

Article

Coating Gold Nanoparticles Synthesized from Spent Coffee Ground Extract to Enhance Their Stability

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Abstract. Gold nanoparticles (AuNPs) were synthesized using the extract from cold-brew spent coffee grounds. The colloids were characterized by UV-vis spectrophotometry and transmission electron microscopy. When using 1.8 mM and 4.0 mM HAuCl₄, the average diameter of AuNPs was 10.51 and 13.75 nm, corresponding to the peak at 533 and 539, respectively. The colloidal stability was studied using the results from spectrophotometry. The aggregation of as-synthesized AuNPs, reflected by the red shift of the spectrum, occurred after the centrifugation at 10000 rpm for 10 minutes but this was prevented after coating with 5%w/v PEG400, 5%w/v PEG6000, 0.1%w/v alginate, and 0.5%w/v SDS solutions. Electrostatic stabilization using alginate and SDS could withstand up to three times of centrifugation, while steric stabilization using PEG400 and PEG6000 could withstand the disturbance of Ca²⁺ more effectively. The smaller AuNPs were more affected by Ca²⁺ than the larger ones. In highly acidic solutions, all coated AuNPs showed the red shift and SDS-coated AuNPs were the most effective against acid disturbance, and in highly basic solutions, all coated AuNPs showed the blue shift. Besides, the coated AuNPs were still stable at 52°C. This study could be applied in situations where mechanical, chemical, thermal and acid disturbances are concerned.

Keywords: Gold nanoparticles, localized surface plasmon resonance, coffee ground extract, steric stabilization, electrostatic stabilization.

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1. Introduction

Nowadays, gold nanoparticles (AuNPs) have been widely studied and applied in various research fields such as medical treatment, catalysis, and chemical analysis. Due to the nanoscale sizes, AuNPs exhibit different properties from bulk gold, especially its localized surface plasmon resonance (LSPR). This phenomenon is observed around metallic nanoparticles whose conductive electrons could be excited in electromagnetic fields. The resonance wavelength of the oscillating electrons is determined by the dielectric properties of the surrounding medium and the metal, and by the nanoparticle size and shape. Different metallic nanoparticles, thus, have their own characteristic absorbance peaks which could be applied in several applications such as sensing probes, targeted drug delivery, and imaging in a biomedical and pharmaceutical applications [1-5]

Usually, AuNPs are synthesized by a colloidal method using some reducing chemicals to reduce the gold ion precursors together with certain stabilizing agents to control the growth. However, the increasing concern of reducing usage of chemicals and energy leads to the intensive study of green synthesis of nanoparticles. Many plant extracts have been investigated as a source of reducing and stabilizing agents in the synthesis of AuNPs. For examples, cinnamon bark [6], mimosa tenuiflora bark [7], euphrasia officinalis leaf [8], and mariposa christia vespertilionis leaf [9]. In addition to the plant extracts, wastes from some processes are also of interest. Previously, the skim natural rubber latex, a by-product from the concentrated rubber production, was used in the synthesis of silver nanoparticles (AgNPs) [10] and later only the serum part, basically a waste, was used in the synthesis of AuNPs [11].

In Thailand, coffee consumption has become more popular in recent years. The coffee consumption in 2018 reached 120,000 tons per year, and it is expected that it will reach 300,000 tons per year by 2022. As a result, the quantity of spent coffee grounds (SCG), wastes from brewing coffee, will be more than 290,000 tons per year in 2022 [12]. Furthermore, it was reported [13] that 100 g dry SCGs contained approximately 12.40% cellulose, 39.10% hemicellulose, 23.90% lignin, 2.29% fat, 17.44% protein and 1.30% ashes from minerals. Basically, cellulose could be further hydrolyzed during extraction to yield glucose and hemicellulose to various sugars including approximately 3.60% arabinose, 19.07% mannose and 16.43% galactose. In addition, a report on recovery of phytochemicals from SCGs [14] found that total phenolic contents of SCGs was 17.75-21.56 mg GAE/g of SCG. These phenolic compounds were related to a polyphenol family of chlorogenic acids which were the esters of caffeic acid in SCGs. All the sugars could act as reducing agents, proteins as stabilizing agents [10] and polyphenols as both reducing and stabilizing agents [15]. Thus, in this work, the extract of

the SCG was used in the synthesis of AuNPs, which has not been studied before.

For reliable applications of AuNPs in either biomedical field or sensors, it is necessary that the gold nanocolloids are of high stability. Very fine nanoparticles have high surface area to volume ratios leading to high surface energies [16]. To minimize their surface energies, the nanoparticles agglomerate, affecting the stability of the colloid. There are two main mechanisms for colloidal stability, electrostatic stabilization and steric stabilization. Electrostatic stabilization is caused by the electrostatic repulsion of particles on which surfaces, the ions or charged molecules are adsorbed. For example, an anionic surfactant such as sodium dodecyl sulfate (SDS) was used to coat AuNPs surfaces in many applications requiring the surface charges to be negative due to the sulfate group. Thus, it can control the size and the dispersion of AuNPs [17]. Naturally, particles without charges attract one another through van der Waals interaction. The charges on a particle surface induce the generation of double layers of the surrounding medium, the inner compact layer (the Stern layer) fixed on the surface containing the counter ions and the outer diffuse layer containing both types of ions. This double layer interaction prevents two particles from overlapping, thereby, generating the so-called Debye length, a center-to-center distance between two particles. The greater is the number of charges, the longer the Debye length [18].

On the other hand, steric stabilization is generated by the repulsion of bulky molecules such as polymers adsorbed on nanoparticles to prevent nanoparticle aggregation [19-20]. For instance, polyethylene glycol (PEG) has been used for dispersion and stabilization of gold nanocolloids [21]. Changing the chain length of PEG from PEG200 to PEG20000 could tune the surface charge of AuNPs ranging between -28 and -2 mV [22]. The steric stabilization is thermodynamically favorable because the negative entropy change from the adsorption of polymer chains on the particle surfaces is much exceeded by the positive entropy change from the dispersion of nanoparticles. In addition, alginate is a natural anionic polysaccharide found in seaweed, which can also be used as a coating polymer [23-25]. In the case of nanoparticles coated with alginate, their colloidal stability could be enhanced through both electrostatic and steric stabilizations, sometimes called electrosteric stabilization.

The colloidal stability in case of electrostatic interaction could be interpreted from zeta potential results based on the electrophoretic mobility of ions. The zeta potential is the electronic potential at the shear plane, i.e. the boundary of the Stern layer. The greater is the absolute value of zeta potential, the larger the thickness of double layers, and the higher the colloidal stability. However, this technique is sensitive to the condition of the measurement. For example, the sample has to be diluted to an appropriate concentration before testing. Therefore, the results did not reflect the real values of the synthesized product and those of the samples

prepared for the applications. Besides, it could not be applied for the effect of steric stabilization.

In this work, the colloidal stability of the synthesized AuNPs was interpreted from the LSPR results instead. A theoretical study of the LSPR showed that different type of material, the clearance between particles in the clusters and the size of nanoparticles affected the LSPR results [26]. Particle aggregation caused a red-shift, a shift of the peak to a higher wavelength, in the UV-visible absorbance spectra because of decreased interparticle distance [27]. Therefore, in this work, the stability could be monitored through the peak position. Moreover, the synthesized AuNPs with two different size ranges (or two average sizes) were compared for their enhancement of colloidal stability to withstand various conditions including mechanical, chemical, thermal, and acid-base disturbances. The AuNPs would be coated with polyethylene glycol 400 and 6000 (PEG400 and PEG600) to enhance the steric stability, with SDS to enhance the electrostatic stability, and with sodium alginate to enhance both the steric and electrostatic stabilities.

2. Experimental

2.1. Chemicals and Reagents

Cold-brew spent coffee grounds (SCG) were obtained from Starbucks, a branch store at TUH Park Plaza, Pathum Thani, Thailand. Tetrachloroauric acid (HAuCl_4) as a precursor for gold ions, sodium alginate, polyethylene glycols 400 and 6000 were supplied by Sigma Aldrich CO., USA. Sodium dodecyl sulfate, and potassium sulphate were purchased from Ajax Finechem Pty Ltd., Australia. Sodium hydroxide, acetic acid, and hydrochloric acid were purchased from Merck Ltd., Germany. Calcium chloride was purchased from Kemaus., Australia.

2.2. Instruments

The absorbance spectra of the synthesized AuNP colloids were recorded using UV-Vis spectroscopy (Model UV-5100, Metash, China). The collection of AuNPs and the test against mechanical stability was performed with a microcentrifuge (Wise Spin CF-10). The mixing of substances was done with a vortex mixer (Gemmy Industrial Corp. VM-300). Furthermore, morphology of the synthesized AuNPs was characterized by using transmission electron microscopy (JEOL JEM-2010).

2.3. Synthesis of Gold Nanoparticles (AuNPs)

2.3.1. Preparation of cold brew SCG extract

Fresh spent coffee grounds were dried at 80°C for 12 h. They were added to 75 mL of reverse osmosis water and soaked for 18 h in a beaker. Then, the mixture was

filtered to obtain an aqueous extract. The extract was characterized by UV-visible spectrophotometry.

2.3.2. Synthesis of AuNPs and their characterization

Two milliliters of HAuCl_4 solution at different concentrations, 1.8 and 4.0 mM, were mixed with 2 ml of SCG extracts. Each mixture was heated in a microwave at 80 Watts for 10 seconds until the color change from light brown to red or purple was observed. The temperature measured at the end of the heating was around 80°C . The AuNP colloids were characterized by UV-visible spectrophotometry, using the scanning range from 400 to 600 nm. All UV-visible absorbance spectra were read against distilled water.

For the case of uncoated AuNPs, they were collected after centrifugation at 10000 rpm for 10 minutes. The precipitate was redispersed in distilled water of the same volume (4 mL) and checked for its absorbance by UV-visible spectrophotometry. In another experiment, the precipitate was checked for the morphology by transmission electron spectroscopy. It is also interesting to check the colloidal stability of the as-synthesized AuNPs so it was tested by adding 0.75 mL of 1%w/v CaCl_2 solution or 1%w/v K_2SO_4 solution to 0.75 mL of the as-synthesized AuNP colloid to investigate the effects of divalent positive and divalent negative charges, respectively. The LSPR results from UV-visible spectrophotometry were used to interpret the colloidal stability.

For the case of stability-enhanced AuNPs, the synthesized colloids in the original medium of SCG extracts were coated according to the procedure described in 2.4.

2.4. Coating AuNPs with Various Substances

The effect of the concentrations of the coating materials was investigated with the AuNPs synthesized from the 1.8 mM HAuCl_4 solution. Later the optimal concentration was chosen and then applied to the AuNPs prepared from the 4.0 mM precursor solution.

The AuNPs coated with PEG400 and PEG6000 (denoted as PEG400-AuNPs and PEG6000-AuNPs) were prepared by mixing 0.75 mL of AuNP colloid with 0.75 mL of PEG400 and PEG6000 aqueous solutions, in a 1.5 ml micro-centrifuge tube. The concentration of each polymer was varied to be 2.5, 5 and 10% w/v. Similarly, for coating with alginate (Alg-AuNPs), sodium alginate solutions were prepared at 0.025, 0.05 and 0.1%w/v and 0.75 mL of each alginate solution was mixed with 0.75 mL of AuNP colloid in a micro-centrifuge tube. To coat AuNPs with SDS (SDS-AuNPs), 0.75 mL of AuNP colloid was mixed with 0.75 mL of SDS at various concentrations, which were 0.25, 0.5 and 1%w/v, in a micro-centrifuge tube. It should be noted that the range of concentration was chosen differently for different coating material based on practical purposes of minimizing the usage of coating materials and keeping

the viscosity of the mixture not so high that it could obstruct the centrifugation.

The obtained mixtures were mixed rigorously by a vortex mixer to disperse the coating materials and expedite their adsorption on to nanoparticles. The coated AuNPs were collected after centrifugation at 10000 rpm for 10 minutes. The supernatant liquid was removed and the precipitate was redispersed in distilled water and these coated AuNPs were ready to be used in subsequent experiments. The spectra of the coated AuNP colloids were obtained by UV-visible spectrophotometry.

2.5. Stability Tests of the Coated-AuNPs

Firstly, only the AuNP samples synthesized using 1.8 mM precursor and coated with each coating material (PEG400-AuNPs, PEG6000-AuNPs, Alg-AuNPs, and SDS-AuNPs) at an optimal concentration were chosen for further study in the following stability test. The criterion for choosing is that the spectra of the optimally coated AuNPs should be disturbed the least by the centrifugation at 10000 rpm for 10 minutes. Therefore, all samples were centrifuged, redispersed in the same volume of water and checked for their spectra. The coating materials at these chosen concentrations were then applied to the AuNPs synthesized using 4.0 mM HAuCl₄. After being chosen, those coated AuNP samples underwent further disturbances as described below. The samples were diluted with distilled water to yield the maximum absorbance around 0.5 before the stability tests against chemical, pH, and thermal disturbances, whereas the undiluted samples of AuNPs were used for the mechanical stability test.

2.5.1. Mechanical stability test

The mechanical stability test was done by applying a mechanical force, which in this case is the centrifugal force, to 1.5 mL nanocolloid samples in the micro-centrifuge at 10000 rpm for 10 minutes. The centrifugation and redispersion were performed repeatedly and the spectra of the samples from each round were compared.

2.5.2. Chemical stability test

0.75 mL of each coated-AuNP colloid was added with 0.75 mL of 1%w/v CaCl₂ solution and mixed by the vortex mixer. After that, the LSPR results were obtained by UV-visible spectrophotometry to monitor AuNP aggregation.

2.5.3. pH stability test

0.75 mL of HCl solution or NaOH solution at various concentrations was added to 0.75 mL of each coated-AuNP colloid to adjust the pH of the mixture in the range from 1 to 13. The mixture was rigorously

stirred by the vortex mixer. After that, the LSPR results of all samples were obtained by UV-visible spectrophotometry to monitor AuNP aggregation.

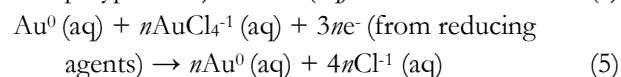
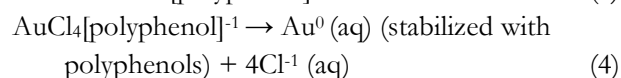
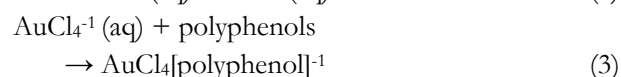
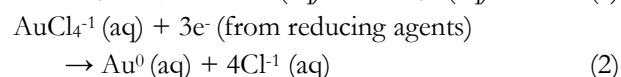
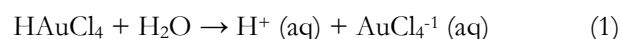
2.5.4. Thermal stability test

0.75 mL of each coated-AuNP colloid was added with 0.75 mL of water at different temperature, which was 25°C, 40°C, and 80°C, and then mixed by the vortex mixer. After mixing, all samples were checked immediately by UV-visible spectrophotometry to obtain LSPR results.

3. Results and Discussion

3.1. Characterization of the As-synthesized AuNPs Using SCG Extracts

As mentioned in the introduction that the SCG extract contains reducing and stabilizing agents for the synthesis of AuNPs. The reaction could be proposed as shown in Eq. (1) to (5).



The chloroauric acid (HAuCl₄), when dissolved in water, gave H⁺ and AuCl₄⁻¹ as in (1). It was reported that at acidic conditions below 100°C, the gold ions were in the form of AuCl₄⁻¹ and they could be reduced to AuCl₂⁻¹ at the temperature greater than 100°C [28], implying that the reduction of gold ions needs an energy input. These ions could react with reducing agents such as sugars in SCG extract as in (2) and some might form a complex ions with polyphenols as in (3) [15]. Upon reduction, gold seeds were formed as in (4). The steps would be repeated for the growth of gold seeds (Au⁰) to obtain gold particles in nanoscales (*n*Au⁰ or AuNPs) as in (5), for example.

UV-visible spectrophotometry and TEM were employed to characterize the AuNPs synthesized in cold-brew SCG extracts. The absorbance spectra of the AuNPs synthesized from 1.8 mM and 4.0 mM HAuCl₄ were presented in Fig. 1 along with the color of the two colloids which appeared red wine and purple, respectively. The two samples were denoted as 1.8AuNPs and 4.0AuNPs, hereafter. The spectra of 1.8AuNPs and 4.0AuNPs colloids exhibited absorbance peaks, or λ_{max}, at 533 and 539 nm, respectively, corresponding to characteristic values of AuNPs

reported in a literature around 520-540 nm [29]. The position of the peak gave information about the average particle size of the AuNPs, while its full width at half-maximum (FWHM) could be related to the particle size polydispersity [30]. The larger λ_{\max} of 4.0AuNPs indicated that the average size of 4.0AuNPs was larger than that of 1.8AuNPs. The FWHM values of the 1.8AuNPs and 4.0AuNPs colloids were approximately 58 nm and 69 nm, respectively, indicating a wider size distribution was obtained for 4.0AuNPs colloid [30]. These LSPR results from UV-vis spectrophotometry could be confirmed with the results from TEM micrographs as displayed in Fig. 2.

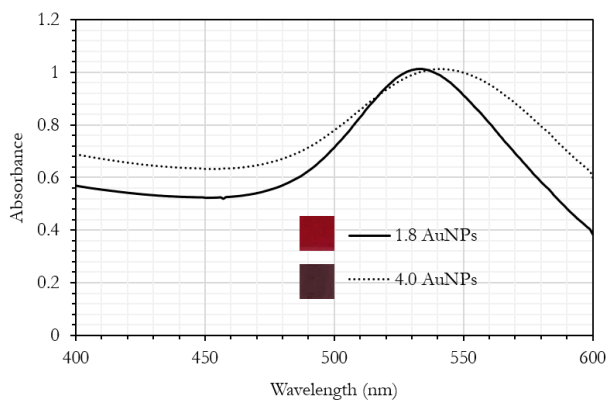


Fig. 1. Absorbance spectra and colors of 1.8AuNP and 4.0AuNP colloids.

TEM micrographs exhibited that AuNPs synthesized from cold-brew SCG extracts were polydisperse. The 1.8AuNP colloid contained nanoparticles mostly spherical together with some triangular, and rod ones, while the 4.0AuNP colloid contained mostly spherical nanoparticles and some triangular ones. Statistics from at least 100 particles gave that the average sizes of 1.8AuNPs and 4.0AuNPs were $10.51 \pm 3.79 \text{ nm}$ and $13.75 \pm 4.01 \text{ nm}$, respectively.

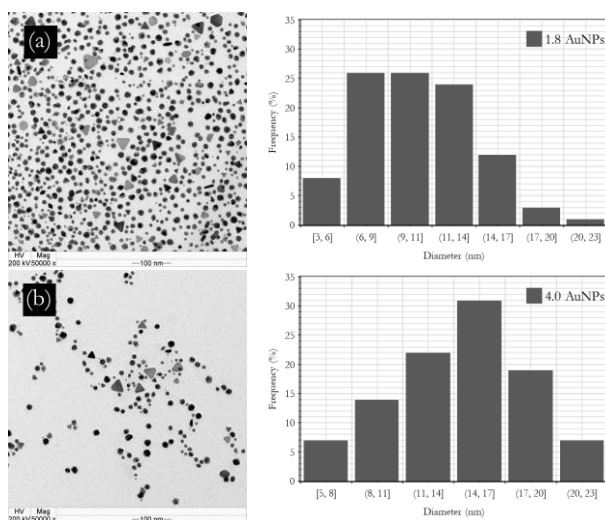


Fig. 2. TEM micrographs and size distribution of (a) 1.8AuNP and (b) 4.0AuNP colloids.

The above evidence of size polydispersity of the AuNP colloids synthesized in SCG extracts implied that there might not be enough stabilizing agents to control the growth during the synthesis or there might be temperature gradient occurring inside the reaction volume causing the particle growth at different rates. It was reported that polyphenols in 1% black tea could stabilize the AuNPs and the average size obtained was around 15 nm. Similar to this work, that colloid contained AuNPs with a similar degree of size polydispersity [31]. Because SCG extracts contain some amount of polyphenols [14, 32], it is possible that they could act as stabilizing agents for the synthesis of AuNPs. However, these stabilizing agents seemed not to yield the synthesis of monodisperse nanoparticles. Instead, they might help prevent the aggregation of AuNPs. Therefore, the stability test was done to clarify this assumption.

The as-synthesized AuNPs were subjected to two types of disturbances, mechanical disturbance by centrifugation and chemical disturbance by adding divalent ions which were CaCl_2 and K_2SO_4 . The results were shown in Fig. 3 and Fig. 4, respectively.

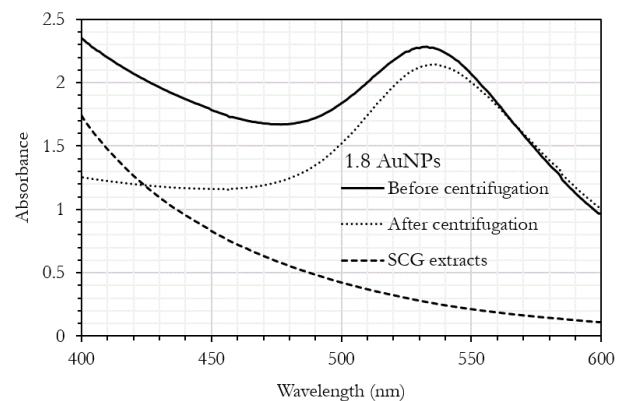


Fig. 3. The spectra of the as-synthesized 1.8AuNP colloid before and after centrifugation at 10000 rpm for 10 minutes along with that of the SCG extract.

In Fig. 3, after centrifugation out of the SCG extracts and redispersion in water with the same volume, a decrease of the absorbance peak of 1.8AuNPs was seen which could be related to the effect of aggregation so strong that particles might precipitate. However, there was no precipitate to be seen and the colloid appeared homogeneous. Therefore, the decrease of the peak should be due to an unnoticeable degree of aggregation and mostly from the deduction of the spectrum of the remaining SCG extract. Fig. 3 also showed that the λ_{\max} shifted from 533 to 535 nm, implying that a slight degree of aggregation occurred upon centrifugation. A simulation of LSPR of AuNPs suggested that not only an increase of the nanoparticle size, but a decrease of clearance distances among nanoparticles (i.e. close clearance distance yielded clustering of particles) also resulted in a red shift [26].

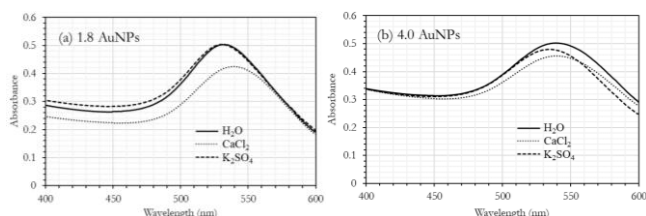


Fig. 4. The spectra of as-synthesized (a) 1.8AuNP and (b) 4.0AuNP colloids after adding CaCl_2 and K_2SO_4 , compared with adding water as a blank.

Comparing Fig. 3 and 4, the effect of mechanical disturbance on AuNPs stability was found not as strong as the effect of chemical disturbance. Fig 4. compared the effect of adding 1%w/v CaCl_2 and 1% w/v K_2SO_4 with the dilution with distilled water of the same volume for both 1.8AuNPs and 4.0AuNPs. For both AuNP colloids, upon adding CaCl_2 , the peak shifted to a longer wavelength. The FWHM values were increased to 63 and 70 nm for 1.8AuNP and 4.0AuNP, respectively. The λ_{max} of 1.8AuNPs shifted from 533 to 539 nm, and the intensity obviously decreased. The color changed from red to purple. All these implied the aggregation of nanoparticles. In the case of 4.0AuNP, the λ_{max} slightly shifted from 539 to 541 nm, and the intensity also decreased, corresponding to the precipitation of AuNPs as could be observed in the experiment. It was reported that adjusting the pH of the medium containing AuNPs resulted in different zeta potential. For AuNPs at pH around 7.0 as in this work, the zeta potential was -15 mV to -27 mV, indicating that the ions adsorbed on the surface of AuNPs had negative charges [33]. Therefore, the aggregation occurred because of neutralization between calcium ions and negative charges on the AuNP surfaces, causing the Debye length to be shorter. It was also possible that one calcium ion could link two monovalent anions together, facilitating the aggregation of particles.

In addition, after adding K_2SO_4 to AuNP colloids, the LSPR peak shifted to a shorter wavelength for both samples. The λ_{max} of 1.8AuNPs shifted from 533 to 530 nm while that of 4.0AuNPs shifted from 539 to 535 nm. The FWHM values were 57 and 63 nm for the 1.8AuNPs and 4.0AuNPs, respectively. The color of both samples changed slightly. It seemed that SO_4^{2-} could adsorb on the AuNP surfaces, increasing negative surface charges, increasing the Debye length and increasing the clearance distance among nanoparticles.

From the discussion above, the as-synthesized AuNPs possessed a good degree of mechanical stability but were obviously disturbed with divalent cations. In the applications involving the presence of multivalent cations such as chemical sensors, coating with some materials could help enhance the chemical stability of AuNP colloids.

3.2. The Effect of the Concentration of Various Coating Materials

In this section, various materials were experimented as the coating material for 1.8AuNPs, including PEG400, PEG6000, sodium alginate and SDS. The effect of the concentration of each coating material was presented in Fig 5. All the coated AuNPs were subjected to the centrifugation at 10000 rpm for 10 minutes and then redispersion in water. It was observed that when applied with at least 5%w/v PEG400, 5%w/v PEG6000, 0.1%w/v sodium alginate and 0.5%w/v SDS, the precipitate could be wholly redispersed in water, and the λ_{max} remained at 533 nm, confirming that the stability was enhanced for the coated 1.8AuNPs. Thus, these concentrations of the coating materials were chosen to be the optimal concentrations for coating AuNPs.

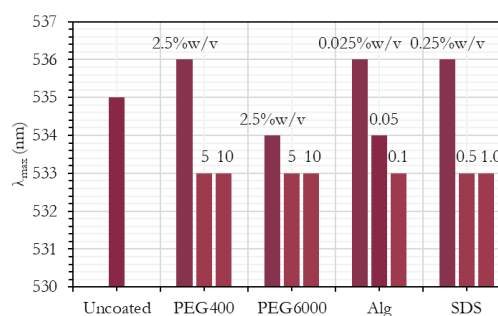


Fig. 5. The λ_{max} of uncoated 1.8AuNPs and those of AuNPs coated with PEG400, PEG6000, alginate and SDS at different concentration when the colloids were subjected to centrifugation at 10000 rpm for 10 min.

The coated AuNPs were also prepared from adding the coating materials in the reacting medium in the first place. It was reported that λ_{max} and FWHM of PEG400-AuNPs using 1.1 mM HAuCl_4 was at 533 and 50 nm, comparable to our case of 1.8AuNPs (0.9 mM HAuCl_4 in the overall mixture with SCG extract), and those of PEG6000-AuNPs was at 519 and 47 nm, respectively [22]. So PEG could help control the growth of AuNPs during the reactions but the concentration was as high as 12%wt/v. When 1% alginate, ten times our use, was added during the reaction using 0.5 mM HAuCl_4 , the λ_{max} of the alginate-AuNPs was found to be 526.5 nm [34]. In addition, upon using up to 2.88% SDS solution and 0.42 mM HAuCl_4 , the λ_{max} of the resulting SDS-AuNPs was 520 nm [17]. From all these literatures, the coating materials were efficient in controlling the growth of AuNPs during the reaction but they had to be applied at high concentrations along with lower concentrations of HAuCl_4 .

3.3. The Effect of Coating on Mechanical Stability of the Coated AuNPs

The experiments in this section could be considered the extended version of those done in the previous

section. The 1.8AuNPs were coated with the coating material at the optimal concentrations chosen in the previous section. The coated 1.8AuNPs were repeatedly centrifuged at 10000 rpm for 10 minutes and redispersed in water and the spectra were compared for all coating materials as shown in Fig. 6.

Comparing PEG400 with PEG6000-AuNPs, it was found that for PEG400-AuNPs, the λ_{\max} did not shift after the second centrifugation but shifted from 533 to 535 nm after the third one, whereas for PEG6000-AuNPs, the λ_{\max} shifted from 533 to 536 nm after being centrifuged twice. Therefore, this experiment showed that being exploited at the same concentration, PEG with shorter chains promoted higher stability. For steric stabilization, the steric layers around the nanoparticles are formed from the polymer chains attached on the surface. It was reported that PEG with a lower molecular weight (or with a shorter chain) was more polar so it could interact with surrounding water molecules more strongly via hydrogen bondings [35], leading to a high degree of swelling of the chain. If the chain is more swollen, the steric layer could be thicker, leading to higher stability of the nanoparticles.

Also shown in Fig. 6, the λ_{\max} of Alg-AuNPs was maintained at 533 nm when centrifugation was repeated up to three times. Due to alginate is an anionic polymer and the alginate polymer chains are relatively long [23], it can increase both electrostatic and steric stability of nanoparticles. In case of SDS-AuNPs, the negative charges of the SDS molecules, once adsorbed on the surfaces of nanoparticles, would generate double layers for electrostatic stabilization. The repulsion between nanoparticles was so strong that even after three times of centrifugation, the λ_{\max} of the SDS-AuNPs colloid was still at 533 nm.

All these results indicated that coating AuNPs with these materials, especially the ones providing electrostatic repulsion, could increase the mechanical stability of the AuNPs when being subjected to repeated centrifugation.

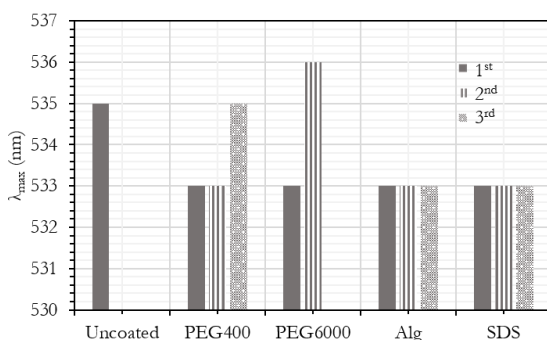


Fig. 6. The λ_{\max} of 1.8AuNPs coated with PEG400, PEG6000, alginate and SDS after repeated centrifugation.

3.4. The Effect of Coating on Chemical Stability of the Coated AuNPs

The changes of absorbance spectra of the 1.8AuNPs coated with PEG400, PEG6000, sodium alginate, and SDS after being disturbed with 1%w/v CaCl_2 solution were presented in Fig. 7(a). After adding CaCl_2 , the colors of PEG400-AuNPs and PEG6000-AuNPs changed from red to purple. The λ_{\max} shifted from 533 nm to 535 and 536 nm, respectively, implying that the aggregation slightly occurred, less than the uncoated-AuNPs whose λ_{\max} shifted to 539 shown in Fig. 4. It would be expected that coating with non-ionic polymers such as PEG, the coated AuNPs should not be affected by the electrolyte solution. However, the change was observed in this work. This could be attributable to the fact that water molecules were able to surround and interact with both cations and anions via ion-dipole interactions. These could cause the local reduction of the number of water molecules involved in swelling of the polymer chains, leading to a smaller degree of swelling and thinner steric layers.

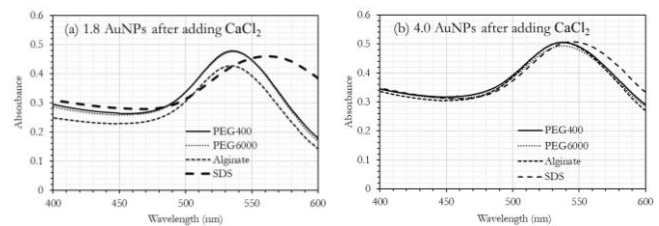


Fig. 7. The spectra of (a) 1.8AuNPs and (b) 4.0AuNPs coated with PEG400, PEG6000, alginate, and SDS after the addition of 1% w/v CaCl_2 solution.

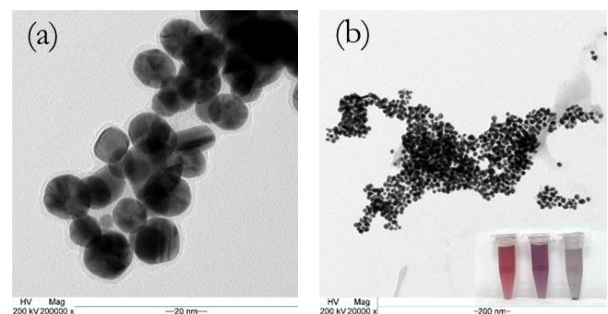


Fig. 8. The SDS-AuNPs after being disturbed with 1% w/v CaCl_2 solution showed (a) the aggregation of AuNPs seen with coating layers and (b) the connectedness of structures making a large cluster of nanoparticles. The appearance changed as shown in the inset of (b).

As expected, the greater changes were observed for Alg-AuNPs and SDS-AuNPs. Because alginate is an anionic polymer that can form a hydrogel with divalent cations. In this case, Ca^{2+} would act as crosslinkers between the functional groups of alginate chains [36]. The result showed that the λ_{\max} shifted from 533 nm to

535 nm and the color of Alg-AuNPs colloid changed abruptly from red to very light purple, and precipitation occurred immediately, leading to the decrease in absorbance of the peak.

In addition, the SDS-AuNPs colloid exhibited a color change from red to purple and then the colloids were coagulated as purple precipitate as displayed in Fig. 8(b). Because SDS is an anionic surfactant, it could interact with calcium ions. As a result, the λ_{\max} shifted greatly from 533 nm to 563 nm, and the intensity dropped pronouncedly, corresponding to the precipitation of nanoparticles occurring in the solution. The results of SDS-AuNPs were confirmed with TEM micrograph as shown in Fig. 8(a) and (b).

The same experiment was also performed with 4.0AuNP colloid. In Fig. 7(b), it was seen that the λ_{\max} of PEG400-AuNPs and PEG6000-AuNPs did not shift, whereas that of Alg-AuNPs slightly shifted from 539 to 537 nm and that of SDS-AuNPs greatly shifted from 539 to 545 nm but did not precipitate.

Furthermore, the FWHM values of the coated 1.8AuNPs changed from 56 nm to 58 nm, except for SDS-AuNPs whose value changed to 88 nm. In case of the coated 4.0AuNPs, the FWHM value was still 67 nm, excluding SDS-AuNPs whose value changed to 74 nm. These results showed that coating 4.0AuNPs with all these coating materials could enhance their stability more effectively than the case of 1.8AuNPs, probably because smaller nanoparticles possess higher surface activity due to the greater surface area to volume ratio.

3.5. The Effect of Coating on Stability against pH of the Coated AuNPs

At pH 7, the initial λ_{\max} of all the coated 1.8AuNPs was at 533 nm. Fig. 9 showed the λ_{\max} when the pH of the media was adjusted from 1 to 13. The colors of bar charts were the real color of the colloids after pH adjustment. It could be observed from the previous experiments that if the aggregation occurred, the color would change from red wine to purple and then it turned to lighter purple if precipitation occurred.

For all the coated 1.8AuNPs, the same trend was found. In a highly acidic media (pH 1 and 2), the red shift was observed, whereas in a highly basic media (pH 12 and 13), the blue shift was observed. Varying the pH from 1 to 13, the λ_{\max} ranged from 546 to 527 nm for PEG400-AuNPs, from 542 to 527 nm for PEG6000-AuNPs, from 541 to 527 nm for Alg-AuNPs, and from 536 to 527 nm for SDS-AuNPs. The range of pH in which the nanoparticles was not affected could also be observed in Fig. 9. It was 3-10 for PEG400-AuNPs, 3-9 for PEG6000-AuNPs, 3-7 for Alg-AuNPs, and from 3-10 for SDS-AuNPs.

Considering PEG400-AuNPs and PEG6000AuNPs, it was possible that the polymer chains were hydrolyzed and finally degraded by an acidic medium at a high concentration [37]. The cutting-off sections might be lost to the surrounding medium, leading to a thinner steric

layer and lowering the stability of the colloids. When these two colloids were subjected to a basic medium at a high concentration, the interaction between hydroxide ions and all hydroxyl groups of PEG could highly promote the formation of hydrogen bondings between polymer chains and those between chains and water molecules, facilitating gelation of PEG chains [37] and resulting in stronger steric layers which could effectively prevent aggregation of nanoparticles. Comparing PEG400 with PEG6000, it was seen that PEG400 was more affected by both the acid and the base, probably because of the higher polarity of the shorter chains [35] which could be degraded more easily by acid hydrolysis at a low pH and entangled more easily by a lot of hydrogen bondings at a high pH.

In case of Alg-AuNPs at a low pH, the neutralization occurred between H^+ and COO^- on alginate chains, leading to a decrease in electrostatic stabilization and the hydrophobic part of the chains coiled up to avoid contact with water and the attraction between particles occurred via this hydrophobic interaction, leading to less stability. The color of the colloid changed to purple and a trace of purple gel was observed. It was reported that the alginate gel crosslinked with Ca^{2+} was shrunk at a low pH and swollen at a high pH [38]. So, at a high pH, Alg-AuNPs were more stable as could be observed that its color was bright red.

For SDS-AuNPs, the electrostatic repulsion from negative charges was lowered by neutralization with H^+ at a low pH as reported that the zeta potential of SDS was less negative at a lower pH value ranging from 10.5 to 3. Similar to the alginate, it was also proposed that hydrophobic interaction was responsible for attraction of nanoparticles at a low pH value and SDS was more stable at a high pH value [39].

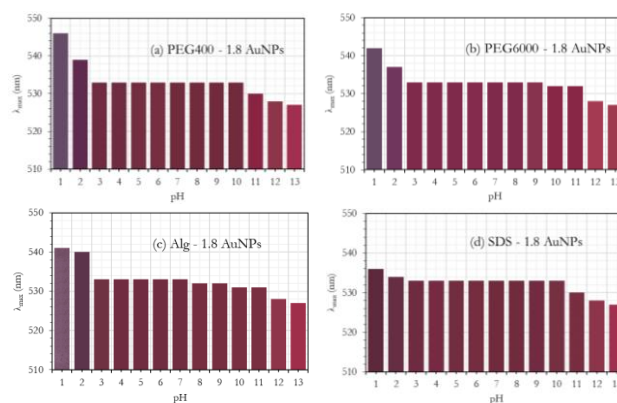


Fig. 9. Effect of the pH value on the λ_{\max} of 1.8AuNPs coated with (a) PEG400, (b) PEG600, (c) alginate, and (d) SDS.

The same experiments were performed for 4.0AuNPs and the results were shown in Fig. 10. They showed the same trends as for 1.8AuNPs. The initial position of λ_{\max} of all the coated 4.0AuNPs was at 539 nm at pH 7. Upon varying the pH from 1 to 13, the λ_{\max} ranged from 543 to 527 nm for PEG400-AuNPs, from

543 to 527 nm for PEG6000-AuNPs, from 544 to 528 nm for Alg-AuNPs, and from 542 to 527 nm for SDS-AuNPs. The range of pH in which the nanoparticles was not affected was 3-10 for all coated AuNPs. This range was the same as the one reported for the PEG2000-coated AuNPs [21].

Compared with 1.8AuNPs, 4.0AuNPs seemed to be more affected by the acid, considering that the pH range of not being affected, which was 4 to 10, was narrower than that of 1.8AuNPs (3 to 10) and all coating materials were similarly effective, except for the alginate, in which the precipitation of nanoparticles was obviously seen at pH 1.

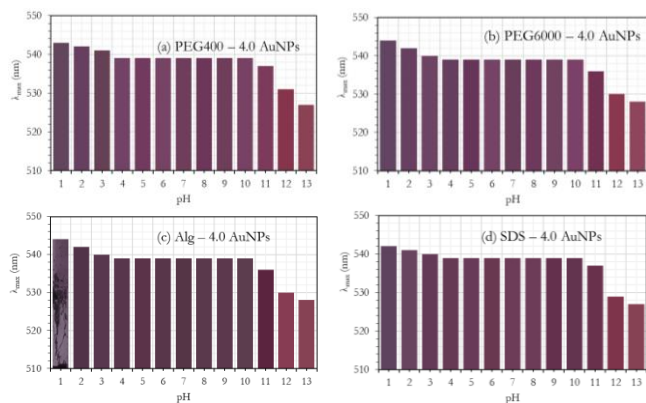


Fig. 10. Effect of the pH value on the λ_{\max} of 4.0AuNPs coated with (a) PEG400, (b) PEG600, (c) alginate, and (d) SDS.

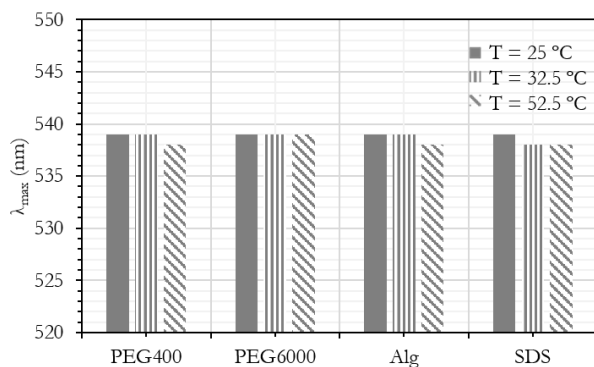


Fig 11. The λ_{\max} of 4.0AuNPs coated with PEG400, PEG6000, alginate, and SDS at 25, 32.5 and 52.5°C.

3.6. The Effect of Coating on Thermal Stability of the Coated AuNPs

The mixing temperature after adding water at 25, 40 and 80°C to the colloids at 25°C with the volume ratio of 1:1 was approximately 25, 32.5 and 52.5°C. It was found that for 1.8AuNPs, the λ_{\max} at different temperature was still at 532 nm while for 4.0AuNPs, a slight blue shift from 539 nm to 538 nm was seen in some samples as shown in Fig. 11. PEG6000-4.0AuNPs was not affected by the temperature up to 52.5°C but the λ_{\max} of PEG400-4.0AuNPs shifted to 538 at 52.5°C. Alg-4.0AuNPs were

affected at 52.5°C, whereas SDS-4.0AuNPs were affected at 32.5°C.

As the temperature increases, the attractive forces among particles are weakened so it is possible that some particles could get loose from the original aggregates, leading to the blue shift. This experiment showed that there should not be any problem if AuNPs were applied at a higher temperature such as around 40 °C, a temperature of photothermal therapy of AuNPs [40].

4. Conclusion

The AuNPs were successfully synthesized using SCG extracts. Using 1.8 mM and 4.0 mM HAuCl₄, yielded AuNPs with the average sizes of 10.51 and 13.75 nm, respectively. They were then coated with various materials. This work could be useful in the selection of coating materials for different purposes.

For medical purposes, PEG and alginate are appropriate thanks to their biocompatibility while SDS is toxic. The collection of nanoparticles via centrifugation could effectively replace nanofiltration. All coating materials could be applied for this purpose. However, if they were subjected to repeated centrifugation, 0.1%w/v alginate and 0.5%w/v SDS solutions were better.

When subjected to Ca²⁺, 5%w/v PEG400 and 5%w/v PEG6000 could prevent the aggregation more effectively than anionic coating materials. However, the Alg-AuNPs and SDS-AuNPs showed a potential for their sensing abilities towards cations. Besides, At pH 1 to 3, the red shift occurred for all coated AuNPs while the blue shift at pH 12 or 13 was observed. Finally, when heated to 52.5°C, all the coated 1.8AuNPs were stable. A slight blue shift was observed for 4.0AuNPs.

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