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Biosynthetic Gold Nanoparticles as Sensitive and Selective Colorimetric Method for Mercury Ions

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Abstract: Gold nanoparticles (AuNPs) are widely studied materials that have intense applications in various fields. Different methods use to synthesize the AuNPs, one of them is eco-friendly biological method, known as "green synthesis" a simple and inexpensive method. The Synthesis of AuNPs, using ginger extract is described here. The components found in ginger work as a stabilizing and reducing agent. Hydrogen tetrachloroaurate ($HAuCl_4$) are mixed with the ginger extract to produce AuNPs. The Surface Plasmon Resonance (SPR) measured at $\lambda max = 540$ nm. UV-Vis, FTIR and Zeta potential are used to identify AuNPs, as well as AFM and SEM. Data indicate that AuNPs has a spherical structure. The results demonstrate that AuNPs stabilized by ascorbic acid were used as a colorimetric probe for Hg^{2+} ions, based on the production of (Au-Hg) amalgamate which made changes in their absorbance, due to its ability to reduce Hg^{2+} to Hg^0, to enhance amalgamation. Practically, this procedure has successfully worked to detect Hg^{2+} in tap water as a sensitive and selective probe with upper limit of detection equal to 120 µM as well as the limit of detection (LOD) equal to 0.65 µM, so the method proved to be sensitive and selective probe.

Keywords: Gold Nanoparticles, Ginger extract, Phytochemicals, Mercury detection, Selective sensing.

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1. INTRODUCTION

Gold nanoparticles (AuNPs) are widely studied materials that have intense applications in various fields. Recently, the need to nanomaterials that are used in many industries has increased, because they have much better properties than other large-sized molecules and materials due to they have a larger surface area than large molecules(1). Several methods including physical, chemical and biological approaches have been described for preparation of NPs. The wide range of uses for metal nanoparticles in sensing, catalysis, electronics, and photonics has a huge interest (2, 3). These NPs are in colloidal forms suitable for biological applications as they do not contain any harmful chemicals (4, 5). On the other hand, suitable selection is appropriate in this case, to find out which parts of the plant have excellent reducing or stabilizing components (6, 7). Biological methods are among the best, cheapest, safest and easiest methods of preparation(8-10). Therefore, ginger extract contains many compounds that act as antioxidants which are able to reducing gold ions as Au+3 and converting them to AuNPs as Au^0 (11, 12).

It is well known that mercury (II) ion has been one of the most common heavy metals that induced human poisoning , as well as for the global environment. The accumulation of this metal, and other metals, leads to a diversity of toxic effects on a variety of body tissues and organs (13, 14). According to the World Health Organization (WHO) and the US Environmental&Protection Agency (EPA), the permissible limit of mercury (II) ion in drinking water is approximately low 30 and 10 nanometres, respectively(15). So, it is necessary to design a potent selective and sensitive way that can accurately identify and detect the presence of environmentally mercury ion. Therefore, scientists have developed different techniques for detecting mercury ions attributed on various mechanisms and materials, as atomic emission spectroscopy(16), Surface enhanced Raman scattering(17), Electrochemical methods(18), Fluorescent methods(19) and colorimetric methods(20, 21). Presently, different techniques used for quantitation of Hg^{2+} involve atomic absorption spectrometry(22), inductively coupled plasma mass spectrometry(23) and cold vapour selective fluorescent atomic spectrometry(24). Due to their elevated of toxicity, safety contents in food and the environment of mercury had been set to be exceedingly low. These techniques include commercial tools and are so complicated, time-consuming, expensive, as well as not suitable for onsite applications. In the past time, colorimetric methods using AuNPs have been an alternative technique for Hg^{2+} detection because of their characteristic as simplicity, speediness, selectivity and sensitivity (25-30).

So, this study aimed to biosynthesis of AuNPs using ginger extract and study of its efficiency to detect Hg^{2+} by colorimetric change, as well as, a proved if this procedure helpful and efficient for Hg^{2+} detection in different real aqueous samples.

2. EXPERIMENTAL SECTION

2.1. Materials

Hydrogen tetrachloroauratem (III) (HAuCl4); ascorbic acid (AA, 99%); mercury chloride and all the metal salts from Merck. ginger was taken from local market in Baghdad, Iraq. Double distilled water (D.D.) was used throughout the study. All other reagents are of analytical grade.

2.1.1. Preparation of ginger extract

All dirt and contaminants were removed from ginger by washing it well with distilled water, after that plant (ginger) was peeled, then cut it into a little part and converted into juice using the mixer, later it was filtered through gauze to remove the residues (fibers) and get a clear juice then filtered throughout filter paper No.1 to obtained the ginger extract, set aside at 4°C until used (31).

2.1.2. Preparation of AuNPs

The ginger extract 5 mL was added to 13 mL of 2 mM aqueous solution of $HAuCl_4$ in a beaked in order to reduction of Au^{+3} to Au^0. The bio-reduction for gold ions was observed periodically by measuring the solution by UV-Vis spectroscopy. The reduction of gold chloride to gold ions was observed by changing the color from light yellow to purple, later to dark purple. The obtained solutions were stored at 4℃ in dark container (32).

2.2. Characterization of AuNPs

2.2.1. UV-Vis spectra

The UV-Vis spectrum was performed through spectrophotometer model (PG-Instruments Limited, T80, Germany). The reduction of AuNPs was made by spectroscopy assay at regular intervals in the range of (300 – 800)nm. Three milliliter of the sample was added into a test tube and thereafter analyzed at room temperature (33).

2.2.2. Zeta potential

Zeta potential assay was performed in order to identifying both of Nano-material and the ginger extract. Zeta potential was determined the light dispersion using the Zeta Plus tool. The data were being around with 5 measurements. Measurement of NPs Size were done using Electrophoretic light scattering (ELS) and dynamic light scattering (DLS), while characterizing of nano-materials surfaces and estimated their surface charge through the determination of the zeta potential (34).

2.2.3. Scanning electron microscopy

Scanning electron microscope (SEM) analysis performed to characterize the shape, size, and morphologies of NPs formed. The SEM provides high-resolution images of the sample surface, which makes this technique helpful to characterizing the size distribution of NPs. Energy dispersive spectrometer (EDS) analysis for the confirmation of gold was carried out to detection of elemental gold(5).

2.2.4. Atomic force microscopy

The Atomic Force Microscope (AFM) analysis performed to determining the topography of NPs formed. The AFM generally measure the height of AuNPs. A drop of liquid sample was drained on a glass cover slide (2x6 cm^2), and dehydrated the sample prior to scan (35).

2.2.5. Fourier transmission infrared spectroscopy

Fourier Transmission infrared (FTIR) is an analytical method used to measure the intensity of infrared radiation against its wave number or wavelength light of possible bio molecule in the samples. After complete reduction of $AuCl_4^-$ ions by the ginger extract, the AuNPs dried and ginger extract were analyzed by FTIR (Shimadzu) using potassium bromide (KBr) discs in the range 400-4000 cm^{-1}. The AuNPs were centrifuging for 15 minutes at 5000 rpm. This method was repeated 3-5 times, then proceeds the precipitate and dry it in oven at 40 °C for 4 hours (36).

2.3. Detection of Mercury

Standard stock solution of Hg^{+2} ions (120 µM) was prepared by serial of dilution. The selectivity and sensitivity of this procedure to the Hg^{2+} ions, *p*H of the reaction, time of incubation, LSPR of AuNPs and ascorbic acid content were adjusted preliminarily. To detect Hg^{2+} ions, approximately 1.5 mL AuNPs solution at *p*H 5.0 and 32 µL from 0.1 M of ascorbic acid was added into 2mL centrifuge tube. After gently shaking, aliquots 320 µL of Hg^{2+} ions solution (final concentrations: 5, 10, 15, 20, 25, 30, 35, 40, 45,50, 55,60) µM were added, one at a time, into all tube. After a while the solutions were mixed and later, placed for 50 min at room temperature, the photos and UV-Vis spectra were taken.

The selectivity of this method was investigated toward Hg^{2+} by repeated the method mentioned above, but Hg^{2+} ions was replaced with 720 µM of other metallic ions, including (Co^{+3}, Cr^{+3}, Cu^{+2}, Mg^{+2},Fe^{+3}, Ni^{+2} , Mn^{+2}, Sn^{+2}, Ca^{+2} and Zn^{+2}).

2.4. Analysis of Real Samples

Before starting the assay, each one of water samples used in this study was filtered through membrane with a thickness of 0.2 µm. The Hg^{2+} standard solution of different concentrations was added to the spiked water samples. or Hg^{2+} standard solutions of different concentrations were added to the spiked water samples.. Later, all of the tubes with water samples were mixed with 1.5 mL of AuNPs at *p*H 5 and 32µL of ascorbic acid 0.1M for assay.

3. RESULTS AND DISCUSSION

3.1. Biosynthesis of AuNPs

In this study, the biosynthesis of AuNPs was carried out using ginger extract as a reducing agents to aqueous gold solution of $HAuCl_4$, and the results indicated a clear change in solution color from yellow into reddish-purple, and this indicate to producing of AuNPs. As shown in (Figure 1) the change in color obtained, which is in agreement with a number of other studies (37, 38) .

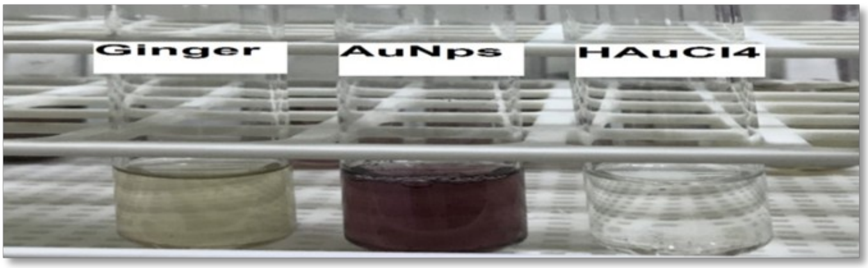

Figure 1: The color change during the synthesis of AuNPs.

3.2. Characterization of AgNPs

3.2.1. UV-Vis Study

The features of AuNPs were known via UV-Vis spectra. The data indicated that there was no a clear band for the ginger extract, while when $HAuCl_4$ was added; a broad peak appears in the range 530-540 nm as illustrated in (Figure 2). As a further definite by other characterizations, this band appoints the formation of mono-dispersed spherical form of AuNPs(39). This reaction occurs in a period of (50 minutes) with the obvious color change.

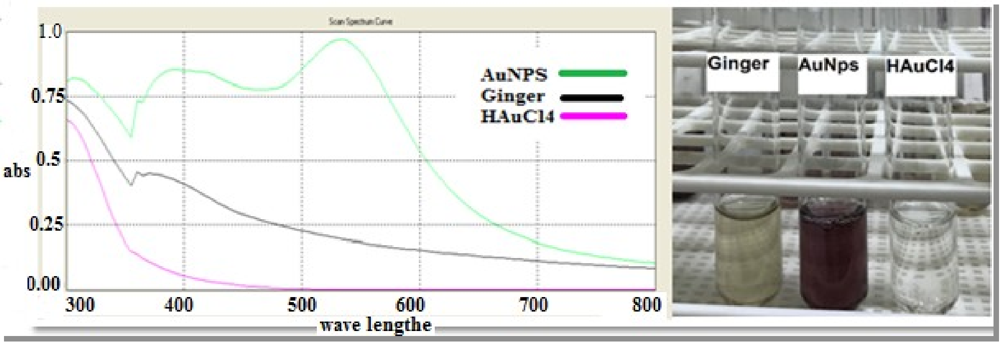

Figure 2: UV–VIS spectra and change in color of HAuCl4, ginger extract and AuNPs.

3.2.2. FTIR study

The FTIR spectra was using for the identifying the probable bio-reducing components in the ginger extract and in the prepared AuNPs following reaction with $HAuCl_4$, (Figures 3).

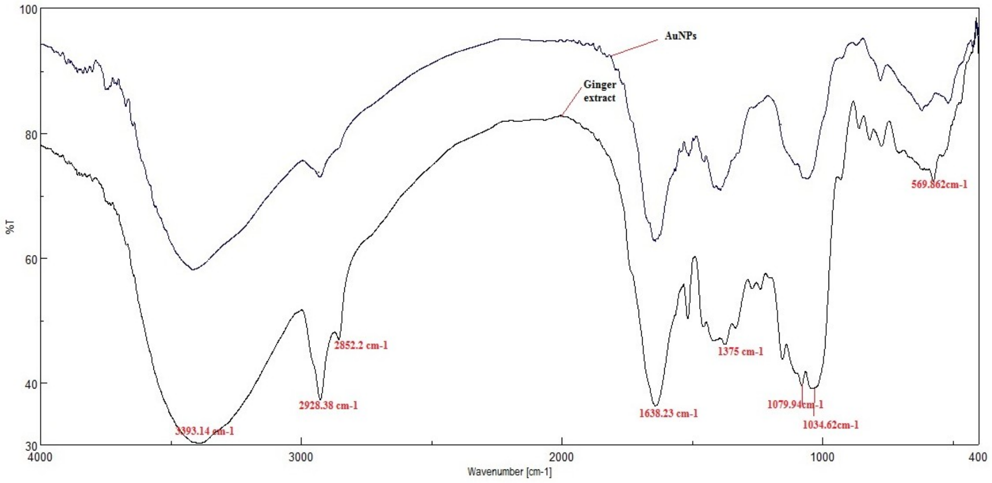

Figure 3: The FTIR for ginger extract and synthesized AuNPs.

The spectra of the used ginger extract and AuNPs were showed bands nearby 569 and 540cm^{-1} which is refer to –CH bend vibrations of substituted ethylene -CH=CH- , while the band at 1034 to 1149 cm^{-1} belong to vibration of C-O stretching. The band of 1409 - 1454 cm^{-1} relates to stretching C-C aromatic group, the main band in 3393.14 cm^{-1} is specified to stretching of -NH and -OH for the phyto-components obtained in ginger extract. The band in 2928.38 cm^{-1} is belonging to stretching of C-H. Bands appear to C=O stretching as well as bending -NH are join together as one and show a broad peak at range of 1863-1516 cm$^{−1}$ with a focus at 1638 cm^{-1}. The peak at 1375 cm^{-1} is equivalent to the side chain vibrations bands which can clarify the ginger extract proteins also carbohydrates. The AuNPs spectra indicated every one of the ginger extract vibrations bands, which recommended that NPs were become stable by the phyto-constituents(39-41). Previous works illustrate that the constituents of phyto-proteins has a tendency to produce a photo-induced electron transfer for reduce metal ions(42), therefore, this study advise that the bio-constituents obtained in this extract referee the photo-induced preparation of AuNPs as well as the stability of NPs in the aqueous media.

3.2.3. AFM Study

The AFM was used to distinguish the surface morphology and to define topography which provides 2D and 3D of NPs surface images at an atomic level with the conforming size distribution of AuNPs. The average particle diameter size is 31.35 nm which was calculated in nano-scale size as indicated in Figure 4. Surface analysis by AFM needs good care because the agents that might be effect on the results like tip or contaminations(43).

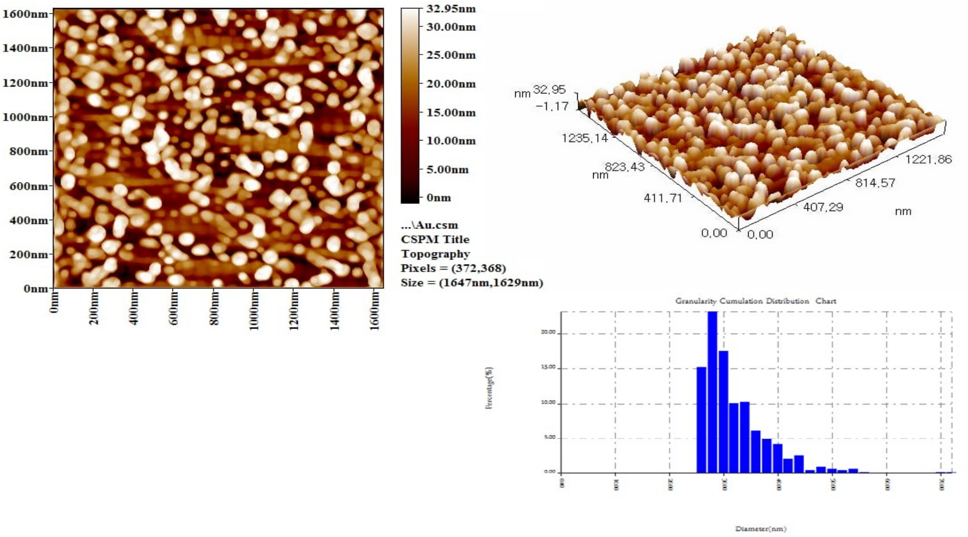

Figure 4: AFM images and size distributions of prepared AuNPs.

3.2.4. SEM Study

The SEM was applied to study the NPs structure and morphology (44), as well as obtained further knowledge of the AuNPs gained from the suggested biosynthesis process using ginger extract. The SEM results explained different shapes, such as linear and spherical, but the chief shape or the largest quantity of the NPs shape is the spherical (Figure 5).

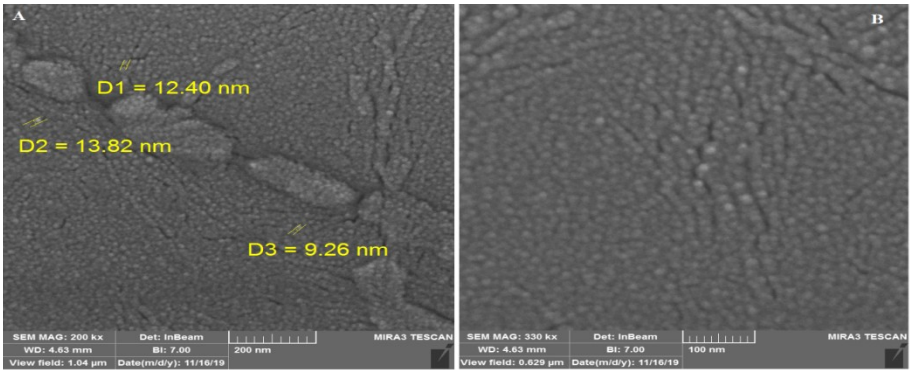

Figure 5: SEM images of AuNPs (A=200 nm, B=100 nm).

The average particles size and distribution are firm arbitrarily using SEM technique. The surface of NPs is smooth with good crystallinity, in agreement with other study(45). The chief particles size of the AuNPs between 9.26 to 13.82 nm. Notable, this size gotten from SEM measurements is meaningfully lesser than.

Existence of elemental gold in formation of AuNPs was definite by EDS micro-analysis, (Figure 6). The spectrum shows an indication of gold area. Metallic gold nano crystal presentation an absorption peak near 3 keV belongs to SPR(46, 47). Another signs for other metals C, N, O, Na, Mg, P, S, Cl, K and Ca existing during reaction media established that the additional cellular organic components from ginger extract were initiate on the AuNPs surface or the nearness.

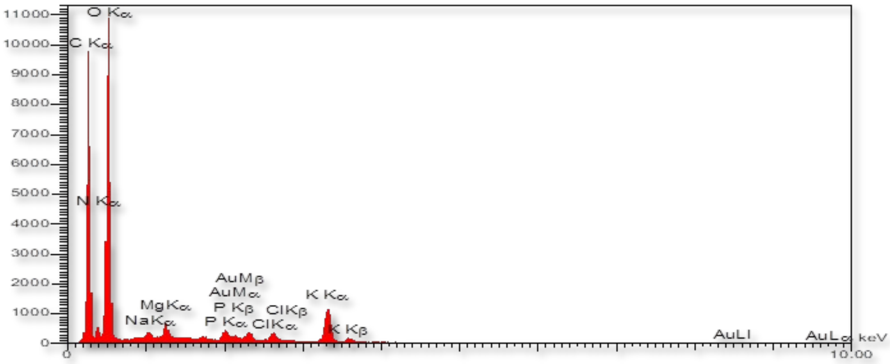

Figure 6: The EDS pattern of spherical synthesized AuNPs using ginger extract.

3.2.5. Zeta potential study

Zeta potential measured the stability of NPs in the suspension material (colloidal solution). Its achieved by ELS to ginger equal to -17.88 mV and the mobility value equal to -1.40 (μ/s) / (V/cm) as illustrated in (Figure 7 A), while the reading for AuNPs was -26.89 mV and -2.10 (μ/s) / (V/cm), respectively, (Figure 7 B). These suggest that the electrical boundary of the AuNPs is comparatively separate from each other and reduced the aggregation of NPs. Also, ELS is initially applied to measure the charges belong to the colloidal particles found on the surface or other macro-molecules in an electric field of fluid media(48). So, the value showed that the particles present in fluid media has less stable because the value of zeta potential has less than ±30 mV and it has further stable as colloidal materials (found as NPs) than it has initiated in ginger extract one.

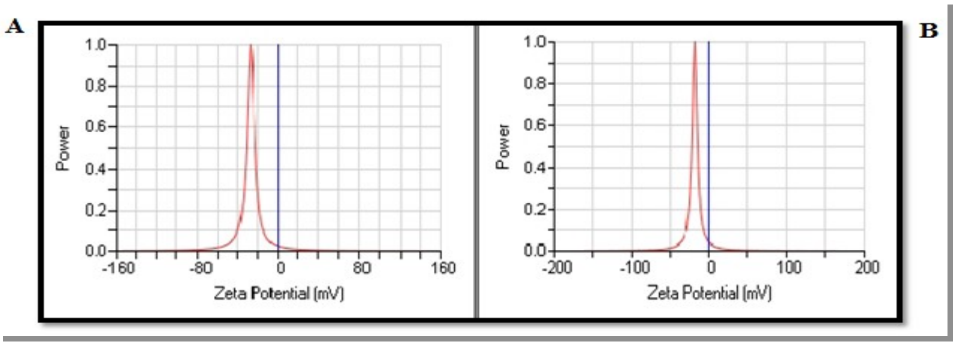

Figure 7: Zeta potential distribution ofAuNPs (A) and ginger extract (B).

3.3. Selectivity of the Detection Probe

The selectivity for AA-AuNPs was tested in concentration 720 µM of Hg^{2+}, as well as various concentrations 120µM of different metal cations (Zn^{2+}, Cu^{2+}, Cr^{3+}, Mg^{2+}, Co^{+3}, Mn^{2+}, Sn^{2+}, Fe^{3+}, Ni^{2+}, and Ca^{2+}), were also measured. Each one of the metal cations didn't show any changes in color except the mercury ions Hg^{2+}, as shown in (Figure 8).

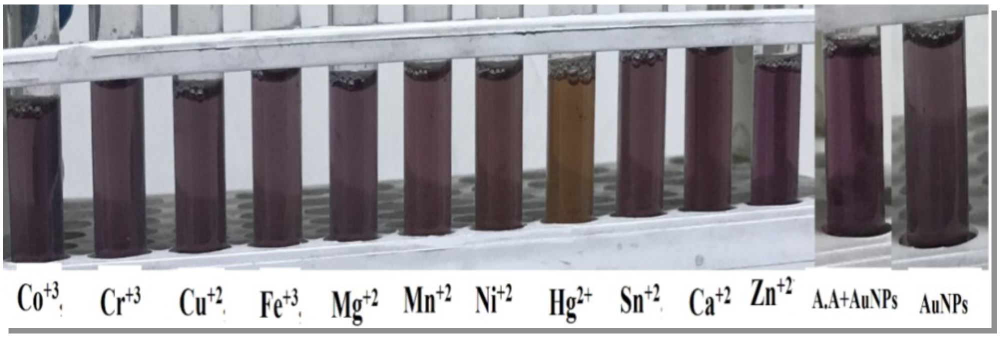

Figure 8: Photo-image of selectivity test to AuNPs after the addition of different metal cations.

The UV-Vis absorption of AA-AuNPs solutions in *p*H 5 and 50 ºC, were measured in different metal cations as illustrated in (Figure 9). The high peak appeared near 540 nm belong to Hg^{2+}, this allows easy distinction between different metal cations. Ratio of absorbance (ΔAbs/Abs 540) for the liquid component of AA-AuNPs through addition of each one of cation was recorded to study the selectivity test for Hg^{2+}ion. The elevation in ratio of absorbance for Hg^{2+}-AA-AuNPs was attributed to the aggregation of component AA-AuNPs, while a decreasing in ratio of absorbance for AA-AuNPs in existence of different metal cations illustrated the keeping of well-dispersed forms in AA-AuNPs component, so that, the selectively coordinated of Hg^{2+} ion must be done with a specific site of AA-AuNPs. The interaction effect in existence of different metal cations was tested in AA-AuNP component through addition of Hg^{2+} ions which mixed with other ions. These ions didn't interfere with Hg^{2+} ions detection, despite of their concentrations were ten times higher than that of Hg^{2+} ions.

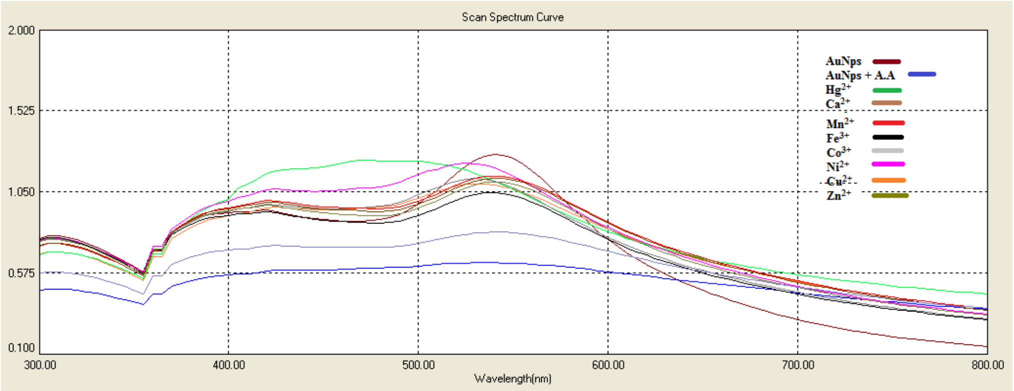

Figure 9: UV–VIS spectrum of selectivity test to AA-AuNPs after the addition of different metal cations.

3.4. Sensitivity of the Detection Probe

The sensitivity study was conducted by interacting between different concentrations of Hg^{+2} with AA-AuNPs solution. The color of Hg^{+2} ion solution with concentration 120 µM, work as a control solution. The purple color of AA-AuNPs solution changed to brown color with elevating in concentration of Hg^{+2} ions as shown in (Figure 10). This visible color changing in M-AA-AuNPs solution refer to interaction of potential and effective components present on the surface of NPs with Hg^{+2} ions through metal-ligand interactions. These interactions together with Hg^{+2} ions in solution might be responsible for M-AuNPs aggregation.

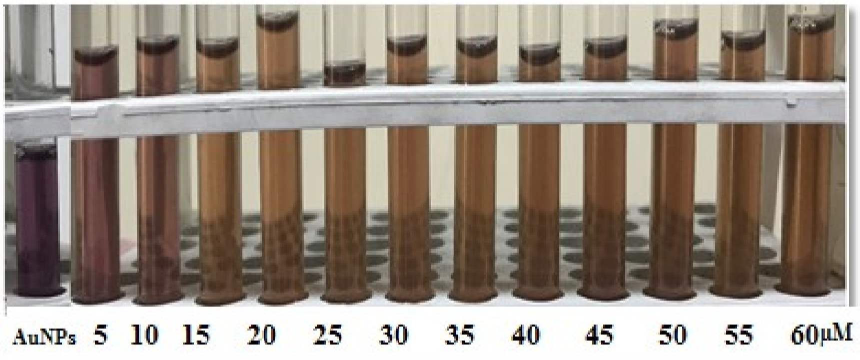

Figure 10: Changing in visible color of AA-AuNPs with different concentrations of Hg^{+2} ions.

Furthermore, the alterations in band intensity, position as well as width were studied through UV-Vis analysis. Various concentrations were prepared to study SPR spectra. The band intensity at 450 nm will reduce related to Hg^{2+} ion concentrations (Figure 11).

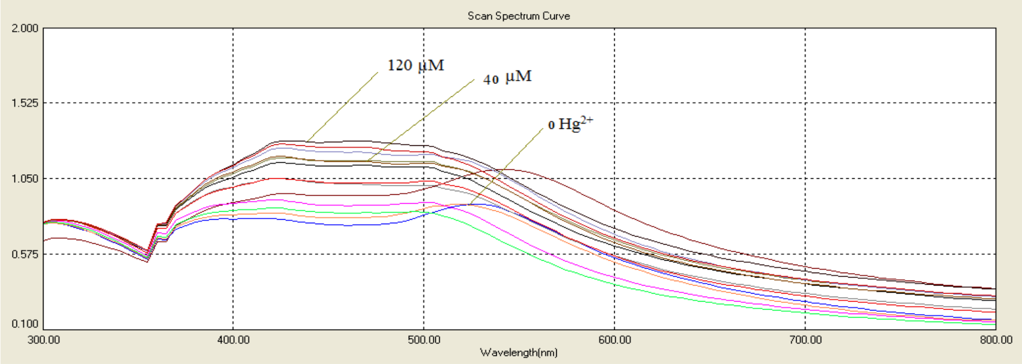

Figure 11: UV-Vis spectrum of AA-AuNPs through addition of different concentrations of Hg^{2+} ions.

The color changes obvious and could be seen with the unaided eye as the concentration of Hg^{2+} is increase more than 15 µM. A linear relation-ship was established between ΔA/A0 and the concentration of Hg^{2+} A(AU) = 0.0035-0.007c (µM) as well as the coefficient value R2 = 0.9919. The upper limit of detection for this procedure equal to 120 µM as well as the limit of detection (LOD) equal to 0.65 µM determined by 3S, the limit of quantification equal to 2.14 lowering than the limit set by both WHO and EPA. As compared to the probe using gold nanorods, the data found in this study is more sensitive for Hg^{2+} estimation, these results plotted in (Figure 12).

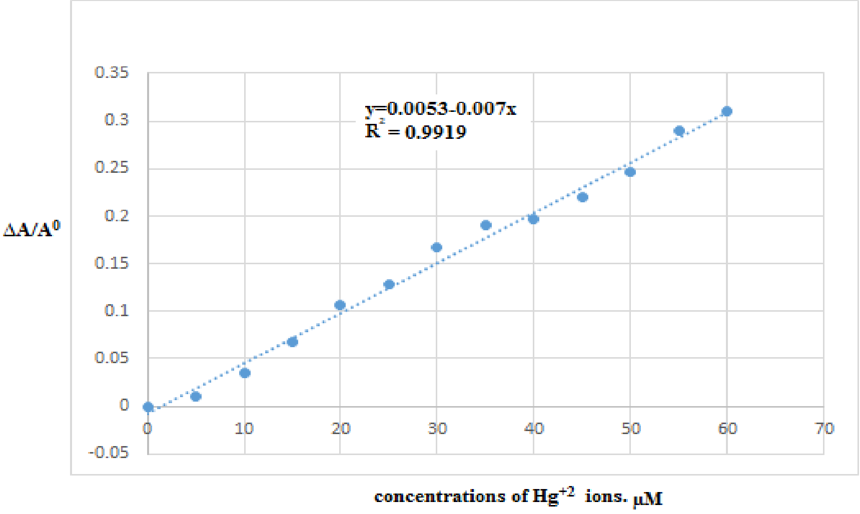

Figure 12: Plot of ΔA/A0 at 540 nm versus different concentrations of Hg^{2+} ions.

In contrast to most other reported NPs-based methods that depend on disaggregation or aggregation, the assay in this study is depend on Hg^{2+} induced morphological transformation of AuNPs. Also, the data found in this study is further sensitive than atomic absorption spectroscopy (AAS) (22), as well as fluorescence methods(49). As compar to other study, the data indicated that LOD equal to (0.53 µM) and linear range equal to (0-398 µM) for Au-Ag Bimetallic NPs(50). Furthermore, our assay has an extremely good selectivity for Hg^{2+} equal to 5µM, over the measured metal ions (20 µM), including (Zn^{2+}, Cu^{2+}, Cr^{3+}, Mg^{2+}, Co^{+3}, Mn^{2+}, Sn^{2+}, Fe^{3+}, Ni^{2+}, and Ca^{2+}) as shown above.

3.5. Detection of Hg^{2+} in Water Samples

To examine the colorimetric method for environmental systems, different samples of tap water were chosen and Hg^{2+} ions were added with known concentration. The spiked samples had been tagged with the proposed detection system. The results indicated that none of the tap water samples caused a change in visible color change of AA-AuNPs probe, pointing to the quantity of Hg^{2+} ions of these samples are below 180 µM. Conversely, when the tap water samples spiked with Hg^{2+} with concentration 120 µM, change to brown color of water was shown. The data assure that the detection assay established here can be used to detection of Hg^{2+} ions in real environmental and water samples.

So, by taking a benefits and advantage of the colorimetric method using AuNPs toward Hg^{2+} ion, a very simple colorimetric probe was developed to detect Hg^{2+} ion depending on ascorbic acid with AuNPs. This proposed method has many remarkable advantages, as compared to other known sensors or probe to Hg^{2+} ion, like: simplicity to use AuNPs, easily to synthesis its complex; short detection period with suitable condition (room temperature, aqueous solution); the method proved to be sensitive and selective probe toward Hg^{2+} ion in presence of other metal ions; easily monitoring the end point of reaction by color change; and finally low cost(51, 52).

4. CONCLUSION

In this work, we describe a simple, fast and reproducible method of environment. Our data revealed that the novel colorimetric AuNPs sensor was successfully developed. Easy synthesis of AuNPs without the need for expensive reducing agents. Gold ions Chemically reduced to NPs by ginger extracts. AuNPs stabilized by ascorbic acid can be used as a colorimetric probe for Hg^{2+} ions with detection limit equal to 0.65 µM. Other countered metal ions exist in the solution didn't interact or effect in detection of Hg^{2+} ions. Practical applicability of AuNPs stabilized by ascorbic acid exhibits successfully using to detection of Hg^{2+} ions as highly sensitive and selective probe in different water samples like tap, and river water. The method also provided to be highly sensitive and selective probe to detection of Hg^{2+} ions, so other samples should be used in the future to indicated that our newly developed assay might open a new approach to detect Hg^{2+} levels .

5. CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

6. ACKNOWLEDGMENTS

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