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Research Article

Synthesis of silver nanoparticles as a reagent for colorimetric detections of creatinine

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Keywords

Aggregation
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Abstract

In this study, Silver Nanoparticles (AgNPs) was synthesized using natrium citrate as a stabilizing agent and ascorbic acid as a reducing agent. The aim of this study is to investigate the optimum condition of creatinine colorimetric detection using silver nanoparticle synthesized. The reaction was carried out at temperature within an alkaline environment with a pH of 10,5. The concentration of reducing and capping agent showed a significant effect to absorption spectra via UV-Vis spectrophotometer. The produced citrate-capped AgNPs exhibited SPR absorbance within the range of 390–410 nm. The detection mechanism relies on the aggregtion of nanoparticles with analytes, resulting in a shift in Localized Surface Plasmon Resonance (LSPR) towards a longer wavelength. The experiment showed that the pH condition of the medium played an essential part in the interaction between creatinine and silver nanoparticles (AgNPs). Development of creatinine detection methods is based on the ability of tautomerization of creatinine to its anionic amino species at alkaline pH led to cross-linking with the negatively charged AgNPs through hydrogen bond networks, facilitating the aggregation mechanism. This aggregation resulted in particle resulted a color shift from yellow (with a maximum wavelength of 403nm) to dark blue (with a maximum wavelength of 670 nm) within 5 minute of reaction at pH 12. With this strategy, a linear relationship between the A670/A403 extinction ratio and creatinine concentrations was obtained in the range of 10–50μM with a coefficient of determination of 0.935.

Introduction

Creatinine (2-imino-1-methyl-2-imidazolin-4-one) is an important analyte as the concentration of creatinine in human blood or urine serves as a biomarker for the early detection of kidney dysfunction and clinical muscle damage (Tonomura et al., 2015). Low levels of creatinine may indicate poor nutritional status, whereas elevated levels in blood serum can serve as an indicator of various kidney damages such as tubular necrosis (a cause of acute kidney failure), glomerulonephritis (damage to the glomerulus), and a marker of reduced glomerular filtration capacity. The physiological content of creatinine under normal conditions ranges from 3.5 to 34.6 mM in urine and 50 to 140 μM in blood (Thompson, 2015).

The determination of creatinine levels in samples can be conducted using electrochemical and chromatographic methods. However, the instruments required for these methods entail high costs, complex sample preparation, and skilled analysts, making them less effective for routine analyses. Currently, the most commonly used method for creatinine determination in numerous healthcare settings is the Jaffé reaction-based method. In this method, creatinine reacts in an alkaline medium with picric acid, and the resulting color change in the solution (from yellow to orange) can be measured with a spectrophotometer. Interference of picric acid with other biomolecules, such as glucose, urea, uric acid, and ascorbic acid, compromises the sensitivity and selectivity of this method (Küme et al., 2017).

Various methods, including electrochemistry and colorimetry (Yilong et al., 2015), have been developed to determine creatinine concentrations. Enzyme-based methods, while exhibiting high selectivity and sensitivity,

require sophisticated equipment and are impractical for routine use. As an alternative, the use of sensor technology for rapid analyte detection is currently under rapid development. Colorimetric sensors have gained wide acceptance due to their sensitive response and high selectivity to various analytes. The colorimetric method offers advantages in terms of cost-effectiveness and practicality. Research on colorimetric sensors, particularly those utilizing nanoparticles, has been extensively reported (Abdullah et al., 2018). The small size of nanoparticles enhances their reactivity, allowing their atoms to directly interact with other materials and contribute to the assembly of materials with new properties or functions. Nanoparticles exhibit distinctive properties such as electromagnetic, catalytic, electrochemical, and plasmonic properties (Taei et al., 2015).

The development of colorimetric sensors for creatinine has previously been explored using gold nanoparticles capped with sodium citrate (Findari et al., 2022). Colorimetric analysis has become crucial in recent years to enhance the use of plasmonic-based sensors. Colorimetric applications based on metal nanoparticles have been developed in previous research for detecting proteins, DNA, anions, metal ions, and organic molecules in various samples. Colorimetric sensing of drugs, proteins, DNA, microorganisms, and other biological targets is typically achieved through nanoparticle aggregation and non-aggregation strategies, relying on inter-particle distances and size/morphology-dependent principles. Both approaches provide visually detectable colorimetric responses without the need for chromogenic substrates. Colorimetric strategies based on nanoparticle aggregation in previous research have demonstrated sensitive and accurate measurements for a variety of biomolecules by exploiting nanoparticle coupling (Tonomura et al., 2015).

Noble metals commonly used in colorimetric sensors are gold and silver. The application of gold nanoparticles (AuNPs) is widespread due to their stability compared to silver nanoparticles (AgNPs). However, AgNPs are often preferred due to their relatively higher extinction coefficients than AuNPs with nearly the same size (Sabela et al., 2017), resulting in high visibility and good sensitivity. Silver nanoparticles tend to undergo aggregation due to Van der Wascorbic acidls forces, requiring stabilizing agents such as carbohydrates or proteins (amino acids) to prevent aggregation (Ramadhani et al., 2021). Citrate can be used as a capping agent since this compound acts as a reducing and stabilizing agent for AgNPs (Park and Shumaker-Parry, 2014). To address these issues, In this research, silver nanoparticle synthesized with citrate as a capping agent and natrium ascorbate as a reductor will be used as a reagent for detecting and quantifying creatinine.

Method

Chemicals

Silver nitrate, trisodium citrate, ascorbic acid, sodium hydroxide, creatinine (≥98%), glucose, uric acid, urea, glutamic acid. All reagents were analytical grade without additional purification and prepared using distilled water. Characterization of samples was carried out using a UV–Vis spectrophotometer, The distribution of AgNPs particle size was calculated by using imageJ software. Dynamic Light Scattering (DLS).

General Procedure

Silver nanoparticles (AgNPs) were synthesized using the following procedure: Initially, an 18 mL freshly prepared aqueous solution containing 4.0 mM sodium ethylenediaminetetrascorbic acidcetate and 3.6 mM ascorbic acid was adjusted to a pH of 11 by adding 1.0 mL of 0.6 M NaOH under stirring at 600 rpm at room temperature. Subsequently, 1.0 mL of a 0.010 M AgNO3 solution was gradually introduced while stirring continuously for 30 minutes. Consequently, the color of the solution shifted from colorless to yellow. The resulting AgNPs colloid was then examined using a UV–VIS spectrophotometer, with scans conducted in the range of 300–800 nm.

The detection and quantification of creatinine were carried out at room temperature. In brief, the pH of undiluted AgNPs was adjusted to 12 by adding 10 mL of a 600 mM NaOH solution to 480 mL of the colloids. Subsequently, various concentrations of creatinine (10 mL) were introduced into the alkaline AgNPs colloids. After a 1-minute incubation, 50 mL aliquots of the resulting mixtures of creatinine/AgNP colloids were diluted with deionized water to reach a final volume of 1 mL and then analyzed using UV-visible spectrophotometry).

Results and Discussion

UV-Visible Study of the Effect of Ascorbic Acid dan Citrate on the Synthesized AgNPs

Fig.-1 displays the UV-Vis spectra of AgNPs generated at different concentrations of ascorbic acid. The UV-Vis spectrum of the colloid produced with 150 mM ascorbic acid solution exhibits the narrowest peak and smallest

Full Width at Half Maximum (FWHM). Ascorbic acid and silver possess reduction potentials of 0.008 V and 0.80 V, respectively. The notable contrast in reduction potential promotes the redox reaction, wherein ascorbic acid undergoes oxidation to dehydroascorbic acid, and Ag+ is concurrently reduced to Ag°. Optimum of ascorbic acid concentration is carried out by observing the effect of ascorbic acid concentration variation within the range of 25 to 200 mM. The effect of ascorbic acid concentration on the UV-Vis spectrum data of Silver Nanoparticles can be seen in the Fig.-1.

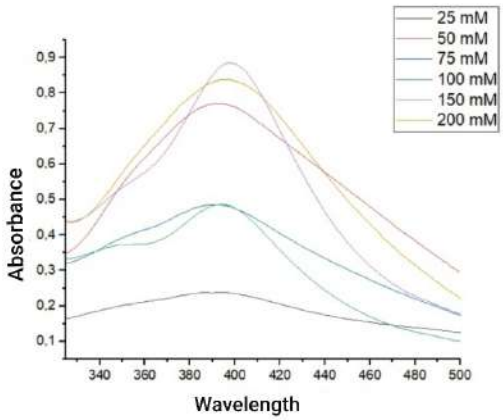


Fig.-1. The UV-Visible spectrum of AgNPs with various ascorbic acid concentrations

Ascorbic acid can interact with different metal ions in an aqueous solution, forming complex ions (Zümreoglu-Karan, 2009). When the concentration of ascorbic acid in the solution is limited, some silver ions will not undergo complete reaction. Conversely, an excess of ascorbic acid concentration can lead to rapid nucleation. Typically, the silver particle core grows into primary nanoparticles and aggregates or agglomerates into microspheres to minimize the overall energy of the system. The resulting nanoparticles exhibit structural defects, serving as sites for the subsequent deposition of silver. Throughout this process, ascorbic acid undergoes oxidation to produce its byproduct, DGA (2,3-diketo-1-gulonic acid), which significantly influences crystal growth (Dewhirst and Fry, 2018). Effect concentration of citrate with variation concentration 3-25Mm on UV-Vis spectrum data of silver nanoparticles can be seen in the spectrum below (Fig.-2).

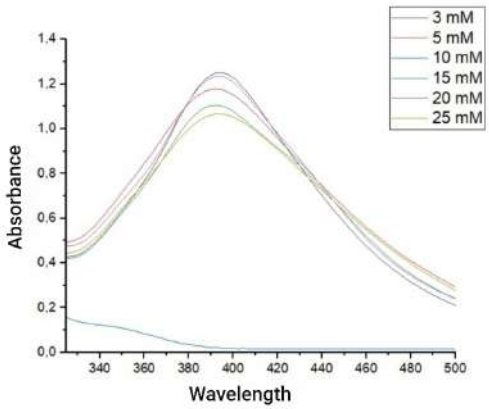
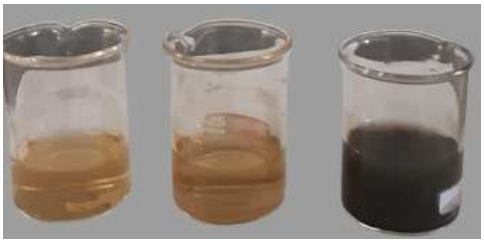


Fig.-2 UV-Vis spectrum of AgNPs with various sodium concentration of citrate as capping agent

The spectrum above indicates that the higher the concentration of sodium citrate, the lower the absorbance. The optimum concentration of sodium citrate obtained was 3mM. This concentration was then used for further experiments. The role of the stabilizing agent is crucial in the creation of uniformly sized nanoparticles. Fig. 2 illustrates the adjustment of citrate concentration from 3mM to 25 mM. At 3,0 mM concentracion of citrate, the colloid exhibits a singular and well-defined peak in LSPR absorbance. However, at higher concentrations than 3,0 mM, the peak absorbance diminishes and broadens. A diminished UV-Vis peak suggests a lower colloid concentration or larger particle size. The emergence of a peak at a longer wavelength is indicative of larger particle formation, particularly evident at low capping agent concentrations (<4.0 mM). Faster crystal growth occurs with lower capping agent concentrations, leading to larger particle sizes. At 8.0-10.0 mM, this manifests as decreasing and broadening peaks.

Interaction between AgNP and Creatinine

UV–Visible spectrophotometer was carried out to analyze the colloidal solution of AgNPs within the wavelength range of 300–800 nm. The solution resulted displayed a yellow color, characteristic of plasmon resonance absorption centered at 403 nm. The single narrow band indicated uniformly spherical nanoparticle structures. Upon exposure to an alkaline environment (pH 12), the colloid transitioned from yellow to dark blue within a minute, and the addition of creatinine induced a red-shifted peak. The decrease in absorbance intensity was attributed to the aggregation phenomenon, nanoparticle field interactions, and plasmon coupling, resulting in a longer wavelength and broadening peak of the localized surface plasmon resonance (LSPR). The color shift of AgNPs before and after addition of creatinine can be seen in Fig.-3.



(a) (b) (c)

Fig.-3. (a) Nanoparticles AgNP-citrate, (b) AgNp-citrate+NaOH (c) AgNp-Citrate +NaOH+ Creatinine

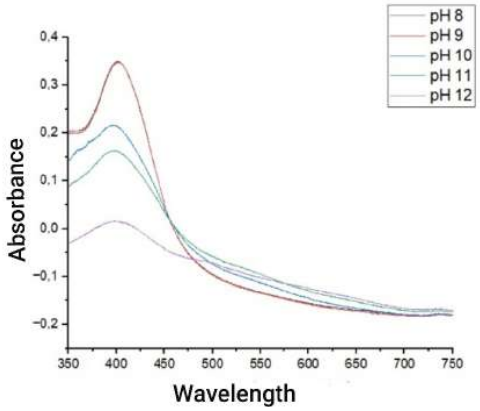


Fig.-4. Comparison between spectrum Nanoparticles AgNP-Citrate, AgNp-Citrate+NaOH, and AgNP-citrate+NaOH+creatinine

pH Optimum

The effect of pH on the interaction between AgNP-citrate and creatinine was determined by the ratio of absorbance decrease (A670/A403). pH variations were carried out from 8 to 12. At pH 12, The result show that at pH 12 exhibit the largest decrease of absorbance at 403nm. The UV-Vis spectrum optimum of pH can be seen in the Fig.-5.

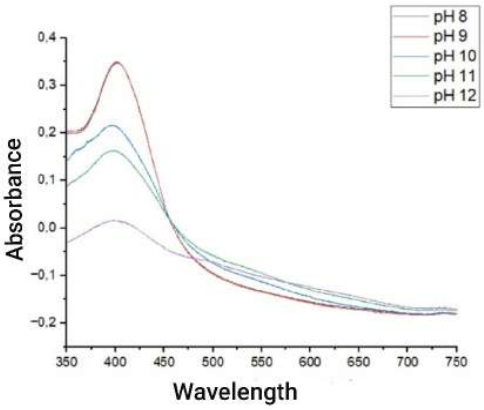


Fig.-5. Effect of pH on absorption in the UV-VIS spectrum

Creatinine exists in its tautomeric state, oscillating between amino and imino forms and maintaining equilibrium with the creatinium cation tautomer at pH 6 (Gao et al., 2013). Consequently, the appearance and

increase of anionic creatinine forms are observed with rising pH levels beyond neutrality. This emergence of species leads to the interaction between AgNPs and creatinine through hydrogen bonding, triggering aggregation (Antoniou and Dionysiou, 2007). The progressive rise in the number of anionic forms indicates the gradual formation of carbanion and oxoanion species from the amino tautomer of creatinine. These forms reach their peak ion concentration at pH 11.1, followed by a decrease at higher pH levels (Gao et al., 2013).

Optimum Reaction Time

The study aimed to examine how the interaction between AgNPs and creatinine is influenced by reaction time. Despite the creatinine concentration, the aggregation of AgNPs reached a plateau within the initial minute of the reaction. After 1 min, no significant changes in spectral or colorimetric features were observed for the tested creatinine concentrations. This effect was most notable at 5 and 10 mM creatinine, where the A670/A403 extinction ratios exhibited a rapid and linear increase to different intensities within the first 30 seconds as illustrated in Fig.-6.

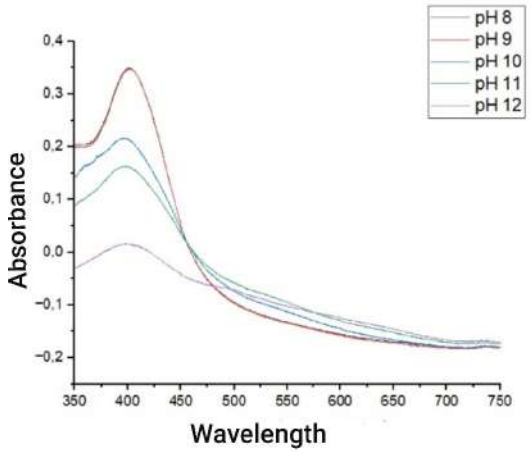


Fig.-6. Effect time reaction to absorption in the UV-VIS spectrum

Due to the rapid aggregation rate, discerning color changes at various time intervals beyond 1 minute became challenging through colorimetric means. Furthermore, prolonged reaction times could lead to the precipitation of AgNPs at elevated creatinine concentrations, thereby negatively impacting the analytical linear range. Consequently, a reaction time of 1 minute was chosen as the standard for subsequent creatinine assays in aqueous solution.

Creatinine Concentration Effect

In this stage, concentration creatinine varied from 5µM to 50µM. The creatinine determination method validation took place under optimal conditions. Solutions of AgNPs were prepared as described earlier. UV–Visible spectra of the mixtures were recorded, generating a series of A670/A403 ratio values. Linearity was assessed by creating a curve depicting the correlation between creatinine concentration and the A670/A398 ratio. In optimal conditions, the relative change in absorption ratio increased proportionally along with the increasing of creatinine concentration. The effect of concentration illustrated in Fig.-7.

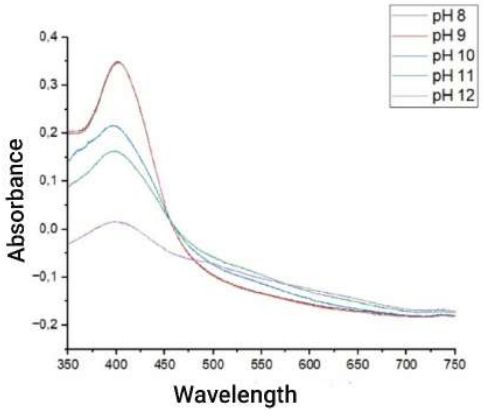


Fig.-7. Effect concentration to extinction spectra in UV-VIS

From the spectrum profile in the above image, an equation depicting the relationship between creatinine concentration and the extinction coefficient can be plotted. The decrease in absorption at the wavelength of 403nm is attributed to the agglomeration of AgNPs. This phenomenon gives rise to the emergence of new peaks around 670 nm. The linear relationship between these variables yields a correlation coefficient of 0.935.

Conclusion

The concentration of ascorbic acid dan citrate plays an important role in controlling the absorption intensity of AgNPs. The optimal A670/A403ratio was achieved by AgNPs at pH 12.0. The observed color change is attributed to the aggregation of AgNPs facilitated by creatinine molecules. The plot depicting A670/A403 against creatinine concentration exhibits a sigmoid shape within the 5–50 μM range. The detection mechanism relies on the creatinine-mediated aggregation of the AgNPs at pH 12, which elicits a visibly-detectable color change. The AgNP exhibited a linear correlation between the A670/A403 extinction ratio and creatinine concentration. This correlation enables the potential of AgNPs synthesized for detecting and quantifying creatinine concentration.

Conflict of Interests

The author (s) declares that there is no conflict of interest in this research and manuscript.

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