Chapter 3

Calix[4]pyrrole Octahydrazide Stabilized Gold Nanoparticles as Selective and Sensitive Fluorescence Sensor for Co(II) Ions
Resume

Water dispersible stable gold nanoparticles (AuNps) have been synthesized by using calix[4]pyrrole octahydrazide (CPOH) as a reducing as well as stabilizing agent. CPOH-AuNps have been characterized by surface plasmon resonance, particle size analyzer and transmission electron microscopy. CPOH-AuNps are water dispersible, highly stable for more than 150 days at neutral pH with a size of less than 10 nm and zeta potential of 15 ± 2 MeV. Ion sensing property of CPOH-AuNps has been investigated for various metal ions Pb(II), Cd(II), Mn(II), Fe(III), Ni(II), Zn(II), Hg(II), Co(II) and Cu(II) by colorimetry and spectrofluorimetry. Among all the metal ions investigated, only Co(II) ions gives sharp colour change from ruby red to blue and is easily detectable by naked-eye. CPOH-AuNps being fluorescent in nature also shows great sensitivity and selectivity for Co(II) ions which can be selectively detected at very low concentration level of 1 nM in a facile way of fluorescence quenching.
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1. Introduction

Nanotechnology may be defined as the design, characterization, evaluation, production and application of structures, devices and systems by controlling the size and shape at a nanometer scale. Au nanoparticles in the size range of ~1–100 nm offer new functions and applications in relation to bulk materials because of their unique electronic, magnetic, optical, mechanical, physical and chemical properties. The AuNps can be prepared by physical methods, chemical methods and supercritical fluid technology [1].

The last decade has seen many reports of chemical reduction routes for synthesis of gold nanoparticles using different reducing agents. One of the most widely used method for the preparation of gold colloid was proposed by Turkevich et al. [2] which was further refined by G.Frens [3, 4] involves the reduction of an aqueous solution of chloroaucric acid (HAuCl₄) by trisodium citrate. Another method of obtaining gold nanoparticles include the Brust-Schiffrin method which usually involves reduction by sodium borohydride (NaBH₄) in an organic solvent in the presence of tetraoctylammonium bromide as capping agent [5, 6]. Other reducing agents such as tannic acid, hydrazine, ascorbic acid, tartaric acid and human cells are also used to reduce the gold halides (HAuCl₄) to get nanoparticles [7-12]. The weakness of these reducing agents is that they yield nanoparticles which are not stable and tend to aggregate with change in their pH and electrolyte environments [13].

Calix[4]resorcinarenes have received much attention in the recent past for their use as reducing as well as stabilising agent for the preparation of gold nanoparticles [14-18]. Gold nanoparticles (AuNps) are the most extensively
investigated nano materials due to their unique tunable optical properties, which can be applied in various applications such as sensing, detecting, and imaging [19, 20]. AuNps also find application in the fields of catalysis [21-23], electronic and electro-optical devices [24] and biomedicine [25-28] etc. Because of characteristic signature of AuNps as surface plasmon resonance band (SPR) [29, 30] in the visible region (510-580 nm) and their fluorescent nature they can be potentially exploited as detection devices for target analytes i.e., a chemical or biological threat agent: radioactive substances, explosive compounds, chemical warfare agents, biotoxins, and biothreat pathogens [31]. Any change in the surface plasmon resonance band (SPR band) or fluorescence intensity of AuNps due the presence of analyte which can be measured spectrophotometrically or spectrofluorimetrically makes them potential chemosensors.

Determination of cobalt is important because of its widespread occurrence in water, earth crust, meteorites, animals, and plants. It is an essential element for nutrition and whose deficiency may cause anemia, retarded growth and loss of appetite. Cobalt ions are the component of vitamin B-12, which is required for good health [32]. But, if consumed in large doses, cobalt may be toxic and cause diarrhea, irritation of gastrointestinal tract and vomiting. Exposure to cobalt may cause lung effects, which include respiratory irritation, coughing, asthma, pulmonary edema and pneumonia [33]. Cobalt is widely used in paint, varnish, and ink industry as a drying agent, in the preparation of pigments like cobalt blue and cobalt green, in ground coats for porcelain enamels, in lithium ion battery electrode, as catalyst in petroleum and chemical industries, in electroplating industry [34].
Calix[4]pyrrole belongs to the family of hetero-calixarene macrocycles, which has four pyrrole units instead of phenolic units. Various reviews have appeared on their synthetic methodologies and applications during the last 10 years [35-38]. In this chapter novel calix[4]pyrrole octahydrazone (CPOH) has been used as reducing agent as well as stabilizing agent for the preparation of water dispersible and stable CPOH protected AuNps. As these nanoparticles having characteristic signature (Surface Plasmon Resonance Band) in visible region and being fluorescent in nature they have been explored for their use as selective and sensitive sensor for metal ions by colorimetry and spectrofluorimetry.

2. Experimental section

2.1. Chemicals and reagents

All metal salts including HAuCl₄ were purchased from Sigma-Aldrich. Other reagents and solvents of analytical grade were purchased from commercial sources and used without further purification. All aqueous solutions were prepared from Millipore water (resistivity, 18 MΩ.cm @25°C; Millipore Systems). TLC plates (F-2009) fluorescence active were procured from the Merck.

2.2. Instruments

The melting points (uncorrected) were recorded from a VEEGO (Model; VMP-DS) melting point apparatus. FT-IR spectra were recorded on Bruker, tensor 27 Infrared spectrometer with samples prepared as KBr pellets. ¹H-NMR spectra were recorded on a FT-NMR model Bruker, Avance II (500MHz) at 298 K with TMS as the internal reference. Mass spectra were recorded on JEOL SX 102/DA 6000
mass spectrometer using Xenon/Argon (6kV, 10mA) as the FAB-MS and ESI-MS were recorded on micromassQuarter2 mass spectrometer. Absorption spectra were studied on a Jasco V-570 UV-Vis recording spectrophotometer. pH of the solutions was measured using pH analyzer LI 614- Elico. The Malvern Zetasizer (Model; ZEN3600) was used for particle size (e.g., hydrodynamic diameter) and the zeta potential measurements by Laser Doppler Electrophoresis (LDE). TEM images were recorded in MACK/model JEOL, JEM 2100 at an accelerated voltage of 200 kV. Fluorescence spectra were recorded on Jasco FP-6500 spectrofluorometer.

2.3. Procedure for the synthesis of calix[4]pyrrole octahydrazide protected gold nanoparticles (CPOH-AuNps)

25 mL (1 mM) aqueous solution of CPOH (Mol. Wt. 1378, synthesis and characterisation already mentioned in chapter 2) was added rapidly into 25 mL (1mM) solution of HAuCl₄ (Mol. Wt. 393.8) in a 100 mL conical flask under vigorous stirring. Calix[4]pyrrole octahydrazide (CPOH) stabilized stable colloids (AuNps) were obtained immediately but vigorous stirring was continued for 10 minutes to ensure complete homogenization. The transparent colourless solution was converted to the characteristic ruby red colour, indicating the formation of gold nanoparticles. This CPOH-AuNps solution was then subjected to repeated centrifugation (3 times) at REMI (R-8C) laboratory centrifuge at 14000 RPM, washed with a copious amount of deionised water and again redispersed in 50 mL of deionised water to get 0.0084 % solution of CPOH-AuNps for further studies (Scheme 1).
2.4. General procedure for the spectrophotometry and spectrofluorimetry measurements

Stock solution of the CPOH-AuNps (0.0084 %) and various metal ions (2 µM), Pb(II), Cd(II), Mn(II), Fe(III), Ni(II), Zn(II), Hg(II), Co(II), Cu(II) were prepared in deionised water. 2.5 mL of CPOH-AuNps and 2.5 mL of each metal ions were taken in a 5 mL volumetric flask, so that the effective concentration of the cations is 1 µM. The interaction behaviour of various metal ions with CPOH-AuNps was recorded on absorption spectrophotometer and spectrofluorimeter.

3. Results and Discussion

3.1. Synthesis and mechanism of formation of gold nanoparticles (CPOH-AuNps)

In this study, water soluble calix[4]pyrrole octahydrazide (CPOH) which has all the power and properties of hydrazide to act as reducing agent and its web type structure with an inherent hollow cavity making it a very effective stabilizing agent has been used for the formation of water dispersible stable gold nanoparticles (CPOH-AuNps). Reduction of HAuCl4 by calix[4]pyrrole octahydrazide occurs via oxidation of the amino group i.e., transfer of electrons from the amine of calix[4]pyrrole octahydrazide to the Au3+ ions (Eq. 1). CPOH can passivate the surface of gold nanoparticles and stabilize nanoparticles owing to the coordination with nitrogen atoms of hydrazide (-NH-NH2) group with Au atoms at the surface of gold nanoparticles. The resulting metallic gold nucleates to form gold nanoparticles and further stabilize it electrostatically as follows [39-41].

\[ \text{HAuCl}_4 + 3NR_3 \rightarrow \text{Au}^0 + 3NR_3^+ + \text{H}^+ + 4\text{Cl}^- \] \hspace{1cm} \text{-------Eq.1}
3.2. Characterization of CPOH-AuNps by PSA, TEM and EDX

A drop of dilute solution of aqueous nanoparticles was placed on carbon coated copper grids and was dried in vacuum and directly observed in the TEM. The morphology and particles size of CPOH-AuNps as shown in figure 1a revealed that the particles are roughly spherical in shape and uniform in size, as well as, well dispersed with a narrow size distribution with an average particles size of 8±2 nm (Figure 1b). The size distribution of the CPOH-AuNps was also determined using particle size analyzer where the average hydrodynamic diameter was found to be 16±3 nm. These higher values were due to the light scattered by the core particle and the layers formed on the surface of the particles. Energy-dispersive X-ray (EDX) analysis spectrum recorded in the spot-profile mode from one of the densely populated CPOH-AuNps regions on the surface of film. Strong signals from Au atoms while weaker signals from C, O, Si, Cu and Ca atoms were observed (Figure 1c). The overall particles charge in a particular medium is denoted as their zeta potential value which is also responsible for deciding the fate of stability. Here, synthesized gold nanoparticles (CPOH-AuNps) had a 15 ± 2 MeV zeta potential values, which is sufficient to keep the particles away from aggregation and maintained the stability, moreover positive value also suggests that hydrazide groups were successfully introduced onto the surface of nanoparticles. CPOH-AuNps which are water dispersible, highly stable for more than 150 days at neutral pH with a size of less than 10 nm and zeta potential of 15 ± 2 MeV makes these nanoparticles very potential candidate for various biological/biomedical applications [42-46].
3.3. Effect of pH and time on stability of CPOH-AuNps

Stability of gold nanoparticles has been investigated by change in their surface plasmon resonance (SPR) band and fluorescence intensity at different pH (3.0 to 11.0). SPR band of CPOH-AuNps shows slight change at pH other than 7.0 and tend to agglomerate. It is noteworthy that when their agglomerated form is sonicated for 10-15 minutes they retain their originality with a negligible compromise in their SPR band and thereby size (Figure 2a). No change in SPR band of the CPOH-AuNps at pH 7.0 was recorded up to 150 days (Figure 2b). Likewise, fluorescence intensity of gold nanoparticles CPOH-AuNps decreases slightly at pH other than 7.0 (Figure 2c). Therefore, pH 7.0 was selected to carry out all experiments on CPOH-AuNps and concluded that CPOH-AuNps shows maximum stability and fluorescence intensity at pH 7.0.

3.4. Colorimetric detection of Co(II) using CPOH-AuNps

The metal nanoparticles are emerging as important type of colorimetric reporters because of their large extinction coefficients and tendency to agglomerate in the presence of analyte. Agglomeration leads to distinct colour change from red to blue [47-51] and thereby making them very useful colorimetric sensing platforms. To investigate the colorimetric response of CPOH-AuNps, various metal ions i.e. Pb(II), Cd(II), Mn(II), Fe(III), Ni(II), Zn(II), Hg(II), Co(II) and Cu(II) in μM concentration were added to these nanoparticles. No visible colour change was observed with any of the metal ions except Co(II), which exhibits a sharp change in colour from ruby red to purple, and finally to blue which can be easily judged by the naked eye (Figure 3). The colour change with Co(II) ions can be easily noticed even at nano molar (nM) concentration in aqueous samples. It
may be concluded that CPOH-AuNps can be used a remarkable selective colorimetric sensor for Co(II) ions.

3.5. Interaction of various cations with CPOH-AuNps by spectrophotometry and spectrofluorimetry measurements

The absorption spectra of CPOH-AuNps with various metal ions i.e. Pb(II), Cd(II), Mn(II), Fe(III), Ni(II), Zn(II), Hg(II), Co(II) and Cu(II) were recorded (Figure 4). No change in the absorption spectra was observed with all the metal ions except Co(II) ions which shows red shift of 55 nm. The red shift is dependent on the number of particles and their spatial arrangement within an aggregate [52, 53]. The red shift and significant broadening of the absorption bands is also due to overlapping of shifted modes of vibration [54, 55]. Because of these reasons, gold nanoparticles offer significant potential for detection applications due to (i) their high sensitivity to perturbations in the local dielectric constant (or refractive index) of the surrounding media and (ii) aggregation-induced colour changes, which result from the plasmon peak shift and broadening [56, 57]. It is concluded that the shift to higher wavelength (red shift) is related to a change in the particles size (Figure 5).

The emission spectra of the CPOH-AuNps were recorded at 698 nm. The fluorescence emission of CPOH-AuNps was studied over a wide range of pH (3.0 to 11.0). The maximum emission intensity was observed at pH 7.0. The selectivity of CPOH-AuNps for Co(II) ions was also examined in the presence of various metal ions i.e. Pb(II), Cd(II), Mn(II), Fe(III), Ni(II), Zn(II), Hg(II) and Cu(II) at 7 pH (Figure 6). The linear range of minimum and maximum detection of Co(II) ions was determined by means of fluorescence titration of CPOH-AuNps (0.0084%) with increasing concentration of Co(II) ions (Figure 7). It was noted that
fluorescence intensity, at 698 nm, gradually decreases with an increase in the concentration of Co(II) ions (inset Figure 7). The minimum and maximum fluorescence quenching (90%) (Figure 8) was observed at 1 nM and 1 µM concentration of Co(II) ions, respectively, (Figure 9), which is considered as the minimum and maximum detection limit CPOH-AuNps. Therefore these nanoparticles can be used as a highly sensitive and selective fluorescence “turn-off” sensor for Co(II) ions without further modification. This highly sensitive and selective sensing properties may result from the aggregation of CPOH-AuNps induced by the cross-link complexation between and Co(II) ions [58]. Conclusion

In this chapter a novel calix[4]pyrrole octahydrazide (CPOH), which has properties of hydrazine, to act as reducing agent and its web type structure with inherent hollow cavity, to encapsulate/cap/engulf/stabilize the reduced nanoparticles, has been used for the preparation of nanoparticles. Characterization has been done by surface plasmon resonance, particle size analyser and TEM. CPOH-AuNps were found to be colorimetric and fluorometric chemosensors for remarkable selective signalling of Co(II) ions. Among various ions i.e. Pb(II), Cd(II), Mn(II), Fe(III), Ni(II), Zn(II), Hg(II), Co(II) and Cu(II), only Co(II) can be selectively detected in nanomolar concentration in a facile way of fluorescence quenching. CPOH-AuNps which are water dispersible, highly stable for more than 150 days at neutral pH with a size of less than 10 nm and zeta potential of 15 ± 2 MeV makes these nanoparticles very potential candidate for various biological/biomedical applications.
Scheme 1: Schematic representation showing the preparation of gold nanoparticles (CPOH-AuNps)
Figure 1: a) TEM image of gold nanoparticles (CPOH-AuNps), b) Particle size distribution graph and c) Energy-dispersive X-ray (EDX)
Figure 2: a) Effect of pH on surface plasmon resonance, b) Stability of gold nanoparticles (CPOH-AuNps) with respect to time and c) Effect of pH on fluorescence intensity
Figure 3: Visual colour change of gold nanoparticles (CPOH-AuNps) upon addition of different metal ions (1 µM) at pH 7.0

Figure 4: UV-Vis spectra of CPOH-AuNps after the addition of various metal ions
**Figure 5:** Fluorescence spectra of CPOH-AuNps on addition various metal ions (1 µM).

**Figure 6:** Fluorescence response of CPOH-AuNps on addition of Co(II) solution (1 nM to 1 µM). The inset shows a stern volmer plot of relative intensity versus concentration of Co(II).
**Figure 7**: Graph shows minimum and maximum fluorescence quenching of CPOH-AuNps after the addition of Co(II) ions.

**Figure 8**: Graph shows % quenching of CPOH-AuNps after the addition of Co(II) ions.
Schematic representation showing fluorescence enhancement of **CPOH-AuNps** in presence of Co(II) ions resulting in a turn off fluorescent assembly.

**Graphical Abstract**
Chapter 3

References


