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ORIGINAL ARTICLE

Colorimetric Sensing of Toxic Metal and Antibacterial Studies by Using Bioextract Synthesized Silver Nanoparticles

A. Kalam[1,2] · A. G. Al-Sehemi[1,2] · S. Alrumman[3] · G. Du[4] · M. Pannipara[2] · Mohammed Assiri[1] · Hussain Almalki[5] · Mahmoud F. Moustafa[3]

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Abstract Here, we report the simple and cost effective colorimetric technique for the determination of toxic metals (Hg^{2+}) in aqueous sample by using bioextract silver nanoparticles (AgNPs). The indigenous AgNPs were synthesised by green and ecologically friendly style using extract of fig (Ficus carica) leaf. The synthesized AgNPs were confirmed by UV–vis spectroscopy, FT-IR spectroscopy, and scanning electron microscopy methods. The synthesis of AgNPs was observed by its colour changing from light yellow to dark brownish. The existence of furanocoumarins bioactive materials in the fig leaf extract, which act as bio-reducing and capping agent, help in the formation of stabilized silver nanoparticles. In addition, the bacterial activity of the synthesized silver nanoparticles was tested against gram-negative (*Klebsiella oxytocam*, *Pseudomonas aeruginosam*, *Shigella flexneri* and *Proteus mirabilis*), gram-positive (*Staphylococcus aureus* and *Micrococcus luteus*) and one Candida (*Candida albicans*) human pathogen and the results showed moderate activity.

Keywords Silver nanoparticle · Green synthesis · Electron microscopy · Colorimetric sensing · Antibacterial studies

Introduction

Metal nanoparticles have attracted more attention because of their potential applications in various fields such as biology, medicine, chemistry and physics [1, 2]. Predominantly, silver nanoparticles (AgNPs) have gained significant interest due to their application towards therapeutics, biomolecular, drug delivery, waste management [3, 4], catalysis [5], eco-friendly and cost effective synthesis. In general, these properties mostly depend on the particle size, capping sheet, surface area and morphology of nanoparticles [6–8]. Therefore, it is necessary to develop a cost effective and environmental friendly synthetic route to prepare AgNPs. Innumerable bacteria [9], fungi [9, 10] and plant leaves [9, 11–16] are employed for the biosynthesis of AgNPs which act as reducing as well as capping agents. Even though the exact mechanisms are still under dispute, using plant extracts for biosynthesis of AgNPs have received greater attention in recent years over microorganisms due to the ease of handling, simplicity, environmental friendly and cost effective nature compared to the elaborate process of maintaining cell cultures [11–15].

Currently, the functionalised silver nanoparticles (AGNPs) have attracted much more attention due to their exclusive optical and active sensor properties [17, 18]. Nanoparticle surfaces have to be functionalized with

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✉ A. Kalam
abul_k33@yahoo.com

1 Department of Chemistry, Faculty of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia

2 Research Center for Advanced Materials Science (RCAMS), King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia

3 Department of Biology, Faculty of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia

4 Zhejiang Key Laboratory for Reactive Chemistry on Solid Surfaces, Institute of Physical Chemistry, Zhejiang Normal University, Jinhua 321004, China

5 Department of Chemistry, University College in Al-Qunfudah, Umm Al-Qura University, 1109 Makkah Al-Mukarramah, Saudi Arabia

relevant metal networking chemical units for the selective sensing of heavy metal ions, which is not an eco-friendly style [19–21]. The extract obtained from plants contain various metal networking functional groups like –OH, –COOH, $–NH_2$, –CN and heterocyclic ring which act as stabilising and reducing agent during the synthesis of AgNPs. Therefore, we propose a prospect to advance ecologically benevolent and effective colorimetric sensors for harmful metal ions in aqueous solution [22]. Moreover, AgNPs show great antimicrobial potential activity and biocompatibility than other nanoparticles [3, 4]. We selected this particular plant because it is available and easily accessible all over the world, and acts as a green catalyst, bioreductant and solvent. These properties might be tuned to deliver the appropriate sensing and antimicrobial properties. Herein, we report the green synthesis of AgNPs using fig leaf extract and demonstrate the ability of green synthesized AgNPs to detect heavy metal ions in aqueous solution and antibacterial studies. To the best of our knowledge, there has been no report available for colorimetric sensing of Hg^{2+} by using fig leaf extract AgNPs.

Materials and Methods

Materials

All the reagents and solvents were used as received without additional purification. Silver nitrate ($AgNO_3$) (BDH reagent, ≥99.0%) was purchased from Sigma–Aldrich Co. Double distilled water was used in all experiments. The stock solution of $3CdSO_4.8H_2O$, $NiCl_2.6H_2O$, $CoCl_2.6H_2O$, $Pb(CH_3COO)_2.3H_2O$, $Mn(CH_3COO)_2.4H_2O$, $Hg(NO_3)_2.H_2O$ and $FeSO_4.7H_2O$ were prepared in doubled distilled water and test solutions for metal ion detection were prepared by diluting proper aliquot of each metal ion stock and AgNPs stock solution to preferred concentration. The clinical isolates were obtained from the Microbiology Laboratory, Biology Department, Faculty of Science and Microbiology Laboratory, Faculty of Medicine, King Khalid University, Kingdom of Saudi Arabia.

Extraction and Synthesis of AgNPs

Fig leaves were procured from Abha near to King Khalid University and washed several times with doubly distilled water to eliminate dust particles and kept at dark place for drying. 5.0 g of sieved dry fig (*F. carica*) leaves were mixed with 250 ml distilled water and left to stand for 24 h at room temperature. Earlier to an experiment, leaf extract (bio-extract) was centrifuged at 35,000 rpm for 5 min to isolate any solid particles from it. The extract was stored and was used for the synthesis of AgNPs. In a conical flask, 10 ml of 0.01 molar silver nitrate solution was added drop wise into the 1 ml of bio-extract at 25 °C. After 30 min, the colour of the solution changed from light yellow to dark yellowish but the final permanent colour was dark brown with increase in time, which is the clear sign for the creation of silver nanoparticles (Fig. S1). The prepared nanoparticles solution was stored at room temperature for further study.

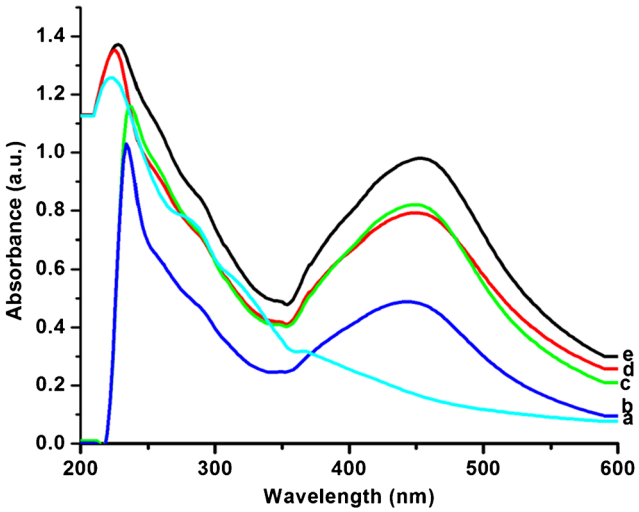

Fig. 1 UV–visible spectra of (**a**) extract and AgNPs prepared from (**b**) 1 (**c**) 1.2 (**d**) 1.4 and (**e**) 1.6 ml fig extract

Colorimetric Sensing of Hg^{2+}

The prepared AgNPs were assessed as a colorimetric sensor for numerous metal ions (Cd^{2+}, Ni^{2+}, Co^{2+}, Pb^{2+},

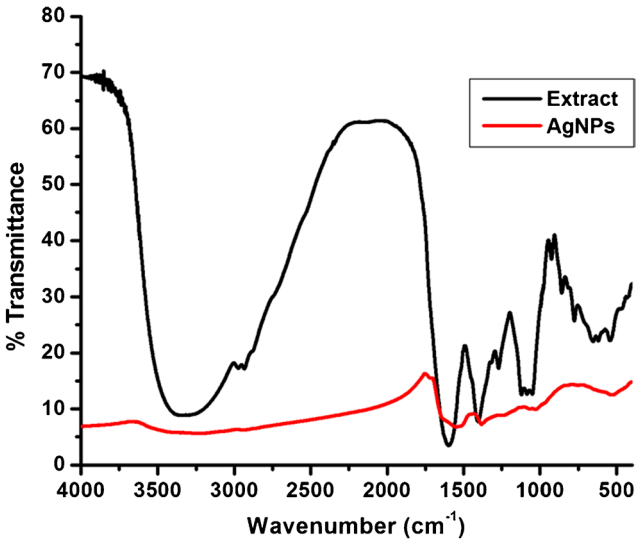

Fig. 2 FT-IR spectrum of Extract and AgNPs prepared from 1 ml fig extract

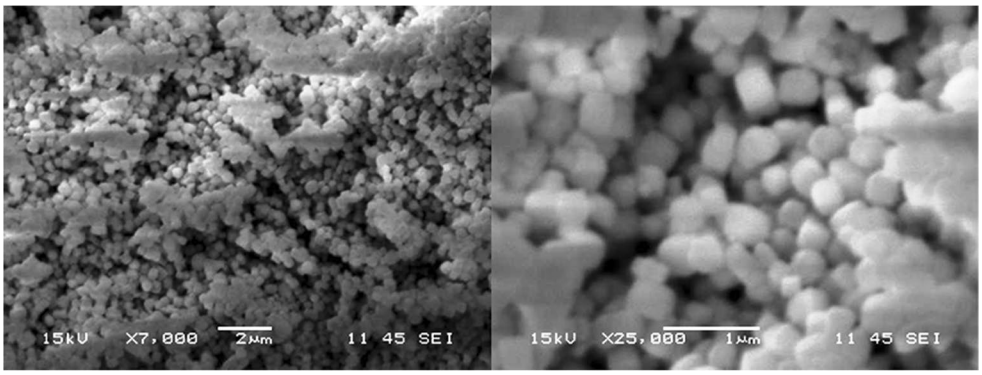

Fig. 3 SEM micrograph of AgNPs prepared from 1 ml fig extract

Mn^{2+}, Hg^{2+}, Fe^{2+}) by metal salt solutions (10^{-3} M) into a dilute solution of green synthesized AgNPs (final volume 10 ml) which displayed selective decolorization only for Hg^{2+}ion (Fig. 4b). We added different concentrations of Hg^{2+} ion solution to the solution containing 0.1 ml AgNPs in a 10 ml volumetric flask for colorimetric sensing of Hg^{2+} ion. The spectral measurements were recorded by using UV–visible spectrophotometer after completion of the reaction.

Biological Studies

The silver nanoparticle was tested for antibacterial activity against both gram-negative (*Proteus bacilli* and *Klebsiella pneumoniae*) and positive (*Staphylococcus aureus*) bacteria by using the standard agar well diffusion method [23] at the concentration of 100 mg/mL in dimethyl sulfoxide (DMSO). Antibiotic chloramphenicol used as standard for antibacterial activity. The zone of inhibition was measured in millimetres and the activity was compared with the standard at the concentration of 30 µg/mL.

Characterizations

The progress of the reaction was monitored by its colour changing from colourless to brown. The absorption spectra were recorded on UV–visible spectrophotometer (PG instrument) and the scanning electron microscopy (SEM; Hitachi S4800) was used to study the surface morphologies of AgNPs. Fourier transform infrared spectroscopy (FTIR) was used to identify the functional groups and bio-reductants. It was carried out in the range of 4000 – 500 cm^{-1} at room temperature by using JASCO 460 plus.

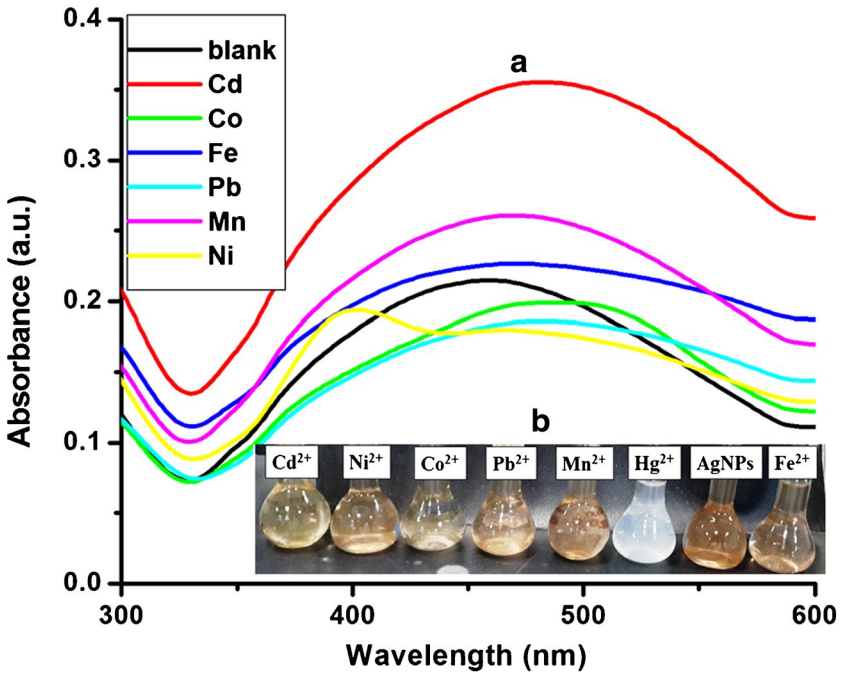

Fig. 4 **a** UV–visible spectra of AgNPs with different metal ions (10^{-3} M) in aqueous solution **b** Images of selective sensing of Hg^{2+} ion by AgNPs in aqueous solution

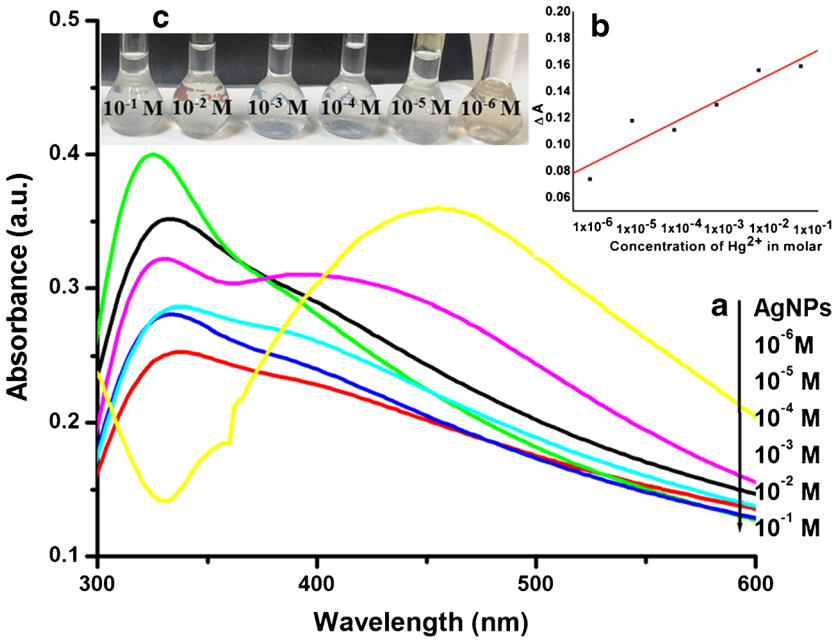

Fig. 5 **a** UV–visible spectra of AgNPs with different concentration of Hg^{2+} (10^{-1} to 10^{-6} M) in aqueous solution. **b** Variation of the absorbance of AgNPs solution as a function of Hg^{2+} ion concentration **c** Images of colour variations of AgNPs with different concentration of Hg^{2+} (10^{-1} to 10^{-6} M) in aqueous solution

Results and Discussion

The progress of AgNPs synthesis was monitored by its colour changing from light yellow to brownish black. Figure 1 displays the absorption spectra of synthesized AgNPs with different volumes of bio-extract (1, 1.2, 1.4 and 1.6 ml) along with pure fig extract. The pure extract showed the narrow absorption peak at 224 nm endorsed to the polyphenol that would be responsible for reduction of Ag^{+} ion to Ag^{o} and stabilizing agent [24]. The pure extract did not exhibit any absorption peak between 400 and 500 nm which is a clear indication that there are no nanoparticles. Figure 1 proved the synthesis of AgNPs at room temperature and broad absorption spectra obtained between 440 and 452 nm, which is the characteristic spectra of AgNPs due to Surface Plasmon Resonance (SPR). As the volume of bioextract decreases, the absorption band becomes blue shifted which revealed that the AgNPs could be small size and monodisperse, respectively. AgNP prepared from a lower volume of fig extract with constant volume of $AgNO_3$ showed a small blue shift in the SPR absorption; a fact that could be endorsed to the availability of more capping molecules. Therefore, we selected only the AgNPs which is prepared by using 1.0 ml bioextract for further studies.

The FT-IR spectrum of pure fig extract and prepared silver nanoparticles (AgNPs) are shown in Fig. 2. The fig extract and AgNPs both showed an absorption band at ~3333 cm^{-1} due to the presence of O–H stretching vibration but in the case of AgNPs the intensity decrease which indicates the interaction of O-H on the surface of silver nanoparticles [25, 26]. For fig extract, the bands appeared at 1033, 1086, 1117 and 1278 cm^{-1} which may originate from the stretching vibration of C–O and C–N bonds of phenol, alcohols, amines, carboxylic acid and their derivatives. The bands at ~1380, 1547 cm^{-1} are ascribed to the bending mode of alpha CH_3 in aldehydes and ketones and mostly by the bending mode of O–H bonds in alcohols and phenols, respectively. A stretching vibration band which appears at ~521 cm^{-1} may be due to the adsorption or interaction of O–H on the surface of silver nanoparticles. FTIR spectra of fig extract and AgNPs exhibit almost same peaks which suggest that their capping molecules are same. Therefore, the FTIR results revealed that the biomolecules of extracts are present on the surface of silver nanoparticles [16]. The FTIR results demonstrated that synthesized AgNPs might be stabilized by the existence of furanocoumarins bioactive materials in the fig leaf extract, which act as bio-reducing and capping agent, and therefore help in the formation of stabilized silver nanoparticles.

Table 1 Antibacterial activity of silver nanoparticles

Chemicals name	*S. aureus*	*M. luteus*	*K. oxytocam*	*P. aeruginosam*	*S. flexneri*	*P. mirabilis*	*C. albicans*
AgNPs with 1 ml extract	3.13 ± 0.06	2.6 ± 0.42	4.06 ± 0.10	2.22 ± 0.15	4.10 ± 0.10	3.37 ± 0.15	3.23 ± 0.21
AgNPs with 1.6 ml extract	2.90 ± 0.10	3.6 ± 0.35	2.06 ± 1.27	1.90 ± 0.10	3.78 ± 0.15	3.10 ± 0.10	2.78 ± 0.25
Cefoxitin- 30 µg	2.87 ± 0.37	2.6 ± 0.42	3.12 ± 0.28	2.22 ± 0.15	2.9 ± 0.17	2.72 ± 0.19	2.93 ± 0.21

Figure 3 displays the micrographs of AgNPs using Scanning electron microscopy (SEM) at various magnifications. The SEM images of AgNPs show cubic shapes with uniform size distribution. The small degree of agglomeration could be seen in SEM investigations that may be due to the effect of bioextract during synthesis. The SEM studies clearly depicts that the bioorganic extract play a momentous role for producing the cubic AgNPs materials.

Figure 4a illustrates the selective colorimetric sensing of various metal ions (Cd^{2+}, Fe^{2+}, Co^{2+}, Pb^{2+}, Ni^{2+}, Mn^{2+}, Hg^{2+}) upon addition of the green synthesized AgNPs which exhibited slightly red shifted SPR peak except Hg^{2+} which caused the blue shift in the absorption spectra. The brown colour of AgNPs solution becomes colourless upon addition of Hg^{2+} solution and validated the selective sensing of Hg^{2+} ion (Fig. 4b). Figure 5a shows the absorption spectra of AgNPs in the presence of different concentration of Hg^{2+} ion (10^{-1} to 10^{-6} M). The results display that by increasing the concentration of Hg^{2+} ions, the absorption decreases simultaneously as can be seen from Fig. 5a. Figure 5c shows that the brown black colour of AgNP scattering diminishes in changing degree or deviations to colourless contingent on the concentration of Hg^{2+} ion. The mechanism of colorimetric sensing of Hg^{2+} ion by fig mediated synthesis of AgNPs could be explained on the basis of electrochemical series. According to the electrochemical series, metals with higher electrochemical reduction potential act as better oxidising agents. As we know that, the standard reduction potential of Ag^{+} is 0.80 V whereas for Hg2+ is 0.92 V. A good linear correlation ($R = 0.86484$) existed between the net adsorption (ΔA) value and the concentration of Hg^{2+} over the concentration range from 10^{-1} to 10^{-6} M (Fig. 5b) where, $\Delta A = A_0 - A$. A_0 represents the initial absorption intensity of AgNPs and A stands for the absorption intensity of AgNPs after reaction with Hg^{2+}.

Bio-synthesized AgNPs display effective antimicrobial activity as compared to the other metal salts. Table 1 demonstrates the antibacterial activity of AgNPs against *S. aureus*, *M. luteus*, *K. oxytocam*, *P. aeruginosam*, *S. flexneri*, *P. mirabilis*, *C. albicans* and Cefoxitin in which 30 µg was used as a positive control. The results showed that the prepared AgNPs exhibits maximum inhibition zone against *S. flexneri* (4.10 ± 0.10) and minimum inhibition zone against *P. aeruginosam* (2.22 ± 0.15). It was noted that AgNPs prepared from less volume of bioextract has stronger activities due to small size against all tested microbes. The mechanism of effect of AgNPs inside the microorganism is vague but, it is presumed that due to the small average size of AgNPs, they may have the capability to pierce through the innermost bacterial cell and decrease the respiration due to reduction of energy or impairment of cell membrane.

Conclusion

In the current study, we established the green synthesis of cubic AgNPs from fig leaf extract which is expedient and eco-friendly. The formation of AgNPs during reaction was confirmed by UV–visible spectroscopy and colour changes. The SEM micrographs of prepared AgNPs showed cubic shapes which are mono dispersed. Here, we report for the first time the selective colorimetric sensing of Hg^{2+} ion and antibacterial study by using AgNPs prepared from fig extract. The AgNPs displayed colorimetric sensing property only with Hg^{2+} and could be examined by bare eye or with UV–vis spectrophotometer. The prepared AgNPs display moderate activity against pathogen which can be attributed to the larger size and low surface area.

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