ijlpr 2020; doi 10.22376/ijpbs/lpr.2020.10.2.L76-82

International Journal of Lifescience and Pharma Research

Research Article

Chemistry for better environment

Colorimetric Sensing of Hg^{2+} and Fe^{3+} Ions in aqueous solution using Green Synthesized Silver Nanoparticles

Pinaki Mandal*[1] and Samir Maji[2]

[1] *Department of Chemistry, Bankura Sammilani College, Kenduadihi, Bankura-722102, West Bengal, India.*

[2] *Department of Chemistry, Ramananda College, Bishnupur, Bankura-722122, West Bengal, India*

Abstract: Plant extracts have a very important role for the synthesis of different nanoparticles due to its reducing capability, availability and nontoxicity. Biosynthesized silver nanoparticles were investigated for colorimetric sensing of metal ions at different concentrations. Aqueous leaf extract of *Plumeria alba* was used as bio reduction to synthesize stable Silver nanoparticles (AgNPs). A possible interaction between AgNPs and metal ions at different concentrations was proposed on the basis of UV-Vis spectral analysis. The produced silver nanoparticles were characterised by different technique which includes UV–vis spectroscopy and transmission electron microscopy (TEM). The intensity of the localized surface plasmon absorption band at 438 nm in UV-visible spectrum confirmed the presence of silver nanoparticles. TEM analysis showed the spherical and irregular particles shape and size of nanoparticles ranging from 20 to 100 nm. Metal ion sensing properties of these AgNPs in aqueous solution has been explored. In the detection of different metal ions using these green synthesized AgNPs, the promising colorimetric response was observed with naked eyes and spectroscopic changes were examined by using a UV-Visible spectrophotometer. The colorimetric sensor studies showed selective sensing of Fe^{3+} and potentially hazardous Hg^{2+} ions in aqueous medium with a limit of detection of about 0.2 mM concentrations. This method may be applied as low cost and high potential colorimetric sensor for the selective recognition and monitoring of Hg^{2+} and Fe^{3+} ions in biological, pharmaceutical samples as well as in water.

KeyWords: *Plumeria alba*, Green synthesis, silver nanoparticles, metal ion, colorimetric Sensing

***Corresponding Author**

Pinaki Mandal , Department of Chemistry, Bankura Sammilani College, Kenduadihi, Bankura-722102, West Bengal, India. Email id : p_naki@rediffmail.com

Recieved On **21 January 2020**
Revised On **06 February 2020**
Accepted On **24 February 2020**
Published On **06 April 2020**

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Pinaki Mandal and Samir Maji , Colorimetric Sensing of Hg2+ and Fe3+ Ions in aqueous solution using Green Synthesized Silver Nanoparticles.(2020).Int. J. Life Sci. Pharma Res.10(2), 76-82 http://dx.doi.org/10.22376/ijpbs/lpr.2020.10.2.L76-82

1 INTRODUCTION

Pollution by heavy metal is one of the most serious environmental problems, which undermines global sustainability. Due to increasing industrial activities heavy metals enter the environment and have been found to be potential pollutants even at trace concentrations[1]. They are persistent pollutants because they do not breakdown in the environment and can bio-accumulate through a food web which serves as a treat to the environment and human health[2]. Numerous analytical methods for heavy metal measurement include atomic absorption spectroscopy (AAS)[3], inductively coupled plasma/ mass spectrometry, high performance liquid chromatography (HPLC)[4], inductively coupled plasma/atomic emission spectrometry, flame photometry[5], ultraviolet−visible spectroscopy, etc.[6] Although these techniques are highly sensitive, selective and have good accuracy they still have several major limitations. These methods require sophisticated instrumentation, laborious sample pre-treatment processes, and well-trained operators etc., which makes them unsuitable for rapid detection[7-8]. Therefore the advancement of real-time, rapid, highly sensitive, selective and cost-effective tools is important for detection, measurement of heavy metals concentration in environmental, drinking water, food and biological samples under aqueous conditions. Recent literature reports on the utilization of nanotechnology in the analytical or biological sciences have started a new phase for designing novel nanoprobes for heavy metal detection[9]. Particularly, colorimetric sensors have been widely used to monitor metal ions due to their simplicity, cost-efficiency, high sensitivity, rapidity and applicability for on-site monitoring[10-11]. In Particular, functionalized metal nanoparticles of silver (AgNPs) have attracted much attention for their effective sensing ability towards heavy metal detection due to their unique optical properties[12]. Generally, metal nanoparticles can be synthesized by two methods: chemical method (bottom-up) and the physical method (top down)[13-14]. The most reported method is the chemical reduction method which involves the reduction of metal ions using different reducing agents like borohydrides, hydroxylamine hydrochloride, trisodium citrates and dimethylformamide[15]. However most of the methods are quite expensive and potentially hazardous to the environment, requiring high energy consumption[14]. In such a situation, biological approach for synthesis of nanoparticles appears to be very appropriate. Recently the green synthesis of silver nanoparticles using different plant extract like *Bombax ceiba* flower[16] Latex of *Plumeria rubra* [17], *Carica papaya* leaf[18], Flower of *Butea monosperma*[19] and *Plumeria rubra* flower[20] is available. For the selective sensing of heavy metal ions, Nanoparticle surfaces have to be functionalized with receptors with specific binding affinity for metal ions which is not an eco-friendly approach[21-22]. Different phytoconstituents as well as metal interacting multi-functional groups like hydroxyl, carboxyl groups etc. were present in the plant (flower, leaf, bark, fruit etc) extracts, supposed to involve in the reduction and capping of silver nanoparticles offer an opportunity to develop cost effective and environmentally benign colorimetric sensors for metal ions in aqueous solution. The green synthesized AgNPs using different plant extracts showed colorimetric sensing of Hg^{2+}, Pb^{2+}, Co^{3+} Zn^{2+} and Fe^{3+}metal ions at micromolar concentrations [7,23-24]. In the present study, we showed that an aqueous extract of *Plumeria alba* leaf was used in reduction of Ag(I) and demonstrate the ability of this green synthesized AgNPs to detect $Hg^{2+}$and Fe^{3+} ions in aqueous solution.

2 MATERIALS AND METHODS

2.1 Preparation of leaf extract

Fresh leaves of *Plumeria alba* were collected from the Bankura town, West Bengal, India. To remove the dirt and dust particles, the collected leaves were cleaned by washing many times with double distilled water. Then, they were dried in sunlight and ground to make powder. 10g of powder taken in 250 ml beaker and 100 ml double distilled water was added and allowed to sit in a hot water bath for 30 minutes. Then filtered and a very pale yellow transparent extract solution was collected. This leaf extract was used for nanoparticle synthesis.

2.2 Chemicals

0.5M $AgNO_3$(Silver nitrate) ($AgNO_3$, Sigma Aldrich, USA) solution was prepared for Silver nanoparticles synthesis. All the heavy metal salt solutions (10 mM, stock) were prepared by mixing the requisite amount of salt in Double distilled water. From stock solution different concentration metals solution used for experiments were prepared.

2.3 Bio-Synthesis of Silver nanoparticles using leaf extract

The green Synthesis of Silver Nanoparticles using leaf extract was done according to procedure described by Mandal 2018[20] who reported synthesis of Silver nanoparticles using flower of *Plumeria rubra* flower. Here we have synthesized Silver nanoparticles using aqueous *Plumeria alba* leaf extract. Digital photographs were taken for these nanoparticles at different time intervals.

2.4 Instruments for Characterization

The obtained AgNPs were characterized using UV-vis spectroscopy and their particle sizes were measured using Transmission electron microscope (TEM). Initially colour change was detected using naked eye. UV-Vis absorption spectra were recorded on Shimadzu UV–visible spectrophotometer (UV-1800) within the 200–700 nm range using 1 cm quartz cell. The morphology and particle sizes of the biosynthesized silver nanoparticles were examined using a high-resolution transmission electron microscope (TEM, JEOL JEM1400 plus microscope, operated at an accelerated voltage of 120 kV). TEM's grid was prepared by spreading 5μl of the silver nanoparticles solutions on carbon-coated copper grids followed by air-dried and then analyzed.

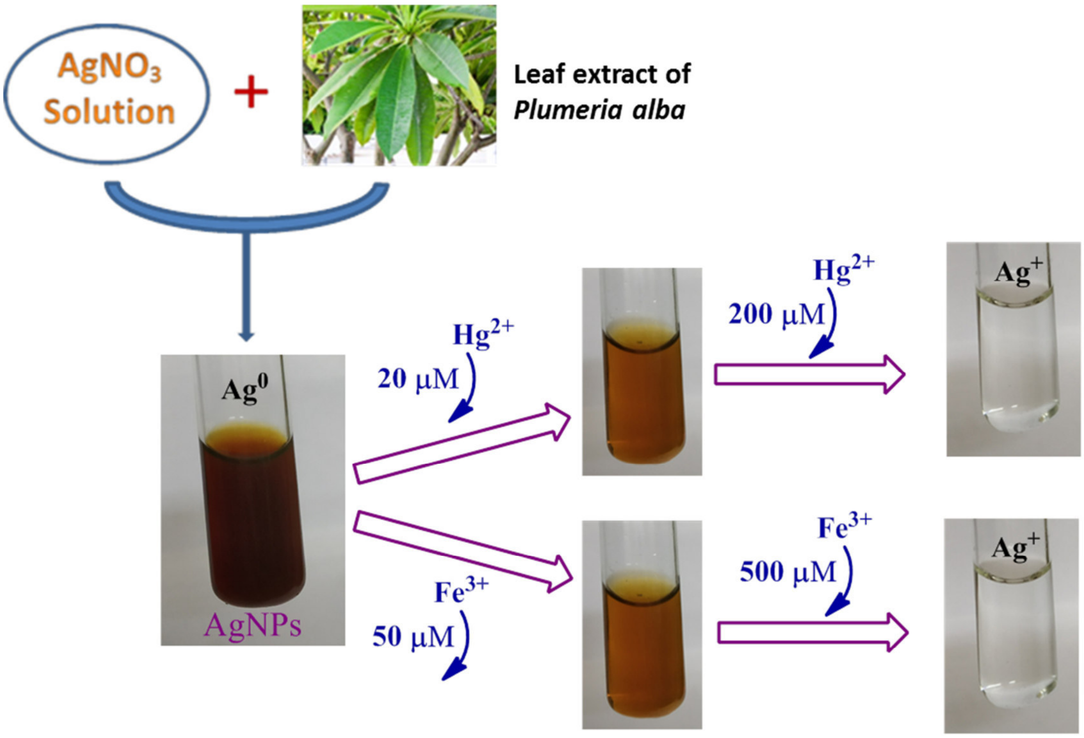
2.5 Colorimetric determination of Hg^{2+} and Fe^{3+} ions

For colorimetric detection of mercury (II) and iron (III) ions in aqueous samples, as-prepared AgNPs were diluted three times by double distilled water and transferred to test tubes and then known concentration of Hg^{2+}, Fe^{3+} and other metals ions (Pb, Cu, Mg and K) were added into the test tube. The changes in the UV–vis absorption spectra were monitored and performed at room temperature. The photographs were taken with a digital camera after 5 min of mixing. All the experiments were carried out in triplicate to confirm reproducibility.

3 RESULTS AND DISCUSSION

3.1 UV-Vis Spectroscopy and TEM analysis of AgNPs

The present study reports the green synthesis of silver nanoparticles (AgNPs) from leaf extract of *Plumeria alba* and synthesized AgNPs were investigated for colorimetric sensing of metal ions at different concentrations as described in Scheme 1.



Scheme 1. Schematic representation for green synthesis of AgNPs and sensing strategy of metal ions at different concentrations.

Nanoparticles (NPs) are generally characterized by their size, shape, surface area, and dispersity. When the leaf extract was mixed with $AgNO_3$ at room temperature, no colour change observed. However, as the reaction proceeded, within 15 min of the reaction, the solution starts colour change and turned its colour from very light yellow to dark brown (Figure. 1) due to the reduction reaction of Ag^+ ions to Ag^0, indicating the formation of AgNPs of different particle sizes.

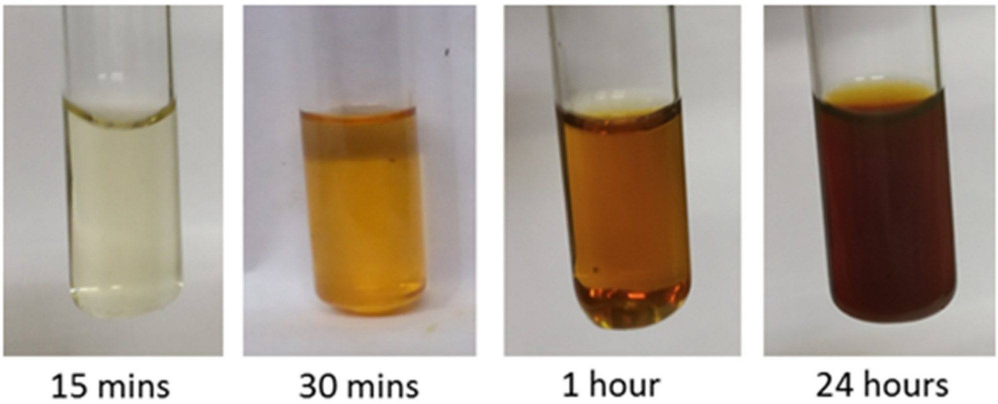


Fig 1. Digital photograph of green synthesized AgNPs from a mixture of aqueous *Plumeria alba* leaf extract and $AgNO_3$ solution at different time intervals.

This bio-reduction is an efficient, one-pot, rapid and cost-effective method which was very well explained by other researchers [25-26]. Change in colour was due to the excitation of surface plasmon vibrations in metal nanoparticles[27]. Our results similar to Mandal et al., 2018 [19-20], who reported the formation of AgNPs within 15 min of incubation. It is believed that water-soluble phenolic acid and flavonoid compounds which are present in *Plumeria alba* leaf [28-29] play a major role in bio-reduction reaction, though the possible mechanism is still unknown. The characteristic dark brown colour of silver nanoparticles provided a convenient spectroscopic signature to indicate nanoparticles formation. Figure 2 shows the UV-Vis spectra of synthesized AgNPs using aqueous leaf extract of *P. alba* taken at different reaction time intervals.

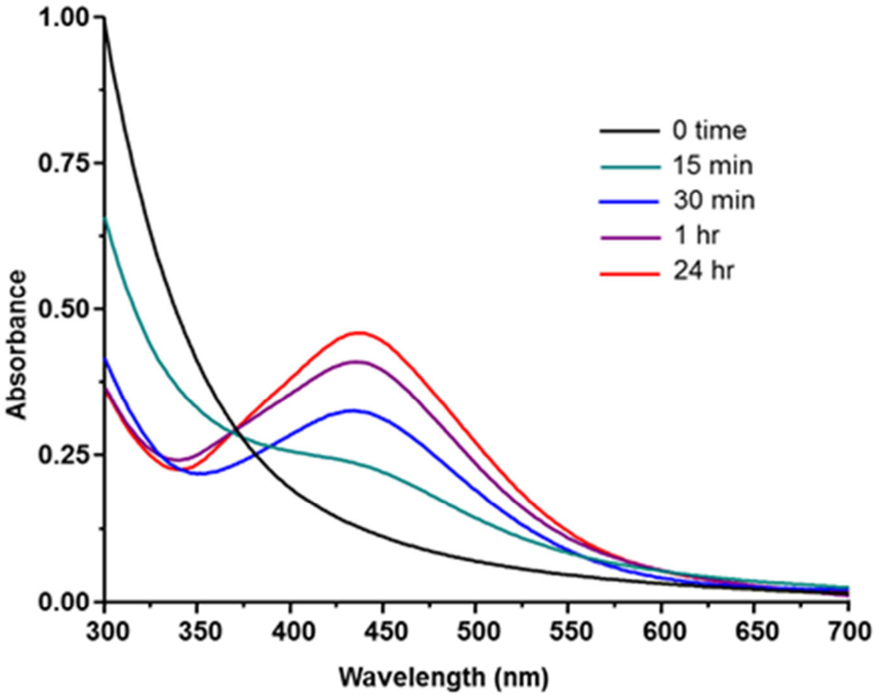


Fig 2. UV–vis absorption spectra of mixture of aqueous *Plumeria alba* leaf extract and $AgNO_3$ at different reaction time.

After addition of freshly prepared silver nitrate solution into the aqueous leaf extract, the consequent colour changes of the reaction mixture was monitored from very light yellow to dark brown within 24 hours. The maximum absorbance peak at 438 nm in the UV-absorption spectrum, confirmed the presence of surface plasmon resonance peak of silver nanoparticles (Figure 2). The spectra of silver nitrate solution and leaf extract did not show any surface plasmon resonance (SPR) band. The intensity of the SPR peak increases with reaction time (15 mins, 30 mins, 1 hr, 24 hrs). However, the wavelength of SPR peaks does not change. The increase in intensity could be due to an increase in the number of AgNPs form as a result of reduction of silver ions present in the aqueous solution. UV-vis spectra of synthesized nanoparticles showed a single SPR band which indicates the presence of spherical shaped nanoparticles in the reaction mixture[30]. Morphological characterization of formed silver nanoparticles was performed with transmission electron microscopy (TEM). Micrograph revealed roughly spherical shapes with a diameter range from 20 to 100 nm (Figure 3) which agree to the shape of single SPR band in the UV–visible spectrum. However, some of the nanoparticles were found to be having structures of irregular shape.

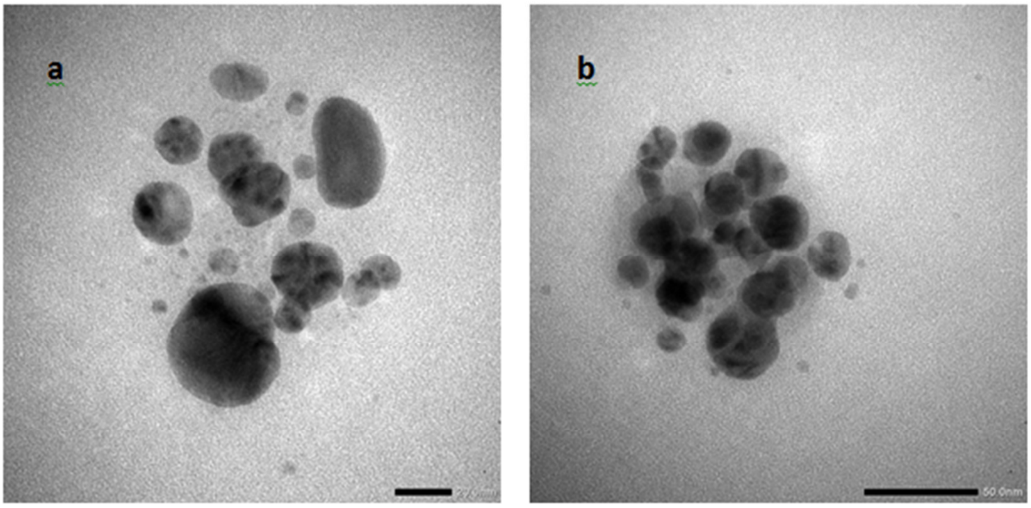


Fig 3. Different magnifications (a,b) of Transmission Electron Microscopy images of Silver Nanoparticles synthesized using *Plumeria alba* leaf extract.

3.2 UV-Visible spectral study and optical sensing of Hg^{2+} and Fe^{3+} ions

Hg^{2+} is one of the most hazardous metal pollutants, and is widely distributed in water, soil and even food. Toxic effect of mercury includes damage to the brain, lungs, kidney and especially nervous system[31]. The sensitivity and selectivity of the leaf extract reduced AgNPs towards various metal ions were monitored by UV-visible spectral change and also the colour change was visualized with naked eyes. As-prepared AgNPs were characterized before and after addition of metal ions using UV–vis spectroscopy. As shown in Figure 2, the characteristic SPR band of the AgNPs after diluting 4 times was observed at 438 nm and the solution was almost colourless in the absence of metal ions. Out of several number of metal ions tested, only Hg^{2+} and Fe^{3+} ions exhibited significant spectral change indicating that the AgNPs have very high selectivity and specificity toward Hg^{2+}, Fe^{3+} ions and AgNPs are not significantly sensitive to other metal ions under similar conditions. The addition of Hg^{2+}solution into AgNPs decolourised the solution from dark brown and demonstrated the selective sensing of Hg^{2+} ions. The minimum detectable concentration of Hg^{2+}ions in aqueous solution (visible observation of colour change) was determined by adding different concentrations of mercury ions into AgNPs and monitoring the absorbance change (Figure 4 A).

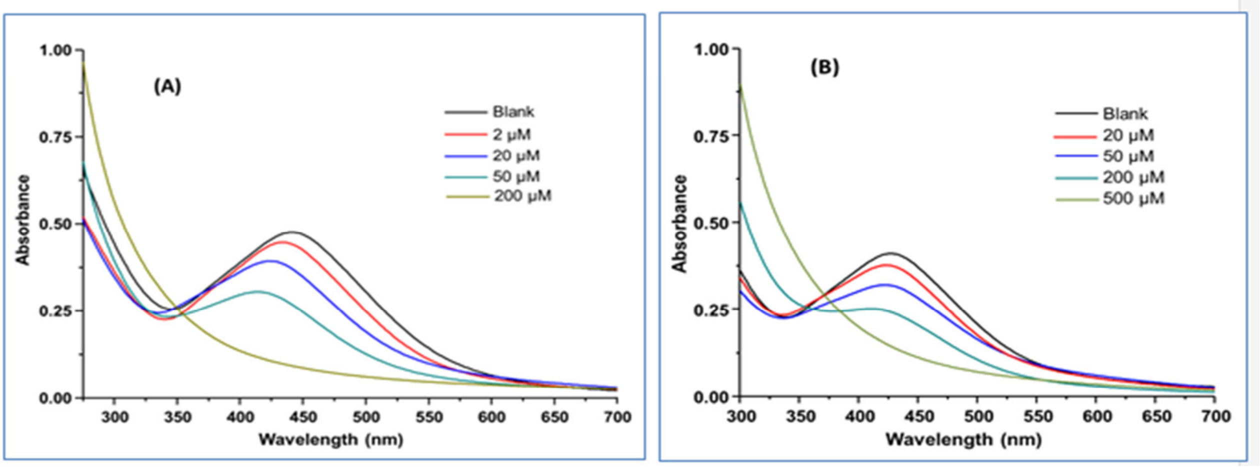


Fig 4. UV-visible spectra of AgNPs interaction with various concentrations of metal cations (A) change of AgNPs absorption in presence of Hg^{2+} ions (B) change of AgNPs absorption in presence of Fe^{3+} ions

Mercury (II) ion with a closed-shell d^{10} configuration has no optical spectroscopic signature. After addition of 2 μM Hg^{2+} to Ag NPs, the colour of solution decreases accompanying with the broadening and blue shifting of the SPR band and finally turns to colourless after addition of 200 μM of Hg^{2+} at room temperature. This may be stated that the observed phenomena is probably related to a redox reaction between zero-valent silver and Hg^{2+}. The oxidation of AgNPs is due to electrochemical reduction potential differences. According to the electrochemical series, metals with a higher reduction potential act as better oxidising agents. The standard reduction potential for Ag is +0.80 V ($Ag^+ + e^- = Ag^0$) whereas for Hg^{2+} it is +0.92 V ($2Hg^{2+} + 2e^- = 2Hg^0$). Therefore the AgNPs were oxidised to form Ag^+ and Hg^{2+} reduced to Hg^0 or Hg_2^{2+} [32-33]. Similarly, quantitative Fe^{3+} assays for direct colorimetric visualization were done by adding various concentrations of Fe^{3+} to the AgNPs solution and the changes in the SPR band were noted using UV−visible spectroscopy (Figure 4 B). The colour of AgNPs solution changes from yellowish-brown to pale yellow and its colour decreases gradually with increasing concentration of Fe^{3+} and finally changes to colourless after addition of 500 μM of Fe^{3+}. The addition of iron (III) solution resulted in a redox interaction between the iron(III) and AgNPs present in the solution. After the addition of iron(III) into the solution, the AgNPs reduced the Fe^{3+} to zero valent iron. Annadhasan et al., 2014 [7] reported similar observations. It is noted here that the intensity of the SPR band decreases remarkably with a blue shift in the λ_{max} and showed preferably strong interactions between Hg^{2+} and AgNPs when compared to that of Fe^{3+} and AgNPs.

4 CONCLUSION

We have reported a simple, fast and an eco-friendly method for synthesis of silver nanoparticles. The water soluble bioactive compounds from leaf extract of *Plumeria alba* that act as bio reduction of Ag(I) to form AgNPs. The formation of Ag-NPs was confirmed by UV-Visible spectroscopy and TEM. Importantly, a simple, cost effective, portable, selective and sensitive detection method has been developed using this biologically prepared AgNPs as a colorimetric probe that allows rapid detection of Hg^{2+} and Fe^{3+} ions. The metal ion sensing is based on the optical response (change in the absorbance strength of LSPR band) of silver nanoparticles. The mechanism probably relies on simple redox reaction between AgNPs and metal ions in solution. The presence of metal ions in the mixture will re-oxidise Ag(0) of AgNPs to Ag(I) ions. The colour of AgNPs solution changes from yellowish-brown to pale yellow and colour decreases to colourless linearly with increasing concentration of metal ions. The sensor reaction showed good sensitivity for Hg^{2+} and Fe^{3+} ions with a limit of detection of about 0.2 mM concentrations in aqueous medium. Our results indicated that apart from the usual pharmacological properties, the green synthesized AgNPs can be effectively employed for selective sensing of Hg^{2+} and Fe^{3+} ions in biological, environmental and pharmaceutical samples.

5 ACKNOWLEDGEMENT

Authors acknowledge Dr. Samaresh Ghosh, Assistant professor, Dept. of chemistry, Bankura Sammilani College, Bankura, for his support in this work. Authors are also grateful to the scholars, authors whose articles are cited and included as references in this manuscript.

6 AUTHORS CONTRIBUTION STATEMENT

P Mandal and S Maji carried out the research study and performed the experiments. P Mandal evaluated the results and drafted the manuscript. Both authors discussed the final version of the manuscript.

7 CONFLICT OF INTEREST

Conflicts of interest declared none.

8 REFERENCES

1 Firdausi ML, Fitriani I, Wyantuti S, Hartati YW, Khaydarov R, Mcalister JA, Obata H, Gamo T. Colorimetric Detection of Mercury(II) Ion in Aqueous Solution Using Silver Nanoparticles. Anal. Sci. 2017; 33(7): 831-83.
DOI: 10.2116/analsci.33.831

2 Nolan EM, Lippard SJ. Tools and Tactics for the Optical Detection of Mercuric Ion. Chem. Rev. 2008; 108(9): 3443−3480.
DOI: 10.1021/cr068000q

3 Yang Q, Tan Q, Zhou K, Xu K, Hou X. Direct detection of mercury in vapour and aerosol from chemical atomization and nebulization at ambient temperature: exploiting the flame atomic absorption spectrometer. J. Anal. At. Spectrom. 2005;20:760–792.
DOI; 10.1039/B505298J

4 Ichinoki S, Kitahata N, Fujii Y. Selective determination of mercury (II) ion in water by solvent extraction followed by reversed-phase HPLC. J Liq.Chromatogr. Rel. Tech. 2004; 27(11): 1785–1798.
DOI; 10.1081/JLC-120037371

5 Kuswandi B, Dam HH, Reinhoudt DN, Verboom W. Development of a disposable mercury ion-selective optode based on trityl-picolinamide as ionophore. Anal. Chimica Acta. 2007; 591(2):208–213.
DOI: 10.1016/j.aca.2007.03.064

6 Lorber KE. Monitoring of heavy metals by energy dispersive X ray fluorescence spectrometry. Waste Manag. Res. 1988; 1−11.
DOI; 10.1016/B978-0-12-207690-9.50004-0

7 Kannadhasan M, Muthukumarasamy vel T, Babu VRS, Rajendiran N, Campus M. Green Synthesized Silver and Gold Nanoparticles for Colorimetric Detection of Hg^{2+}, Pb^{2+} and Mn^{2+} in Aqueous Medium. ACS Sustain. Chem. Eng. 2014; 2(4): 887–896.
DOI: 10.1021/sc400500z

8 Ravi SS, Christena LR, Sai Subramanian N, Anthony SP. Green synthesized silver nanoparticles for selective colorimetric sensing of Hg^{2+} in aqueous solution at wide pH range. Analyst. 2013;138(15): 4370–4377.
DOI: 10.1039/C3AN00320E

9 Rosi NL, Mirkin CA. Nanostructures in biodiagnostics. Chem. Rev. 2005; 105(4): 1547-1562.
DOI: 10.1021/cr030067f

10 Noh KC, Nam YS, Lee HJ, Lee KB. A colorimetric probe to determine Pb^{2+} using functionalized silver nanoparticles. Analyst. 2015; 140(24): 8209–8216. DOI: 10.1039/C5AN01601K

11 Lee S, Nam YS, Lee HJ, Lee Y, Lee KB. Highly selective colorimetric detection of Zn(II) ions using label-free silver nanoparticles. Sensors Actuat. B: Chem. 2016; 237: 643–651.
DOI: 10.1016/j.snb.2016.06.141

12 Li L, Li BX, Qi YY, Jin Y. Label-free aptamer-based colorimetric detection of mercury ions in aqueous media using unmodified gold nanoparticles as colorimetric probe. Anal. Bioanal. Chem. 2010; 393(8): 2051–2057.
DOI; 10.1007/s00216-009-2640-0

13 Khandel P, Yadav RK, Soni DK, Kanwar L, Shahi SK. Biogenesis of metal nanoparticles and their pharmacological applications: present status and application prospects. J Nanostructure Chem. 2018;8(3): 217–254.
DOI; 10.1007/s40097-018-0267-4

14 Ahmed S, Ahmad SM, Swami BL, Ikram S. Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. J. Radiat. Res. Appl. Sci. 2016; 9(1):1–7.
DOI: 10.1016/j.jrras.2015.06.006

15 Lee PC, Meisel D. Adsorption and surface-enhanced Raman of dyes on silver and gold sols. J. Phy. Chem. 1982; 86(17): 3391–3395. DOI; 10.1021/j100214a025

16 Mandal P. Green Synthesis and Characterization of Polyvinyl Alcohol Embedded Silver Nanoparticles using Simul (*Bombax ceiba*) Flower Extract. Int. J. Curr. Res. Rev. 2017; 9(14): 3-6.
DOI; 10.7324/IJCRR.2017.9142

17 Banala RR, Nagati VB, Karnati PR. Green synthesis and characterization of *Carica papaya* leaf extract coated silver nanoparticles through X-ray diffraction, electron microscopy and evaluation of bactericidal properties. Saudi J. Biol. Sci. 2015;22(5): 637–644.
DOI: 10.1016/j.sjbs.2015.01.007

18 Chandrashekhar D, Patil & Satish V., Patil & Hemant P., Borase & Bipinchandra K, Salunke & Rahul B. Larvicidal activity of silver nanoparticles synthesized using *Plumeria rubra* plant Latex against *Aedes aegypti* and *Anopheles stephensi*. Parasitol. Res. 2012;110(5):1815–1822.
DOI: 10.1007/s00436-011-2704-x

19 Mandal P, Ghosh S. Green synthesis of poly(vinyl alcohol)–silver nanoparticles hybrid using Palash (*Butea monosperma*) flower extract and investigation of antibacterial activity. Poly. Bull. 2018; 75(5): 1949–1955.
DOI: 10.1007/s00289-017-2137-5

20 Mandal P. Biosynthesis of Silver Nanoparticles by *Plumeria rubra* Flower Extract: Characterization and Their Antimicrobial Activities. Int. J. Eng. Sci. Inv.2018; 7(1): 01-06

21 Zhou Y, Zhao H, Li C, He P, Peng W, Yuan L, Zeng L, He Y. Colorimetric detection of Mn^{2+} using silver nanoparticles co functionalized with 4-mercaptobenzoic acid and melamine as a probe. Talanta.2012; 97(15): 331-335.
DOI: 10.1016/j.talanta.2012.04.041

22 Mehta VN, Kailasa SK, Wu HF. Sensitive and selective colorimetric sensing of Fe^{3+} ion by using p-amino salicylic acid dithiocarbamate functionalized gold nanoparticles. New J. Chem. 2014; 38(4): 1503–1511. DOI: 10.1039/C3NJ01468A

23 Swarnali M, Gadadhar B, Jayasree KL. Detection of heavy metals (Cu^{+2}, Hg^{+2}) by biosynthesized silver Nanoparticles. Appl. Nanosci. 2016;6(4): 529–538. DOI: 10.1007/s13204-015-0452-4

24 May BMM, Oluwafemi OS. Sugar-Reduced Gelatin-Capped Silver Nanoparticles with High Selectivity for Colorimetric Sensing of Hg $^{2+}$ and Fe $^{2+}$ Ions in the Midst of Other Metal Ions in Aqueous Solutions. Int. J. Electrochem. Sci. 2016; 11: 8096 – 8108.
DOI: 10.20964/2016.09.29

25 Singhal G, Bhavesh R, Kesariya K, Sharma AR, Singh RP. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. J. Nanopart. Res. 2011;13(7): 2981–2988.
DOI; 10.1007/s11051-010-0193-y

26 Pugazhendhi A, Prabakar D, Jacob JM, Karuppusamy I, Saratale RG. Synthesis and characterization of silver nanoparticles using *Gelidium amansii* and its antimicrobial property against various pathogenic bacteria. Microb. Pathogenesis. 2018; 114: 41–45. DOI: 10.1016/j.micpath.2017.11.013

27 Rajkumar G, Rahuman AA. Acaricidal activity of aqueous extracts and synthesized silver nanoparticles from *Manilkarazapota* against *Rhipicephalus* (Boophilus) microplus. Res. Vet. Sci. 2012;93(1): 303−309.
DOI; 10.1016/j.rvsc.2011.08.001

28 Radha R, Kavimani S, Ravichandran V. Antitumor Activity of Methanolic Extract of *Plumeria alba* L. Leaves Against Dalton Lymphoma Ascites in Mice . Int. J. Health Res. 2008; 1(2): 79-85.
DOI: 10.4314/ijhr.v1i2.47919

29 Byamukama R, Jane N, Monica J, vying M A, Bernard T K. Anthocyanins from ornamental flowers of red frangipani, *Plumeria rubra*. Hort. Sci. 2011;129(4): 840-843.
DOI: 10.1016/j.scienta.2011.03.053

30 Kumar B, Smita K, Cumbal L, Debut A. Synthesis of silver nanoparticles using Sacha inchi (*Plukenetia volubilis* L.) leaf extracts. Saudi J. Biol. Sci. 2014; 21(6): 605–609.
DOI: 10.1016/j.sjbs.2014.07.004

31 Tchounwou PB, Ayensu WK, Ninashvili N, Sutton D. Environmental exposure to mercury and its toxicopathologic implications for public health. Environ. Toxicol. 2003;18(3) 149–175.
DOI: https://doi.org/10.1002/tox.10116

32 Atkins P, Paula J de. Physical Chemistry. 9th ed. Edition, W H Freeman, Oxford, 2009.

33 Gao X, Lu Y, He S, Li X, Chen W. Colorimetric detection of iron ions (III) based on the highly sensitive plasmonic response of the *N*-acetyl-l-cysteine-stabilized silver nanoparticles. Analytica chim. acta. 2015; 879: 118–125.
DOI:10.1016/j.aca.2015.04.002.