OPTICAL AND COLORIMETRIC SENSING OF TOXIC MERCURY ION USING GREEN SYNTHESIZED SILVER NANOPARTICLES

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ABSTRACT. In ancient, human used silver and silver salts but silver nanoparticles (AgNPs) have recently been found to be accepted as the result of green synthesis. Now a day, silver nanoparticles (AgNPs) have become extremely popular in the field of the sensor due to their remarkable and significant optical, chemical, and electrical properties. In this paper, we have reported the green and reasonably priced synthesis of silver nanoparticles using *Acacia confusa* leaf extract which acts as both capping as well as reducing agents. The optical sensing and morphology of the as prepared AgNPs were determined using ultraviolet-visible (UV-Visible) and scanning electron microscopy (SEM) analysis. The sensing of Hg^{2+} ion through colorimetric and optical have been studied by UV-Vis spectroscopy as well as the naked eye. Moreover, the SEM analysis showed that the volume of the extract had no considerable effect on the morphology except for size. The colorimetric and optical sensing study revealed that the as-synthesized AgNPs exhibited a good response with colorimetric and optical sensing activity specifically towards the toxic Hg^{2+} ion.

KEY WORDS: Green synthesis, AgNPs, Optical sensor, Ultrasonic method

INTRODUCTION

Nowadays, heavy metal ion is one of the most global environmental problems [1] due to the severe toxicity. It damages the ecological systems even at relatively low concentrations [2]. Conversely, certain types of metals are crucial for the typical physiological processes of the human body [3]. However, the presence of a trace quantity of heavy metals such as lead, cadmium, chromium, nickel, and mercury in the environment is extremely hazardous for mankind as well as the ecosystem [4, 5]. In addition, these are non-biodegradable, extremely carcinogenic, and have a strong tendency to accumulate with food consumed by both humans and animals [6]. For example, several latest research works have been shown that mercury (Hg) is one of the most fatal and prevalent metals for human lives and marine lifespan. Presence of Hg can have distressing effects on human as well as animal body parts such as inhibiting ligand-receptor binding, impairment of DNA, kidney, liver, and annoying the immune system [7-9]. Moreover, Hg^{2+} is one of the most stable inorganics solvated mercuric ion forms and even at exceedingly low concentrations, it can harm the brain, kidney, stomach, heart, intestines, vision, and hearing loss as well as even death [1, 10]. Hence, considering the adverse effects of Hg^{2+}, it is most important to develop methods that must be rapid, very sensitive, inexpensive, and simple for detecting Hg^{2+} [11, 12]. The inductively coupled plasma-atomic emission spectrometry (ICP-AES), atomic absorption

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spectrometry (AAS) [13], square wave anodic stripping voltammetry (SWASV) [14], surface-enhanced raman spectroscopy [15], inductively coupled plasma-mass spectrometry (ICP-MS) method, gas chromatography (GC)-atomic fluorescence, high performance liquid chromatography (HPLC), fluorescence sensor, conjugated polymers, ratiometric, oligo-nucleotides, proteins, and electrochemical sensing for the selective detection of Hg^{2+} have been discussed in the literature but all methods are time taking, very expensive, hard, and need highly qualified employees [14-24]. Therefore, everyone is interested to establish simple, quick, and real time sensors to make a distinction between heavy metal ions. In this respect, colorimetric sensor remains the centre of attraction for scientists because it gives quantitative and qualitative facts by the naked eye without using instruments that are classical and expensive [25-27] as well as it is simple, highly sensitive, and cost-effective [28]. Thus, silver nanoparticles (AgNPs) have attracted much much greater interest in the area of biosensing and colorimetric sensing due to their unique properties for instance; high excitation coefficient, strong biocompatibility, definite optical properties [29-33], great stability, and water solubility [34].

Usually, nanoparticles were synthesized via three different methods: physical, chemical, and green synthesis [35, 36]. The physical methods of AgNPs synthesis require expensive equipment, high temperature, and pressure. In the chemical synthetic methods of AgNPs, toxic and hazardous chemicals can harm the living creature in the environment and atmosphere. Due to these drawbacks, green synthesis of AgNPs using leaf, root, and fruit extract is favored over the traditional synthesis because it is environment friendly, low cost effective, single step that can be managed to a large extent of preparation and does not need hazardous chemical, high temperature, pressure and energy [37-40]. Proteins, polyphenols, amino acids, carbohydrates, and flavonoids, etc. present in the extract play a significant role in the synthesis of silver nanoparticles as reducing and stabilizing agents [41]. Previously, we have reported the completely green synthesis of AgNPs using various extract which act as reducing as well as capping agent and results showed that the antimicrobial activity and sensing properties enhanced [42-44].

For the first time, we are reporting here the green synthesis of AgNPs using acacia leaf extract to sense the Hg^{2+} ion with the optical sensing technique and colorimetric with different concentrations. The results showed that the prepared AgNPs were a specific colorimetric sensor for Hg^{2+} ion only.

EXPERIMENTAL

Materials

Acacia confusa leaves were collected from Abha, Assir region of Saudi Arabia. All chemicals comprising $Pb(CH_3COO)_2.3H_2O$, $Mn(CH_3COO)_2.4H_2O$, $Hg(NO_3)_2.H_2O$, $Sr(NO_3)_2.6H_2O$, $AgNO_3$, $CdSO_4.8H_2O$, $NiCl_2.6H_2O$, $CoCl_2.6H_2O$, $CuCl_2.H_2O$ and $ZnCl_2$ were purchased from Merck or Sigma-Aldrich and used as received. The stock solutions were prepared in double distilled water by the calculated amount of $AgNO_3$ and each metal salt to the preferred concentration.

Extraction and synthesis of AgNPs

The leaves of *Acacia confusa* were washed several times with double distilled water for removing dust particles and after that dried in a shadow place. About 5.0 g of sieved dry *Acacia confusa* leaves were mixed with 100 mL distilled water and left to stand for 24 h at room temperature. Earlier to an experiment, leaf extract (bio-extract) was filtered using Whatman filter paper and centrifuged at 3000 rpm for 20 min to isolate any solid particles from it. The extract was stored and used for the synthesis of AgNPs.

The green approach was used for the synthesis of AgNPs without using any toxic chemicals as reducing or capping agents. In a simple reaction, *Acacia confusa* leaves extract and $AgNO_3$

(0.01 M) solution were mixed in different ratios (1:10, 2:10 and 3:10 v/v) and the final mixture was kept in an ultrasonic bath for 30 min at room temperature (Table 1). The formation of AgNPs was endorsed by getting brown coloured final products which were abbreviated by AgNP-1, AgNP-2, and AgNP-3 (Figure 1).

Figure 1. Photo image (a) bio-extract, (b) before and (c) after reaction.

Table 1 represented the preparation of AgNPs samples (AgNPs-1, AgNPs-2 and AgNPs-3) with different volume of bio-extract.

Table 1. Synthesis of AgNPs samples (AgNPs-1, AgNPs-2 and AgNPs-3).

Sample code	Composition of reaction mixture	Time to change colour (min)
AgNPs-1	10 mL AgNO₃ + 1 mL Extract	7
AgNPs-2	10 mL AgNO₃ + 2 mL Extract	5
AgNPs-3	10 mL AgNO₃ + 3 mL Extract	4

Characterization of nanoparticles

The optical sensing application and characterization of AgNPs were attained by using UV-Visible double-beam spectrophotometer (PG Instrument). Morphology and sizes of the prepared AgNPs were analysed by using scanning electron microscopy (JSM 6360 SEM).

General procedures for sensing metal ions

Green synthesized AgNPs were used for the sensing of various metal ions (Co^{2+}, Cd^{2+}, Hg^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Sr^{2+}, Zn^{2+}, Pb^{2+} and Ag^{+}). Generally, 1 mL of AgNPs was mixed with 1 mL solution of several metals at 0.1 mM or 10^{-3} mol. For the evaluation of the sensitivity of AgNPs towards different concentrations of Hg^{2+} ion. For colorimetric sensing of Hg^{2+} ion, however, we added about 0.1 mL of AgNPs in a 10 mL volumetric flask. The reaction mixtures were kept at room temperature for few minutes and then UV–Visible absorption spectra were recorded to find out different absorption intensities.

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RESULTS AND DISCUSSION

UV-Visible studies

The formation of AgNPs facilitates visual reflection when the colour change tracks the conversion of silver ions to AgNPs. Here, the reactions were monitored by the naked eye to examine the colour change and stop the reaction when the colour of reaction mixture was stable. Figure 1 showed that the colour of the reaction mixture gradually changes from colourless to brown in 5 to 15 min due to the formation of AgNPs. Finally, the reaction mixture achieved the highest intense colour after 30 min of sonication, which can be recognized to the excitation of surface plasmon resonance (SPR) in AgNPs [45]. Additionally, the formation of AgNPs was also observed by UV–Vis spectral analysis.

Figure 2 displays the absorption spectra of extract, $AgNO_3$, and all prepared AgNPs with different volumes of extract (AgNP-1, AgNP-2 and AgNP-3). The results of UV-Vis spectra revealed that extract and $AgNO_3$ did not exhibit any absorbance peak between 350 to 700 nm. But, in our case, all prepared AgNPs had absorbance peaks between 460 to 462 nm which endorsed to both the SPR band and formation of AgNPs in presence of *Acacia confusa* leaves extract. The SPR takes place after light imposes on the solution of AgNPs. In general, the SPR band depends on the shape, size, capping agents and dielectric constant of AgNPs and is used as a convenient tool for an optical sensor [46]. Krishnaraj *et al.* reported that spherical NPs exhibits a SPR band whereas anisotropic molecules exhibit two or more SPR band [47]. In the current study, only one SPR band was observed for all prepared AgNPs which point toward the spherical shape. Here, we studied the effect of volume of extract (concentration) on the synthesis of AgNPs. The formation of AgNPs started with all three volumes of the extract at different times and the intensity is maximum with 3 mL of extract due to the continuous increase in the number of AgNPs formation. The absorption spectra of AgNP-1 and AgNP-3 were observed at 462 nm whereas for AgNP-2 was 460 nm. The results indicated that 2 mL extract is most appropriate to get small size NP and blue shifted with other two AgNPs [42]. Thus, AgNP-2 was employed for additional studies.
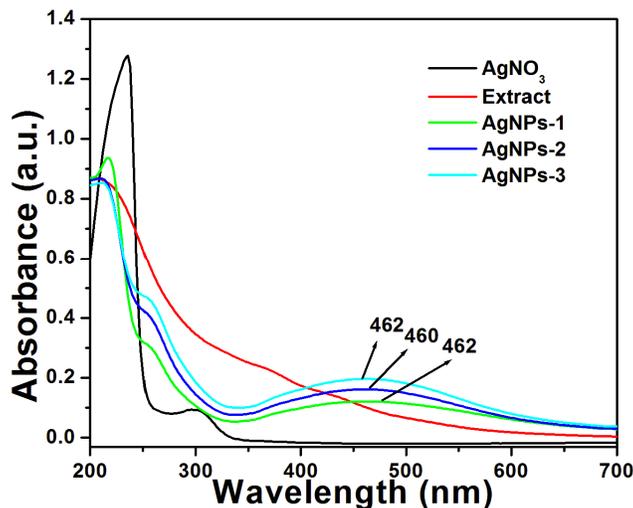

Figure 2. UV-visible spectra of silver nitrate, extract and AgNPs.

Morphological studies

SEM studies were performed to determine the micrographs and size of prepared AgNPs. Figure 3a-c represented the SEM micrographs of extract and AgNPs which indicated that all prepared AgNPs are almost spherical and size in the range of 90, 155, and 160 nm for AgNP-2, AgNP-1, and AgNP-3, respectively. In addition, it is also clear from images that particles were delimited by biomolecules of extract which control the shape and size of NPs. The results of SEM studies revealed that except for size, the volume (concentration) of extract had no remarkable influence on the morphology of AgNPs.
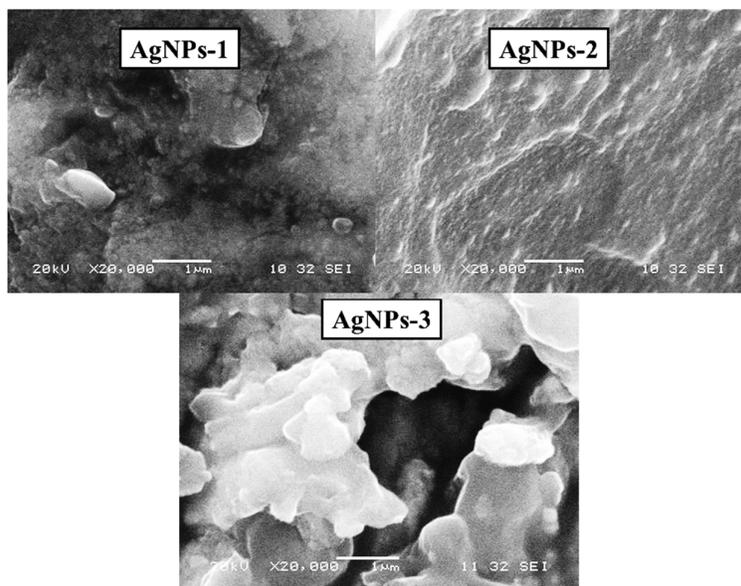

Figure 3. SEM micrographs of (a) AgNPs-1, (b) AgNPs-2, (c) AgNPs-3.

Colorimetric sensing and absorption studies of AgNPs with various metal ions

In this part, we have sightseen the SPR band of AgNPs to identify the different metal ions (Cu^{2+}, Cd^{2+}, Hg^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Sr^{2+}, Pb^{2+}, Ag^+ and Zn^{2+}) in an aqueous solution. A detailed absorption titration and colorimetric sensing were carried out in presence of different metal ions to explore the aptitude of green synthesized AgNP-2 (Figure 4a and b). Figure 4a illustrated the naked eye color observation after the interaction of different metal ions with AgNP-2 which endorsed that there was no colour change detected with Pb^{2+}, Sr^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Mn^{2+}, and Zn^{2+} ions. But a momentous change in colour was observed after the interaction of Hg^{2+} ion with AgNPs. The colour of prepared AgNP-2 with Hg^{2+} ion changes from brown to colorless and we can say that the AgNP-2 is more specific and selective for mercury ions only.

On the other hand, Figure 4b showed the UV-Vis absorption spectra of various metal ions with AgNP-2 and bare AgNP-2. Absorption spectra results showed that SPR bands of Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+} ions are blue shifted whereas Pb^{2+} and Mn^{2+} are red shifted as compared to the AgNP-2. The SPR band of Sr^{2+} ion was same as the AgNP-2, except Hg^{2+} ion, which showed no SPR band upon addition of AgNP-2. The SPR band of AgNP-2 obtained at 460 nm but after

adding Hg^{2+} ion, the band finally disappeared, and colour also changed from brown to colourless. Therefore, the prepared AgNP-2 is very specific and selective sensor for Hg^{2+} ion. Furthermore, the aggregation properties of AgNP-2 did not happen with other metal ions except Hg^{2+} over catalytic responses between Hg^{2+} ions and AgNP-2.
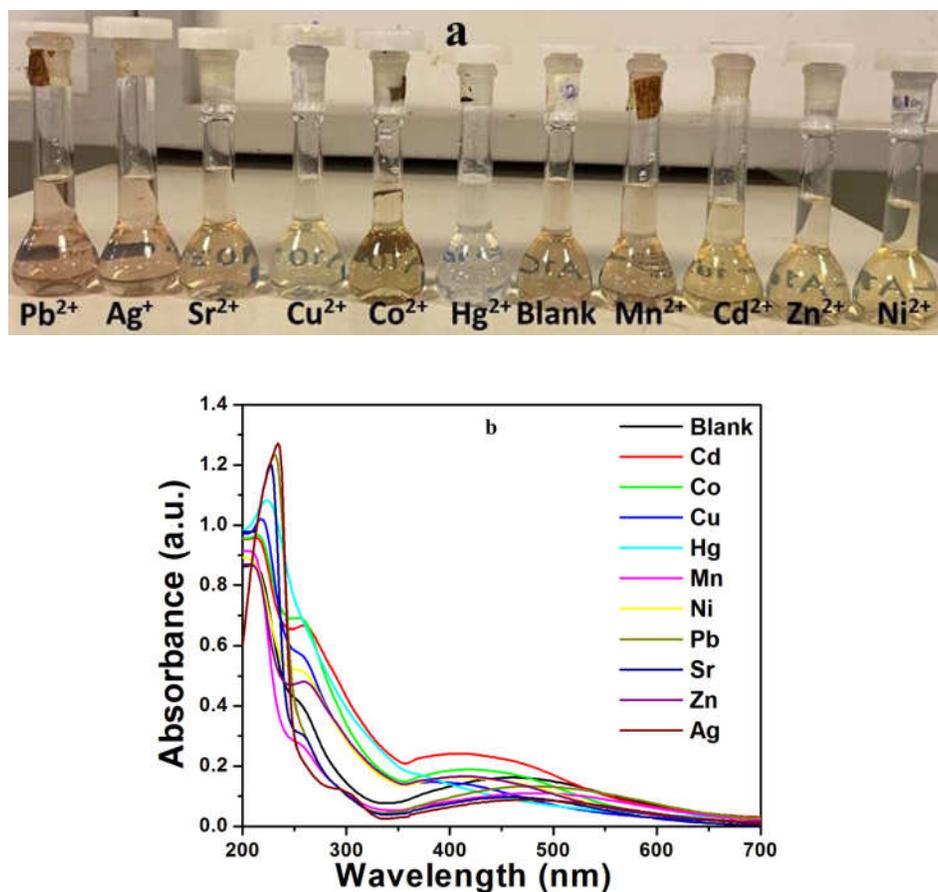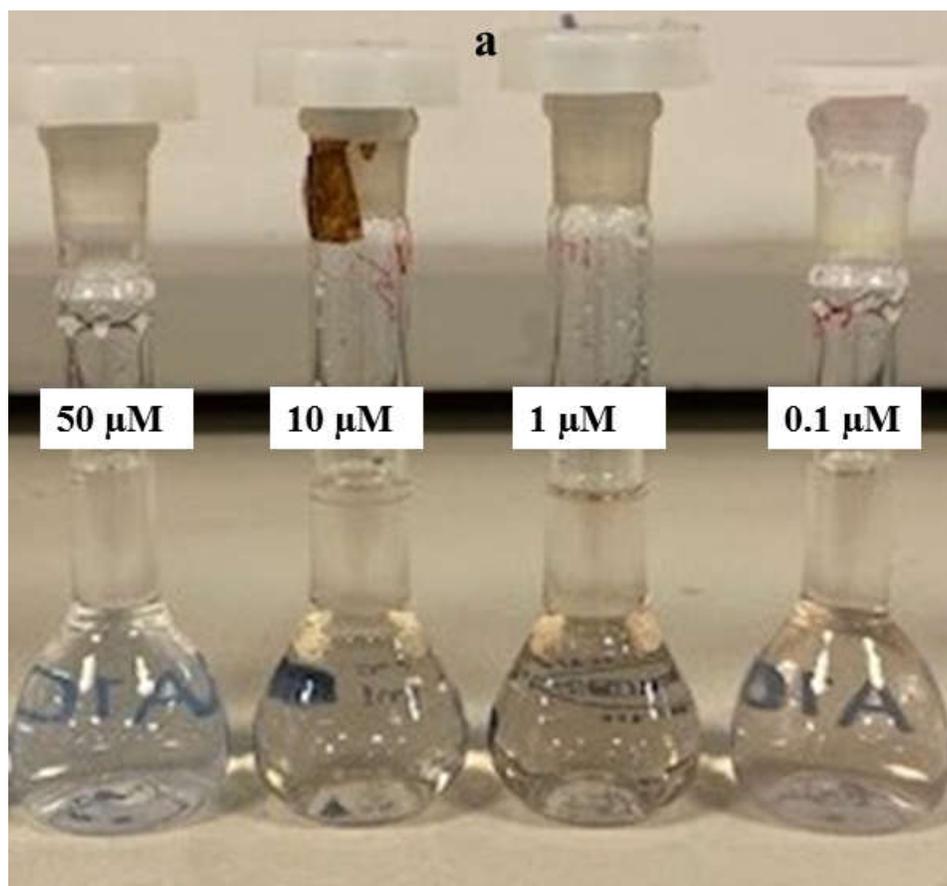

Figure 4. (a) Images of color variations of AgNPs with various metal ions. (b) UV-Vis absorption spectra of various metal ions with blank (AgNPs-2) and blank.

Effect of mercury (Hg^{2+}) concentration

UV-Vis spectra were recorded to carefully analyse the sensitivity and limit of detection (LOD) of Hg^{2+} ion with different concentrations varying from 50 µM to 0.1 µM. We noticed that colour changes from brown, light brown to clear by increasing the concentration of Hg^{2+} ion, as mentioned in the Figure 5a. Figure 5b represented the absorbance spectra of AgNP-2 against Hg^{2+} ion, demonstrating that by increasing the concentration of Hg^{2+} ion the SPR band intensity decreases. Furthermore, by increasing the concentration of Hg^{2+} ion, the SPR band becomes blue shifted. The decolorization and decrease the intensity of SPR band of AgNP-2 by introduction of

Hg^{2+} ion can also be enlightened by standard electrochemical redox potential difference (Ag^+ = 0.8 V and Hg^{2+} = 0.92 V). Due to high electrochemical redox potential of Hg^{2+} ions, this oxidized Ag to Ag^+ ions [48].

Figure 5c represented a linear relationship between concentration and absorption intensity of Hg^{2+} with correlation factor (R^2) is 0.85 and estimated the limit of detection (LOD) and observed 44.3 µM, which is lower than previously reported green synthesized AgNPs [49]. The results revealed that the green synthesized nanoparticle is a competent, environmental-friendly as well as economical sensor for the detection of the concentration (µM) of Hg^{2+} ion.

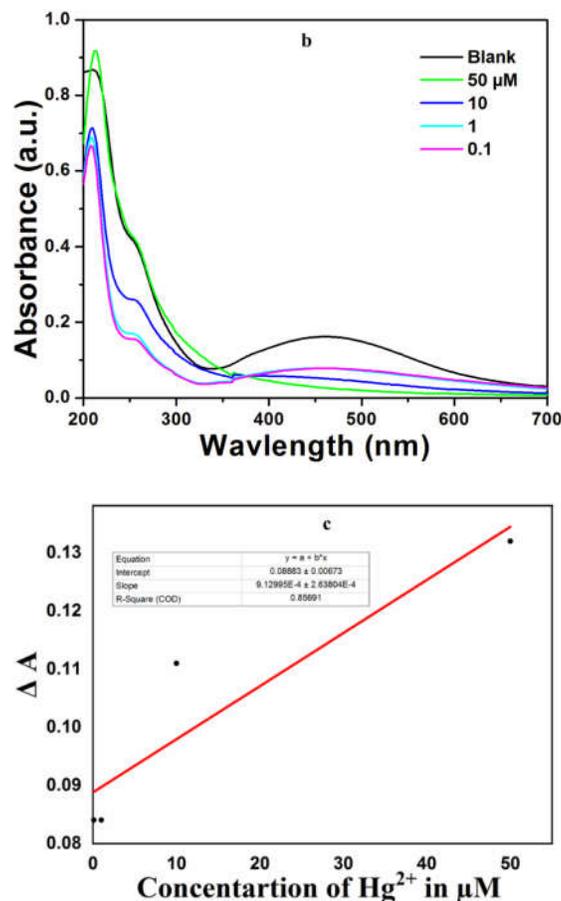Figure 5. (a) images of color variations of AgNPs with Hg^{2+}, (b) UV-Vis absorption spectra of Hg^{2+} (50 μM to 0.1 μM) with blank (AgNPs-2), (c) linear relationship between concentration and absorption intensity.

CONCLUSION

In this work, we efficaciously prepared a series of AgNPs using acacia confusa leaf extract which is eco-friendly and cost effective at ambient room temperature by ultrasonic method. Importantly green synthesized AgNPs derived from plant extracts have been successively exhibited as efficient colorimetric sensor for potential hazardous metal ion (Hg^{2+}). The presented biomolecules in the extract which are responsible for the reduction of silver ions into silver metal and then form nanoparticles after nucleation. The UV-Visible spectroscopy and color changes confirmed the formation of AgNPs during the reaction. From the SEM micrographs, the results revealed that extract volume is not significantly effect on the morphology except for size. Moreover, the prepared AgNPs nanoparticles showed encouraging colorimetric and optical sensing activity precisely towards toxic Hg^{2+} ion and we hope that prepared AgNPs may be a different tactic for finding of Hg^{2+} ions.

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