Chapter 6

Behaviour ofSynthesized Nanoparticles towards Biomolecules: A Comparative Study
Resume

In this chapter, the interaction of the synthesized nanoparticles (CPH-AuNps) and (CPH-AgNps) with different amino acids like arginine, aspartic acid, glutamic acid, glycine, leucine, methionine, histidine, tryptophan and cysteine has been studied by using UV-Visible and fluorescence spectroscopy. CPH-AuNps and CPH-AgNps were found to be the selective and sensitive fluorescent probe for L-Dopa and histidine, respectively. The minimum detectable limit for both L-Dopa and histidine was found at 10 nM by a facile way of fluorescence quenching i.e. by a turn off mechanism. CPH-AuNps and CPH-AgNps have also been studied for their antioxidant activity and interaction with DNA by UV-Visible spectrophotometry. Furthermore, electrophoresis technique confirmed the interaction of EtBr intercalated CT-DNA and S-DNA with CPH-AuNps and CPH-AgNps, respectively. The simplicity, sensitivity and specificity for the detection of amino acids by the CPH-AuNps and CPH-AgNps, indicates that the synthesized nanoparticles can be used as nano biosensor, with a potential prospect in the biomedical analysis.
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1. Introduction

Design, synthesis and manipulation of nano-sized materials have been found of tremendous interest in recent times because of its wide application in biology and medicine. The unique size-dependent properties of nanoscale materials have significantly impacted all spheres of human life making nanotechnology a promising field for biomedical applications. The advances in the synthesis and characterization of nanoscale materials [1-3] allow scientists to understand and control the interactions between nanomaterials and biological entities (e.g., nucleic acid, proteins, or cells) at molecular or cellular levels. These advances promise major achievements in the life sciences [4-7]. Due to the biological importance of amino acids; the development of optical probes for these molecules has been an active research area during recent years [8].

L-Dopa is one of the essential precursors used for the biosynthesis of dopamine. The enzyme aromatic-l-amino-acid decarboxylase present in brain converts L-Dopa into dopamine [9]. L-Dopa (3,4-dihydroxy-l-phenylalanine) is widely used as a source of dopamine in the treatment of most patients with Parkinson’s disease and epilepsy [10]. Several methods have been reported in literature for the determination of L-Dopa, such as titration [11], spectrophotometry [12, 13], high-performance liquid chromatography (HPLC) [14], photo kinetic method [15], capillary zone electrophoresis [16] and glass carbon electrode [17].

Histidine, one of the 20 natural amino acids, plays an important role as a neurotransmitter or neuromodulator in the mammalian central-nervous system. It could also control the transport of metals in biologically important bases [18, 19] and minimize internal bleeding from micro trauma [20]. Histidine deficiency usually causes Friedreich
ataxia, epilepsy, Parkinson’s disease, and the failure of normal erythropoiesis development [21], while ingestion of high levels can result in symptoms of intoxication [22].

DNA-based nanotechnology has generated interest in a number of applications due to the specificity, programmability, and reproducibility of DNA interaction with noble metal nanoparticles. The study of biopolymers such as negatively charged nucleic acids (DNA or RNA) on colloidal systems has recently gained considerable attention, owing to their biological importance and applications [23].

Antioxidant compounds in food play an important role of a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases, including cancer and heart disease. Primary sources of naturally-occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk [24]. The gold nanoparticles coated with torolex and chitosan enhance the DPPH radical scavenging activity. Silver, selenium, copper and platinum nanoparticles also exhibit the DPPH radical scavenging activity [25-28].

Here, the application of nanoparticles (CPH-AuNps and CPH-AgNps) as sensitive and selective fluorescent sensors for detection of amino acids has been explored. Among various amino acids like arginine, cysteine, aspartic acid, glutamic acid, glutamine, leucine, methionine, threonine, histidine, L-Dopa and tryptophan, fluorescent CPH-AuNps and CPH-AgNps were found to be highly selective and sensitive for L-Dopa and histidine, respectively. The present study also attempts to evaluate the interaction of
nanoparticles with CT-DNA, S-DNA by UV-Visible and electrophoresis techniques. Furthermore, the antioxidant activity of CPH-AuNps and CPH-AgNps with the DPPH assay has been studied.

2. Experimental section

2.1 Materials and methods

All the reagents and amino acids of AR grade were purchased from Sigma-Aldrich and used without further purification. Solvents used for spectroscopic studies were purified and dried before use. All aqueous solutions were prepared from quartz distilled deionized water which was further purified by a Millipore Milli-Q water purification system (Millipack 20, Pack name: Simpak 1, Synergy). CT-DNA and S-DNA were purchased from Hi media and 1-1-diphenyl-2-picrylhydrazyl (DPPH) purchase from Sigma–Aldrich. The agarose gel electrophoresis required 1 X TAE Buffer, Agarose powder, bromo Phenol blue, Xylene cyanol, Ethidium bromide chemical and studied on Electrophoresis Tank Power Pack. The colloidal solutions were centrifuged in REMI, Model No. R-8C laboratory centrifuge. Absorption spectra were studied on a Jasco V-570 UV-Visible recording spectrophotometer. Fluorescence spectra were recorded on Jasco FP-6500 spectrofluorimeter.

2.2 Interaction of various amino acids with CPH-AuNps and CPH-AgNps by absorption and emission spectroscopic measurements

1 mL (1 mM) aqueous solution of amino acids like arginine, cysteine, aspartic acid, glutamic acid, glutamine, leucine, methionine, thryonine, histidine, tryptophan and L-Dopa was added to 1mL solution of each CPH-AuNps and CPH-AgNps. Furthermore, fluorescence intensity of CPH-AuNps and CPH-AgNps quenched rapidly in presence of
L-Dopa and histidine, respectively. To evaluate their minimum detectable limit, L-Dopa, and histidine were titrated against nanoparticles.

2.3 Casting and running the gel

To prepare 100 mL of 1.0% gel, 1.0 g agarose powder was weighed accurately and sprinkled into 100 mL of suitable electrophoresis buffer (1xTAE or 1xTBE) in 250 mL flask. The agarose is boiled to dissolve by placing the flask in a boiling-water bath or heating mantle for about 15 minutes, once the agarose is completely dissolved the flask is removed from the water bath and left at room temperature to cool (50-55°C). Then the platform was washed with distilled water wiped dry with tissue paper, and the open ends were sealed with cello tape. The comb was placed 1 cm from the top end and made sure that the tip of the comb does not touch the surface of the platform i.e. 1 to 2 mm gap was maintained so that the DNA sample does not leak into the platform. The platform was placed on a smooth horizontal surface. Once the agarose solution was cooled to about 50°C, the solution was gently poured to cover the entire surface of the platform and left undisturbed for about 30 minutes. After the gel was formed, the comb was removed by gently pulling up and the cello tape was also removed from both ends. Then the gel along with the platform (casting tray) was kept inside the gel tank and the electrophoresis buffer was poured through one side of the tank to just cover the gel surface. Sample prepared in 1:3 ratio of EtBr intercalated CT-DNA and S-DNA and various concentration (10, 50, 100 μL) of nanoparticles were gently mixed followed by 30 min incubation. The sample was mixed with the loading dye on a paraffin wax sheet and then 10 μL samples were loaded carefully on the well. Once the sample was loaded into the well, the cathode was connected towards the top end of the gel and the anode was connected towards the
bottom end of the gel. The electrophoresis was started by switching on the power pack. After about 30-45 minutes, the current was switched off and the casting tray was removed. The gel was gently pushed onto a UV trans-illuminator by keeping the platform in a slanting position. And the UV light was passed over it to observe the bands.

2.4 Interaction of CT-DNA, S-DNA with CPH-AuNps and CPH-AgNps by UV-Visible absorption measurements.

(10 mg/10 mL) solution of each CT-DNA and S-DNA were prepared by using 0.1 M Tris buffer (pH 7.4) in a 100 mL standard flask. CPH-AuNps/AgNps (500 µL of each nanoparticles) was mixed with 2 mL of CT-DNA and S-DNA each to study the interaction of nanoparticles with DNA. This mixture was then left undisturbed for 2 hours before analysis.

2.5 Interaction of a Nanoparticle with EtBr intercalated CT-DNA and S-DNA by electrophoresis techniques.

For gel electrophoresis studies, EtBr intercalated CT-DNA, S-DNA (10 mg/10 mL) were prepared in 1:3 ratio, 100 µL EtBr intercalated CT-DNA, S-DNA and various amount of (10, 50, 100 µL) nanoparticles were gently mixed followed by 30 minutes incubation. Then 10 µL samples were loaded on the well, and electrophoresis in the Agarose gel at 50 Volts for 1.5 hours. The band of control DNA and DNA interacted nanoparticles were observed then resolved bands in the gel were documented using bio imaging system [29, 30].

2.6. In vitro antioxidant assay by (1-1-diphenyl-2-picrylhydrazyl) DPPH radical quenching method

The antioxidant property of gold and silver nanoparticles was evaluated by monitoring the ability of quenching synthetic stable DPPH radical into non-radical
form[31]. The reaction mixture containing 2.7 mL of 2.5 mM DPPH was mixed with a solution containing (10, 50, 100, 200, 250, 300 μL) amount of nanoparticles and the total volume was kept constant, i.e. 3.0 mL. The reaction mixture was incubated at 37°C for 30 minutes and the colour change was measured spectrophotometrically at 517 nm. The radical-scavenging activity (RSA) was expressed in percentage of inhibition using the following equation:

\[
\%RSA = \frac{(A_{\text{DPPH}} - A_x)}{A_{\text{DPPH}}} \times 100
\]

(Eq. 1)

3. Results and discussion

**CPH-AuNps and CPH-AgNps as a fluorescent sensor for L-Dopa and histidine**

The synthesized gold nanoparticles (CPH-AuNps) and silver nanoparticles (CPH-AgNps) were found to be the selective and sensitive fluorescent probe, for the detection of L-Dopa (Scheme 1) and histidine (Scheme 2), respectively. From the absorption spectra, it was observed that CPH-AuNps showed a red shift in surface plasmon resonance only in the presence of L-Dopa in Fig. 1, while CPH-AgNps gives change in their absorption spectra in the presence of histidine in Fig. 2. The maximum emission of CPH-AuNps and CPH-AgNps was observed at 540 nm and 560 nm, respectively. To ensure high selectivity of synthesized nanoparticles for amino acids, the interaction of nanoparticles with different amino acids such as arginine, cysteine, aspartic acid, glutamic acid, glutamine, leucine, methionine, thryonine, histidine, L-Dopa and tryptophan has been studied under similar conditions Fig. 3 and Fig. 4. The initial concentration of amino acids was taken 1 nM, which gradually increased to 100 μM and it was observed that the fluorescence intensity of CPH-AuNps and CPH-AgNps
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decreased with the increasing concentration of L-Dopa Fig. 5 and histidine Fig. 6, respectively. Several mechanisms have been proposed to explain the fluorescence quenching of nanoparticles, including inner filter effects, non-radiative recombination pathways, and electron transfer process [32]. Quenching often proceed via the formation of complex at excited state i.e. an exciplex (collisional quenching) or by ground state complex formation (static quenching). In the case of interaction of CPH-AuNps with L-Dopa, the excited state is more stabilized than the ground state so that both the absorption and emission spectra are red-shifted. There is an excellent linear relationship of fluorescence quenching with increasing concentration of L-Dopa and histidine and abide by Stern-Volmer equation. (Eq.2)

\[ \frac{F_0}{F} = 1 + K_{sv} [Q] \]  

Eq. 2

Where \( F_0 \) and \( F \) are the steady state fluorescence intensities in the absence and presence of quencher, respectively. \( K_{sv} \) is the Stern-Volmer quenching constant which defines the quenching efficiency and \([Q]\) is the concentration of quencher, i.e. concentration of L-Dopa/histidine

The fluorescence data were analyzed by plotting \( \frac{F_0}{F} \) versus concentration of L-Dopa/histidine over the range of 10 nM to 10 \( \mu \)M, exhibits a good linear relationship with a correlation coefficient \( R^2 = 0.9874 \).

**Interaction of CPH-AuNps and CPH-AgNps with CT-DNA and S-DNA**

Nanoparticles are well established test system to investigate DNA linking. For the investigation of the DNA mediated coupling of the nanoparticles, mainly absorption spectroscopy is employed [33]. The absorption spectra of CT-DNA and S-DNA was monitored at 250 nm in Fig. 7. The SPR band of CPH-AuNps was observed at 524 nm
then, after conjugating with CT-DNA and with S-DNA it shifted towards higher wavelength at 545 nm Fig. 8a, whereas, in case of CPH-AgNps, after conjugating with CT-DNA and S-DNA, wavelength shifted from 408 nm to 420 nm Fig. 8b. The successful interaction of the particles leads to a red shift and broadening of the UV-Visible spectrum as observed in the present case [34].

Emission of intense fluorescence takes place by the electrophoresis studies of DNA in the presence of EtBr due to its strong intercalation between the adjacent DNA base pairs [35]. The extent of fluorescence quenching of EtBr bound to DNA is utilized to determine the extent of binding to the second molecule like nanoparticles [36]. In the present study, we report the decrement in fluorescence by the addition of EtBr intercalated CT-DNA and S-DNA to CPH-AuNps and CPH-AgNps, respectively.

This hypothesis is further examined by electrophoresis studies. It was observed that for both control DNA, with an increase in the concentration of nanoparticles CPH-AuNp and CPH-AgNps (10 µL, 50 µL, 100 µL) Fig. 9 and 10. The bands intensity have become faint. It has been noted that the binding affinity has increased with an increase in the concentration of the nanoparticles [37-39], which in turn competes with the hydrophobic intercalator, EtBr on the DNA binding sites either directly (via intercalative insertion) or indirectly through probably protein moieties associated with DNA. Thus, it can be concluded that the fluorescent quenching of EtBr by nanoparticles may be due to the interaction between the nanoparticle and DNA [40]. Thus, CPH-AuNps, CPH-AgNps would be highly useful in the quantitative detection of DNA samples even at very low concentration.
Antioxidant activity

Free radical is an unstable atom or molecule with an outermost electron unpaired and is highly reactive. The free radicals always strive to form a stable bond, by gaining or losing an unpaired electron. Nanoparticles of gold and silver based on reaction conditions are ready to accept/donate an electron to quench radicals [27, 31, 41].

The DPPH is a stable and well-characterized radical for evaluation of antioxidant potential of compounds. The DPPH is reduced by accepting the hydrogen or electron, the DPPH reducing ability of gold and silver nanoparticles was quantified spectrophotometrically by changing the DPPH colour from purple to yellow Fig. 11. A comparative study of antioxidant activity with standard antioxidant reference materials, quercetin and ascorbic acid (AA) were conducted Fig. 11a. Although the antioxidant activity of CPH-AgNps Fig. 11b and CPH-AuNps Fig. 11c is slightly lower than that of standard antioxidant quercetin and ascorbic acid (AA), it is therefore, reasonable to propose that CPH-AuNps and CPH-AgNps hold the potential of their use as a good antioxidant agent Fig. 12.

The percent DPPH radical inhibition with effect of time is indicated in Fig. 13. Inhibition was found to be low in gold nanoparticles Fig. 13a, when compared with silver nanoparticles Fig. 13b, which may be due to the facts that silver act as a good oxidant can easily lose electrons. The resulting decolourization is stoichiometric with respect to the number of electrons captured [24, 26, 28, 31].
Conclusion

In this chapter, a new highly sensitive and selective turn-off fluorescent sensor has been developed for detection of L-Dopa and histidine, over the range of 10 nM to 10 μM using CPH-AuNps and CPH-AgNps, respectively. Spectroscopic and electrophoresis studies of the present investigation also confirmed the interaction between the nanoparticles and DNA. This method can also be adapted for biodetection in clinical samples. In addition, the antioxidant activity of CPH-AuNps and CPH-AgNps were also successfully evaluated to show free radical scavenging activity up to 70% in 45 minutes, which is relatively higher in comparison to other metal nanoparticles.
Scheme 1 Schematic representation for the formation of CPH-AuNps and fluorescence turn off mechanism of CPH-AuNps in presence of L-Dopa

Scheme 2 Schematic representation for the formation of CPH-AgNps and fluorescence turn off mechanism of CPH-AuNps in presence of histidine
**Fig. 1** Absorption spectra of CPH-AuNps in presence of various amino acids

**Fig. 2** Absorption spectra of CPH-AgNps on addition of various amino acids
Fig. 3 Fluorescence spectra of CPH-AuNps in presence of various amino acids

Fig. 4 Fluorescence spectra of CPH-AgNps on addition of various amino acids
Fig. 5 Fluorescence response of CPH-AuNps in presence of L-Dopa (10 nM to 10 μM)

Fig. 6 Fluorescence response of CPH-AgNps on addition of histidine (10 nM to 10 μM)
the inset shows a Stern-Volmer plot of intensity versus concentration of histidine
Fig. 7 Absorption spectra of CT-DNA and S-DNA

Fig. 8 Absorption spectra of a) CPH-AuNps and b) CPH-AgNps with CT-DNA and S-DNA
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1: Calf thymus-DNA + ErBr (3:1)
2: Calf thymus-DNA + ErBr (3:1) + 10 μl Nano
3: Calf thymus-DNA + ErBr (3:1) + 50 μl Nano
4: Calf thymus-DNA + ErBr (3:1) + 100 μl Nano
5: Herring sperm -DNA + ErBr (3:1)
6: Herring sperm -DNA + ErBr (3:1) + 10 μl Nano
7: Herring sperm -DNA + ErBr (3:1) + 50 μl Nano
8: Herring sperm -DNA + ErBr (3:1) + 100 μl Nano

Fig. 9 Agarose gel-electrophoresis image showing the different concentration (10μL, 50 μL, and 100 μL) of CPH-AuNps with CT-DNA and S-DNA
Fig. 10 Agarose gel-electrophoresis image showing the different concentration (10μL, 50 μL, and 100 μL) of CPH-AgNps with CT-DNA and S-DNA
Fig. 11 Antioxidant assay of DPPH with: a) Standard quercetin and ascorbic Acid, b) different concentration of CPH-AgNps, c) different concentration of CPH-AuNps

Fig. 12 DPPH antioxidant assay at (λ max 517) of CPH-AuNPs and CPH-AgNps with compared to standard
Fig. 13 Effect of time on free radical scavenging by a) CPH-AuNps and b) CPH-AgNps
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References


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