



Bio-Colloidal Silver Nanoparticles Prepared via Green Synthesis Using Sandoricum koetjape Peel Extract for Selective Colorimetry-Based Mercury Ions Detection

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Keywords

Bio-colloidal; Colorimetric; Mercury ions; Sandoricum koetjape; Silver nanoparticles

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RESEARCH PAPER

Bio-Colloidal Silver Nanoparticles Prepared via Green Synthesis Using *Sandoricum koetjape* Peel Extract for Selective Colorimetry-Based Mercury Ions Detection

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Abstract

Hazardous waste from mercury is a critical problem that requires serious attention. Herein, we report bio-colloidal silver nanoparticles (AgNPs) synthesized via green synthesis route from AgNO₃ aqueous solution and *Sandoricum koetjape* (SK) peel extract and demonstrate their sensitivity at detecting mercury ions (Hg²⁺) using colorimetry. AgNPs were synthesized by varying the volume ratio of AgNO₃ solution to SK peel extract, namely AgNPs(4:1), AgNPs(3:2), AgNPs(1:1), AgNPs(2:3), and AgNPs(1:4). Bio-colloidal AgNPs were tested for their ability to detect toxic heavy metal mercury ion by adding different concentrations of Hg²⁺. All samples have successfully detected Hg²⁺ at varying levels of sensitivity. This investigation revealed that AgNPs(4:1) is the most sensitive colloid for detecting Hg²⁺ up to a concentration of 1 ppm.

Keywords: Bio-colloidal, Colorimetric, Mercury ions, *Sandoricum koetjape*, Silver nanoparticles

1. Introduction

Mercury is the most toxic heavy metal that naturally occurs in small amounts on earth. Human activities such as battery and electronic devices waste disposal, the manufacturing industry, and gold mining have contributed to the increase in levels of mercury ions (Hg²⁺) in soil and aquatic ecosystems [1]. Ingestion of mercury by humans will increase the risk of kidney, brain, and lung damage [2,3]. Excessive levels of heavy metal mercury ions can seriously harm human health, pollute the land and water, and represent a big threat to the environment. Therefore, a technique for detecting Hg²⁺ in the environment is urgently needed. The available techniques for determining Hg²⁺ concentrations in the environment include vapor atomic fluorescence spectroscopy [4], high-performance liquid chromatography [5], and inductively coupled

plasma mass spectrometry [6]. However, these methods are not efficient due to the difficult and time-consuming process as well as costly [7,8]. The colorimetric method is an effective way of detecting Hg²⁺ by changing the color of the indicator when reacted with the analyte. In addition, the detection process with this method is simple and fast and also produces high sensitivity [9–11].

Colorimetric indicators using silver nanoparticles (AgNPs) have proven to be promising materials for monitoring Hg²⁺ levels. It is due to their high efficiency of surface plasmon resonance (SPR) [2,12]. AgNPs have great optical properties than gold nanoparticles. It has received more attention regarding color changes with visible SPR [13]. AgNPs have high electrical and thermal conductivity, chemical stability, and catalytic activity. In addition, AgNPs also exhibit a broad spectrum of bactericidal and fungicidal activity which makes

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them very popular in various products including plastics, soaps, pastes, food, and textiles [14,15].

AgNPs can be synthesized through three approaches, namely physics, chemistry, and biology. The synthesis of nanoparticles by physical and chemical methods acquires toxic compounds. Also, the processing techniques are complicated, time-consuming, expensive, and require sophisticated working conditions [13,16]. Thus, biological methods are developing as an environmentally friendly alternative to produce non-toxic nanoparticles with low cost and low energy consumption [14,17]. In general, green synthesis or biosynthesis is the biological method for preparing nanoparticles involving microorganisms, plants, and viruses. Biosynthesis using microbes is less efficient because the breeding process requires complicated procedures and is prone to contamination. Meanwhile, the use of plant extracts can overcome the problem of environmental pollution, is more cost-effective, and has tremendous potential in heavy metal detoxification [18]. The existence of phytochemical substances, primary and secondary metabolites which are responsible for reducing metal salts, drives the use of plant extract for the synthesis of nanoparticles [19,20].

In this paper, we discussed the biological synthesis of AgNPs using the peel extract of sentul fruit or in Latin it is *Sandoricum koetjape* (SK) as a reducing agent. This extract was chosen because SK has high antioxidant activity. In addition, sentul fruit is a tropical fruit native to Southeast Asia which is located in maritime areas such as Indonesia, Malaysia, Philippines, Brunei Darussalam, and Timor-Leste [21]. The synthesized AgNPs were used as a selective colorimetric sensor to investigate the presence of Hg^{2+} . The optical properties, functional group, morphology, and structure of AgNPs will also be discussed based on UV–Vis spectroscopic, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and transmission electron microscopy (TEM).

2. Experimental details

2.1. Materials

The materials used were freely available and purchased for experimental work. Organic materials such as SK peel are obtained directly from fruit that falls from SK trees which are widespread around the University of Riau, Pekanbaru, Indonesia. The chemicals used included silver nitrate ($AgNO_3$) (EMSURE®) as a silver precursor, sodium hydroxide (NaOH) (EMSURE®) as a stabilizing agent, mercury (II) chloride ($HgCl_2$) (Smart Lab) as an oxidizing agent

in colorimetric method, distilled water, and acetone as a solvent, all of which were purchased from Kimia Pro Analyze, East Jakarta, Indonesia.

2.2. Synthesis of bio-colloidal AgNPs

A 20 g/L of SK peel extract was prepared by dissolving 5 g of SK peel powder that has been through the processes of sterilization, drying, and blending into 250 mL of DI water. The biosynthesis of AgNPs was produced by adding $AgNO_3$ solution 2.5 mM to SK peel extract in a 50 mL glass beaker. Then, NaOH was dropped as a catalyst until reached pH 10. These mixtures were heated for 30 min at 80 °C until bio-colloidal AgNPs were formed. In this work, the volume ratio of $AgNO_3$:SK was varied with samples name AgNPs(4:1), AgNPs(3:2), AgNPs(1:1), AgNPs(2:3), and AgNPs(1:4). The schematic procedure of synthesis AgNPs can be seen in Fig. 1.

2.3. Characterizations

UV–Vis spectroscopy analysis was carried out using an Agilent Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Malaysia, G68860A) to determine the peak absorbance of nanoparticles. Functional groups that describe the type of bonding structure of the sample were characterized using FTIR. The crystal structure was examined using an X'Pert-type diffractometer. The morphology of AgNPs was observed using Tecnai-20F Lorentz TEM.

2.4. Colorimetric-based mercury ions detection

A sensitivity test of AgNPs to Hg^{2+} was carried out by adding 1 mL of $HgCl_2$ solution into 0.5 mL of synthesized AgNPs solution into vials. The $HgCl_2$ was prepared in different concentrations of 1, 20, 40, 60, 80, 100, 150, 200, and 250 ppm. The concentrations determine the limits of the detection ability of AgNPs as indicator colorimetry. The color change of the solution was observed (see Fig. 1) and the absorbance was measured using a UV–Vis spectrophotometer.

3. Results and discussion

3.1. Optical properties

UV–Vis absorbance measurement was carried out on a mixture of $AgNO_3$ solution and SK peel extract at various volume ratios. The absorbance spectrum in Fig. 2 shows the SPR peak which corresponds to the characteristics of AgNPs at a wavelength of 407 nm [22].



Fig. 1. The biological synthesis scheme of AgNPs.

The color change shows a reduction process for silver ions (Ag^+) to form Ag due to the SK peel extract acts as a reducing agent [23]. The SPR peak confirms the effect of SK peel extract volume in reducing Ag^+ to AgNPs from AgNO_3 [14]. The absorbance peak decreases as the volume ratio of AgNO_3 reduces. The shift of the absorbance peak occurs towards a wavelength greater than 400 nm which is known as the SPR redshift. In addition, there are also other peaks in the wavelength of less than 350 nm which indicates the effect of SK peel

extract compound. It is caused by the electronic transition of the metal Ag appearing in the spectral range of 250–330 nm previously reported using the Gum karaya extract [24].

3.2. FTIR analysis

Fig. 3 depicts the FTIR spectrum used to identify the functional groups present in a sample of AgNPs(1:1) and pure extract of SK peel. The spectrum consists of aromatic (C–H), alkene (C=C),

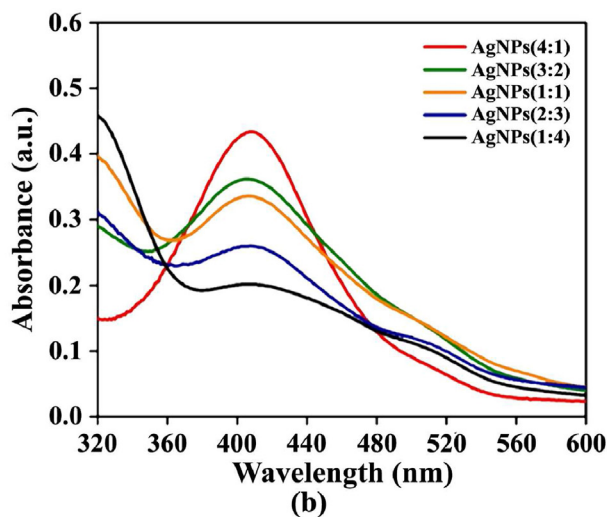
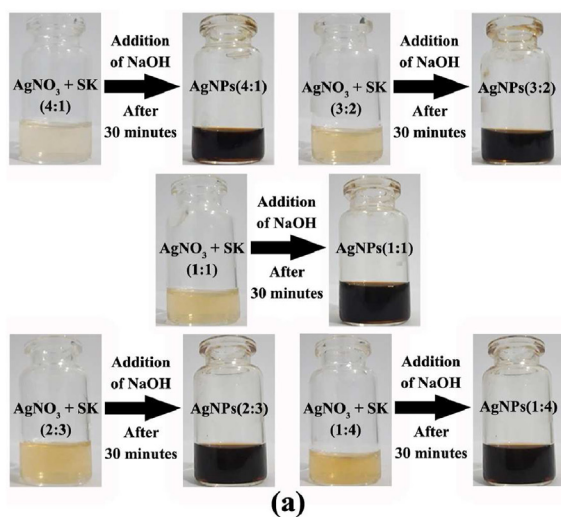


Fig. 2. Several samples of bio-colloidal AgNPs of various volume ratios of AgNO_3 :SK had uniform visual color as shown in (a), and UV-Vis absorption characteristics as shown in (b) where each sample exhibited an SPR peak in the range of 405–407 nm indicating the formation of the AgNPs structure.

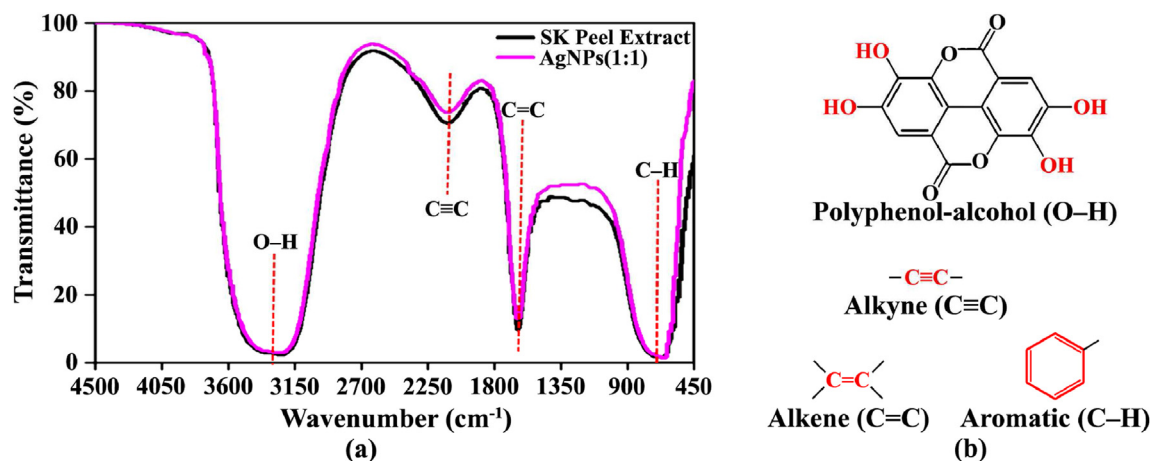


Fig. 3. The minimum FTIR spectrum shows the characteristics of functional groups for SK peel extract samples and AgNPs(1:1) as shown in (a), and the general structure of several identified functional groups as shown in (b) except for the specific polyphenol-alcohol form only arose from the Rosidae subclass in the SK peel extract.

alkyne groups ($C\equiv C$), and polyphenol-alcohol ($O-H$). AgNPs(1:1) sample and SK peel extract have the same functional groups. Each group has a different number range which is shown in Table 1.

The standard for determining the functional groups and bonds of the sample was compared with data from the literature [25]. Both samples have at most 4 functional groups with the smallest wavenumbers in the range of $632-720\text{ cm}^{-1}$ and $641-711\text{ cm}^{-1}$. The alkyne group is shown in the spectral region $1619-1638\text{ cm}^{-1}$ and $1617-1642\text{ cm}^{-1}$. The alkene group derived from SK peel extract can reduce and act as a stabilizer of Ag^+ to Ag. It has similarities based on the results of FTIR characterization of AgNPs of red dragon fruit peel extract [25]. The alkene groups can encapsulate AgNPs and bind to metals to prevent particle agglomeration [26]. The IR absorbance at the wavenumber ranges of $2110-2135\text{ cm}^{-1}$ and 2108 cm^{-1} show the connection between alkyne functions. Absorbance at the wavenumber ranges of $3204-3481\text{ cm}^{-1}$ and $3179-3575\text{ cm}^{-1}$, shows polyphenol-alcohol in AgNPs (1:1) and SK peel extract.

Table 1. Infrared spectrum data from FTIR analysis.

Wavenumber (cm^{-1})		Functional Group	Chemical Bond
AgNPs(1:1)	SK Peel Extract		
632–720	641–711	Aromatic	C–H
1619–1638	1617–1642	Alkene	C=C
2110–2135	2108	Alkyne	C≡C
3204–3481	3179–3575	Polyphenol-Alcohol	O–H

3.3. Structural analysis

XRD investigation revealed the crystalline nature of Ag nanoparticles. The XRD pattern of dried nanoparticles derived from colloid samples is shown in Fig. 4. The XRD peaks show Bragg reflections at diffraction angles of 38.36° , 44.06° , 64.72° , and 77.42° indicating the (111), (200), (220), and (311) planes of silver, respectively. The peaks are consistent with the crystalline phase of the face-centered cubic structure and agree with the standard powder diffraction card of JCPDS, silver file no. 04-0783 [27].

The observed peak broadening was most likely caused by macromolecules contained in the plant extract, which might be responsible for silver ion reduction. As a result, the XRD pattern clearly showed that the silver nanoparticles produced in this current synthesis are crystalline in nature. There are additional peaks detected in addition to the Bragg peaks that represent silver nanocrystals, showing that bio-organic phase crystallization occurs on the surface of the silver nanoparticles [28]. This peak is denoted by an asterisk (*).

The structure information, such as crystallite size (D), the distance between crystal planes, and lattice constant (a) were determined using Debye–Scherrer formula in Equation (1), Bragg formula in Equation (2), and formulation lattice constant on the cubic-type system in Equation (3), respectively.

$$D = \frac{0.9\lambda}{\beta \cos \theta} \quad (1)$$

$$2d_{hkl} \sin \theta = n\lambda \quad (2)$$

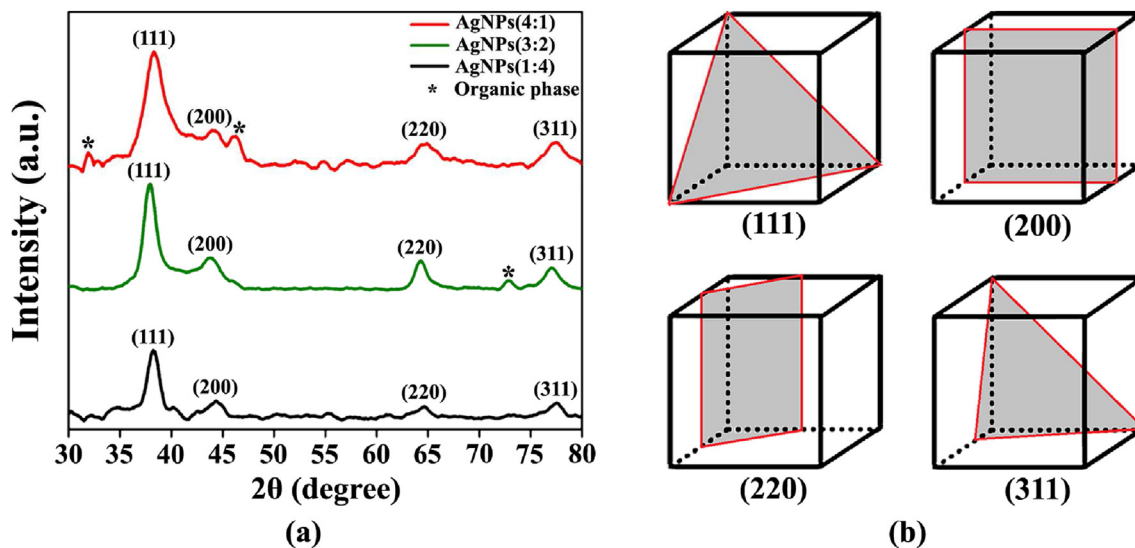


Fig. 4. The XRD spectra of each AgNPs sample show the presence of a crystal lattice shape and the diffraction angle at the peaks is almost the same as that shown in (a), and the crystal lattice plane representation of each AgNPs sample as in (b) shows its consistency with the face-centered cubic structure.

$$a = d \times \sqrt{h^2 + k^2 + l^2} \quad (3)$$

where, λ is the wavelength of X-ray (1.5406 Å), β is the full width at half maximum (FWHM), θ is the diffraction angle, d_{hkl} is the interplanar spacing, and hkl is the Miller indices [29]. The XRD parameters including the lattice parameter and crystal sizes are shown in Table 2.

The sample with the highest crystal size is AgNPs(3:2) in the (111) and (311) planes of 6.906 nm and 7.488 nm, respectively. Meanwhile, the planes (200) and (220) are found in the AgNPs(1:4) sample. These samples have the largest crystal sizes of 5.998 nm and 10.92 nm, respectively. These crystal sizes include the size standard for AgNPs which are usually 2–10 nm [30]. The average size lattice parameter of nanoparticles was calculated at

4.086 nm. The estimated lattice constant is in accordance with the previously reported [31].

3.4. Morphology of AgNPs

Fig. 5 displays the morphology and particle size histogram of the AgNPs samples. The TEM image revealed that the majority of the particles generated by AgNPs were spherical, with some being oval. This particle form is identical to what has been observed in earlier research utilizing *Citrullus lanatus* peel extract [32]. AgNPs synthesized by biological systems tend to have a similar shape and size [33].

Particles in AgNPs(4:1) appear to have the smallest size while the largest particle size is obtained at AgNPs(1:4). These results are in accordance with the

Table 2. Characteristic XRD parameters of AgNPs.

Sample	Plane (<i>hkl</i>)	2θ (degree)	FWHM (degree)	Crystallite Size (nm)	Interplanar Spacing (nm)	Lattice Parameter (nm)
AgNPs(4:1)	(111)	38.37	1.659	5.070	2.344	4.060
	(200)	44.06	1.821	4.705	2.054	4.107
	(220)	64.72	1.391	6.763	1.439	4.070
	(311)	77.42	1.684	6.046	1.232	4.085
AgNPs(3:2)	(111)	37.94	1.216	6.906	2.370	4.105
	(200)	43.78	1.728	4.956	2.066	4.130
	(220)	64.30	1.247	7.526	1.447	4.094
	(311)	77.06	1.356	7.488	1.237	4.101
AgNPs(1:4)	(111)	38.24	1.264	6.653	2.352	4.073
	(200)	44.32	1.430	5.998	2.042	4.084
	(220)	64.58	0.861	10.92	1.442	4.079
	(311)	77.44	1.615	6.305	1.232	4.084

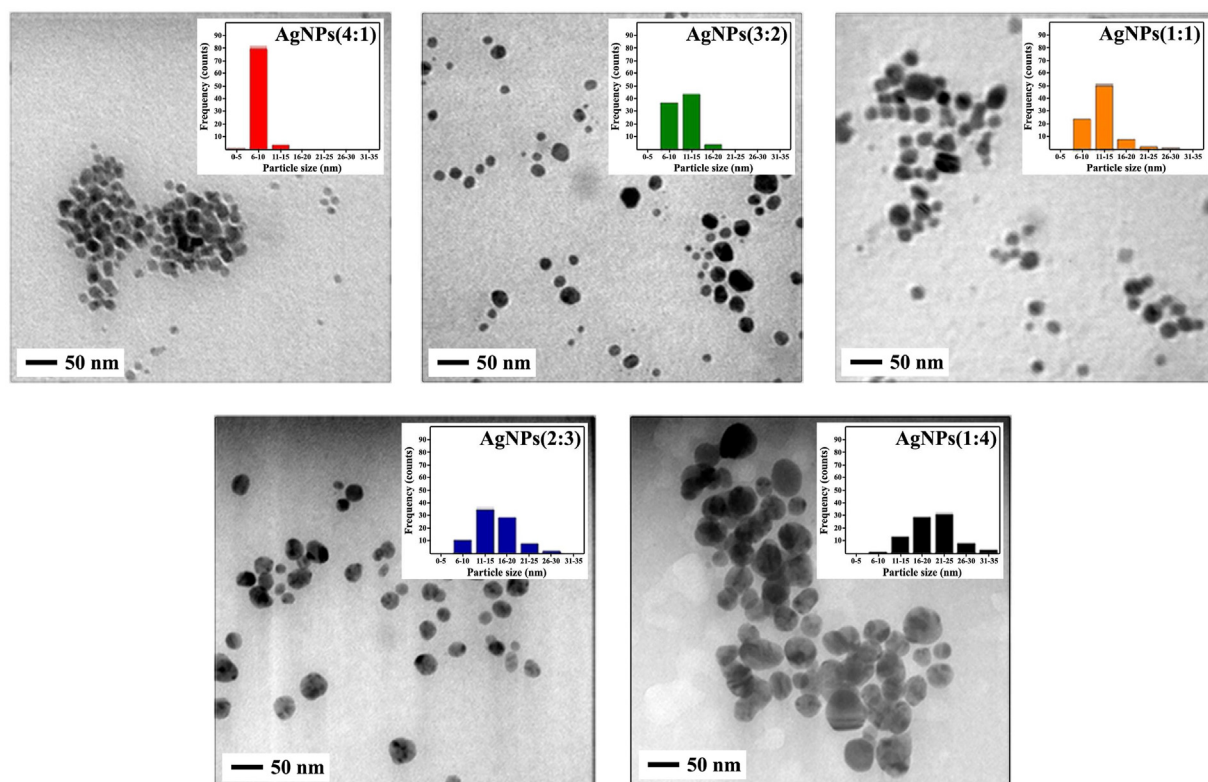


Fig. 5. Morphology and histogram of AgNPs with variations in volume ratio.

SPR band from UV–Vis spectroscopic characterization. The absorption peak at lower wavelengths indicates a smaller size of AgNPs formed, while higher wavelengths indicate a larger size of AgNPs formed [34]. In addition, the difference in particle size is also caused by the volume used where the larger the volume of AgNO_3 in the sample, the smaller the particle size. The distribution of particle sizes in each sample is shown in the histogram graph inserted in Fig. 5. The decrease in the volume of AgNO_3 resulted in a more uneven distribution of particle size as seen from the larger standard deviation value. It means the sample with the highest SPR peak has an even distribution of particle size.

3.5. High-sensitivity mercury ions detection

The sensitivity of AgNPs to detect Hg^{2+} with varying concentrations of HgCl_2 can be seen from the UV–Vis spectrum in Fig. 6. Detection of Hg^{2+} was determined from the SPR peak of AgNPs. It can be seen that the SPR peak decreases with increasing Hg^{2+} concentration. A blue shift occurs at the absorption peak of the AgNPs curve from 407 nm to 384 nm due to an increase in the concentration of HgCl_2 . This phenomenon indicates that the distance

between nanoparticles gets closer during the reduction of HgCl_2 by AgNPs. So, the frequency increases and at the same time shortens the wavelength [7].

The reaction of AgNPs and HgCl_2 produces a color change which indicates the ability of the AgNPs sensor to detect the presence of Hg^{2+} . AgNPs(4:1) after reacted changed color from yellowish brown to pale yellow to a concentration of 150 ppm which was observed for 30 min. The color of the reaction degraded and turned colorless gradually with increasing concentration of HgCl_2 and finally same to water. This sample shows a significant color change compared to other AgNPs samples. AgNPs(3:2), AgNPs(1:1), and AgNPs(2:3) showed a less significant change in the color of the reaction products. It was the highest concentration of HgCl_2 resulting in a change in the color only to pale yellow. In addition, the AgNPs(1:4) also produced an insignificant change where the final color change at the largest concentration of HgCl_2 of the bio-colloidal AgNPs to a solid yellow color limit. It is supported by the highest absorbance peak in the AgNPs(4:1) where the SPR properties are better. The resulting color change corresponds to the SPR properties of AgNPs with an increase in the

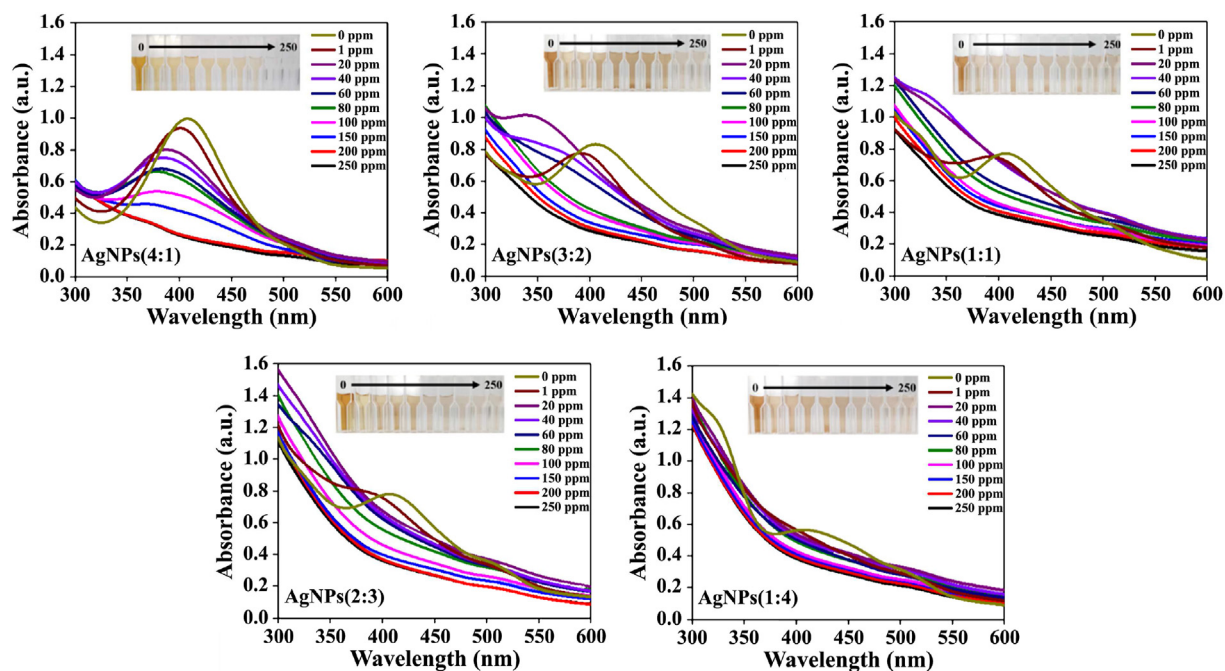


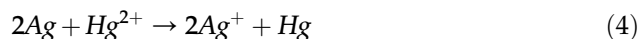
Fig. 6. UV-Vis spectra of AgNPs using different concentrations of Hg^{2+} .

concentration of HgCl_2 which can be seen clearly. In addition, bio-colloidal AgNPs change color due to aggregation by the Hg analyte and due to the strongly oxidizing properties of HgCl_2 . Color loss occurs due to the release of Ag^+ and subsequent reactions with Cl^- to form AgCl [35].

AgNPs(4:1) in Fig. 6 shows a significant decrease and widening of the absorption peak of the SPR band with an increase in the concentration of HgCl_2 . This result is associated with a significant color change. SPR curve at AgNPs(3:2) (Fig. 6) which experienced a significant decrease occurred only at concentrations of HgCl_2 from 1 to 80 ppm. Meanwhile, the larger concentration only decreased slowly indicated by the adjacent curve. The same results were also found in the AgNPs(1:1), AgNPs(2:3), and AgNPs(1:4) (Fig. 6) which the SPR curve experienced a significant decrease only to HgCl_2 concentrations of 60 ppm, 20 ppm, and 1 ppm, respectively. At large concentrations, there is only a slight decrease in the curve. The SPR curves for large HgCl_2 concentrations appear to coincide. It indicates there is no more change in the color of the solution. From these results, it can be seen that increasing the volume ratio of the extract reduces the mercury ions detection ability in AgNPs. So, AgNPs(4:1) is considered the most sensitive sample in detecting mercury ions compared to other either from a change in color solution and optical absorption.

The linear relationship between absorbance intensity and HgCl_2 concentration is shown in Fig. 7.

The regression value for AgNPs(4:1), AgNPs(3:2), AgNPs(1:1), AgNPs(2:3), and AgNPs(1:4) samples are 0.9141, 0.8337, 0.8357, 0.8325, and 0.9094, respectively. The greatest regression value was obtained at AgNPs(4:1) with a steep slope. AgNPs(3:2) and AgNPs(1:1) also have a steep slope but the regression value obtained is lower. AgNPs(2:3) and AgNPs(1:4) regression values obtained are quite large but the slope of the linear line is very small. Based on these results, AgNPs(4:1) can be concluded as the most sensitive sample to Hg^{2+} in colorimetric detection. Overall, the absorbance of AgNPs decreased with increasing Hg^{2+} concentration. It indicates AgNPs sample synthesized with the bio-reductant of SK peel extract can be used as a colorimetric sensor for mercury ions. The redox reaction is the principle of colorimetric detection of mercury ions by AgNPs. Most of the transition metals, alkalis, and alkaline earth metals cannot oxidize Ag from AgNPs to Ag^+ due to their lower reduction potential than Ag^+ . Therefore, it enables highly selective analysis of Hg^{2+} . Hg^{2+} has a higher reduction potential than Ag^+ , so the redox reactions that occur can be seen in Equation (4) [6].



Oxidation of Ag to Ag^+ changes the color of AgNPs from yellowish brown to colorless, as observed in the initial colorless AgNO_3 solution.

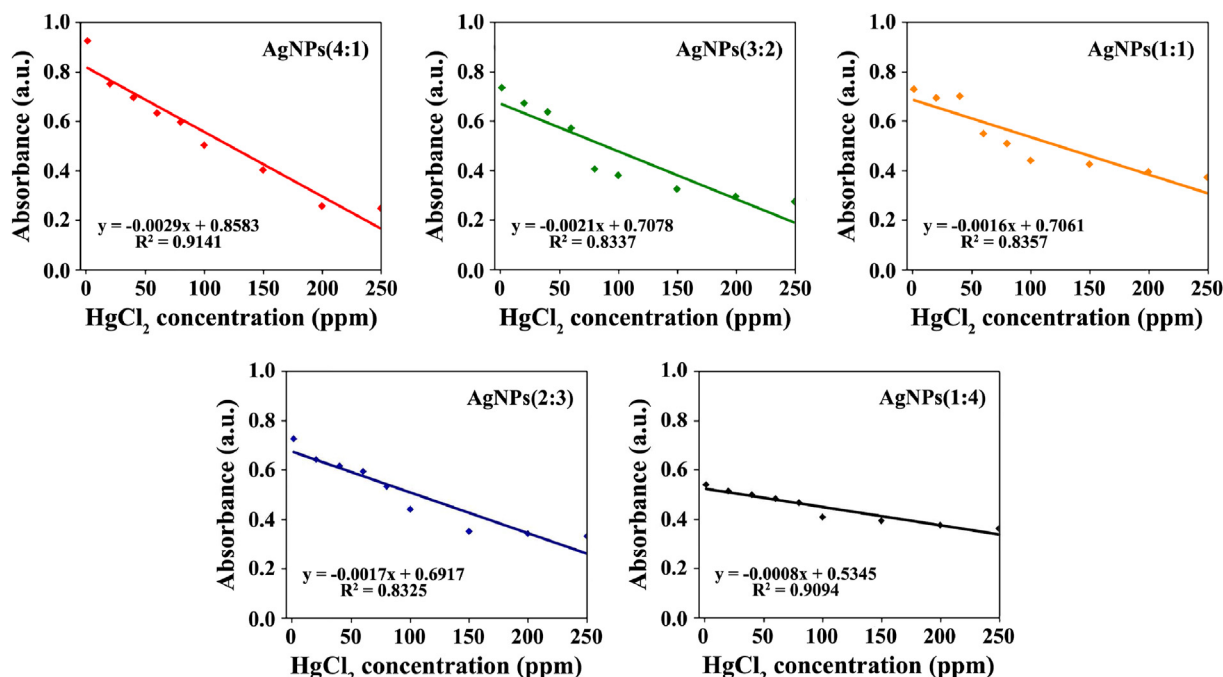


Fig. 7. The plot of absorbance intensity at 407 nm versus Hg^{2+} concentration.

4. Conclusions

The green synthesis approach is cost-effective and environmentally benign to produce AgNPs. Here, the SK peel extract acts as both a reducing and stabilizing agent. The surface plasmon resonance of green-synthesized silver nanoparticles confirmed the existence of AgNPs. The results of X-ray diffraction demonstrate clearly that the silver nanoparticles generated by the reduction of Ag^+ ions by the extract of SK peel are crystalline in nature. Using Debye-Scherrer's formula, the average crystalline size of AgNPs is calculated to be less than 10 nm. The particle size of AgNPs from TEM was found to be less than 20 nm. This study demonstrated that AgNPs are highly sensitive colorimetric sensors for detecting Hg^{2+} due to their ability to induce large colloid color changes.

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