemical sensors. AgNPs exhibit properties that can be harnessed for the development of analytical methods, particularly through the phenomenon known as surface plasmon resonance (SPR). SPR occurs when light interacts with the conduction electrons of metal nanoparticles, resulting in a spectrally observed absorption peak in the visible region. Typically, the characteristic absorption peak of AgNPs appears around 400 nm, although it can vary depending on the size of the particles (Ajitha et al., 2016; Frost et al., 2017).

AgNPs have been used to develop analytical methods for detecting various metal ions. Several studies have reported colorimetric detection of metal ions such as Cd(II) (Tamilselvan et al., 2022), Pb(II) (Samuel & Rao, 2023), and Fe(III) (Bellingeri et al., 2024) using AgNPs. The principle behind the colorimetric detection of metal ions with AgNPs relies on the aggregation of the nanoparticles when the metal ion is introduced. This aggregation results in changes to the SPR spectra, with variations in the absorption peaks corresponding to the concentration of the analyte. The aggregation of

AgNPs induced by analytes can be triggered by modifying the nanoparticles surface with ligands. When ligands are bound to the surface of AgNPs, they form complexes with metal ions, promoting the aggregation of the nanoparticles. Aside from enhancing aggregation, the ligands also improve the selectivity of the AgNPs. AgNPs synthesized by reducing silver ions using sodium borohydride show different responses depending on the functionalizing agent used. For example, AgNPs modified with tartaric acid exhibit selectivity toward Cr(III) and Cr(VI) (Shrivas et al., 2016), while those modified with 5-sulfosalicylic acid demonstrate selectivity for Cd(II) (Jin et al., 2015).

Several studies reported that AgNPs have been used for Cu(II) colorimetric detection using different methods for AgNPs synthesis and sensing platforms. For example, AgNPs were synthesized by reducing silver ions with sodium borohydride and then functionalized with humic acid. Humic acid served as a functional group that interacted directly with Cu(II) ions, leading to aggregation (Lopez et al., 2020). In another study, AgNPs were synthesized using carrageenan as a reducing and capping agent. Detection of Cu(II) was attributed to interactions between Cu(II) and the sulfate and hydroxyl groups present in carrageenan (Wang et al., 2020).

AgNPs functionalized with homocysteine and dithiothreitol were used for colorimetric sensing of Cu(II) by paper-based analytical devices (Ratnarathorn et al., 2012). More recently, AgNPs were synthesized using sodium borohydride as the reducing agent and trisodium citrate as the capping agent. Chitosan served as a functionalizing agent. These AgNPs were utilized to create a paper-based sensor integrated with smartphone detection, highlighting the development of a portable sensing platform rather than focusing on the mechanism of nanoparticle aggregation in solution (Gomeceria et al., 2024).

Despite extensive development of colorimetric methods based on AgNPs, challenges remain in understanding how different capping agents and ligands influence nanoparticle stability and aggregation behavior during sensing processes. In particular, the interaction between ligand and nanoparticle aggregation has not been fully explored for AgNPs capped with *p*-hydroxybenzoic acid. Our previous studies have reported the synthesis, characterization, and stability of AgNPs capped with *p*-hydroxybenzoic acid and their applications in the determination of organic analytes, such as paraquat (Gusrizal et al., 2018, 2020). However, their potential for metal ion detection via ligand-assisted aggregation mechanisms has not yet been investigated.

In this study, we explore the use of AgNPs capped with *p*-hydroxybenzoic acid in combination with EDTA as a coordinating ligand to induce aggregation for colorimetric detection of Cu(II). EDTA is known to form stable complexes with Cu(II) due to the presence of multiple donor atoms capable of coordinating with the metal ion, consistent with Pearson's hard soft acid base (HSAB) concept (Pearson, 1968). The formation of Cu(II)–EDTA complexes is expected to influence the interparticle interactions of the AgNPs, leading to aggregation and a corresponding optical response. Therefore, this study investigates the optical response, aggregation behavior, and analytical performance of AgNPs capped with *p*-hydroxybenzoic acid toward Cu(II), providing insight into the role of ligand-mediated interactions in the aggregation-based detection mechanisms of these AgNPs. The findings contribute to a better understanding of ligand-mediated aggregation processes in AgNPs capped with *p*-hydroxybenzoic acid for metal ion detection.

EXPERIMENTAL SECTIONS

Materials

The materials used in this study included *p*-hydroxybenzoic acid (Merck), silver nitrate (Merck), sodium hydroxide (Merck), copper nitrate (Merck), and distilled water.

Instruments

The instruments employed in this study comprised standard laboratory glassware, a hotplate, a magnetic stirrer, an analytical balance, a pH meter, and a UV-Visible spectrophotometer (Shimadzu 1280). The size and surface charge of the nanoparticles were measured using a particle size analyzer (Horiba Scientific SZ-100)

Experimental Procedure

Synthesis of AgNPs Capped with p-hydroxybenzoic Acid

AgNPs were synthesized by reducing Ag^+ ions to Ag^0. A total of 5 mL of 2×10^{-4} M silver nitrate solution was added to 5 mL of *p*-hydroxybenzoic acid solution with a concentration of 4×10^{-3} M, whose pH had been adjusted to 11 by adding sodium hydroxide solution. The mixture was then heated in a boiling water bath for approximately 30 minutes until the solution changed from colorless to yellow, after which it was cooled and stored at room temperature (Gusrizal et al., 2018).

Characterization of AgNPs Capped with p-hydroxybenzoic Acid

The synthesized AgNPs were analyzed using a UV-Visible spectrophotometer in the wavelength range of 300-800 nm to determine the absorption pattern and the position of the absorption peak of the nanoparticles. The nanoparticle size and surface charge were measured using a particle size analyzer.

Detection of Cu(II) Using AgNPs Capped with p-hydroxybenzoic Acid

The solution of Cu(II) was added to a mixture of AgNPs and EDTA. The volume ratio of Cu(II), AgNPs, and EDTA was 1:8:1. The resulting color change in the solution was then measured using a UV-Visible

spectrophotometer. Several performance parameters of the method, including linearity, limit of detection (LoD), limit of quantification (LoQ), precision, and accuracy, were subsequently determined.

RESULTS AND DISCUSSION

Synthesis of AgNPs Capped with *p*-hydroxybenzoic Acid

AgNPs capped with *p*-hydroxybenzoic acid were synthesized using a chemical bottom-up approach. This was achieved by reducing silver nitrate with *p*-hydroxybenzoic acid at a pH of 11, which was adjusted by adding sodium hydroxide solution. In this process, silver nitrate served as the silver precursor, while *p*-hydroxybenzoic acid functioned both as a reducing agent and a capping agent. During the reaction, the combination of silver nitrate and *p*-hydroxybenzoic acid produced a yellow-colored solution, indicating the successful formation of AgNPs. This yellow coloration is due to the SPR phenomenon, which occurs when free electrons on the metal surface polarize and oscillate collectively at a specific frequency in response to electromagnetic radiation. Resonance occurs when the oscillation frequency of these electrons matches the frequency of the incoming electromagnetic radiation, resulting in light absorption. The SPR phenomenon can also be confirmed through UV-Visible spectrophotometric measurements, which typically show a distinct absorption peak (Alzoubi et al., 2023).

The synthesis of AgNPs using *p*-hydroxybenzoic acid has been previously documented (Gusrizal et al., 2018). It is known that the pH level of *p*-hydroxybenzoic acid significantly affects the nanoparticle formation process, with an optimal pH identified as 11. In basic conditions, sodium hydroxide ionizes *p*-hydroxybenzoic acid and facilitates the formation of silver oxide, which then hydrolyzes to produce silver ions. Therefore, silver oxide serves as the source of silver ions, and the concentration of available silver ions for reduction depends on the concentration of the base or the pH of the solution.

Characterization of AgNPs Capped with *p*-hydroxybenzoic Acid

The successful synthesis of AgNPs capped with *p*-hydroxybenzoic acid was confirmed through UV-Visible spectrophotometry and dynamic light scattering (DLS) measurements. These methods were used to determine the particle size and surface charge. UV-Visible spectrophotometry provided insights into the SPR spectral pattern and the maximum absorption wavelength, as illustrated in **Figure 1**. The synthesized AgNPs exhibited a maximum absorption peak at 420 nm, which is consistent with findings from previous studies (Gusrizal et al., 2018).

The maximum absorption peak of AgNPs typically occurs around 400 nm, although this can vary based on the shape and size of the nanoparticles. These characteristics are influenced by the reducing and capping agents used during the synthesis process (Rycenga et al., 2011). Even when using the same reducing agent, employing different capping agents can result in variations in the absorption peak position. AgNPs synthesized with sodium borohydride as the reducing agent and polyvinyl alcohol, polyvinylpyrrolidone, ethylenediaminetetraacetic acid (EDTA), and polyethylene glycol as capping agents exhibited absorption peaks at 425, 431, 438, and 445 nm, respectively (Ajitha et al., 2016).

DLS analysis offers insights into the average size and size distribution of AgNPs. The size distribution illustrated in **Figure 2** indicates an average particle size of 60 nm, with a polydispersity index (PDI) of 0.369. Based on this PDI value, the dispersion of the nanoparticles is classified as moderately uniform (Bhattacharjee, 2016).

Another important characteristic of nanoparticles is their zeta potential, which indicates the surface charge in a colloidal medium. The zeta potential reflects the electrostatic interactions between dispersed particles and indicates their electrostatic stability. In the case of AgNPs synthesized with *p*-hydroxybenzoic acid, a zeta potential of -43.8 mV was observed, as illustrated in **Figure 3**. This value suggests a high level

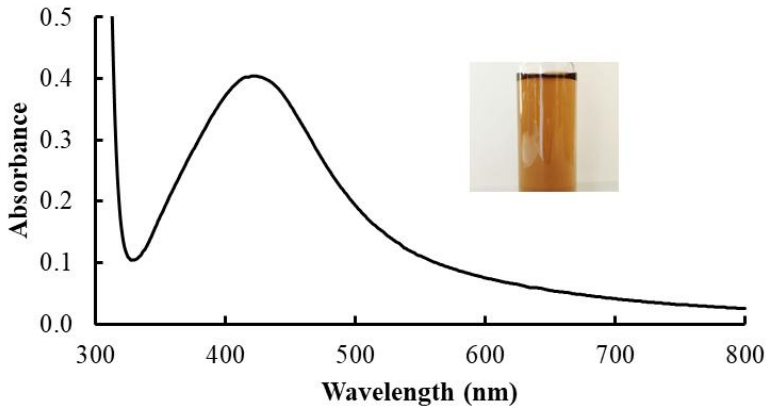

Figure 1. SPR spectra of AgNPs capped with *p*-hydroxybenzoic acid

of electrostatic stability (Bhattacharjee, 2016; Pochapski et al., 2021), which aligns with previous studies that have demonstrated the high storage stability of these nanoparticles (Gusrizal et al., 2018). The negative zeta potential indicates that the AgNPs have a negative surface charge in aqueous dispersions. This charge originates from the *p*-hydroxybenzoic acid molecules that cap the nanoparticle surface. Hydroxybenzoic acid contains carboxylate and hydroxyl groups that serve as reducing and capping agents for the AgNPs (Gusrizal et al., 2018).

The reducing and capping agents used in AgNPs synthesis significantly influence the nanoparticle characteristics (Ajitha et al., 2016). **Table 1** summarizes various properties of silver nanoparticles synthesized with different reducing and capping agents.

Detection of Cu(II) Using AgNPs Capped with *p*-Hydroxybenzoic Acid

The synthesized AgNPs were used to detect Cu(II) in solution. The detection principle relies on the aggregation of AgNPs induced by the presence of metal-ion analytes. To initiate this aggregation, the AgNPs were combined with an EDTA ligand. At a concentration of 1×10^{-3} M, the addition of EDTA did not cause any changes in color or alterations in the SPR spectra of the AgNPs, as shown in Figure 4a. Similarly, when 1×10^{-3} M Cu(II) was added to the AgNPs, there were no significant effects on the SPR spectra, as illustrated in Figure 4b. However, when 1×10^{-2} M Cu(II) was introduced into a mixture of AgNPs and 1×10^{-3} M EDTA, a notable color change to grey occurred, along with a significant alteration in the SPR spectra, as depicted in **Figure 4c**. The absorption intensity at 420 nm decreased, while a new peak emerged at 680 nm. These changes in peak intensity suggest that the addition of EDTA enhances the responsiveness of AgNPs to Cu(II).

AgNPs aggregation occurs due to the formation of complexes between Cu(II) and EDTA ligands that are attached to the surface of the AgNPs. This aggregation can be monitored by observing changes in the SPR spectra. The appearance of an absorption peak at 680 nm indicates the presence of larger aggregated particles (Gusrizal et al., 2020). **Figure 5** illustrates the aggregation mechanism of nanoparticles driven by the interactions between AgNPs capped with *p*-hydroxybenzoic acid and Cu(II) mediated by EDTA.

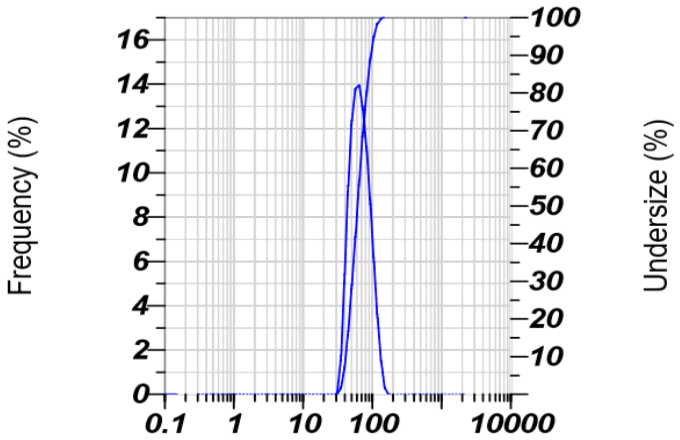

Figure 2. Size distribution of AgNPs capped with *p*-hydroxybenzoic acid

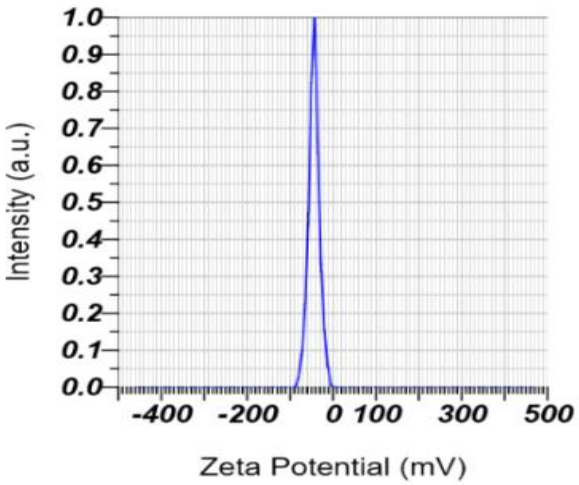

Figure 3. Zeta potential of AgNPs capped with *p*-hydroxybenzoic acid

Table 1. Characteristics of AgNPs synthesized with various reducing and capping agents

Reducing agent/ Capping agent	Particle Size (nm)	PDI	Zeta potential (mV)	Reference
Carrageenan	10	-	-54,4	(Wang et al., 2020)
Humic Acid	101,4	0,447	-	(Lopez et al., 2020)
Soursop Leaf Extract/PVP	71,5	0,364	-	(Pratiwi et al., 2024)
p-hidroksibenzoic acid	60	0,369	-43,8	This study

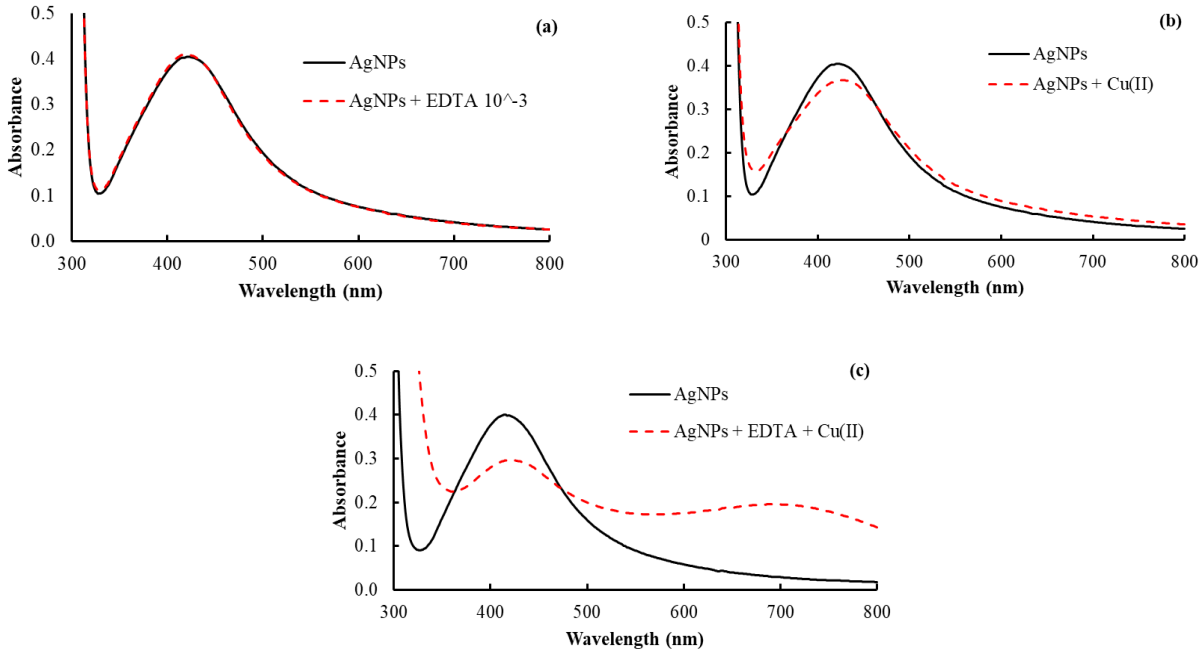

Figure 4. Effect of the Cu(II) addition to the spectra of AgNPs capped with *p*-hydroxybenzoic acid

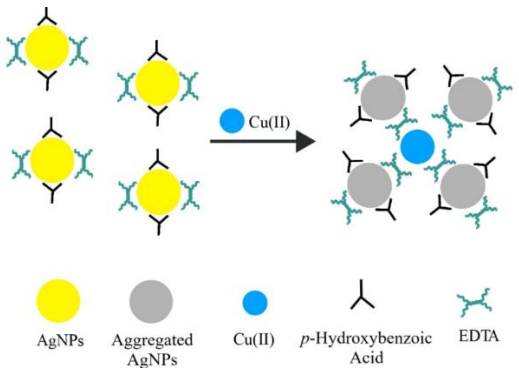

Figure 5. Proposed model for the interaction of Cu(II) and AgNPs capped with *p*-hydroxybenzoic acid

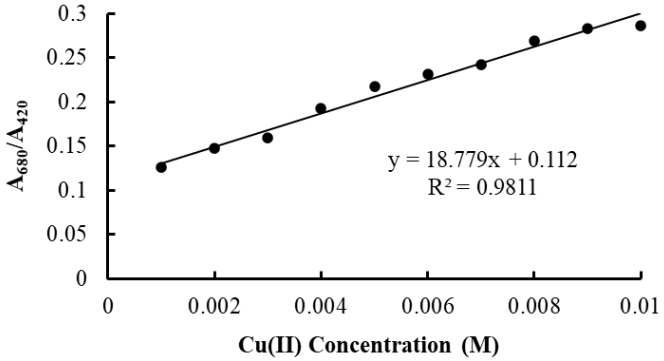

Figure 6. Correlation between Cu(II) concentration and the ratio of absorbance at 680 and 420 nm

The colorimetric sensing mechanism proposed in this study is based on the aggregation behavior of AgNPs capped with *p*-hydroxybenzoic acid in the presence of Cu(II) and EDTA. The synthesized AgNPs are stabilized by *p*-hydroxybenzoic acid molecules adsorbed on the nanoparticle surface. The presence of carboxylate groups in *p*-hydroxybenzoic acid provides electrostatic stabilization, as reflected in the relatively high negative zeta potential of the nanoparticles. As a result, the nanoparticles remain well dispersed in solution and exhibit a characteristic SPR absorption band around 420 nm.

When EDTA and Cu(II) ions are introduced into AgNPs, EDTA forms stable coordination complexes with Cu(II). Cu(II) is classified as a borderline acid and can form complexes with donor atoms, such as oxygen and nitrogen, in EDTA. This concept is consistent with previous studies, which reported that the oxygen and nitrogen atoms from polyvinylpyrrolidone (PVP) can also form complexes with Cu(II) (Maiti et al., 2016; Xu et al., 2020). Additionally, the interaction between AgNPs and Cu(II)-EDTA likely involves electrostatic interactions, specifically with the oxygen donor atoms. An electrostatic attraction arises between the electropositive core of the AgNPs and the electron-rich (electronegative) oxygen atoms. This interaction reduces electrostatic repulsion between nanoparticles and promotes interparticle association. This process leads to nanoparticle aggregation, resulting in plasmon coupling between adjacent nanoparticles. Consequently, a new absorption band appears at longer wavelengths (680 nm) and the solution color changes from yellow to grey.

The observed spectral shift and color change are therefore attributed to aggregation-induced optical changes of the silver nanoparticles. Although additional characterization techniques such as TEM imaging or post-reaction zeta potential measurements would provide further confirmation, the proposed mechanism is consistent with the UV–Vis spectral behavior observed in this study and with previously reported aggregation-based nanoparticle sensing systems (Samuel & Rao, 2023; Tamilselvan et al., 2022).

The effectiveness of AgNPs capped with *p*-hydroxybenzoic acid for the colorimetric detection of Cu(II) was evaluated using linearity, limit of detection (LoD), limit of quantification (LoQ), precision, and accuracy.

Linearity Test

Linearity was evaluated by measuring the absorbance of mixtures containing AgNPs, 1×10^{-3} M EDTA, and Cu(II), with concentrations ranging from 1×10^{-3} to 1×10^{-2} M. A curve was created by plotting the concentration of Cu(II) against the absorbance ratio at wavelengths of 680 nm and 420 nm. **Figure 6** demonstrates a linear relationship between Cu(II) concentration and the absorbance ratio at these wavelengths. The linear equation derived from the data is $y = 18.779x + 0.112$, with a coefficient of determination (R^2) of 0.9811 and a correlation coefficient (r) of 0.9905. These results confirm a linear relationship over the Cu(II) concentration range of 1×10^{-3} to 1×10^{-2} M. Further optimization of AgNPs and ligand concentration may potentially broaden the analytical range.

Determination of LoD and LoQ

LoD and LoQ were determined by repeatedly measuring the absorbance of a mixture containing AgNPs, EDTA, and a blank (distilled water) at wavelengths of 420 nm and 680 nm. The calculated LoD and LoQ values were 0.4×10^{-3} M and 1.4×10^{-3} M, respectively. A comparison of the performance of *p*-hydroxybenzoic acid-capped AgNPs with other silver nanoparticle-based methods for detecting Cu(II) is presented in **Table 2**. This comparison indicates that the developed method demonstrates a linear range comparable to other studies (within the millimolar concentration scale),

Table 2. Performance of various AgNPs in Cu(II) detection

AgNPs	Linear range (M)	Limit of Detection (M)	Reference
AgNPs were synthesized with carrageenan as a reducing and capping agent	$2.5\times10^{-6} – 1.0\times10^{-3}$	1.7×10^{-6}	(Wang et al., 2020)
AgNPs were synthesized with humic acids as a reducing and capping agent	$0.0 – 1.25\times10^{-3}$	6.99×10^{-5}	(Lopez et al., 2020)
AgNPs were synthesized with $NaBH_4$ as a reducing agent and trisodium citrate as a capping agent	$2.0\times10^{-3} – 2.8\times10^{-2}$	1.49×10^{-3}	(Gomeceria et al., 2024)
AgNPs were synthesized with *p*-hydroxybenzoic acid as a reducing and capping agent, and EDTA as a ligand	$1.0\times10^{-3} – 1.0\times10^{-2}$	0.4×10^{-3}	This study

while the LoD and LoQ values are within the parts per million scale. The differences in the LoD and LoQ between our experimental results and previously published data are likely due to variations in the ligands and reducing agents used to synthesize the AgNPs. These variations can impact the size distribution and surface characteristics of the AgNPs, which in turn affect plasmon sensitivity.

Precision Test

Precision, which refers to the repeatability of an analytical method under the same conditions (including operator, equipment, time, and location), was evaluated by repeatedly measuring mixtures containing AgNPs, 1×10^{-3} M EDTA, and Cu(II) at concentrations of 1×10^{-3} M and 1×10^{-2} M. Precision was expressed as the relative standard deviation (%RSD). For the 1×10^{-3} M Cu(II) concentration, the %RSD was 4.08%, which is lower than two-thirds of the Horwitz coefficient of variation (CV) of 26.72. Similarly, the %RSD for the 1×10^{-2} M concentration of Cu(II) was 5.47%, also below two-thirds of the Horwitz CV (21.47). Therefore, the precision test met the acceptance criteria, indicating good repeatability (Riyanto, 2014).

Accuracy Test

The accuracy of the proposed method was assessed by calculating the percentage recovery of standard solutions (1×10^{-2} M) spiked into a real sample collected from a small canal in a peatland area. Since the sample was collected from a peatland, the primary matrix component is naturally organic matter. The recovery value obtained was 104%, indicating that the colorimetric detection method for Cu(II) using AgNPs capped with *p*-hydroxybenzoic acid and EDTA addition demonstrates good accuracy. This result is considered acceptable, as the deviation is below 5% (Riyanto, 2014). However, recovery rates may vary for different real samples due to differences in the sample matrix.

While the promising colorimetric response observed in this study is noteworthy, several limitations must be acknowledged. The detection limit achieved in this work is relatively high compared to the requirements for trace levels of Cu(II) in environmental monitoring. Additionally, the linear detection range spans approximately one order of magnitude, which may restrict its applicability for quantitative analysis across broader concentration ranges. Another significant limitation is the lack of a systematic interference study involving competing metal ions such as Zn^{2+}, Fe^{3+}, Pb^{2+}, Cd^{2+}, and Hg^{2+}. Furthermore, the aggregation mechanism proposed here is mainly inferred from UV-Vis spectral changes and considerations of coordination chemistry. Stronger mechanistic confirmation would benefit from additional characterization techniques, such as TEM imaging of the aggregated nanoparticles, FTIR analysis, or zeta potential measurements after the addition of Cu(II). Future studies should focus on improving analytical sensitivity, evaluating selectivity against competing ions, and further elucidating the aggregation mechanism through complementary characterization techniques.

CONCLUSIONS

AgNPs capped with *p*-hydroxybenzoic acid were successfully synthesized, resulting in a yellow colloidal solution. These nanoparticles exhibited a SPR absorption peak at 420 nm, an average particle size of 60 nm, a polydispersity index (PDI) of 0.369, and a zeta potential of -43.8 mV. The detection of Cu(II) using these nanoparticles, along with the addition of EDTA, showed good sensitivity. The method demonstrated a linear response over a range of Cu(II) concentrations from 1×10^{-3} to 1×10^{-2} M. The limits of detection (LoD) and quantification (LoQ) were 0.4 $\times 10^{-3}$ M and 1.4 $\times 10^{-3}$ M, respectively, with a recovery rate of 104%.

Experimental results indicate that AgNPs capped with *p*-hydroxybenzoic acid can be effectively utilized for the quantitative colorimetric determination of Cu(II) through ligand-mediated nanoparticle aggregation. Although the analytical sensitivity of the proposed method is relatively limited and the selectivity toward competing metal ions has not yet been systematically evaluated, the study offers valuable insight into the aggregation behavior of AgNPs capped with *p*-hydroxybenzoic acid in the presence of Cu(II)-EDTA complexes. These findings enhance our understanding of the mechanism involved in colorimetric determination using AgNPs and may serve as a basis for further development and optimization of AgNPs-based metal-ion determination.

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