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Utilizing a tripeptide conjugated fluorescent hybrid nanoparticles as a fluorescence sensor for the determination of copper ions

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ABSTRACT

In this communication, the new, simple and sensitive fluorescent hybrid nanoparticles sensor has been designed for the copper ions detection. For this study, the fluorescent hybrid nanoparticles were synthesized by a single step approach and functionalized with tri peptide. And their size can range from about 70 to 75 nm in diameter. In the presence of copper ions, the imidazole rings of the histidine in tripeptide conjugated fluorescent hybrid nanoparticles can form six membered chelate rings complex, triggered energy transfer, and it leads fluorescence quenching. The results indicated that, minimum up to 0.5 μM of copper ion caused the fluorescence quenching with excellent selectivity response over other interferences at the micro gram level and the quenching kinetics also discussed.

1. Introduction

In recent years, fluorescent silica nanoparticles have emerged as attractive nanomaterial for diverse range of biological and biomedical applications. The properties of silica-coated fluorescent nanoparticles, such as biocompatibility, easy preparation, and less aggregation, make them very attractive [1–8]. The polymer mediated hybrid nanoparticles preparations have some unique advantages because of the flexibility in the control of chemical compositions and characteristics of the nanoparticles. Peptides are well known to be very effective specific ligand for a variety of metal ions, because they contained variety of donor atoms [9–15]. The implementation of fluorescent sensing materials based small sensors play an important role in chemistry, biology, clinical biology and environmental science. Recently large variety of nanoparticles probes containing covalently linked dyes have been developed by researchers, and it includes fluorophores attached silica, poly-(methyl)styrene, and poly(ethylene glycol) (PEG) nanoparticles. Copper is an essential element for the formation of hemoglobin, red blood cells and bones of the human system. At the same time, the over dose of copper ions leads cellular toxicity, promotes free radicals generations and it leads Alzheimer's disease. Past few years, variety of methods has been developed by researcher for the quantification of copper ions, these include voltammetry, chemiluminescence, atomic absorption spectroscopy, ICP mass spectroscopy, potentiometry, and absorption spectro photometry based detections [16–24]. All of these methods are time consuming, laborious and require specialized equipment. The physical absorption of fluorescent dye in the polymer matrix produced inhomogeneity in the materials, reduced the life time and affects the reproducibility. To overcome these problems, the single step polymer–silica hybrid network was reported in this work, and then the organic fluorescent dye covalently attached with polymer silica hybrid network. So the dye leaching effect was completely avoided in this work. For the selective copper ion detections, the synthesized nanoparticles were functionalized with selective tripeptide ligand (Gly–Gly–His). The characteristic of the synthesized and tripeptide conjugated nanoparticles were studied by using atomic force microscopy (AFM), Fourier transform infrared (FTIR) spectroscopy and a spectrofluorometer. The peptide conjugated fluorescent hybrid nanoparticles sensor exhibited the selective fluorescence quenching via the energy transfer from the peptide which formed six membered chelate rings complex with copper ion, and the quenching kinetics was studied by Stern–Volmer equation. The fluorescent hybrid nanoparticles sensor preparation method was new, simple and it may find applications in copper ion detection in the biological and environmental testing.

2. Materials and methods

For the preparation of fluorescent hybrid nanoparticles, 20 mL of ethanol, 10 mL of 2 wt.% polyvinylpyrrolidone solution, 1 mL of tetraethyl orthosilicate was taken. And this solution was heated at 80 °C for 60 min with stirring. After that, the flask was allowed to cool, and then, 5 mL of 6×10^{-3} mol of Tris (2,2′-bipyridyl)

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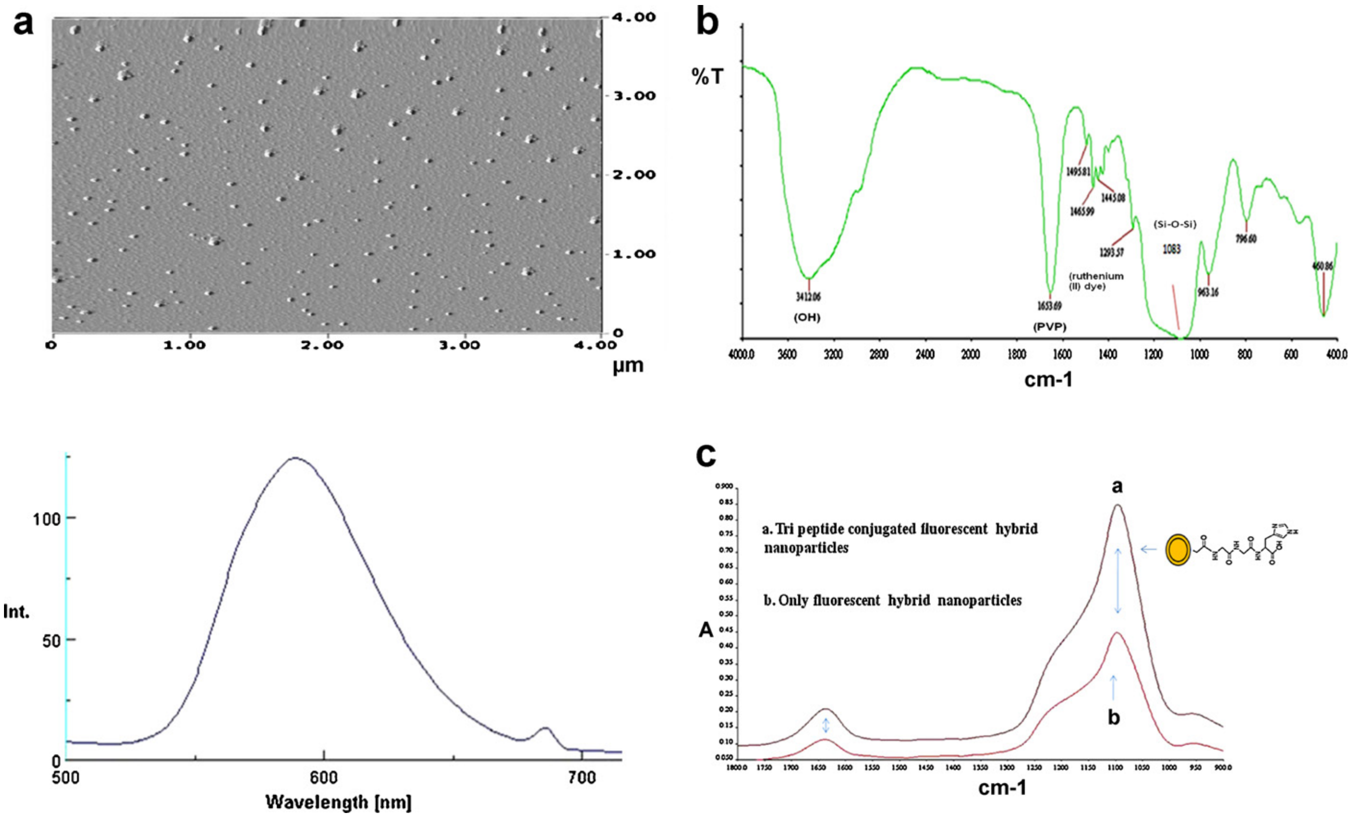

Fig. 1. (a) Particle size measurements and emission spectra of the synthesized nanoparticles. (b) Fourier transform infrared spectroscopy (FTIR) spectra confirmation of nanoparticles. (c) Fourier transform infrared spectroscopy (FTIR) spectra based confirmation of peptide conjugation on nanoparticles.

dichlororuthenium (II) hexa hydrate, 1 mL of ammonium hydroxide solution was added. This solution was stirred for another 3 h at room temperature. Thereafter, the particles were collected by using centrifugation at 6000 rpm for 10 min and then 0.2 mL of amino propyl triethoxy silane (APTES) was added and the reaction was allowed to continue for another 3 h. For peptide conjugation, 5 mL of amine-modified nanoparticles, 5 mL of 1×10^{-3} mol *N*-hydroxy succinimide, 5 mL of 8×10^{-4} mol 1-ethyl-(dimethylaminopropyl) carbodiimide and 5 mL of 1.11 mg/mL tri peptide solutions were taken and stirred for 2 h at room temperature. After that, the particles were collected by using centrifugation at 8000 rpm for 5 min. For the copper ion detection, 50 mg/mL of peptide conjugated fluorescent hybrid nanoparticles were taken in eppendrof tube, centrifuged at 6000 rpm for 5 min, and then 50 mM aqueous ammonium acetate buffer solution (pH 7.0) at various concentrations of copper ions were added, mixed for 15 min at room temperature. And then, the fluorescence intensity was measured with 1.0 cm quartz cells of the F-4500 spectrofluorometer. The excitation and emission wavelength were set at 455 nm and 600 nm, respectively. The slits of excitation and emission were set at 5.0 nm.

3. Results and discussion

For the fluorescent hybrid nanoparticles synthesis, the single step approach was developed based on complexes of polyvinylpyrrolidone, tetraethyl orthosilicate with organic fluorescent dye molecules. The hybrid fluorescent nanoparticles showed high fluorescent intensity and stability, the sizes of the synthesized nanoparticles were confirmed by using atomic force microscopy, and it was shown in Fig. 1a, based on that, the fluorescent hybrid nanoparticles exhibited the size around 70–75 nm. To examine the fluorescence property of these particles, the nanoparticles solution was excited at 455 nm and emission of fluorescence was observed at 590 nm and it was shown in Fig. 1a. The Fourier transform infrared spectroscopy (FTIR) of the nanoparticles were shown in Fig. 1b, the nanoparticles exhibited characteristic bands around 3412.06 cm^{-1} (OH), 1653.69 cm^{-1} (PVP), 1294.37 cm^{-1} (ruthenium (II) dye), 1083.48 cm^{-1} (Si—O—Si). The results indicated that, the hybrid network was successfully coated with ruthenium (II) dye. For tripeptide labeling, the surface chemistry of the nanoparticles were changed by the introduction of aminopropyl functionalities using 3-aminopropyltriethoxysilane (APTS). Subsequently, the peptide was mobilized on to the surface of the nanoparticles using the EDC-NHS method. The FTIR is a powerful tool for peptide conformation, the secondary structure of peptides can be derived from the analysis of the strong amide I band. And the analysis was also applied to monitor structural modification of peptides with a relative decrease of the unordered structure, turns and a substantial increase of a-helix. So the peptide conjugation on nanoparticles surface was confirmed by using FTIR in MID-IR region, and the results were displayed in Fig. 1c, based on that, the peptide coated fluorescent hybrid nanoparticles showed the peak shift around on amide (1645 cm^{-1}) as well as PO_2 stretching region (1100 cm^{-1}). The different concentrations effect of copper ion in standard fluorescence of tripeptide modified fluorescent hybrid nanoparticles were studied using spectrofluorometer. The reaction scheme was shown in Fig. 2a. The imidazole ring of the histidine in tripeptide conjugated fluorescent hybrid nanoparticles to form six membered chelate rings with copper ions. The binding between the copper ions with tripeptide, induced the energy transfer from the fluorescent hybrid nanoparticles to the copper–tripeptide complexes and it may leads selective fluorescence quenching [fluorescent hybrid nanoparticles–Gly–Gly–His + Cu^{2+} (solution) ↔ fluorescent hybrid nanoparticles–Gly–Gly–His–Cu^{2+}], it was shown in Fig. 2b. The fluorescence quenching kinetics were studied by Stern–Volmer equation $F_0/F = 1 + K_{SV}[Q]$, F_0 and F are the fluorescence intensities