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Optimization of a Colorimetric Sensor for Mercury (II) Ion Detection Using Albumin/Silver Nanoparticles

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ABSTRACT

Metal nanoparticles have unique properties and different scientific, industrial, and environmental applications. In this study, an optical sensor has been optimized for mercury (II) ion detection. This sensor is based on the redox interaction of Hg (II) ions with Albumin/Silver Nanoparticles (Alb/AgNPs) in an aqueous solution. The addition of Hg (II) ions caused a visual color change from dark brown to colorless. The color change was proportion to the concentration of mercury ions. Different parameters affecting the Hg (II) and Alb/AgNPs interaction, such as the concentration of Alb/SNPs and the time of reaction, were carefully studied and optimized. Under the optimal experimental conditions, the difference absorbance (ΔA) at 440 nm was linearly proportional to Hg (II) ion concentration in the range from 0.293 μM to 2.340 μM with a limit of detection of 0.141 μM . The optimized sensor was used to detect mercury (II) ions in samples of lightening cream products.

Introduction

Exposure to heavy metals in ionic forms is dangerous to both human health and the environment. Some metals are essential and others are nonessential for the biological processes, but in both cases, they are toxic at certain levels depending on metallic ions. Therefore, rapid and simple quantification is needed and critical especially when the toxic level of these metals is too low. In addition, the cost of detection techniques and analysis is important (Amirjani & Haghshenas, 2018). Up to the present time, the common and accurate methods for heavy metallic ions detection include; inductively coupled plasma mass spectroscopy (ICP-MS), atomic absorption spectroscopy (AAS), and inductively coupled plasma atomic emission spectroscopy (ICP-AES). These traditional methods are expensive, time-consuming, and need complex instrumentations (Sarkar & Mondal, 2021). Subsequently, new techniques based on an exceptional characteristic of nanoparticles were recently proposed and optimized for rapid, cost-effective, sensitive, and selective detection of different analytes (Yu *et al.*, 2020).

The handling of atoms or/and molecules to generate materials working at the submicroscopic level is called nanoscience. These individual atoms and molecules with a scale below the nanometer or up to 100 nm, need chemical,

physical and biological knowledge at the nanoscale. The properties of obtained nanoparticles are new, different, improved, and affected by the distribution, size, and morphology of these particles (Ahmed & Ikram, 2015). Recently, the nanoparticles of noble metals have received the attention of scientists as a result of their electronic, characteristic, catalytic, and optical properties (Annadhasan *et al.*, 2014). The synthesis of metal nanoparticles has been achieved using numerous chemicals, physical and biological methods. Among these methods, the most trusted ones were the chemical methods using different organic and inorganic reducing materials (Fu *et al.*, 2021). Among these noble metals, silver nanoparticles have received the interest and special attention of researchers nowadays because of several factors including the eco-friendly and cheap synthesis of these nanoparticles (Ihsan *et al.*, 2015), and various in-vivo and in-vitro applications (Ahmed & Ikram, 2015). One of AgNPs' applications is developing a colorimetric optical sensor for mercuric ions detection (Hg II) (Akhondi *et al.*, 2020).

Mercury is a widely distributed, non-essential, and poisonous metal to a human being. Universally, one of the major pollutants to the environment is mercury, which has a wide range of uses in medicine, agriculture, and industry. Generally, mercury appears in three forms including

organic, inorganic, and metallic circulating in ecosystems and never demolished (Park *et al.*, 2012). Nevertheless, the organic forms of mercury, as methylmercury, have been reported recently to be used as an ingredient in skin lightening or skin bleaching products (Jose & Ray., 2018). These products are used mainly to minimize melanin (The responsible for skin color) to give a lighter skin appearance. Generally, the skin lightening products are recommended to be used for a specified and short period of time in order to slowly reduce the pigmentation due to various skin conditions such as acne marks, eczema, and psoriasis. But these products may contain harmful and poisonous materials like steroids and hydroquinone in addition to mercury (Ricketts *et al.*, 2020). These active ingredients cause a range of adverse side effects affecting users' health that can be minor such as dermatitis to a mild condition like exogenous ochronosis to severe damage of kidney due to mercury poisoning on long term use (Benn *et al.*, 2019). Accordingly, in this work, a simple, rapid, and sensitive colorimetric sensor for mercuric ion detection will be developed. The sensor is based on the monitoring of the reduction of unmodified Albumin/Silver Nanoparticles (Alb/AgNPs) using absorption measurements. The developed sensor will use to detect mercury (II) ions in skin lightening cream products.

Experimental

Materials and Reagents

All reagents were of analytical grade. Nitric acid (65%), perchloric acid 70%, and mercuric nitrate were supplied from Merck (Darmstadt, Germany). Albumin/Silver nanoparticles solution (0.125M) was photochemically synthesized by the reduction of silver nitrate using UV-radiation, in presence of Albumin, as a green stabilizer, to prevent agglomeration of nanoparticles. The procedure of nanoparticles synthesis, spectroscopic analysis, morphology, particle sizes, and other structural details of the synthesized nanoparticles was described and published by Elsupikhe *et al.* (Elsupikhe *et al.*, 2020). All the glassware used in these analyses was soaked in 10% v/v HNO₃ overnight.

The containers were rinsed thoroughly with deionized water and dried. Deionized water was used for all reagents and solutions preparations

Instruments

The UV-Vis absorption spectra and the absorbance measurements of Alb/AgNPs were recorded using Single Beam CECIL Spectrophotometer (CECIL Instruments Limited, Cambridge, England). The absorption spectra of Alb/AgNPs at three different concentrations (0.0125M, 0.015M, and 0.025M) were measured within 260-780 nm range, using matched quartz cuvettes with 1cm optical path length. All spectroscopic measurements were carried out using double deionized water as a blank or reference sample.

Procedure

Colorimetric detection of mercury ions

For detection of Hg (II) ions using Alb/AgNPs, the optimal contact time between different concentrations of mercury (II) standard solution ranged from 1nM to 10⁵ nM and fixed concentration of Alb/AgNPs (0.015M) was investigated after three different times (0, 5, 10, and 30 min). The

calibration curve was constructed by transferring 1mL of different concentrations of mercury nitrate solutions ranging from (0 to 4.69 μM) to test tubes, then 1 mL of 0.03M of Alb/AgNPs was added in which the concentration of Alb/AgNPs in the final solution mixture is equal to (0.015M). The mixture was stirred by vortex for 1 minute, then the absorbance of each solution was measured at 440 nm. A calibration curve was drawn by plotting the difference absorbance (ΔA) of unmodified Alb/SNPs against the concentration of mercuric ion solutions, and the regression equation was generated. The difference absorbance was calculated as $\Delta A = A_0 - A$, where A_0 represents the absorbance of unmodified Alb/AgNPs at 440nm (Blank solution) and A is the absorbance of unmodified Alb/AgNPs after reaction with mercuric ion solution at different concentrations.

Analysis of Mercury (II) in skin lightening creams

Two lightening creams (S1 and S2) were randomly purchased from a local market in the city of Benghazi. The two samples were digested by an open flask procedure (Akagi & Nishimura, 1991; Agorku *et al.*, 2016). Two grams of each lightening cream sample were weighed out in a test tube, then 10 mL of nitric acid and perchloric acid were added. The digestion tube was heated at 100°C for 30 minutes until a clear solution was obtained. After the digestion process, the sample solution was allowed to cool and then poured into a 100 mL volumetric flask. The sample remaining in the tube was removed by rinsing several times with deionized water. The rinsing water was mixed with the previous sample in the flask and was used to make the solution up to the mark. The sample solution was filtered using Whatman No. 41 filter paper. One milli-liter of the filtrate was mixed with 1 mL of 0.03 M Alb/AgNPs, then the absorbance of the solution was measured at 440 nm. The concentration of mercury in each sample was determined from the regression equation of the standard curve. A blank solution was prepared under identical conditions.

Results and discussion

Colorimetric detection of mercury ions

Many methods have been developed for quantifying the amount of mercury ions due to their harmful effect. The traditional methods for mercury determination are high-performance, inductively coupled plasma mass spectrometry, atomic fluorescence spectrometry, atomic absorption spectroscopy, electrochemical method (Leopold *et al.*, 2010), electrothermal atomic absorption spectrometry, surface-enhanced Raman scattering, and graphite furnace (Zhou *et al.*, 2021). Although, these methods provide an effective and precise determination of low Hg²⁺ concentration. But these methods are costly, need long complicated sample preparation processes, and cannot be carried out in situ. Hence, an optical colorimetric sensor is more suited as it is rapid, easy, and cost-effective (Zhang *et al.*, 2020).

Among various metallic nanoparticles, to develop an optical sensor of mercury, silver nanoparticles gain an extra situation (Zhang *et al.*, 2020). Silver with the dimension of particles in nanosize gives a spectrum in the visible region as a result of optical excitation of the surface plasmon

resonance (SPR) with related scattering and high molar extinction coefficients. The conditions of surrounding media including (analyte concentration and pH), and the interaction with active molecules affect the NPs' SPR that can be used for sensing this difference and reaction (Jouyban & Rahimpour, 2020). The resultant color change can be simply observed with bare eyes excluding the need for costly instruments and appropriate for in-time analysis. The output signal to detect the analyte in this method is due to the change in the absorption of the optical sensor. The analyte can be detected and analyzed using the change in the migration of UV-spectrum or the absorbance measurements (Vasileva *et al.*, 2017).

The metal colloidal dispersion displays broad regions of absorption or absorption bands in the ultraviolet-visible wavelength range. These properties are characteristics of the metallic nature of particles as a result of interband transition or plasmon resonance excitation (Morales-Sánchez *et al.*, 2011). Thus, the first step in the optimization process of the colorimetric optical sensor was the maximum wavelength determination. The SPR band of synthesized Alb/AgNPs was identified by Elsupikhe *et al.*, work, using a UV-Visible spectrophotometer to be between 450-480 nm (Elsupikhe *et al.*, 2020). Therefore, in this work, the maximum wavelength was located by preparing three different concentrations of Alb/AgNPs. The absorbance of the three concentrations was measured using UV-visible in the range between 260-760nm and the absorption curves were illustrated in (Figure 1).

The absorption curves showed the absorbance increased as the concentration of Alb/AgNPs increased. But the maximum wavelength was the same for the three concentrations at 440nm. In which, the first concentration was (0.0125M) showed a maximum absorbance of 0.753 at 440nm, the second concentration was (0.015M) showed a maximum absorption of 0.95 at 440nm and the last concentration was (0.025M) showed maximum absorption of 1.616 at 410nm. Consequently, the maximum wavelength was considered and confirmed to be at 440nm for this synthesized Alb/AgNPs.

Similarly, Fu *et al.*, prepared AgNPs by chemical reduction using silver nitrate, trisodium citrate, and sodium borohydride, and the maximum wavelength for the resultants AgNPs was measured in the range from 350 to 450 nm and located at 400nm. According to different studies, when the silver nitrate used as silver nanoparticles precursor with different reducing and capping agents, the maximum wavelength was determined to be around 400nm (Fu *et al.*, 2021). Also, in Eskandari *et al.*, work, the maximum wavelength for the prepared silver-manganese disulfide/chitosan-polyvinyl alcohol Ag-MnS₂/CSPVA nanocomposites was investigated using UV-visible spectroscopy to be at 410nm, which is near to the maximum wavelength of our used Alb/AgNPs (Eskandari *et al.*, 2020). Conversely, Morales-Sánchez *et al.*, stated that the maximum absorption at the SPR band of silver nanoparticles generally occurs at 420nm. While, their prepared silver nanoparticles using albumin had an SPR band at 443nm (Morales-Sánchez *et al.*, 2011).

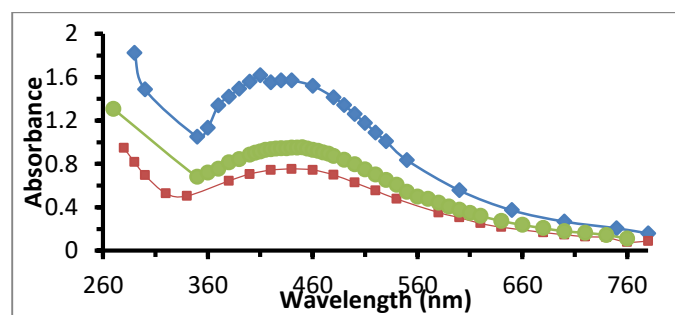


Fig (1): The absorption Spectra of Alb/AgNPs at three different concentrations (♦0.025M, ●0.015M and ■0.0125M).

The second step in the optimization process of the colorimetric optical sensor was the determination of the optimal contact time between the spiked Alb/AgNPs (0.015M) with different Hg (II) standard solution concentrations. In this study, Hg (II) standard solutions of different concentrations were prepared in the range from 1nM to 10⁵ nM. These concentrations spiked with (0.015M) Alb/AgNPs and the optimal contact time was accessed by measuring the absorbance at 0 min, 5 min, 10 min, and 30 min as shown in (Figure 2).

This figure showed a neglected change in absorption as time evolved. Correspondingly, Vasileva *et al.*, studied the reaction kinetics between the prepared starch stabilized silver nanoparticles in nitric acid and Hg (II) with respect to time. The developed absorption curve showed that the change in color was observed with naked eyes within 5 minutes and no change in the intensity of absorption occurs with times (Vasileva *et al.*, 2017).

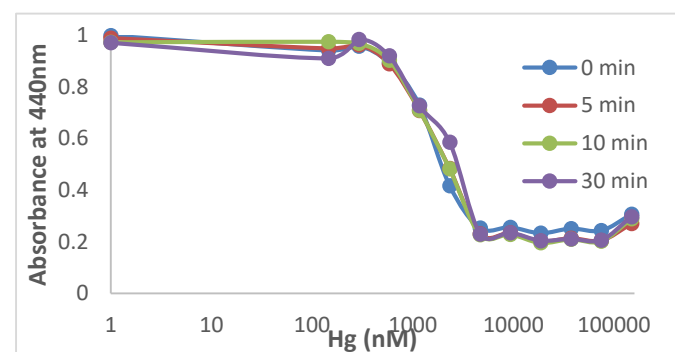


Fig (2): The effect of time on the reaction between 0.015 M of Alb/AgNPs and Hg (II) ion at different concentrations.

The third step in the optimization process of the colorimetric optical sensor was the construction of the calibration curve and determination of the limit of detection (LOD). In this work, the calibration curve was constructed by the serial dilution method. Then, 1ml of 0.03M Alb/AgNPs solution was added to 1 ml of Hg (II) standard solution of different concentrations ranging from 0 to 4.69 μ M. The colour of synthesized Alb/AgNPs was brown, this color decreased as the concentrations of added Hg (II) increased indicating the success of the sensor. Then, the absorption of prepared solutions was measured at 440nm and plotted as in Figure 3. Firstly, the absorbance of these solutions is directly blotted against the concentration of Hg (II) standard

solutions. The curve showed a linear plot with a negative slope representing the decrease in absorption.

This decrease in absorbance values of Alb/AgNPs solution increases linearly with increasing Hg (II) solution until reaches a 2.340 μ M concentration of Hg (II). Similarly, Devadiga *et al.*, got a plot with negative slope as a result of absorbance decrease as the concentration of mercuric ion solution increased. This was part of the optimization of silver nanoparticles for Hg (II) detection in an aqueous solution (Devadiga *et al.*, 2017).

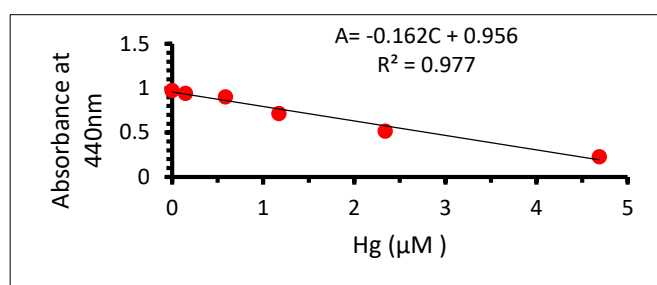


Fig (3): The calibration curve of Hg (II) reacted with 0.015 M Alb/AgNPs, versus the absorbance at 440nm

Secondly, the optimized calibration curve was obtained by plotting the absorbance difference (ΔA) between the absorbance of Alb/AgNPs in blank solution and the absorbance of unmodified Alb/AgNPs in spiked Hg (II) standard solution, as expressed in (Figure 4). The calibration curve showed a linear relationship to Hg (II) ion concentration in the range from 0.146 μ M to 2.340 μ M. The straight-line equation obtained was ($\Delta A = 0.2077C + 0.0023$, where ΔA is the difference absorbance and C is the concentration of Hg (II) ion), and the linear regression coefficient was ($R^2 = 0.9941$). The limit of detection (LOD) was calculated by establishing the minimum concentration at which Hg (II) ion can be detected. The LOD was found to be 0.141 μ M, according to the $3SD/slop$ of the calibration graph definition (Miller J. C. and Miller J. N., 1993), where SD is the standard deviation of blank absorbance measurements ($n=5$).

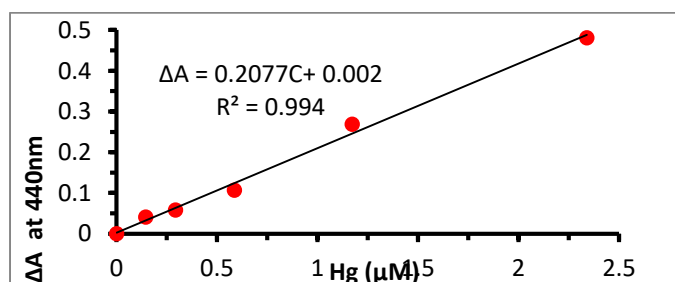


Fig (4): The calibration curve of Hg (II) reacted with 0.015 M Alb/AgNPs versus the difference absorbance at 440nm Likewise, in Devadiga *et al.* study, the prepared AgNPs sensor was brown in color. While, the solution of Hg (II) ions is colorless due to a close-shell d10 configuration. The adding of Hg (II) to the AgNPs colloidal suspension caused a blue shift of spectra and a distinctive decrease in the SPR peak absorption intensity. This can be explained by the occurrence of redox reaction at the surface of AgNPs as a result of the difference in standard potential of silver

($E_{Ag^+/Ag}^0 = 0.8V$ and mercury ($E_{Hg^{2+}/Hg}^0 = 0.85V$ (Devadiga *et al.*, 2017).

In addition, in the work of Vasileva *et al.*, the addition of Hg (II) decreases the intensity of the SPR band at 407nm, the maximum wavelength of prepared starch stabilized AgNPs. The decrease was notable for Hg (II) concentration between 25-500 μ g/L. The blue shift has accompanied this decrease in wavelength. This decrease can be explained by the formation of a layer of Hg (II) around the AgNPs to form an amalgam-like structure which changes the refractive index of silver nanoparticles. The suggestion for the system used in their work is that the redox reaction occurs at the surface of AgNPs but with competition between two oxidizing agents (Hg^{2+}/Hg^0) and (NO_3^-/NH_4^+) with comparable electrode potential that is higher than that of $E_{Ag^+/Ag}^0$ (Vasileva *et al.*, 2017).

In addition, Akhondi *et al.*, successfully prepared two silver nanoparticles named Apple (Ap-AgNPs) and Nigella Sativa (N-AgNPs) seed extracts with β -Cyclodextrin at room temperature without any modification. The SPR of Ap-AgNPs was measured to be 423nm, which decreases on the addition of Hg (II) ions solution up to 200 nm concentration. Also, the color of Ap-AgNPs solution changed from yellow to light yellow to colorless. The color change was easily detected by naked eyes indicating the success of the sensor. A range of Hg (II) concentrations from 60 nm to 4 μ M was added until the color of the solution disappeared. While the linear relationship was obtained for Hg (II) concentration range from 60.0 to 90.0 nM. In the same way, the intensity of located N-AgNPs SPR band at 408 nm was also decreased on the addition of Hg (II) different concentrations from yellow to colorless.

The linear equation was obtained for Hg (II) concentration range from 500.0 to 900.0 nM and LOD equal to 58.0 nM. The proposed AgNPs work well for detecting Hg (II) (Akhondi *et al.*, 2020). In addition, Annadhasan *et al.*, used amino acids to develop an L-tyrosine-stabilized AgNPs to work as an optical colorimetric sensor for different heavy metals detection. Among the tested heavy metals, only Hg (II) and Mn (II) showed a color change of AgNPs from yellow to colorless on the addition of fixed concentration of these metals (Annadhasan *et al.*, 2014). The selectivity of L-tyrosine-stabilized AgNPs to Hg (II) was studied by adding different concentrations of a mercuric solution to AgNPs. The addition of Hg (II) caused a hypsochromic shift in the band of SPR and as the concentration of Hg (II) increased the shift increased in the range from 16 nM to 660 nM with a linear relationship. This can be attributed to the redox reaction between Ag of AgNPs with zero valence and Hg^{+2} ions that was confirmed and compared with previous studies. In other words, the difference in reduction potential between silver and mercury causes reduction of mercury (Hg^{2+} to Hg), which acts as an oxidizing agent, which oxidized AgNPs and formed Ag-Hg amalgam (Ahmed *et al.*, 2020). In fact, the disappearance of color in the previous studies was explained by the formation of amalgam (Ag-Hg) as a result of redox reaction at AgNPs surface which explains our work.

Analysis of Mercury (II) in skin lightening creams

Finally, the last step in this work was to determine the applicability of the optimized Alb/AgNPs colorimetric sensor under the optimal experimental conditions in determining the concentration of mercury in two lighting creams, labeled S1 and S2. A digestion process using strong acids was applied to both S1 and S2. These samples were. Therefore, the absorption curves of Alb/AgNPs in water and in blank acidic digestion solution were measured (**Figure 5**). This figure illustrated that the acidic content of the digestion solution did not affect the SPR band of prepared Alb/AgNPs.

Similarly, Vasileva *et al.*, tested the effect of nitric acid in the optimization process of prepared starch stabilized AgNPs and they have found that nitric acid increases the sensitivity of sensor and it is accepted as sample media (Vasileva *et al.*, 2017)

In addition, Fu *et al.*, mentioned that the acidic pH decreases the absorbance values. Oppositely, the alkaline pH increases the absorption values. This was discussed in earlier work that the values of pH have an effect on AgNPs by changing the surface charges and consequently affecting the aggregation and stability of particles. The AgNPs are not stable in acidic and neutral PH. In which, these conditions make the surface charges denser and more tend to aggregate to large size. While, the cause of absorbance decrease may be due to absorption peaks displacement due to surface plasmon spectroscopy (Fu *et al.*, 2021).

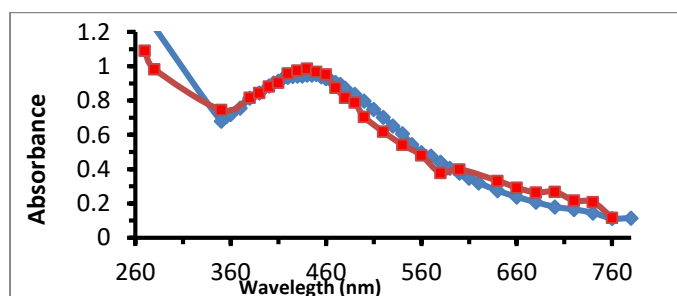


Fig (5): Absorption spectra of 0.015 M Alb/AgNPs in water (♦) and nitric acid solution (■).

To determine the mercury contents in the selected lightening creams, the digestion solution of each sample spiked with Alb/AgNPs. The color of Alb/AgNPs disappeared upon adding to the lighting creams samples. Then, the absorbance of unmodified nanoparticles was measured and mercury contents were calculated using the difference calibration curve. Actually, the concentrations of mercury in the skin lightening creams (S1 and S2) were recorded in (**Table 1**).

In the work of Eskandari *et al.*, the silver-manganese disulfide/chitosan-polyvinyl alcohol (Ag-MnS₂/CSPVA) nanocomposites were prepared for colorimetric detection of Hg (II). The developed method showed good sensitivity and this nanoprobe is effective and applicable for detecting Hg (II) in water samples in the presence of other heavy metals (Eskandari *et al.*, 2020). Also, Akhondi *et al.*, successfully prepared two types of silver nanoparticles, the first-named Apple (Ap-AgNPs). These AgNPs achieved rapid, sensitive, and selective optical colorimetric sensors for mercuric

detection in aqueous solution. The proposed mechanism of Hg (II) detection was a hydrodynamic increase in average size confirmed using SEM and TEM, accompanied by nanoparticles aggregations.

This happens due to strong electrostatic attraction between positively charged Hg (II) ions and negatively charged AgNPs moving any stabilizers on AgNPs surface. This attraction causes oxidation of silver and reduction of mercury with the formation of Ag-Hg alloy. Finally, the decrease in absorption can be due to Hg (II) ions' effect on AgNPs surface with or without reduction in AgNPs size or amalgamation (Akhondi *et al.*, 2020). Additionally, Annadhasan *et al.*, synthesized L-tyrosine-stabilized AgNPs that showed a selective detection and excellent sensitivity for Hg (II) in aqueous solution (Annadhasan *et al.*, 2014).

Table (1): Mercury (II) ion concentration in two skin lightening Cream Samples

Sample	A ₄₄₀	ΔA=A ₀ -A	Hg ion Concentration (ppm)
S1	0.975	0.011	0.574
	0.982	0.004	0.239
Mean ±SD			0.406 ±0.2
S2	0.926	0.06	2.920
	0.938	0.048	2.346
Mean ±SD			2.631 ±0.4

A₀=Absorbance of Alb/AgNPs in Blank solution (0.986), and A=Absorbance of Alb/AgNPs in Sample Solution

Conclusions

The green synthesized Albumin silver nanoparticles (Alb/AgNPs) showed a good colorimetric optical sensing property for Mercuric Hg (II). The colour of the Alb/AgNPs optical sensor changed from brown to light brown to colorless on Mercuric addition. The mechanism of detection was explained. The linear regression equation was obtained with a correlation coefficient (R²) of 0.99641 for Mercuric in the concentration range from (0.293 μM to 2.340 μM). The optimized optical colorimetric sensor was accepted to be used for the detection of Mercuric ions in the selected lighting creams samples.

These results showed that the optimized method is a practical, rapid, and sensitive method and applicable for determining the concentration of mercury in skin bleaching and lightening cream product

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