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Biofabrication of Silver Nanoparticles for Selective and Sensitive Colorimetric Detection of Hg(II) Ions

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The current study explored a green, simple, selective and cost-effective method for the detection of Hg^{2+} ions in the aqueous medium using silver nanoparticles (AgNPs) synthesized from marine macroalgae *Sargassum duplicatum*. The biosynthesized AgNPs were further characterized by using UV-visible spectroscopy, FTIR, HR-TEM, SAED and XRD techniques. The synthesized AgNPs were almost spherical in shape and polydisperse in nature. Due to the intense SPR absorption band, the biosynthesized AgNPs solution is seen as dark brown colour. In the presence of Hg^{2+}, the brown coloured AgNPs solution becomes colourless coupled with the disappearance of absorption maxima at 419 nm. The selectivity and sensitivity of AgNPs towards Hg^{2+} were also investigated and the minimum detection limit was found to be 0.1 μM. Furthermore, a test strip technique for the rapid detection of Hg^{2+} was also devised. Thus, the biogenic AgNPs are expected to be a promising candidate for developing low-cost Hg^{2+} sensors.

Keywords: Biofabrication, Silver nanoparticles, *Sargassum duplicatum*, Colorimetric sensor, Mercury sensor.

INTRODUCTION

Currently, there has been a growing ecological and worldwide public health concern over the environmental contamination caused by heavy metal ions [1]. These contaminants are highly noxious even at the trace level and didn't undergo biodegradation, but can bioaccumulate in the human body through the food chain. Among various heavy metals (*e.g.* Hg^{2+}, Cd^{2+}, Pb^{2+}, *etc.*) mercury(II) ion is one of the deadly heavy metal pollutants since it produces immense hazards to the environment as well as human health [2]. Exposure to mercury at very low concentrations would cause severe damages to the nervous system, the cardiovascular system, the immune system and vital organs such as lungs, liver, kidney, *etc.* [3]. When mercury is discharged into the atmosphere, it can travel vast distances and end up in aquatic habitats, where it can be potentially be methylated to become methylmercury. The most dangerous form 'methylmercury' bioaccumulate in organisms and is passed down the food chain to other organisms and human beings, especially through fish intake [4]. Hence, efficient strategies for quick monitoring of Hg^{2+} in aquatic systems are critical.

Inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectrometry (ICP-OES), atomic fluorescence spectrometry (AFS), cold vapour atomic absorption spectrometry (CVAAS), high-performance liquid chromatography (HPLC), gas chromatography, auger electron spectroscopy and other methods are currently used to detect Hg^{2+} ions in the aquatic medium [5]. These classic methods are excellent in their performance, but the tedious laboratory procedures and expensive instruments complicate the use of such methods. Because of this, there is growing demand for developing ion sensors that could be considered simple, cost-effective, real time and onsite.

Recently, nanoscience offers a potent way for detecting the environmental trace contaminants. Metallic nanoparticles especially silver and gold have piqued the curiosity of researchers due to their intriguing optical features [6,7]. Because of the surface plasmon resonance (SPR) phenomenon, they contribute characteristic colour in aqueous media and exhibit a prominent absorption band in the visible area [8]. Owing to the colour variations associated with surface plasmon resonance capabilities, metal nanoparticles with well-regulated shape and size have lately emerged as colorimetric sensors for the detection of a variety of chemical and biological entities [9-11]. There were several studies on nanomaterials based colorimetric sensors for the detection of toxic heavy metal ion pollutants

[12]. However, they were either synthesized by physical and chemical procedures that are not environmentally conscious due to the use of toxic reducing agents and hazardous stabilizing agents or they had some surface modification with specific expensive ligands. Hence, investigation of ecologically sustainable systems, such as green synthesized metal nanoparticles as colorimetric sensors for the detection of harmful ionic species in aqueous media, is very exciting.

The present work involves the use of marine macroalgae *Sargassum duplicatum* for the first time to synthesize silver nanoparticles owing to their easy availability, presence of active secondary metabolites and cost-effectiveness. Moreover, a test strip approach was developed for real-time detection. It is eco-friendly, free from toxic byproducts and biocompatible.

EXPERIMENTAL

Silver nitrate ($AgNO_3$, 99.9%) purchased from Sigma-Aldrich and 6 different heavy metal ions in the form of nitrate or chloride salts such as lead chloride ($PbCl_2$), cadmium chloride ($CdCl_2$), mercuric chloride ($HgCl_2$), nickel chloride hexahydrate ($NiCl_2{\cdot}6H_2O$), arsenic chloride ($AsCl_3$) and cobalt chloride hexahydrate ($CoCl_2{\cdot}6H_2O$), were precured from Merck India Ltd. All the chemicals were used without any additional purifications and their aqueous solutions were prepared with sterile Milli-Q water.

Sample collection: For the biosynthesis of silver nanoparticles, the seaweed samples of *Sargassum duplicatum* (Fig. 1) were collected from Thirumullavaram coast (8º53′38″N; 76º33′14″E) of Kollam district, India. Thirumullavaram coast is characterized by extensive rocky substratum spread out to shallow sea and well known for rich diversity of seaweeds. The seaweeds were handpicked from their natural habitat during the period of low tides, sorted out and washed thoroughly in seawater and brought to the laboratory in an ice box, identified and confirmed by experts from Kerala University of Fisheries and Ocean Studies, Kochi, India.

Fig. 1. Photograph of seaweed *Sargassum duplicatum*

Biosynthesis of silver nanoparticles: The seaweed samples were washed thoroughly with distilled water followed by Milli Q water, air-dried for 5 days and then chopped into fine pieces. Then grind into a fine powder using an electric mixer and stored it at room temperature for future use. Dried finely powdered *S. duplicatum* (10 g) was stirred with100 mL of distilled water and kept in a water bath at 60 ºC for 20 min and after cooling, the extracts were filtered through Whatman No. 1 filter. For the biosynthesis of silver nanoparticles, seaweed extract (SWE) was mixed with 1 mM $AgNO_3$ solution in 1:9 ratio, stirred well for 15 min at 60 ºC by using a magnetic stirrer and incubated in dark at room temperature under static conditions. A control setup was also maintained without SWE. The synthesis of silver nanoparticles using SWE can be visually observed because of the change in coloration from golden yellow to dark-reddish brown. The bioreduction of $AgNO_3$ into AgNPs was periodically monitored using a UV-Vis spectrophotometer in a range of wavelength between 300 and 700 nm. After 24 h of reaction, the bioreduced reaction mixture was subjected to centrifugation at 15000 rpm for 20 min at 4 ºC. The resulting pellet was redispersed in sterile Milli Q water and freeze-dried. The freeze dried AgNPs were then characterized using UV-visible spectroscopy (Thermo Scientific Evolution 201 Spectrophotometer). The concentration of silver nanoparticles synthesized by using SWE was calculated by using UV-visible absorption data by applying Beer-lambert's law:

$$A = \varepsilon \times l \times c$$

where A is absorbance; ε is the molar extinction coefficient (which depends on the nature of the chemical and the wavelength of the light used); l is the length of the path light must travel in the solution in centimeters; and c is the concentration of a given solution.

Characterization: For the UV-vis spectral studies, a Thermo-Scientific Evolution 201 UV-visible spectrophotometer with a resolution of 1 nm and a wavelength range of 300-700 nm was employed. A Perkin-Elmer Spectrum 100 spectrometer was used to record the FTIR spectrum of freeze-dried AgNPs at wavelengths ranging from 4000 to 550 cm^{-1}. The X-ray diffraction (XRD) studies of drop-coated films of AgNPs on glass slides were performed using a PANalytic X'PERT-PRO X-ray spectrometer with Cu$K\alpha$ radiation (λ = 0.1542 nm). High resolution transmission electron microscopy (HRTEM) images and selected area electron diffraction (SAED) patterns were recorded using Tecnai G2 30 transmission electron microscope.

Heavy metal ions sensing studies

Selectivity studies: To evaluate the heavy metal ions sensing efficiency of silver nanoparticles, the newly prepared AgNPs was first diluted 5 times using HPLC water and to 1.5 mL of this 200 µL of 10 Mm concentration of six different cations such as Cd(II), Pb(II), Co(II), Hg(II), As(III) and Ni(II) were added separately and mixed thoroughly. After 1 min, the colour change of the resulting mixture was observed and the corresponding UV-visible absorption spectra were measured.

Sensitivity studies: To study the sensitivity of AgNPs towards the Hg(II) ions, the test was repeated by adding different concentrations of Hg(II) ions (0.0001 mM to 10 mM) to the AgNP solution. Then the corresponding absorption spectra were recorded. The minimum limit of detection of the analyte in a sample was determined by visual observation and measuring the corresponding UV-visible spectra.

Test strip for practical application: To use this technique in field application for the detection of heavy metal ions, a paper test strip model was developed by dipping Whatman no. 1 filter paper strips in AgNPs solution and then drying them in a vacuum oven. The aqueous solution of various anions (10 mM) was then dropped into it one by one.

RESULTS AND DISCUSSION

UV-Vis studies: The bioreduction of Hg(II) ions into silver nanoparticles by exposing the $AgNO_3$ to seaweed extract (SWE) was traced by monitoring the colour changes with UV-vis spectroscopy. Seaweed extract (SWE) showed a colour change from golden yellow to dark reddish-brown. The colour change was observed within 30 min. The intensity of brown colour increased in direct proportion to the incubation time [13]. The control setup showed no colour change during the entire reaction time. This characteristic brown colour was due to the surface plasmon resonance (SPR) exhibited by free electrons in the noble metals when their size is in the nano-dimension. Because of this size dependant optical properties, AgNPs are known to have an absorption peak in the 300-450 nm. The UV-visible spectrum of the synthesized AgNPs (Fig. 2) showed a characteristic peak at 419 nm.

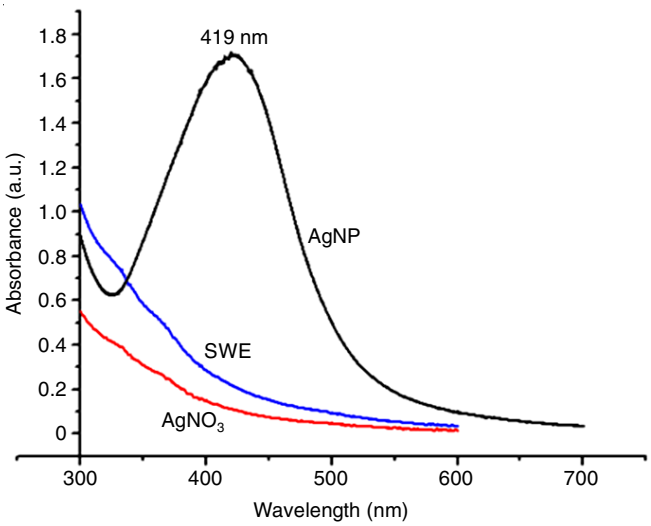

Fig. 2. UV-Visible spectrum of AgNPs in comparison with $AgNO_3$ and seaweed extract (SWE)

Similarly, Kumar & Sudha [14] reported that the SPR band was located at 419 nm for AgNPs synthesized using brown seaweed *Dictyota bartayresiana.* When the duration was prolonged from 20 min to 30 min, the peak area was expanded indicating the significant quantity of nanoparticles production. Though the peak was somewhat narrower. This is an indication of tightly ordered particles size distribution as well as the absence of additional scattering induced by the turbidity of silver colloids. Thus the position, intensity and bandwidth of SPR band are extremely sensitive to morphological characteristics, interparticle interaction and the refractive index of the reaction medium [15]. Thus, the rough details about the size and morphology of the synthesized AgNPs can be understood by monitoring SPR band. The concentration of silver nanoparticles synthesized by using seaweed extract was calculated by using UV-visible absorption data by applying Beer-Lambert's law. The concentration of silver nanoparticles was 1.172×10^{-10} mol/L. Thus, the present study revealed that *Sargassum duplicatum* is one of the most suited biological entities for nanoparticle synthesis, since it not only provides a simple downstream processing for product recovery but also makes biomass handling straightforward.

Morphological studies: Transmission electron microscopy was used to examine the morphology and crystalline nature of biogenic AgNPs. The HR-TEM images (Fig. 3a) at various magnifications demonstrated that the biofabricated AgNPs were almost spherical in shape and polydisperse in nature. The majority of synthesized AgNPs were 20-50 nm in size. Fig. 3b displays the thin coating layer surrounding the nanoparticle, confirmed the role of phytochemicals in SWE as capping agents for the synthesized nanoparticles. The lattice fringes in the HR-TEM image principally demonstrated the nanoparticles' crystalline structure. The selected area electron diffraction pattern (Fig. 3c) exhibit the concentric rings with intermittent bright spots, indicating that these nanoparticles are highly crystalline.

XRD studies: The XRD profile of synthesized AgNPs (Fig. 4) showed various diffraction peaks at 29.43º, 32.22º, 38.10º, 44.25º, 54.70º, 64.48º and 76.60º, respectively. Comparison of obtained XRD spectrum with standard confirmed that the synthesized AgNPs were crystalline in nature. The

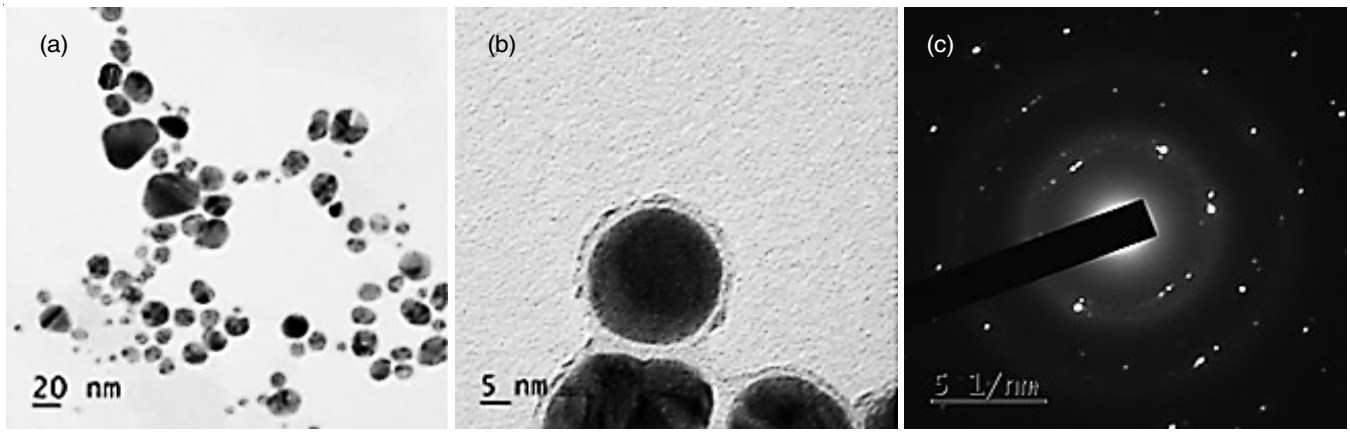

Fig. 3. (a,b) HR-TEM images and (c) SAED pattern of biogenic AgNPs

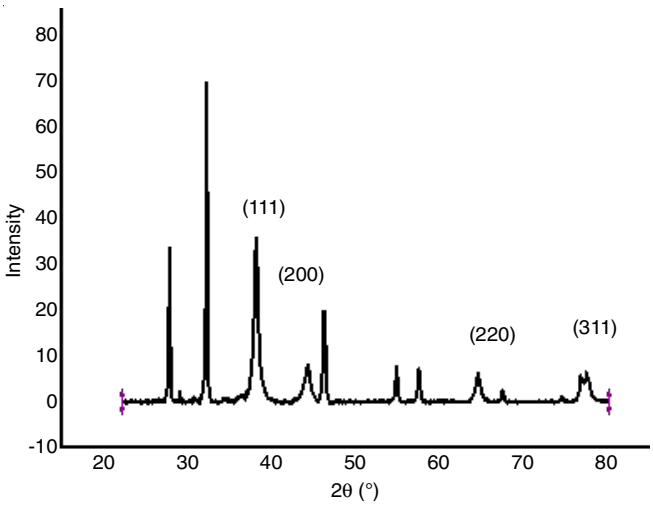

Fig 4. XRD pattern of synthesized AgNPs

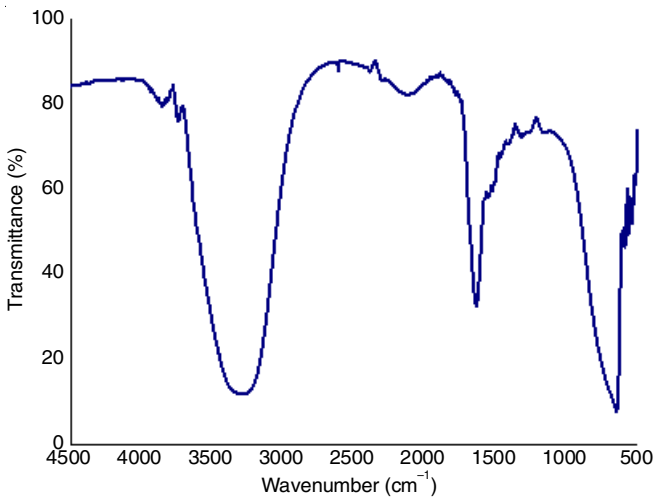

Fig. 5. FTIR spectrum of biosynthesized AgNPs

peaks were assigned to the planes (111), (200), (220) and (311). And the peaks at these planes indicated that silver nanoparticles are face centred cubic [16]. There are some unassigned peaks in the spectrum, due to the presence of bioactive compounds in the seaweed extract. The average crystalline size of biosynthesized AgNPs were calculated by applying Debye-Scherrer's equation,

$$D = \frac{0.94\lambda}{\beta(\cos\theta)}$$

where D is the crystalline size; 0.94 is the value of Scherrer constant; λ is the X ray wavelength (0.1542 nm); β is the full width at half maximum (FWHM) and θ is the Bragg's angle. The average crystalline sizes were determined to be 22.75 nm for AgNPs, which agrees with the particle size obtained from the TEM image.

FTIR studies: The functional groups in seaweed extract involved in capping the nanoparticles were studied using FTIR spectroscopy. The results of the FTIR analysis show several stretches of bonds at different peaks (Fig. 5). The free –OH and –NH stretching vibrations of amino acids are responsible for a strong absorption band at 3372.16 cm^{-1} [17]. A weak peak at 2072.64 cm^{-1} is due to –C≡C stretching [18]. Another strong peak at 1654 cm^{-1} could be attributed to –C=O group derived from carbonyl groups of proteins. Certain small peaks were observed in between 1385-1370 cm^{-1} due to the stretching vibrations of –C-N. A strong absorption band centered at 686.32 cm^{-1} can be attributed to the –C-H bonding, which is confirmed the presence of carbohydrates [19]. These results suggested that proteins containing amide and carboxyl groups and carbohydrates might be involved in the reduction as well as stabilization of AgNPs. But the identification of the exact chemical composition of compounds involved in the reduction and capping of AgNPs needs a detailed analysis.

Potential of AgNPs in selective monitoring of Hg^{2+} ions: To investigate the cation sensing potential of biogenic AgNPs, six different heavy metal ions such as Cd^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, As^{3+} and Co^{3+} were selected and 200 µL of 1×10^{-2} M concentration of each cation was added separately to 2 mL of 5 times diluted AgNPs solution and mixed well. The colour of the resulting combination was reported after 1 min and the associated UV-visible absorption spectra were measured. The study revealed that there is no visible disappearance of brown colour and SPR band intensity observed with metal cations like Cd^{2+}, Ni^{2+}, Pb^{2+}, As^{3+} and Co^{3+} ions were reacted with AgNPs solution. However, when Hg^{2+} ions were added, a remarkable colour change from brown to colourless and the AgNPs' SPR band disappeared in a flash (Fig. 6). This reaction of AgNPs with Hg^{2+} clearly demonstrates selectivity and specificity for colorimetric monitoring of mercuric ions, whereas AgNPs were not found to be sensitive to other heavy metal cations under similar conditions.

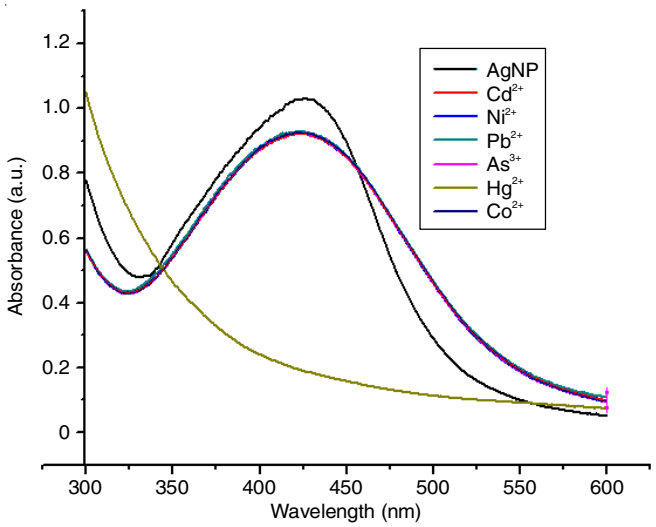

Fig. 6. UV-visible spectra of AgNPs recorded after 1 min of addition of 10 mM metal ions

Sensitivity analysis of biogenic AgNPs in Hg^{2+}: The sensitivity of AgNPs assay towards Hg^{2+} ions was assessed by adding varying concentrations of Hg^{2+} into AgNPs solution and the minimum detection limit was detected. The UV-visible spectra of the interaction of AgNPs and Hg^{2+} recorded after 1 min. Fig. 7 clearly depicts that SPR band intensity decreases gradually with increasing concentrations of Hg^{2+} ions and also

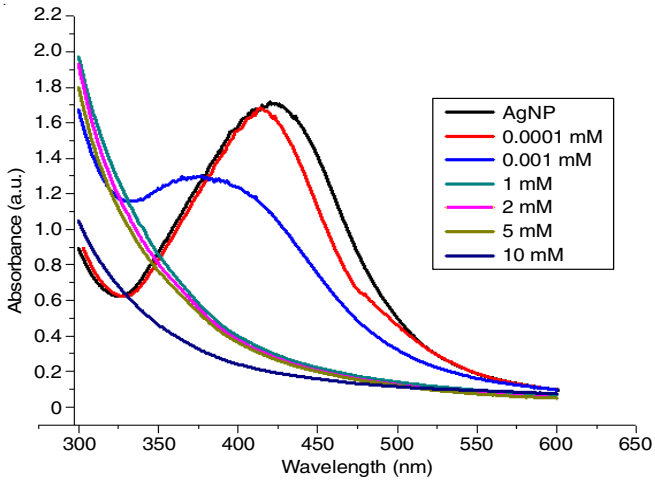

Fig. 7. UV-Visible spectral changes of AgNPs upon interaction with a solution containing cationic mixturewith and without Hg^{2+} ions

observed the blue shifts in the UV-visible spectra when the concentration of Hg^{2+} increases. The minimum detection limit of Hg^{2+} ions in aqueous medium by biogenic AgNPS is 0.1 µM (0.0001 mM).

Mechanism of Hg^{2+} detection: The mechanism of Hg^{2+} ions detection by biogenic silver nanoparticles can be explained by the electrochemical differences between two metal ions; Hg^{2+} and Ag^{+}. The Ag^{+} ion has a reduction potential of +0.80 V, whereas Hg^{2+} ion has a typical reduction potential of +0.92 V. As a result, when AgNPs react with mercury leads to the reduction of Hg^{2+} ions to Hg^{0} along with the partial oxidation of silver in AgNPs. The reduced Hg^{0} get deposited on the surface of AgNPS results in the formation of Hg-Ag alloys. As a result, SPR band intensity of AgNPs declines and the brown colour disappears [20,21].

Applications: A test strip approach for the detection of mercuric ions in an aqueous medium was also proposed. For this, a sensing ability by coating AgNPs on the Whatman filter paper No. strips were immersed them in aqueous solutions of different metal ions. A clear colour change from brown to colourless was observed only with Hg^{2+} ions (Fig. 8). The invention of such a test strip approach is particularly appealing for the real-time measurements, since it does not require any additional equipment.

Conclusion

The present study exploited the sensing potential of biocompatible AgNPs for the rapid colourimetric monitoring of toxic Hg^{2+} ions in the aqueous medium. Biogenic AgNPs gNPs synthesized from marine macroalgae *Sargassum duplicatum* displays high selectivity and sensitivity towards Hg^{2+} ions with a minimum limit of detection of 0.1 µM. Thus, the findings of the study suggested that the biogenic AgNPs are expected to be a promising candidate for developing low-cost colorimetric Hg^{2+} sensors.

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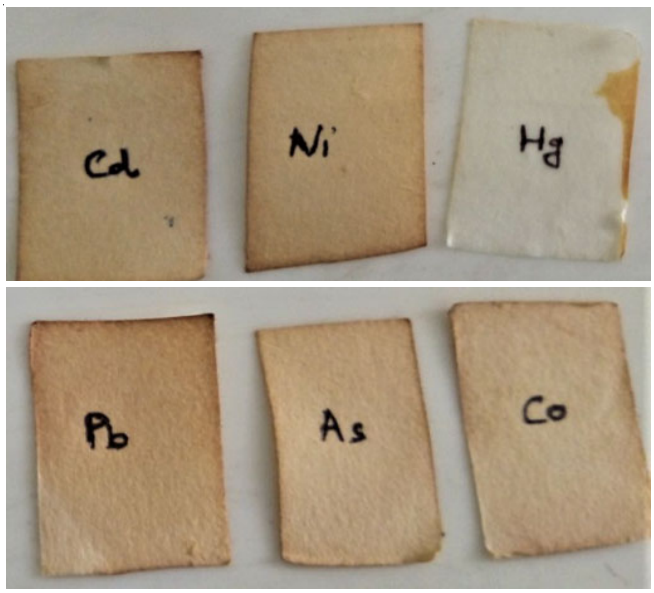

Fig. 8. Images of AgNP coated test strip after applying various metal ion solutions

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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