Biogenic synthesis of silver nanoparticles; study of the effect of physicochemical parameters and application as nanosensor in the colorimetric detection of Hg$^{2+}$ in water

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ABSTRACT

This research demonstrates the ability of biogenic synthesised silver nanoparticles (AgNPs) to sensitively and selectively detect the presence of mercury (Hg$^{2+}$) in water. To achieve this, the following study investigated the synthesis of AgNPs using plant extract from basil and characterised the synthesised AgNPs using scanning electron microscopy, energy dispersive X-ray spectroscopy, UV-visible spectrophotometry, X-ray diffractometry and Fourier transform infrared spectroscopy. We studied the effect of various factors, such as broth concentration, precursor concentration, temperature, contact time and pH, on the synthesis of the nanoparticles. The synthesised AgNPs were then used in the colorimetric detection of Hg$^{2+}$ in water. The as-prepared AgNPs showed high selectivity to detect Hg$^{2+}$ alone compared to other cations and high sensitivity at different concentration of Hg$^{2+}$. The limit of detection for Hg$^{2+}$ was $6.25 \times 10^{-8}$ mol/L (12 µg/L) indicating that these biogenic synthesised AgNPs represent a highly sensitive Hg$^{2+}$ detection tool.

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1. Introduction

Silver nanoparticles (AgNPs) are noble metal nanoparticles that have gained research prominence due to its properties and applications. They can strongly absorb electromagnetic waves in the visible region as a result of their chemical inertness, and they also show biological compatibility, highly stable dispersions, surface plasmon resonance, favourable optical and electrochemical properties; thus, they have been exploited in different fields [1]. Yet, these properties are strongly determined by the size, composition, shape, crystallinity, capping layer and the nature of their nanostructures [2,3]. AgNPs have found application in antimicrobial textile finishes, paint coatings, antibacterial agents, water treatment, catalysis and optics [4–7]. Their broad application has necessitated the development of an efficient, less toxic, cheap and environmentally
friendly approach to synthesis. Conventional methods of synthesising AgNPs include physical and chemical methods. These methods sometimes adopt heat decomposition of compounds, solution reduction, chemical and photochemical reactions in reverse micelles, irradiation, electrochemical and microwave assisted processes [8,9]. These approaches require the use of toxic chemicals that are dangerous both to humans and the environment [10]. Thus, there is now a clear need for developing an efficient, less toxic, inexpensive and environmentally friendly approach for synthesising AgNPs [11]. One viable option is to use biosynthesis techniques, which are not only environmentally friendly but are simple, less time consuming and energy efficient [10].

Previously, the biosynthesis of AgNPs has been carried out using different biological sources as reducing agents. These sources include the use of microorganisms such as Cladosporium cladosporioides [12] Fusarium oxysporum [13], Bacillus licheniformis [14] Bacillus subtilis [15], and plants. The use of plant extract for bio-inspired synthesis of AgNPs is reported to be more energy efficient, requires less purification, less hazardous and demands no culturing or cell maintenance, which can be elaborate in other synthesis methods [14,15]. The biosynthesis of AgNPs has used a variety of different plants, including Jatropha curcas [16], Camellia sinesis [17], Murraya koenigii [18] and Nelumbo nucifera [19]. In this approach, plant extracts are used to reduce silver ion from silver nitrate, which produces the AgNPs. The reduction rate of silver ion, i.e., formation of nanoparticles, shape and size of AgNPs formed, can be altered by changing the pH, substrate concentration, temperature, time of reaction between the plant extract and the silver nitrate, and the volume ratio of the silver nitrate and plant extract [18–20].

One of the applications of AgNPs is their use as a colorimetric sensor for sensitive and selective determination of the presence of mercury (II) in aqueous media. Their unique optical properties, stability, solubility in water and their ability to form an amalgam with mercury (II) provide an exploitable opportunity. In most cases, the AgNPs formed from chemical methods need to be modified with other stabilising agents to increase their sensitivity, since unmodified AgNPs generally have showed low sensitivity of Hg^{2+} in water. Bothra et al. [21] developed a nanosensing system made from the functionalization of AgNPs using β-alanine dithiocarbamide. The system was efficient in the selective detection of Hg^{2+} and Fe^{3+} in the presence of anions. In a similar study [22], although using p-phenylene diamine for functionalization, AgNP was applied for the sensing of metal ions in water.

Thus, the objectives of this research are (1) to determine the effect of physico-chemical parameters such as pH, precursor concentration, temperature, time and broth concentration in the formation of AgNP reduced by basil (Ocimum basilicum) plant extracts and (2) to determine the selectivity and maximum sensitivity of unmodified biogenic-synthesised AgNP for use in the detection of Hg^{2+} in water.

2. Experimental

2.1. Instruments

UV-visible absorption spectroscopy analyses were carried out using a single-beam, Specord 50 UV-visible spectrophotometer (UV-Vis) (Analytik Jena) with a 1.0 cm quartz cell. Surface morphology characterisation was carried out using field emission scanning electron microscopy (Lyra3 TESCAN FESEM). This FESEM was also equipped with an
energy-dispersive X-ray spectroscopy (EDX) detector. Fourier transform infrared spectroscopy analysis of the sample was done using Nicolet 6700 FT-IR (Thermo Electron Corporation). X-ray diffractometry analysis was done using Rigaku Miniflex II desktop X-ray diffractometer with tube output voltage 30 kV.

2.2. Chemicals and materials

The chemicals used were of analytical grade and used with no further purification. Silver nitrate used was purchased from Merck, Germany. The salts used include NaCl, CaCl₂, BaCl₂.2H₂O, Zn(NO₃)₂.6H₂O, NiCl₂.6H₂O, MnSO₄.H₂O, HgCl₂, CuSO₄.5H₂O and KCl, and they were all purchased from Merck or Fischer chemical companies and were also used without further purification. All solutions were prepared using Milli-Q water. Sodium hydroxide (0.1 M) was used in adjusting pH when necessary. All glassware were cleaned with diluted nitric acid and rinsed in Milli-Q water before use.

2.3. Biosynthesis of AgNPs

Basil (Ocimum basilicum) plant (Supplementary Figure S1) was obtained from King Fahd University of Petroleum and Minerals nursery located within the campus. The 40 g of the leaves and flowers were collected and boiled in 300 mL of deionised water. The broth (plant extract) obtained is filtered and stored in the refrigerator at 4°C until further use. Different concentrations and volumes of plant extract and silver nitrate solutions were mixed at different temperatures to study the effects of various factors such as pH, temperature, broth concentration and precursor concentration on the formation of AgNPs. Plant extract solutions were added to the silver nitrate solution drop-wise and a change in the colour of the mixture was observed. The reduction of the silver nitrate solution by the plant extract to form AgNPs was monitored using UV-Vis. The AgNP used for the colorimetry study was synthesised at 40°C, pH 8, 2 mM silver nitrate solution, 1:25 plant extract to silver nitrate volumetric ratios for 240 min after optimisation.

2.4. Determination of the effects of various factors on biosynthesis

The biosynthesis of AgNPs was carried out at varying conditions of pH, time, temperature, broth concentration and precursor concentration. To determine the effect of pH on the synthesis of AgNPs, the experiment was carried out by varying pH values (4, 7, 8 and 10). The determination of the effect of temperature was carried out by allowing the reaction to take place at 25°C, 40°C and 70°C. The effect of time was determined by allowing the reaction to occur for 15, 30, 60, 90, 120, 240 and 480 min. To study the effect of broth concentration, leaf broth (plant extract) was mixed with 1 mM silver nitrate solution at ratios of 1:1, 1:10, 1:20, 1:25, 1:50, 1:100 and 1:200. The effect of precursor concentration was investigated by altering the concentration of silver nitrate solution (1 mM, 2 mM and 5 mM).
2.5. **Characterisation of biogenic synthesised AgNPs**

The biogenic synthesised AgNPs were characterised using different techniques. The reduction of AgNO$_3$ by the plant extract was confirmed using Specord 50 single beam UV spectrophotometer at a resolution of 1 nm set to scan within 300 and 800 nm. The UV-Vis analysis was carried out on a mixture diluted 10×. The shape and sizes of the nanoparticles were analysed using FESEM and transmission electron microscopy (TEM) while the elemental composition of the nanoparticles was determined using elemental dispersive X-ray spectroscopy. AgNPs were characterised using Fourier transform infrared spectrophotometer (FTIR) to determine the biomolecules that took part in reducing AgNO$_3$ to AgNPs and subsequent capping of the nanoparticles. X-ray diffractometer was used to determine the phase purity and the crystal phase identification of the synthesised AgNPs.

2.6. **Colorimetric determination of Hg$^{2+}$**

To demonstrate the detection of Hg$^{2+}$ using the unmodified biogenic synthesised AgNPs, 1 mL of as-prepared AgNPs was added to 9 mL of deionised water to make a 10× diluted solution. This was recorded as blank. This procedure was repeated three times for each solution containing a different concentration of Hg$^{2+}$ ions in order to determine the detection limit of Hg$^{2+}$ ions using unmodified AgNPs. Also to demonstrate the selectivity of the as-prepared AgNPs for colorimetric detection of Hg$^{2+}$, the AgNPs were added to various metal solutions (Ca$^{2+}$, Cu$^{2+}$, Mn$^{2+}$, Ni$^{2+}$, Na$^+$, Zn$^{2+}$, Ba$^{2+}$ and K$^+$) of the same concentration (1 mM) and dilution rate. The analysis was carried out using a UV-Vis.

3. **Results and discussion**

3.1. **Formation of AgNPs**

After adding plant extracts drop-wise into the prepared silver nitrate solution, the colour of the mixture changed to that of the plant extract in an instant (brown) and then the solution became clearer (more transparent or colourless) signifying the start of the reaction. The colour change is shown in Supplementary Figure S2. The steady darkening of the solution from brown to golden brown showed the continuous reduction of silver nitrate to AgNPs and hence the increase in concentration or formation of AgNPs in solution. The formation of AgNPs is characterised by excitation of surface plasmon resonance which revealed a colour change.

3.2. **Effect of various factors on biosynthesis**

The study examined a variety of parameters on the formation of AgNPs. The effect of reaction time on the formation of AgNPs after mixing the silver nitrate solution and the plant extract at neutral pH and room temperature is shown in Supplementary Figure S3. No visible peak was observed within the first 120 min of the commencement of the reaction. A pronounced peak became visible at 240 min. The observed peak increased from 240 min to 480 min. The gradual formation of the peak as shown may be attributed
to the increase in the AgNPs formed in the solution causing surface plasmon resonance vibrations. The difference in the peak formed at time 240 min and time 480 min was negligible, signifying that the reduction reaction had almost reached completion at 240 min. The bands formed at time 240 and 480 min were within the range 430–440 nm, gradually shifting from above 450 nm formed at earlier time. This showed the gradual reduction in the size of AgNPs formed over time.

The effect of broth concentration on AgNPs formation is shown in Supplementary Figure S4 for different mixing ratios of plant extract and silver nitrate. The highest peak was observed at ratio 1:25, which showed that all the silver ions reacted with the plant extracts. The complete reaction of AgNO$_3$ with the plant extract at this ratio therefore showed that the reduction of silver ion and hence the formation of AgNPs is optimum at this combination ratio.

Supplementary Figure S5 shows the effect of the precursor (silver nitrate) concentration at a constant time, temperature, pH and broth concentration. As the precursor concentration was reduced to 1 mM, we observed a shift in band closer to 400 nm. The peak absorbance was obtained at 2 mM AgNO$_3$ indicating that the reaction is optimised at this concentration of the silver nitrate solution. The blue shift noticed may be attributed to the formation of smaller sized AgNP as well as changes in the refractive index of the particles [23].

The effect of temperature on nanoparticle formation was determined at a reaction time of 1 h. Although the absorbance peak obtained on the UV-Vis spectrophotometer when the experiment was carried out at different temperatures was not particularly pronounced, the value of the absorbance at a temperature 70°C was the highest. This shows that the formation of AgNPs increases with increase in temperature. The effect of temperature on nanoparticle formation is shown in Supplementary Figure S6.

The next factor we examined was pH, as the reaction mixture determines the shape and size of the nanoparticles formed. Supplementary Figure S7 shows the effect of pH on the formation of AgNPs. As evident in the figure, the bands obtained are closer to 400 nm compared to the spectra obtained for other effects studied. Also, the values of the absorbance are higher at pH 7, 8 and 10. However, in an acidic medium, the peak was not obvious, signifying that the formation of AgNPs is not supported in an acidic condition. The peak increased with pH and as the solution becomes alkaline. The highest absorbance was obtained at pH 10 with a wavelength of 402 nm. According to Vanaja et al. (2013) [24], the excitation of surface plasmon resonance is supported in alkaline media.

Alkalinity supports the reduction and capping of AgNPs at specific facets and subsequent deposition of silver atom on these facets [25]. They also cause electrostatic repulsion between the nanoparticles as they are formed. However, at acidic pH, the functional groups responsible for reducing the precursor to nanoparticles possess positive charges, due to high concentration of hydrogen ion, which in turn lowers their reducing potential. Nucleation is also promoted at high pH [25].

3.3. Characterisation of AgNPs

We characterised AgNPs that were synthesised during a 2-h reaction using a 2-mM AgNO$_3$ solution at pH 10 and at room temperature. After they were centrifuged at 17,000 rpm, we characterised the AgNPs using FESEM and FTIR.
The TEM and scanning electron microscopy (SEM) images are shown in Figures 1 and 2, respectively. The images illustrated the shape and morphology of the synthesised nanoparticles and gave an estimate of the average size of the nanoparticle. The FESEM image showed an agglomeration of nanoparticles (Figure 2a). The synthesised nanoparticles were spherical and polydispersed, with a particle size of 30–50 nm. The EDX image (Figure 2b) also revealed that the nanoparticle is mainly composed of silver.

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The result of the FTIR analysis is shown in Supplementary Figure S8 (a) and (b). Figure S8 (a) shows the FTIR image of basil leaf. As shown, the bands at approximately 3400 shows either O–H stretching vibrations of hydroxyl groups [26] or N–H stretching of amine and amides [24]. The O–H stretching corresponds to fatty acids and protein structures which may be the constituents of the plant extracts. The bands at 2920 and 2852 may be due to C–H and C–H$_2$ stretching vibrations. Those at about 1637 are assigned to C = O bond stretching mode which also represent protein and fatty acid structures.

After the synthesis process, the nanoparticles were collected and analysed using FTIR to determine the biomolecules responsible for the biogenic synthesis. A FTIR
A comparison of the basil leaf and the AgNP reveals shift, disappearance and increase in the intensity of some bands. In Figure S8 (b), the spectra shows decrease in the intensity at 3400 (O–H stretching) and the appearance of new bands at 1167 (C–C bending) and 557. The band at 1600 shows alkene stretching vibration [27] while that at 1390 is assigned to C–H bending and indicates carboxylates [25]. The band at 1080 shows the absorption peak of –C–O–C– while that at 600 may be due to the C–H bend of alkynes [26]. The appearance, disappearance, shift and increase in the intensity of these bands show that there is a reaction between the functional group contained in the plant and silver precursor which may have resulted in the reduction of the biomolecules [28].

The X-ray diffractometry pattern of the biogenic synthesised AgNPs is shown in Supplementary Figure S9. The peaks are indexed as 111, 200, 220 and 311 planes of face-centred cubic structure of silver according to JCPDS file No. 00-004-0783.

3.4. Sensitivity and selectivity studies

Next, we determine the sensitivity and selectivity of as-prepared AgNP in the detection of Hg$^{2+}$ in aqueous solution. Immediately after the addition of 1 ml of AgNPs to 9 mL of 1 mM Hg$^{2+}$, the colourless Hg$^{2+}$ solution changed to golden brown. However, a gradual change in colour from golden brown to light brown and finally to colourless occurred within 5 min. The change in colour of the mixture indicated that the Hg$^{2+}$ has a large effect on the surface plasmon resonance vibration of the AgNPs. This, however, was not the case for the other metallic cations (Ca$^{2+}$, Cu$^{2+}$, Mn$^{2+}$, Ni$^{2+}$, Na$^{+}$, Zn$^{2+}$, Ba$^{2+}$ and K$^+$) investigated. None of the other metal salts, when subjected to the same treatments, showed any colour change, even after 15 min. Figure 3 shows the UV-Vis of all the metal cations after the same volume of AgNPs was added. As shown in Figure 4, Hg$^{2+}$ showed the highest change in absorbance of all metal cations studied. Also because Hg$^{2+}$ lacked the peak at 400 nm (Figure 3), where all other metal cations and the blank had a broad peak, this confirms that AgNPs could selectively and colorimetrically detect Hg$^{2+}$ in solution. The colour change may be attributed to the

![Figure 3](image_url)

**Figure 3.** UV-vis spectra of a 1 mL AgNPs solution after adding 9 mL of a different metal salt.
aggregation of the AgNP [22]. Rastogi et al. (2014) [29] also reported a similar decolourisation upon the addition of Hg\(^{2+}\) to as-synthesised AgNP. They attributed the decolourisation to the formation of Ag-Hg amalgam. As such, the change in colour illustrated in Figure 7(a) further confirms the selectivity of AgNPs in mercury (II) detection. This selectivity occurs because the AgNPs and the Hg\(^{2+}\) form silver–mercury amalgam, where the mercury II ion is reduced to metallic mercury.

In order to determine the sensitivity of as-prepared AgNPs to Hg\(^{2+}\) and hence the detection limit of Hg\(^{2+}\) in the mixture of Hg\(^{2+}\) and AgNPs, the concentration of Hg\(^{2+}\) was reduced from 18.05 mg/L to 0.03 mg/L (dynamic range). This caused the colour change in the aqueous solution to gradually lessen. As the concentration of Hg\(^{2+}\) drops, the intensity of the colour change diminished; however, the UV-Vis analysis continues to reveal absorbance differences for each of the Hg\(^{2+}\) concentrations examined in Figure 5.

Figure 4. Change in the absorbance of all metallic cations, reflecting their colorimetric response, upon exposure to AgNPs.

Figure 5. UV-vis spectra of 1 mL AgNPs after adding various concentrations of Hg\(^{2+}\) ions.
Thus, the UV-Vis analysis showed that as the concentration of Hg\(^{2+}\) is increased, the change in absorbance is linear from 0.20 mg/L to 18.05 mg/L (linear range), with a regression value of 0.96 (Figure 6). Calculation of the limit of detection was done according to the guidelines of American Chemical Society’s Committee on Environmental Analytical Chemistry and IUPAC using the relation \(S_{LOD} = S_{RB} + 3\sigma_{RB}\), where \(S_{LOD}\) and \(S_{RB}\) are the signal at the limit of detection and of the reagent blank, respectively, while \(\sigma_{RB}\) is the reagent blank’s standard deviation. A comparison of the limit of detection obtained in this work with other similar previous studies is shown in Table 1. Thus, the low detection limit obtained in this study may be attributed to the basil extracts in reducing AgNO\(_3\). Previously, Ahmed et al. [30] reported that basil leaves are suited to be used in the biosynthesis of AgNPs and described their ability to ensure an effective coating on AgNPs enhancing their stability and reducing agglomeration. The stability of AgNPs synthesised using basil leaves has been reported by Ahmed et al. [30] and Philip and Unni [27]. The stability is reported to be due to the formation of a
thin layer of silver electrode, which aids in the conversion of the silver ion to nanosilver. Huang et al. [31] also reported that the stability of as-synthesised is enhanced as a result of the strong interaction between biomolecules and the nanoparticles. Nanoparticles with very small particle sizes are expected to provide a high surface area for the amalgamation of more Hg$^{2+}$. As such, forming very small particles that show less agglomeration may have enhanced the colorimetric detection of Hg$^{2+}$ in our study.

Figure 7(a) confirms the selectivity of AgNPs to detect mercury in aqueous solution. Also, to determine the efficiency of this method to a real-life situation, we formed a complex matrix to simulate a situation where different metals are mixed together. An equal volume (10 mL) of a 1 mM solution of Ca$^{2+}$, Cu$^{2+}$, Mn$^{2+}$, Ni$^{2+}$, Na$^+$, Zn$^{2+}$, Ba$^{2+}$, K$^+$ and Hg$^{2+}$ was mixed together, and 1 mL of AgNPs was added to 9 mL of the complex matrix, making a 10× diluted solution. This was repeated for a matrix without Hg$^{2+}$, for a solution containing only Hg$^{2+}$ and a blank to serve as controls. Figure 7b shows the colour change that occurred during this experiment, while Figure 8 shows the corresponding UV. From the figures, it can be deduced that the presence of other metals poses very little interference to the detection of Hg in the aqueous medium. While a solution containing mercury alone appears to be the clearest, the complex matrix without Hg$^{2+}$ is almost the same as the blank containing de-ionised water and AgNPs only.

| Colorimetric detector used                                | Limit of detection | Work |
|-----------------------------------------------------------|--------------------|------|
| Surface functionalization of silver nanoparticles (AgNPs) with β-alanine dithiocarbamate | $4.89 \times 10^{-6}$ mol/L (981 µg/L) | [22] |
| Silver and graphene oxide nanocomposite (Z)-2-(4-amino-phenyl)-3-(pyridine-4-yl)acrylonitrile (I) and (Z)-2-phenyl-3-(pyridin-4-yl)acrylonitrile (II) | $3.38 \times 10^{-7}$ mol/L (67 µg/L) | [32] |
| Biogenic synthesised silver nanoparticles                  | $1.74 \times 10^{-10}$ mol/L (0.03 µg/L) | [33] |
| Biogenic synthesised silver nanoparticles                  | $6.25 \times 10^{-8}$ mol/L (12 µg/L) | This work |

Figure 8. UV spectra showing how the method responds to interference.
SEM images of AgNPs before and after the addition of mercury (II) are shown in Figure 2(a) and (b). The SEM image before the addition of mercury (II) shows an agglomeration of spherical-shaped AgNPs. Figure 2(b) shows changes in the surface morphology of the AgNPs and a reduction in the amount of nanoparticles noticed in the first image. The presence of mercury in the solution was confirmed in the FESEM and EDX images in Figure 9.

4. Conclusion

Overall, this research presents the biosynthesis of AgNPs using extracts from basil leaves as well as the effects of various physico-chemical factors on the biosynthesis. It also proposes a method for the colorimetric detection of Hg$^{2+}$ in an aqueous medium using unmodified silver nanoparticles. While the formation of biogenic-synthesised AgNPs is enhanced by the increased temperature, pH and time, maximum absorbance was obtained using 2 mM concentration of the precursor and a 1:25 ratio of the broth concentration. The biogenic synthesised nanoparticles were characterised using different characterisation techniques and found to be spherically shaped with particle size less than 50 nm and crystalline. The selectivity of this method for detecting Hg$^{2+}$ when other metallic cations were present in the solution was also determined. It was found that the biogenic synthesised AgNPs were both sensitive and selective. The better detection limit observed for these biogenic synthesised AgNPs, compared to detection limit for similar unmodified AgNPs, may have arisen because of our choice of plant extract (basil), which allows for less nanoparticle agglomeration and smaller particle size. The recorded limit of detection for Hg$^{2+}$ was $6.25 \times 10^{-8}$ mol/L (12 µg/L). Although the Hg$^{2+}$ detection limit for these biogenic synthesised nanoparticles remains higher than the limits for chemically synthesised and modified AgNPs used in colorimetric Hg$^{2+}$, our study still represents a step forward in the development of ‘green’ chemistry to perform various chemical reactions and analysis.

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Disclosure statement

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