Selective sensing of copper biology essay

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Abstract

In this communication, octa-O-methoxy resorcin[4]arene tetrahydrazide (OMRTH) have been used as reducing as well as stabilizing agent for the preparation of gold nanoparticles via simple one pot method. Synthesized gold nanoparticles (OMRTH-AuNps) were characterized by UV-Vis spectroscopy, transmission electron microscopy (TEM) and particle size analyzer (PSA). In addition, the interaction of different cations like $Ag+$, Pb^{2+}, Cu^{2+} , Fe^{2+}, Hg^{2+}, Ni^{2+} and amino acids like Arginine, Cysteine, Aspartic Acid, Glutamic Acid, Glutamine, Leucine, Methionine, Thryonine, Tryptophan with synthesized gold nanoparticles have been studied by UV-Vis and fluorescence spectroscopy. Using OMRTH-AuNps as a selective and sensitive fluorescent probe, Cu^{2+} ions could be detected at a minimum concentration level of 10 μM in a facile way of fluorescence quenching i. e. by a '' turn off'' mechanism. Furthermore, reversible simultaneous fluorescence recovery was also observed when leucine was added in the same solution, which shows the " On-Off" mechanism of OMRTH- AuNPs. Key words: - Gold Nanoparticles, Fluorescence, Resorcinarene, Amino acid

Introduction

Nanotechnology is a collective definition referring to every technology and science which operates on nanoscale and refers to the scientific principles

and pristine properties that can be found and mastered when operative in this range [1]. Gold nanoparticles (AuNPs) have several distinctive physical and chemical attributes; their optical and electrochemical properties, and catalytic activity are not characteristic of bulk gold [2]. AuNPs can be prepared by both chemical and physical methods. Normally, gold derivatives (e. g., chloroauric acid) are reduced and controlled to grow particles with nanometer scale in chemical methods. During the past few decades, a variety of different methods have been reported and reviewed for synthesizing gold colloids of mono disperse and uniform sizes particles (Turkevich, Stevenson et al. 1951; Frens 1973; Hayat 1991; Schmid 1992; Watson, Zhu et al. 1999) [3-7]. The chemical synthesis methodologies include redox synthesis, electrochemical, photochemical, seed-growth, template synthesis, micro-emulsion template synthesis, microwave synthesis, etc. [8-11]. Different reducing agents such as sodium borohydride, trisodium citrate, tannic acid, hydrazine, ascorbic acid, tartaric acid and human cells are used to reduce the gold halides (HAuCl4) to colloid nanoparticles [12-17]. AuNPs can be used as multi labels in simultaneous optical and electron microscopy and as energy transfer assays for the detection of DNA, in optoelectronics, heavy metallic cations determination, protein analysis, cancer treatments [18-21]. Resorcinarenes are compounds made by condensation of resorcinol and aldehydes in the presence of acids. Due to the easy availability and versatile nature of resorcinarenes , they have been extensively used as host molecules and models for receptors as well as building blocks in crystal engineering and self-assembly studies in supramolecular chemistry [22]. Recently, calixarenes and resorcinarenes,

are well known as protective agents to stabilize and functionalize AuNPs in the emerging areas of nanoscience and technology. C4 symmetric tetraalkoxyresorcinarene has emerged as a new member of resorcinarene family and have grabbed obvious attention in versatile application fields [23]. 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 [23-27]. Detection and sensing of heavy and transition metal ions via synthetic receptors are topics of recent interest in supramolecular chemistry because of their significant value in chemical, biological and environmental assays. Most environmental, biological and alloy samples generally contain trace amounts of copper at the level of ng ml-1. Many industrial wastewater streams (such as those used in metal works, semiconductor and copper industries, mining, etc.) contain heavy metals which are of great environmental concern and must be removed prior to water discharge or water recycling [28]. In the determination of copper, various methods including UV/Vis spectrophotometry, FAAS, ICP-AES, ICP-MS, spectrofluorimetry, anodic stripping voltammetry, potentiometry, chemiluminescence and ion chromatography have been used. Among commonly used methods for copper analysis presently, fluorescent methods are more often used because of promising advantages of fluorescence signalling and its intrinsic sensitivity, simplicity and low cost [29]. Xiangqun Guo et al. has developed a highly sensitive and selective fluorescence sensor for the Cu2+ metal ion based on the aggregation-induced fluorescence quenching of glutathione-capped F-AuNPs [30-32]. Biologically active molecules such as amino acids, peptides and proteins are usually attached

to nanoparticles to improve their bio-specificity. To expand application potentialities of these types of systems in biological and medical sciences [33] use of amino acids like, cysteine [34], arginine [35], glutamic acid [27], tryptophan [36], methionine [37] etc as a reducing and capping agent are reported in literature. In futuristic years, research attention in the area of chemical sensors has been focused on further increasing the sensor sensitivity, therefore leucine, an amino acid is used as " turn on" sensor [38-41]. In the present study, we have explored Octa methooxycalixresorcinarene tetra hydrazine (OMRTH) as a reducing, capping and stabilizing agent for one pot synthesis of AuNps and further investigated its applications by florescence. We have reported the effects of different metal cations on the fluorescence of OMRTH-AuNPs and explored their quenching mechanism. Fluorescence's of OMRTH-AuNps was found to selectively respond to Cu2+ ion which quenched the emission. Furthermore, reversible simultaneous fluorescence recovery on addition of leucine was observed, in the same solution, which shows the " Off-On" mechanism of OMRTH- AuNP.

2 Experimental

2. 1 Instrument & Reagents

1H-NMR spectra were recorded at 298 K in CDCl3 at 500 MHz on a FT-NMR model Bruker, Avance II (500MHz). The chemical shifts were recorded in parts per million (ppm) with tetra methyl silane (TMS) as the internal reference. Electro spray ionization (ESI) mass spectra (MS) were determined using Micromass Quarter 2. Advantage mass spectrometer (capillary column temperature 200◦C, spray voltage 400V, anodic ionization mode). Elemental

Analysis was done vario MICRO Variant elemental. The melting points (uncorrected) were obtained from a VEEGO (Model; VMP-DS) melting point detector. FT-IR spectra were recorded on Bruker, tensor 27 Infrared spectro-photometer as KBr pellets and expressed in cm-1. Solvents were dried and distilled before use. Other chemicals were of analytical grade (Sigma Aldrich/ Fluka) and used without further purification. All aqueous solutions were prepared with quartz distilled deionised water which was further purified by a Millipore Milli-Q water purification system. Silica gel & TLC plates (F-2009) fluorescence active will be obtained from the Merck. All the chemicals and reagents were used as received; amino acids were purchased from SRL chemical. All organic solvents were analytical grade and were used as for synthetic purpose. Solvents for analytical studies were freshly purified by standard procedure before use.

2. 2. Synthesis (Scheme 1) and characterization of compounds

2. 2. 1 Synthesis of compound 1[42]

Aq. HCl (9 M, 8 mL) was added drop wise to a stirred solution of 1, 3-dimethoxybenzene (5 mL, 3. 82 mol) and 4-hydroxybenzaldehyde (4. 6 g, 3. 82 mol) in ethanol (EtOH, 62. 5 mL). The mixture was then allowed to stir at reflux for 16 or 72 h. The mixture was allowed to cool to room temperature and then filtered to yield the crude mixture of isomers as a purple powder, which were washed with cold methanol and further recrystallized in DMF-methanol mixture. 1H NMR (500 MHz, DMSO-d6) d 8. 79 (s, 4H, OH), 6. 60 (s, 2H, ArH), 6. 46 (s, 2H, ArH), 6. 36 (m, 16H, ArH), 6. 17 (s, 2H, ArH), 5. 97 (s, 2H, ArH), 5. 49 (s, 4H, ArCH), 3. 64 (s, 12H, OCH3), 3. 59 (s, 12H, OCH3);

νmax/cm-1 3144, 1611, 1511, 1465, 1437, 836, 751 cm_1; mp 320◦C (decomp.); m/z (relative intensity) 968. 4 ((M - H,), 970(M +H), 987(M+H2O), 992 (M + Na,), 1008. 3 (M + K,). Anal. data for C60H56O12 (Found; C, 75. 35; H, 6. 05; O, 18. 54 Calc.; C, 74. 36; H, 5. 82; O, 19. 81)

2. 2. 2 Synthesis of compound 2

Octa -O -alkyl resorcin[4]arene(1) (4 g, 4. 1 mol), anhydrous potassium carbonate (4. 5 g, 4. 7mmol) and potassium iodide (0. 68 g, 4. 0 mmol) in dry acetone (150 mL) was heated to reflux under nitrogen for at least 1 hr. Then ethyl bromoacetate (5. 51 mL, 4. 7 mmol) was added and the reaction mixture was further refluxed for 7 days. After removal of acetone, the residue was dissolved in water, acidified with HCl and extracted with CHCl3. The yellow organic layer was separated and dried with MgSO4. Red oil, yielded after evaporation of the solvent, was treated with alcohol to give yellow product and was further recrystallized from EtOH to give pure yellow solid compound. White solid, 51. 7%; mp 165 °C; IR (KBr) νmax/cm-1 1755 (C= O); 1H NMR (500 MHz, DMSO) d 6. 6(d, 16H, ArH), 6. 2–6. 3(s, 4H, ArH), 6. 15(s, 4H, ArH), 5. 64(s, 4H, Ar3CH), 4. 4–4. 6 (m, 8H, OCH2), 3. 4–3. 6 (m, 24H, OCH3), 1. 0–1. 4 (m, 20 H, C2H5) ; m/z 1313. 4 Analytical data for C76H80O20 (Found; C, 70. 12; H, 6. 05; O, 25. 73 Calc.; C, 69. 50; H, 6. 14; O, 24. 36)

2. 2. 3 Synthesis of compound 3

A mixture of compound 2 (4. 0 g, 2 mmol) and hydrazine hydrate (20 mL, 80%) in 15 mL of EtOH was refluxed for 24 hours and then allowed to cool at room temperature. White coloured solid precipitated out and was washed

with water to get pure compound. White solid, 85. 0%; mp > 300°C; IR (KBr) νmax/cm-1 3261 (-NH), 1503(-CONH) cm-1; m/z 1271. 3 Analytical data for C69H74N8O16 (Found; C, 64. 32; H 5. 95; N, 8. 27; O, 20. 95 Calc.; C, 65. 18; H, 5. 87; N, 8. 81; O, 20. 14)

2. 3 Synthesis and characterization of Octa methoxy calix[4]resorcinarene tetra hydrazine protected gold Nanoparticle (Scheme 2)

10 mL (1mM) solution of HAuCl4 was added to a 50 mL of conical flask containing 10 mL of methanol and then 5 mL (1 mM) solution of Octa methoxy calix[4]resorcinarene tetra hydrazine (3) was added rapidly under vigorous stirring. Octa methoxy calix[4]resorcinarene tetra hydrazine stabilized gold colloids (OMRTH-AuNps) were obtained immediately but vigorous stirring was continued for more 5 minutes to ensure complete homogenization. The colour of the solution changes from yellow to ruby red which indicates the successful formation of gold nanoparticles. Colloidal gold solution was subjected to centrifugation and the conjugate was washed with deionized water to remove any uncoordinated Octa methoxy calix[4]resorcinarene. The conjugate was then dried and subjected to characterization using various physico-chemical techniques. Structure of compound 1A single crystal of (compound 1) suitable for X ray structure analysis was obtained from a solution of DMSO. The diffraction data were collected at 110 (2) K using a Bruker Smart-CCD diffractometer (graphite-monochromated MO Kα radiation: A = 0. 071073 nm). The structure was solved via the omega-phi scan method and refined by means of full-matrix least squares on F2. All the calculations were performed using the SHELXTL

crystallographic software package. A summary of crystallographic relevant data and molecular structure of compound 1 is shown in supplementary data, the four methoxy benzene units in the ring were divided into two groups with two methoxy benzene rings almost perpendicular to the other two methoxy benzene rings, which shows the resorcinarene in Chair (C2h) conformation.

Time, pH and Temperature dependent stability study of OCTH-AuNp by UV/Visible spectroscopy measurements

The time, temperature and pH dependent stability of OMRTH-AuNps was studied by monitoring UV/Vis-spectra. The stability of OMRTH-AuNps was assessed by monitoring SPR band up to 90 days. Generally temperature has a great effect on formation and stability of nanoparticles. Therefore, the stability of gold nanoparticles was studied at different temperatures between 10 to 80 ◦C and it was observed that OMRTH-AuNps were stable at any temperature, whereas the aggregation takes place at higher temperature. The pH related stability study was also carried out at different pH (4 to10) at room temperature.

Interaction of cations with OCMRTH-AuNps by UV/Visible and Fluorescence spectroscopy measurements

The effect of various cations1 mL (1 mM) aqueous solution of (Ag^+ , Zn^{2+}, Cd^{2+}, Hg^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+}, Th^{2+} and Fe^{3+}) and was added to 1mL OCMRTH-AuNps and from the different set of experiments it was observed that OMRTH-AuNps showed red shift in surface plasmon resonance only in presence of Cu^{2+}. Similarly, emission spectra of various cations (Ag^+ , Zn^{2+}, Cd^{2+}, Hg^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Th^{2+} and Fe^{3+}) on

the fluorescence intensity of the OMRTH-AuNPs nanoparticles was investigated. For fluorescence intensity measurements 1mL (0. 1 mM) of individual metal ions was mixed with 1mL aqueous solution of OMRTH-AuNPs. The maximum quenching of emission intensity of OMRTH-AuNPs was observed in the presence of Cu^{2+} ions. Therefore, to determine the detectable limit of Cu^{2+} ion by means of quenching in emission intensity, the Cu^{2+} solution was diluted to 10-10000 times.

Interaction of Amino acids with OCMRTH-AuNps by UV/Visible and Fluorescence spectroscopy measurements

We also investigated the interaction of amino acids with OMRTH-AuNps by UV-Vis and fluroscence spectroscopy method. 1 mL (1 mM) aqueous solution of different amino acids like leucine, glutamic acid, glycine, tryptophan, aspartic acid, proline, cysteine was added to 1mL OMRTH-AuNps. Individual set of experiment carried out with each amino acid reckoned that OMRTH-AuNps showed hyper shift in surface plasmon resonance only in presence of leucine. Similarly, emission spectra of OMRTH-AuNps with 1 mL each of leucine, glutamic acid, glycine, tryptophan, aspartic acid, proline, cysteine (1 mM) were recorded and fluorescence enhancement was observed in presence of leucine. In addition, fluorescence enhancement effect of leucine with various metal ions was investigated by fluorescence spectroscopy. For emission titration studies, the same stock solutions of the OMRTH-AuNps, metal ions and amino acids were used which were used for absorption studies. The addition of 1mL (1 mM) solution of leucine in the presence of metal ions like (Ag^+, Zn^{2+}, Cd^{2+}, Hg^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+}, Th^{2+} and Fe^{3+}) revealed that only Cu^{2+} ion exhibited significant enhancement in

the fluorescence intensity spectra. Under the above conditions, (AuNps +Cu 2+) has also been studied for its interaction with leucine, which shows fluorescence recovery, as the concentration of leucine increases. Therefore, 10-100 times dilution of leucine was done to evaluate minimum detectable concentration of leucine. Since fluorescence changes occur only in the presence of leucine and not with the other amino acids

3 Results and Discussion

Gold nanoparticles can be synthesized by reduction of aqueous solution of HAuCl4 using Octa Methoxy Calix[4]resorcinarene tetra hydrazide as reducing agent (Scheme. 1). The reduction of HAuCl4 occurred through the transfer of electrons from the amine group of hydrazine to the Au3+ ion leading to the formation of Au0. This metallic gold then nucleates and grows to form gold nanoparticles, and is subsequently capped and stabilized by it electrostatically. The amino group of the OMRTH is oxidized to a positive radical due to the transfer of an electron from the amine to the gold ion. The reaction scheme of OCTH reduced and capped gold nanoparticles are shown in (Scheme. 2). OMRTH act as not only as reducing but a capping and stabilizing agent also. The most common technique for characterization of gold nanoparticle is Uv -Vis spectroscopy, which is used for analysis of intensely coloured colloidal dispersions having surface plasmon absorption. Certain metal colloids like Au, Ag and Cu, exhibit strong absorption bands in the visible region and are therefore intensely coloured. The plasmon bandwidth increases with decreasing size in the intrinsic region (mean diameter smaller than 25 nm) and also increases with increasing size in the extrinsic region (mean diameter larger than 25 nm) [43]. The surface

plasmon resonance shows a red- shift in the extrinsic size region and as the particle size increases, the gold nanoparticles colour changes from ruby red to purple and finally blue. The characteristic surface plasmon band for 20 nm gold nanoparticles at 550 nm peak wavelength was observed in the UV-Visible spectrum, confirming the presence of gold nanoparticles and inset of figure shows its purple colour (Fig. 1). The shape and size of particles were determined by Transmission Electron Microscopy (TEM) whose images are shown in (Fig. 5). which also display the morphology of particles. The figure depicts the various particle shapes such as spherical, hexagonal, rods etc., with an average particle size of 20 nm. The presence of a band in EDX spectrum at 2. 1 KeV is conformation of composition of > 99 % of Au metal atom (Fig. 6) [44]. Fig. 7 presents the absorbance spectra of OMRTH-AuNps in the presence of various cations. The experimental results did not show any significant SPR change except for Cu2+. Cu2+caused a shift towards the (red shift) higher wavelength region. The red shift and broadening of the surface plasmon band are observed when Cu2+ is added to the gold nanoparticles, indicating some consequent aggregation to surface modification of gold nanoparticles (Fig. 8). The maximum excitation and emission wavelengths of the OMRTH-AuNps were observed at 480 and 550 nm. (Fig. 9) depicts the fluorescence emission spectra of OMRTH-AuNps in the presence of Cu2+ with varying concentrations. It is observed form the figure that with increasing concentration of Cu2+, the fluorescence intensity decreased gradually. As can be seen in Fig. 10, the presence of Cu2+ gave rise to an obvious quenching of the emission of gold nanoparticle compared to various metal ions such as, (Ag+, Zn2+, Cd2+, Hg2+, Cu2+, Co2+, Ni2+,

Pb2+, Th2+ and Fe3+) and the emission was almost quenched in the presence of 10 µM Cu2+ thus considering it as the remarkable quenching for OMRTH-AuNps fluorescence in the presence of Cu2+. Many divalent metal ions are well-known as a fluorescence quencher and work via numerous mechanisms, including ground-state complexation, collisional conversion of electronic to kinetic energy, heavy atom effects, magnetic perturbations, charge-transfer phenomena, electronic energy transfer, and fluorescence resonance energy transfer [45-47]. So what???? Cu2+ is well known as a strong quencher because of its electronic structure (d9). Quenching by this type of substance most likely involve the donation of an electron from the fluorophore to the quencher, the ion dipole interaction between Cu2+ and the molecule will also be strong due to the large nuclear charge and relatively small size when compared with other metals. Cu+2 usually introduces easily accessible low energy levels, which can give rise to energy and electron transfer processes and is capable of quenching the fluorescent excited state of the molecule [20]. Fig. 11 depicts Cu 2+ as quencher and leucine as analyte. When some amino acids are added to the solution of OMRTH-AuNps - Cu 2+ complex, the fluorescence recovery signal is observed only in presence of leucine (Fig. 14). It was known that amino acids could form stable complexes with some metal ions, i. e., mercury, copper, and zinc ions [48]. There are two main factors affecting the changes on the fluorescence by metal nanoparticles: (1) the plasmon field generated around the particle by the incident light, increases the excitation decay rate of the fluorophore, which in turn enhances the level of fluorescence emission; and (2) the dipole energy around the nanoparticle reduces the ratio of the

radiative to non-radiative decay rate and the quantum yield of the fluorophore, resulting in the fluorescence quenching [49]. The fluorescence emission spectra of OCTH- AuNps (in presence or absence of leucine) were shown in Fig. 2. It could be seen that the fluorescence intensity of OCTH-AuNps was enhanced in presence of leucine. Preliminary measurements indicate that the two modes of binding of the amino acid could be via electrostatic interaction of the protonated amine groups with surface-bound negatively charged $AuCl_4^-$ ions and the other involving direct binding of the amine group to the gold surface.

Conclusion

In summary, an efficient, eco-friendly and simple method has been developed for the preparation of OMRTH-AuNps, where Octa Methoxy Calix[4]resorcinarene tetra hydrazide (OMRTH) acted as both reducing as well as stabilizing agent. Nanoparticles were found to be stable at room temperature, at different pH and also over a long period of time. we have also demonstrated a new type of rapid, highly sensitive, and selective fluorescence turn-on assay for detection of leucine using an OMRTH-AuNps ensemble. This assay is based on the highly specific interaction between the amino acids and the metal ions and the strong fluorescence OMRTH-AuNps probe in a competition assay format. It was also found that OMRTH-AuNps were selective and sensitive fluorescent chemo sensors for Cu 2+ and leucine up to the detection limit of 0. 1 µM. OMRTH-AuNps, This suggests their potential use as fluorescence sensor for amino acids as well as for selective signalling of Cu 2+ ions in presence of other metal ions.

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