



Green synthesis of gold nanoparticles using cinnamon bark extract, characterization, and fluorescence activity in Au/eosin Y assemblies

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Abstract This study reports the synthesis of gold nanoparticles (AuNPs) using an unconventional, eco-friendly method that utilizes cinnamon bark extract as a reducing agent and stabilizer. The synthesized AuNPs were characterized using UV–Vis spectroscopy and transmission electron microscopy (TEM). UV–Vis spectrum shows the surface plasmon resonance (SPR) peak around 535 nm. TEM shows the spherical shape of AuNPs and the particle size distribution at around 35 nm. The ability of the synthesized AuNPs as a quencher of eosin Y dye was monitored using a fluorometer. It was found that in the presence of AuNPs, the fluorescence peak of eosin is quenched. The fluorescence of the mixture of eosin and AuNPs is enhanced in the presence of bovine serum albumin (BSA) protein. TEM shows that the average size of the AuNPs is

reduced from 35 to 5 nm with the addition of eosin, and the size of AuNPs then increased to 26 nm upon adding BSA to the mixture of ANPs and eosin. These fluorescence fluctuations may be used for trace identification and biosensing.

Keywords Au nanoparticles · Green synthesis · Characterization · Fluorescence · Quenching · Enhancement

Introduction

Many different methods for synthesizing metallic nanoparticles have been explored. The conventional method of synthesizing nanoparticles involves chemical and physical processes, which are expensive. The chemicals used in this synthesis are often toxic materials that may pollute the environment and cause health issues (Mafune et al. 2001; Gold 2011). In recent years, biological synthesis has been proposed as an alternative method for the synthesis of nanoparticles. Biological synthesis involves using environmentally friendly materials such as bacteria, fungus, and plant extract. Plant extracts have been shown to perform the biogenic reduction of the metal, thus capping and stabilizing the produced nanoparticles (Zhang et al. 2016; Shah et al. 2010). Extracts from plants usually contain sugars, green terpenoids, polyphenols, alkaloids, phenolic acids, and proteins which are excellent reducing agents; these are useful in the synthesis of silver and gold nanoparticles (Nadaroğlu et al. 2017).

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Synthesis involves using plants and plant extracts such as *Hibiscus cannabinus* leaf (Alaa et al. 2018), curcumin (Abdulwahab et al. 2016), cinnamon [Smitha et al. 2009], and henna (Makarov et al. 2014) that have been successfully used for synthesizing metal nanoparticles. Among all noble metal nanoparticles, the gold nanoparticle has gained huge interests because of its many unique properties and numerous technological and medical applications, such as cancer treatment, medical imaging, and drug delivery (Arvizo et al. 2010). The origin of a unique optical property of gold nanoparticles is a phenomenon known as surface plasmon resonance (SPR) which occurs within the visible region due to combined oscillation of conduction band electrons in resonance with the incident wavelength and results in strong absorption and scattering properties (Myroshnychenko et al. 2008).

The study of the ability of the binding of dye molecules to the surface of the nanoparticles has gained considerable interest in a biochemical assay because they provide many benefits regarding induced quenching efficiency over the classical dye quencher system (Swierczewska et al. 2011). The induced fluorescence quenching of dyes by nanoparticles is reported to be directed by excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation, and collisional quenching.

Gold nanoparticles have shown their ability to quench several fluorophores such as ADS680HO (Raikar et al. 2011) and fluorescein isothiocyanate (FITC) (Loumaigne et al. 2010) and laser dyes (El-Sayed and Gaber 2012). This quenching is essential for a detection system called fluorescence activatable probe. It consists of a fluorophore that acts as a donor and a quencher that acts as an acceptor, e.g. gold nanoparticle (AuNP). When both are placed in close proximity, quenching occurs due to several energy transfer mechanisms. These mechanisms are distance-dependent; the shorter the distance is, the higher the quenching and vice versa. When constructing the probe, both the fluorophore and the quencher are placed near each other to allow the process of quenching to occur. Once the target appears in the vicinity of the probe, the target activates the probe by increasing the distance between the fluorophore and the quencher, allowing the fluorescence to be recovered. The type of the probe can be changed by adding several additional components such as proteins and enzymes, which act as

complementary molecules to the target and cause the activation.

In this study, the extract from cinnamon barks was used as a reducing agent/capping agent to synthesis gold nanoparticles. The UV-visible absorption spectra and TEM images were used to characterize the gold nanoparticles. Gold nanoparticles exhibit a maximum absorption with peaks ranging from 579 to 535 nm, with an average size of 35 nm. The synthesized AuNPs were found to be stable over 1 year. The synthesized nanoparticles were used for investigation of fluorescence quenching of eosin Y dye. The fluorescence quenching mechanism was studied by the Stern–Volmer equation. It was demonstrated that the fluorescence quenching of eosin dye by gold nanoparticles was mainly a result of the formation of a dye-gold nanoparticles complex. According to our knowledge, the ability of synthesis of gold nanoparticles (AuNPs) utilizing cinnamon and in the absence of NaBH₄ for quenching of eosin dye fluorescence is not previously reported. The quenching fluorescence from the eosin and AuNP mixture was recovered in the presence of BSA protein as a probe. The recovery of fluorescence may be used for detection and to identify proteins in buffer and serum.

Methods

Sample preparation

Cinnamon barks were purchased from the local shop and were washed several times and rinsed with distilled water to remove the dust particles. The cinnamon was dried and grinded with a blender. Of finely grounded cinnamon mixed with 100 ml of double-distilled water, 2.5 g was boiled for 5 min and then filtered twice using Whatman filter paper no. 1. To prepare the gold solution, 0.023 g of gold(III) chloride trihydrate powder (HAuCl₄) (Sigma-Aldrich) was added with 60 ml of deionized water to prepare a 1-mM of HAuCl₄ solution. Different volumes of cinnamon extract were added to a fixed volume of 4 ml of 1 mM (HAuCl₄) solution. Each mixed solution was heating for 15 s in the microwave oven of 1000 W power. The stock solution of concentration 3 mg/l of eosin was prepared in ethanol. A stock solution of a concentration of 3.5 g/l of albumin was prepared. Solutions of all coexisting substances were prepared.

Sample characterization

Characterization of the synthesized nanoparticles was performed using a variety of analytical techniques, including UV–Vis Spectrometer (Shimadzu UV-1800) and transmission electron microscope (TEM) (FEI, Morgagni 268, Czech Republic). UV–Vis spectrum was taken after 30 min to allow the completion of the reaction. For TEM, samples were prepared by depositing a droplet of the colloidal nanoparticles onto the TEM grids having carbon support films. The grids were air-dried before to transfer into the TEM. TEM was operated at an accelerating voltage of 80 kV in the bright-field mode. Several images were taken to measure the average size of the nanoparticles. Fluorescence spectra were recorded using a fluorometer (Shimadzu RF-6000).

Results and discussion

The formation of the gold nanoparticles was confirmed by the change of the solution's colour from light yellow to purple-red colour after microwave heating, as well as from the characteristic peak SPR. Figure 1 shows the UV–Vis spectrum of the synthesized nanoparticles. The spectrum reveals a strong SPR peak at around 535 nm. The peak arises from the collective oscillation of free electrons in the conduction band. The presence of a

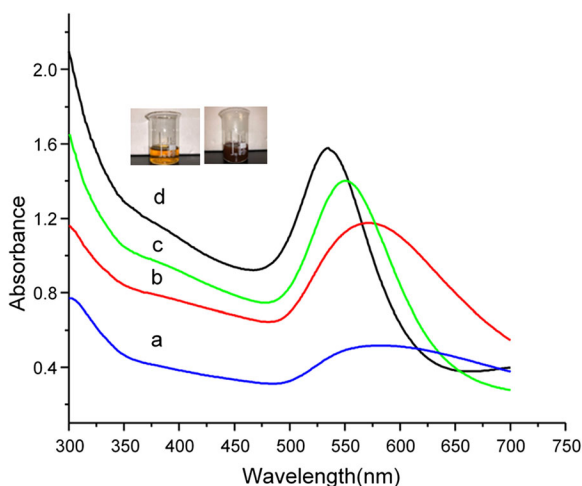


Fig. 1 The UV–Vis absorption spectra of the synthesized nanoparticles, for different volume ratios of cinnamon extract to the fixed volume of the HAuCl_4 solution: (a) 0.14 ml/4 ml, (b) 0.18 ml/4 ml, (c) 0.3 ml/4 ml, (d) 0.4 ml/4 ml; (Insert) colour changes, from yellow (cinnamon extract) to red purple (synthesized AuNPs)

single SPR peak implies that the formed nanoparticles are nearly spherical. The SPR peak is blue-shifted from 579 to 535 nm as the added volume of cinnamon extract increased; the blue shift of SPR peaks is a sign of production of small size nanoparticles. The shift towards shorter wavelengths with decreasing nanoparticle size is associated with frequencies of oscillation of different free electrons in the conduction band (Barnes et al. 2003). The SPR band absorbance increased with an increasing volume of cinnamon extract. The increase of the absorbance with an increasing volume of extract reveals the higher production of Au nanoparticles, which is due to the availability of more reducing agent for the bioreduction of Au ions (Sathishkumar et al. 2009). Cinnamon contains photochemical such as terpenoids, carbohydrates, flavones, and proteins that were reported to be responsible for the bio-reduction of Au^+ to Au^0 . Terpenoids and proteins are believed to play an important role in Au nanoparticle biosynthesis through the reduction of Au ions, and carbohydrates provide a coating of AuNPs (Shankar et al. 2003; Nripen et al. 2010). Synthesizing AuNPs using bark extract have advantages over other plant extracts such as the AuNPs were found to be stable over 1 year without any adding surfactant or PH control to prevent aggregating while most plant extracts need additives to control aggregations. The stability of AuNPs was validated by recording absorption spectra frequently for detecting the SPR peak. The bark extract produces more AuNPs than other plant extracts credited to the availability of the larger amount of reducing agents in the extract, such as flavonoid and its antioxidant activity (Prasad et al. 2009; Shahidi and Hossain 2018). This is detected by the immediate change of colour during preparation. It is worth mentioning that during the experiment, batches of cinnamon bark were purchased randomly from different sources, and their extract was successfully used for synthesis AU NPs; the UV–Vis characterization shows that no significant difference was observed regarding SPR peak position.

The structure and size of the Au nanoparticles were analysed using TEM. Figure 2 displays TEM images for samples A–C. Sample A was prepared according to the procedure explained above from the mixing of 0.24 ml of cinnamon solution with 4 ml of 1 mM of HAuCl_4 , which represents gold nanoparticles alone. Sample B was prepared from the mixing of 50 μl AuNP of sample A added to 4 ml of eosin stock solution (3 mg/l). Sample C was prepared by adding 50 μl of albumin stock

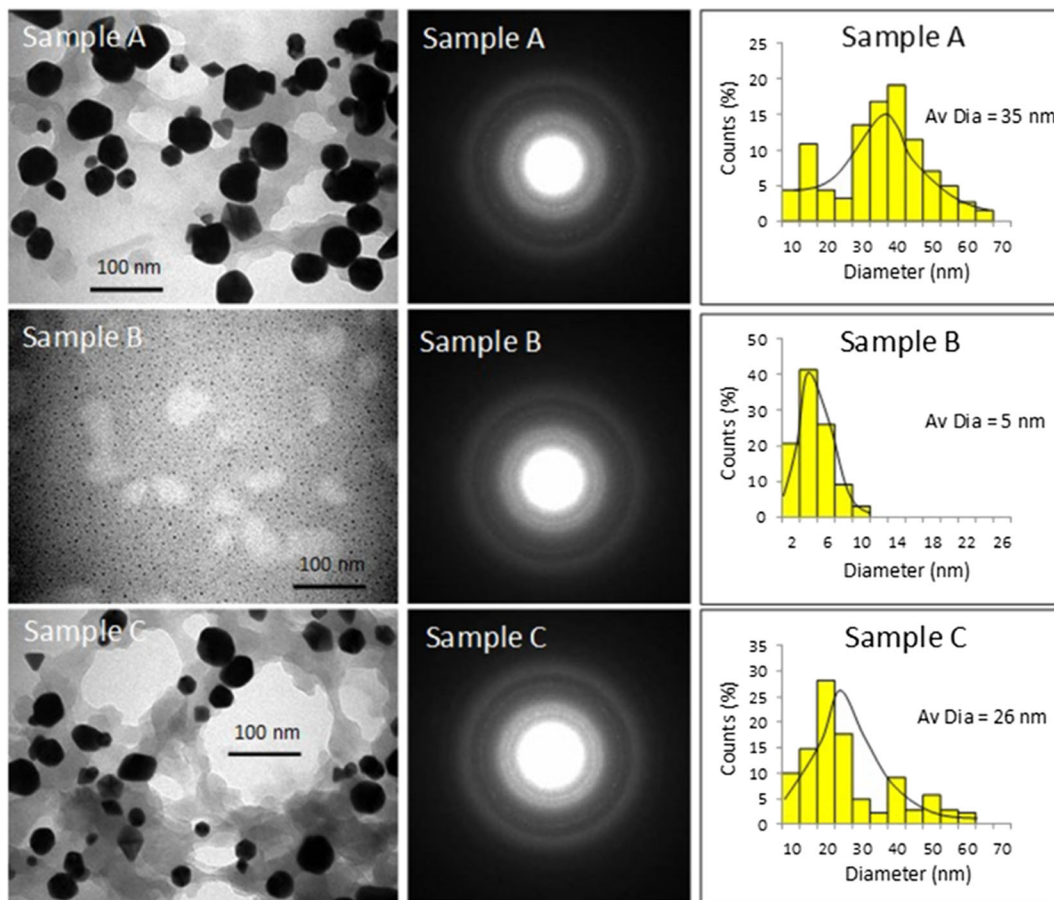


Fig. 2 Sample A—AuNP; sample B—made of a 50- μ l AuNP of sample A added to 4 ml of eosin of stock solution (3 mg/1 l); sample C—made of a 50- μ l of albumin stock solution (3.5 g albumin/1 l) added to sample B

solution (3.5 g/l) to sample B. Figure 2 (left column) shows TEM images of all three samples which mostly have spherical shapes with very little inhomogeneity in shapes.

The morphology of the nanoparticles was analysed by SAED, well-separated rings of the patterns are the signature of the crystalline structure (Fig. 2, middle panel). The gold nanoparticles exhibited a polydispersity in sizes. Figure 2 (right column) shows the particle size distribution of colloidal gold nanoparticles for samples A, B, and C. From size analysis, the average size for AuNPs was found to be in the range of 35 nm for sample A. For sample B (coexistence of AuNPs and eosin solution), the average size of nanoparticles was found to be reduced to the average size of 5 nm. For sample C (coexistence of AuNPs, eosin solution, and albumin), the average size was found to be increased from 5 to 26 nm.

Figure 3 shows the emission spectrum of Au nanoparticles at the excitation wavelength of 336 nm. A sharp fluorescence peak was observed at 413 nm. The emission obtained from gold nanoparticles at 366 nm excitation may originate from the triplet excited states or first excited S state in the sp. band (Shishino et al. 2010). However, the origin of the emission from gold nanoparticles may be established by performing using ultrafast transient absorption spectroscopy.

Fluorescence quenching

Fluorescence quenching involves organic dye and nanoparticles have recently gained considerable interest in bio-photonics (Wang et al. 2017) and material science. Typically, fluorescence quenching experiments compose of a acceptor and acceptor system. A range of

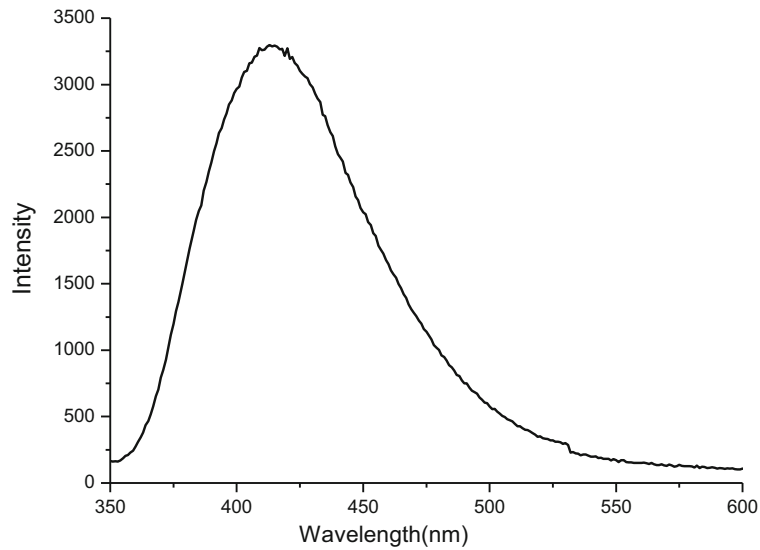


Fig. 3 The emission spectrum of gold nanoparticles at excitation wavelength 336 nm

processes can result in quenching, such as excited state reactions, energy transfer, complex formation, and collisional quenching Förster resonance energy transfer (FRET). FRET is a process by which energy is transferred non-radiatively, via long-range dipole-dipole interactions, from a donor molecule in an excited electronic state to an acceptor molecule. The emission band

of the acceptor molecule must overlap sufficiently with the absorption band of acceptor molecule for FRET to occur, and the rate of transfer of energy is dependent on the separation of the two molecules.

The organic dye molecule used in this experiment was eosin Y dye as a acceptor and green synthesized Au nanoparticles as an acceptor. Figure 4 shows the

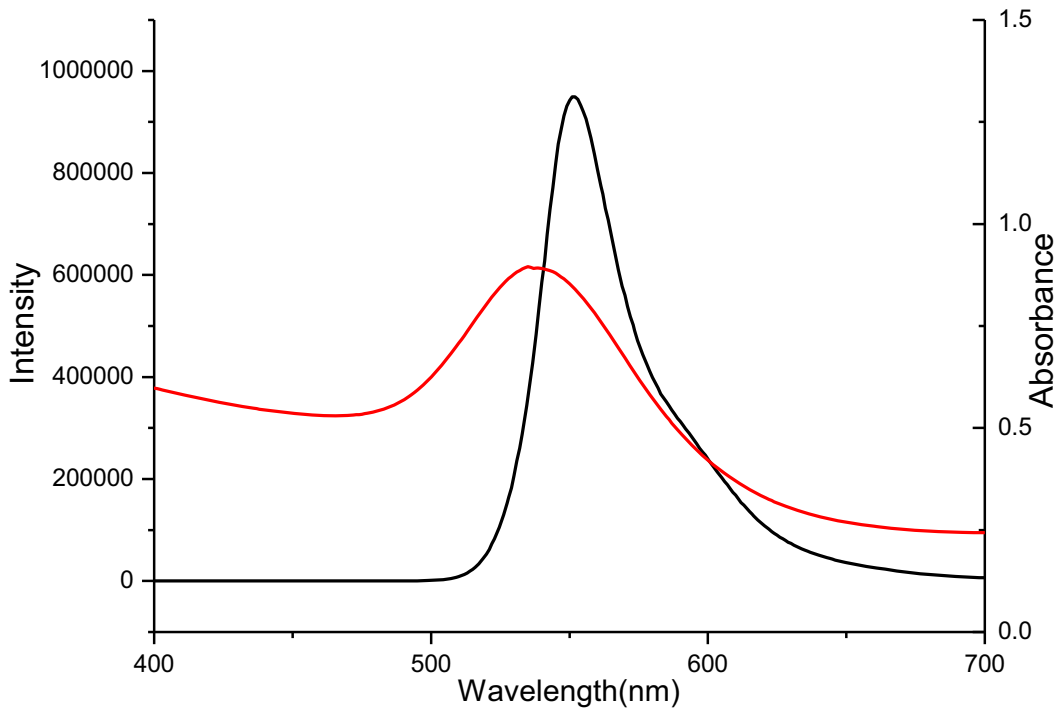


Fig. 4 Overlapping spectra between the absorption spectrum of the acceptor Au nanoparticles (red line) and the emission spectrum of acceptor of eosin dye diluted (black line)

absorption spectrum of gold nanoparticles (red) and the emission fluorescence of the eosin-Y dye molecule (black). As can be seen from the figure, the absorption spectrum of the gold nanoparticles overlaps significantly with the emission from the dye over a wide band 540–610 nm. The overlapping of the absorption spectrum of the acceptor AuNPs and emission spectrum of acceptor eosin dye suggests that the probable mechanism involved in energy transfer is a FRET, with a calculated critical separation distance (R_0) equal 46 Å for AuNP-eosin from the normalized fluorescence and the absorption spectra using the spreadsheet made available by Visser et al. (Visser et al. 2011).

To confirm the quenching, different volumes of the AuNPs were added to a fixed volume of the eosin. It was observed that the fluorescence intensity of eosin dye (acceptor) decreases as the AuNP (accepter) concentration increases. Therefore, one can assume the energy transfer from the eosin molecule to AuNPs. Figure 5 shows the fluorescence quenching of eosin at 550 nm. It was shown that the existence of a fluorophore in the vicinity metal nanoparticles could lead to energy or electron transfer (Lackowicz 1999). As can be seen from Fig. 5, apart from the fluorescence quenching process, the eosin absorption peak exhibited a slight red shift in the peak position as AuNPs

added the eosin dye which may be taken as an indication of the adsorption of eosin on to the AuNPs. The adsorption mechanism is possible due to the columbic attractive force between positively carbohydrate-capped AuNPs and negatively charged eosin dye (Vujačić et al. 2013).

Built on the intensity change as shown in Fig. 5, the ratio of the unquenched fluorescence intensity divided by the quenched fluorescence intensity at different AuNP concentrations is plotted and shown in Fig. 6. The linear relation is analysed by the Stern–Volmer equation $I_0/I = 1 + K_{sv} [Q]$, where I_0 is the emission intensities of dyes in absence of the quencher, I is the emission intensity in the presence of quencher (AuNPs), $[Q]$ is the concentration of quencher, and K_{sv} is the Stern–Volmer quenching constant (quencher rate coefficient). The Stern–Volmer plot of the quenching process of eosin dye emission by AuNPs in the absence and presence of AuNPs is shown in Fig. 6, as can be seen from the figure a relatively linear behaviour between the relative intensity and the quencher concentration. The linear relationship suggested that dynamic quenching is a primary mechanism between eosin molecules and AuNPs. The value of the calculated K_{sv} found to be is 1.10 nM^{-1} which is an indication for the sensitivity eosin molecules to AuNPs as a quencher.

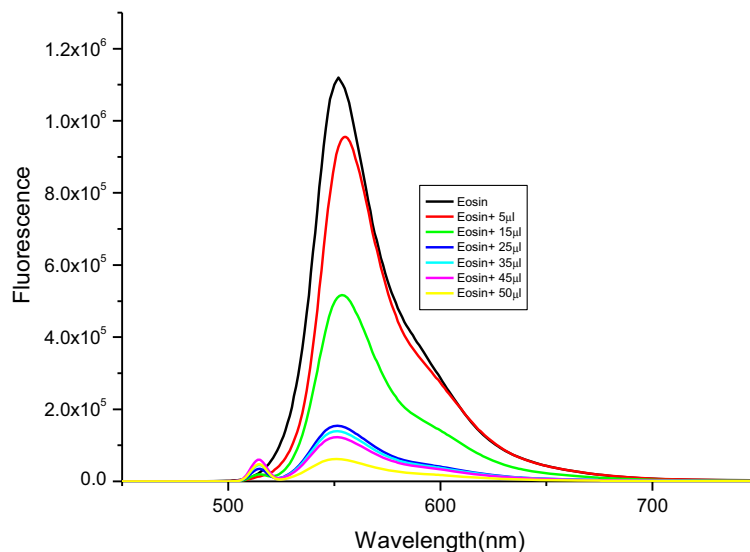


Fig. 5 Quenching fluorescence emission of eosin Y dye as a result of the addition of different volumes of sample Au nanoparticles (volumes are shown on the figure) at excitation 515 nm

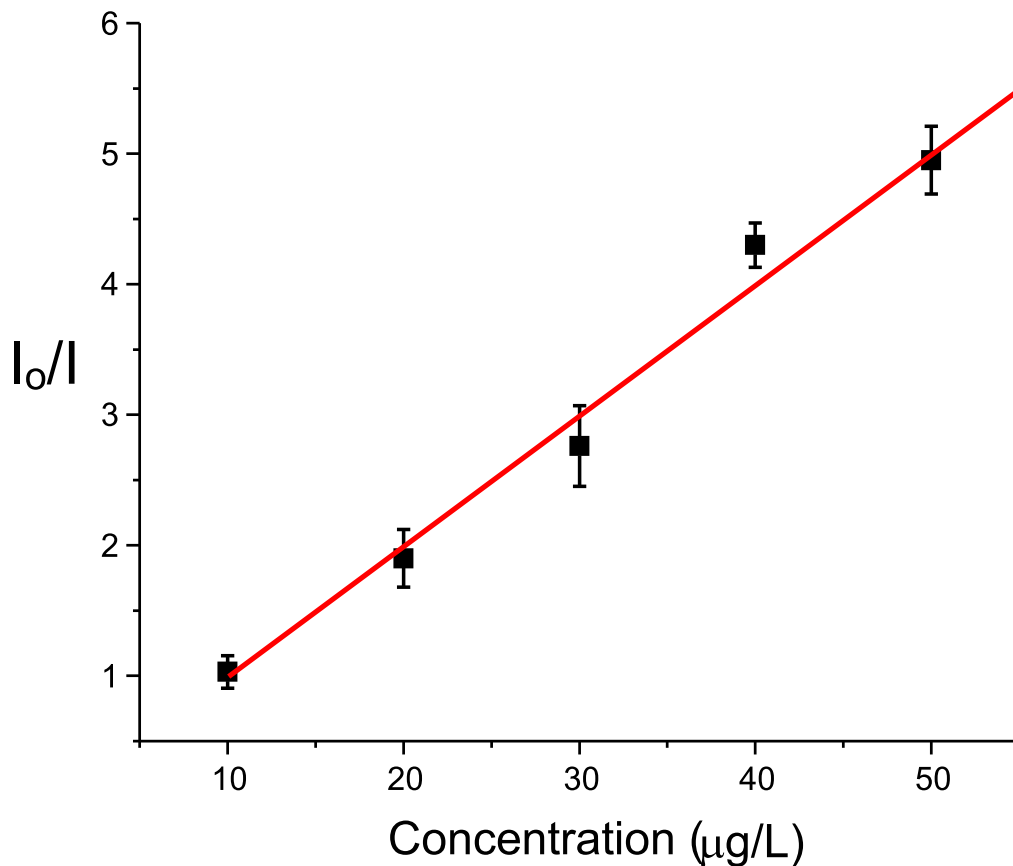


Fig. 6 Stern–Volmer plots for fluorescence quenching eosin dye at different Au concentrations

Augmentation effect of protein on the fluorescence of the eosin–AuNP system

As mentioned above, the adsorbed eosin molecules onto the surface of gold nanoparticles resulted in the quenching of its fluorescent via energy transfer. Upon adding bovine serum albumin (BSA) protein to a mixture of eosin–AuNP, the fluorescence intensity eosin molecules recovered. Figure 7 shows the increase of the recovered fluorescence intensity of the mixture of eosin–AuNP when different concentrations of albumin added to the mixture (concentrations are shown on the figure). The recovered fluorescence intensity may be explained by the possible affinity of albumin with AuNP to form a combination that could detach the eosin molecule from the surface of AuNPs (Visser et al. 2011). The formation of the combination may result in the aggregation of AuNPs and increase the distance between the

AuNPs and eosin (Swierczewska et al. 2011). The aggregation of AuNPs can be confirmed by the size changes from 5 to 26 nm when the albumin is added to the mixture. This means that eosin molecules are detached from the gold nanoparticle surfaces recovering their fluorescence intensity. The procedure may be used for turn-on fluorescence and could be useful for detection and identify proteins in buffer and serum. Further experiments are in progress to establish the reliability of the system for probing different types of proteins.

Conclusion

In this study, gold nanoparticles (AuNPs) were synthesized using an eco-friendly technique that utilizes cinnamon bark extract as a reducing agent and stabilizer. The synthesized AuNPs were characterized by UV–Vis

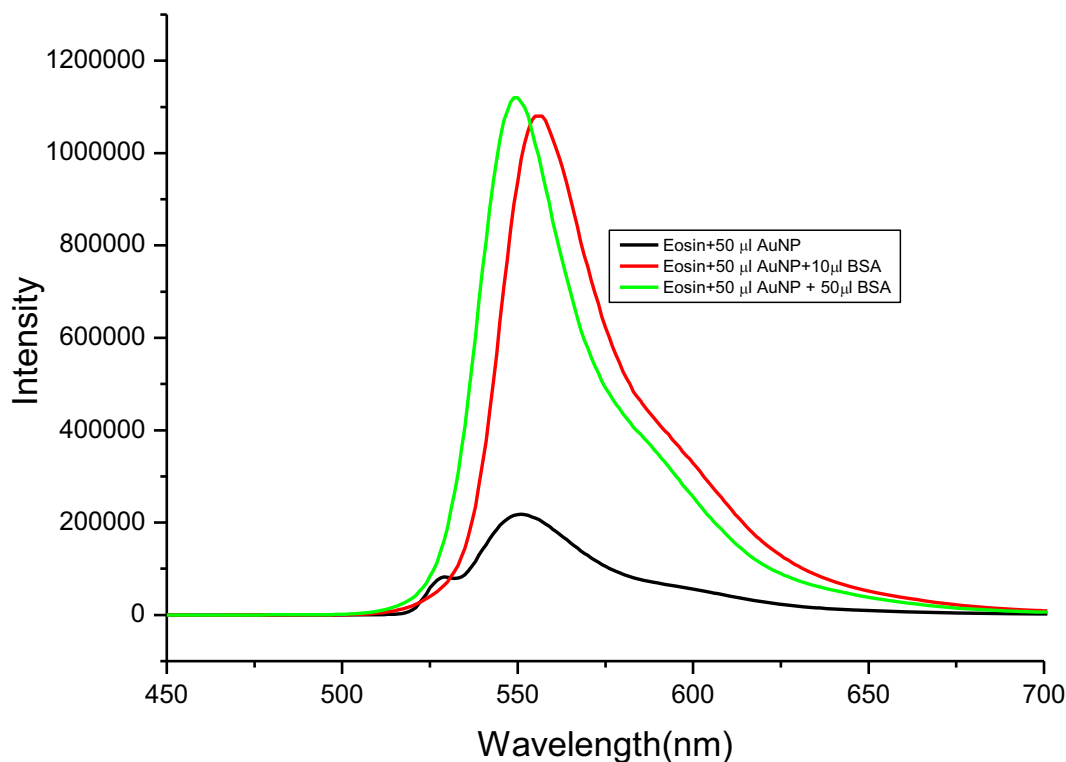


Fig. 7 Recover of fluorescence emission of eosin Y dye and AuNPs mixture as a result of the addition albumin bovine protein (concentrations are shown on the figure)

spectroscopy and transmission electron microscopy (TEM). UV–Vis shows the surface plasmon resonance (SPR) peak around 566 nm. TEM shows the spherical shape of AuNP and the particle size distribution at around 35 nm.

The ability of the synthesized AuNP as a quencher of eosin Y dye was monitored using a fluorometer. It was found that in the presence of AuNP, the fluorescence intensity peak of eosin is quenched. The fluorescence of the mixture of eosin and AuNP is recovered in the presence of albumin bovine protein. This may be attributed to the aggregation of AuNPs when albumin is added. TEM shows that the average size of the AuNP is reduced from 35 to 5 nm with the addition of eosin, and the size of AuNP then increased to 26 nm upon adding albumin bovine protein. The recovery (turn on) of fluorescence may be used for detection and to identify proteins in buffer and serum.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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