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Synthesis of phthalocyanine stabilized rhodium nanoparticles and their application in biosensing of cytochrome c

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ABSTRACT

A single step synthesis route is described for the preparation of rhodium nanoparticles using a cobalt aminophthalocyanine macrocyclic complex as a stabilizer. The results of nanoparticles characterization using electronic absorption, Raman and X-ray spectroscopes as well as transmission electron microscopy are reported. Rhodium nanoparticle modified electrode behavior as examined by cyclic and differential pulse voltammetry is also provided. The nanoparticles were found to be well dispersed and stabilized throughout the macromolecular matrix. TEM studies showed that they have an average diameter of 3 to 5 nm with spherical shape. The colloidal rhodium was then used for electrochemical sensing of cytochrome c using glassy carbon electrode. The results showed that the colloidal rhodium nanoparticles enhanced the electron transfer process between cytochrome c and the electrode. Differential pulse voltammetric measurements of cytochrome c at the colloidal rhodium nanoparticles modified glassy carbon electrode showed a linear relationship with the oxidation peak currents in the concentration range of 100 nM to 3 μM of cytochrome c.

1. Introduction

Since the boom of nanotechnology, macrocycle-metal nanoparticles have achieved a great deal of attention from the researchers, not only because they can be prepared easily by chemical reduction, but also due to their important role in the construction of nanostructured materials for novel technologies. These nanostructured materials can also be used as quantum dots for understanding the quantum size effects [1,2]. Apart from this, they have wide application in catalysis, nanoscale electronics, and magnetic storage [3–6]. The transition metal nanoclusters also serve as a bridge between homogeneous and heterogeneous catalysts and provide new opportunities for catalysis [7]. They are not only active and selective catalysts for various organic reactions but also for electrocatalysis and sensing of biologically important molecules [8–13]. Generally, transition metal nanoclusters with small particle size have high catalytic activity, good light transparency and obvious size dependent properties. The high ratio of surface atoms with free valences to the cluster of total atoms gives rise to high catalytic activation, which has been used in electrochemical catalytic reactions [8]. Although nanoparticles of Au, Ag, Si, SiO_2, Ag, TiO_2 have been proven to be successful for preparation of biosensors [9–13], to explore other nanoparticles, which have good stability, catalytic activity and easy to synthesize, is still a challenge. To prepare stable metal colloidal nanoparticles with small particle size and narrow size distribution, protective agents such as surfactants, polymers or organic ligands were usually used.

There has been a significant amount of research describing the synthesis of metal nanoparticles of the late transition metal nanoparticles in recent years [14]. However, much of this work has focused on synthesizing nanoparticles of Au, Ag, Co, Pd and Pt. A search of the literature confirms that the synthesis of Rh nanoparticles and utilization as biosensor has received much less attention. Because of their potential use as catalysts [4], and interesting optical and magnetic properties [15,16], we were interested to prepare colloidal rhodium nanoparticles with a suitable capping agent. The ligand or capping agent plays a major role in the stabilization of the nanoparticles and can enhance catalytic and sensing properties.

Phthalocyanine macrocycles possess structures similar to chlorophyll and haemoglobin [17]. The structure is highly versatile and can be tuned based on chemical modifications on the ring as well as in the central core [17]. These macrocyclic complexes can act as stabilizing agents for metal nanoparticles. The stabilizers play important roles in not only protecting the nanoparticles but also controlling their functions. The functionalized metal nanoparticles may have improved catalytic and optical properties. As for the use of phthalocyanines

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as capping agents for metal nanoparticles is concerned, there are only limited reports available in the literature [18–22]. Only thiol functionalized phthalocyanines have been used as capping agents to prepare uniformly distributed gold and titanium dioxide nanoparticles and further used as photosensitizers and in photovoltaic applications [18,19].

Heme proteins are important in living cells, which contain the porphyrin complex of iron (II) hemein or iron (III) hemein as a prosthetic group. These proteins perform different physiological functions in the biological system [23]. Electrochemical studies of these proteins have attracted considerable attention on classical as well as nanoparticle modified electrodes [11–13,24–28], since fundamental studies of these proteins can provide, insight into physiological electron transfer processes as well as impetus for the further development of amperometric biosensors and bioelectrocatalytic systems [29]. Horse heart cytochrome c is a simple metalloprotein which is an ideal model for studying biological electron transfer processes. It is difficult for cytochrome c to undergo a facile redox process at conventional electrodes due to the insulated peptide backbone and the protein on adsorbing onto the electrode surface, it may undergo denaturation and loss of both electrochemical and bioactivity. However, direct electrochemistry of redox proteins has great significance as it establishes a model for the mechanistic study of electron exchange among proteins in biological systems and provides a foundation for fabrication of the third-generation biosensors. Nanomaterials are commensurate in size to proteins, and multivalent functionalization holds great promise for controlling biomolecular recognition when they interact with each other [30]. These nanoparticles can act as tiny conduction centers and can facilitate the transfer of electrons. Since Rh nanoparticles are known to have good electrocatalytic properties [4] and the capping agent phthalocyanine macrocycle is having a similar structure like that of cytochrome c which will be highly compatible for its stability and activity, we have used phthalocyanine stabilized rhodium nanoparticles in the electrocatalytic studies of cytochrome c.

2. Experimental details

2.1. Materials

Rhodium chloride ($RhCl_3$), dimethyl sulfoxide (DMSO, 98%), sodium borohydride ($NaBH_4$), sodium sulphide nonahydrate ($Na_2S{\cdot}9H_2O$) were analytical grade reagents purchased from Ranbaxy Chemicals, India. Tetraaminocobaltphthalocyanine (CoPTA) was prepared using a reported procedure [31]. Briefly, slurry of cobalt tetranitro phthalocyanine in water was reduced using sodium sulphide nonahydrate at 50 °C with stirring for 5 h. The bluish green residue is purified and characterized using elemental analysis, UV–Visible spectrum and FT-IR spectroscopy before using it for the preparation of colloidal rhodium nanoparticles (CRN) as capping agent.

2.2. Preparation of colloidal rhodium nanoparticles (CRN)

Rhodium nanoparticles were prepared by a facile route modifying the Brust method in a single step process [32–33]. To a constantly stirred 5 mL 0.025 mM solution of CoPTA in DMSO at 15 °C, 5 mL of 0.05 mM $RhCl_3$ in DMSO was added.

Then, a freshly prepared 5 mL of 0.5 mM sodium borohydride ($NaBH_4$) in water was added in a lot. The color of the solution changed immediately to brownish black. The colloidal solution was stirred for another 30 min at the same temperature. Later on, color of the solution turned brownish and is found to be stable for a long time without undergoing any aggregation or settling in solution. The nanoparticles were subsequently extracted by repeated washing, centrifuging and evaporation.

2.3. Electrode modification procedures

The glassy carbon electrode was mechanically polished to a mirror finish with 0.05 μm alumina, rinsed with doubly distilled deionized water to remove any trapped alumina by ultrasonic waves. Then the modified glassy carbon electrode was fabricated by drop-coating 5 μL CRN dispersed in ethanol onto the electrode surface. The modified electrode was dried in air. It was rinsed with distilled water to remove excess of CRN on the electrode surface.

2.4. Characterization

The UV–Visible spectra were recorded using a Perkin–Elmer model lambda 35 spectrophotometer. UV–Visible absorption spectra were recorded for the nanoparticles prepared freshly using DMSO as blank. The spectra were recorded in the region 300 to 900 nm at a scan rate of 240 nm/min. The samples were prepared by dissolving 1 mg of the nanoparticle in 5 mL DMSO. The FT-Raman measurements were carried out using a Renishaw Raman imaging microscope (WiRE ™ – V1.3). The laser power source was kept at 40 mW and the excitation wavelength used was 532 nm. The Raman spectrum was recorded in the region 200–2000 cm^{-1} for the rhodium nanoparticles. Transmission electron micrographs (TEM) were obtained using a TECNAI electron microscope at an operating voltage of 20 kV. The phthalocyanine stabilized rhodium nanoparticles were characterized using TEM. The colloidal nanoparticles dispersed in DMSO were spread on a Formvar/carbon-coated copper grid, dried and used for taking TEM images, diffraction pattern and small area diffraction. For XRD measurement, the nanoparticles were powdered and spread uniformly on slides. X-ray diffraction spectra were recorded at a temperature of 298 K using a Philips PW 1050/37 model diffractometer, operating at 40 kV and 30 mA. Cu Kα radiation with a wavelength of 1.54178 and a step size of 0.020 in the 2θ range, 5–70° was used. The cytochrome c solutions were prepared in phosphate buffer of pH 7.0 and diluted according to the needs. The stock solution was stored in refrigerator at 4 °C. The cyclic voltammetry and differential pulse voltammetry experiments for redox behavior and sensing were carried out using an electrochemical analyzer (CH Instruments, USA), in a three electrode cell system with saturated calomel electrode as reference, glassy carbon electrode modified with 5 μL of CRN in ethanol as working and platinum foil as counter electrode. The electrochemical experiments were performed in phosphate buffer of pH 7 at a scan rate of 50 mV/s and at different scan rates of 10, 20, 25, 100, 150 and 200 mV/s. The differential pulse voltammetry was performed for different loads of CRN on glassy carbon electrode and a relationship between the amount of CRN onto the glassy carbon electrode surface and the peak current of 0.5 μM cytochrome c has been examined at a scan rate of 50 mV/s.

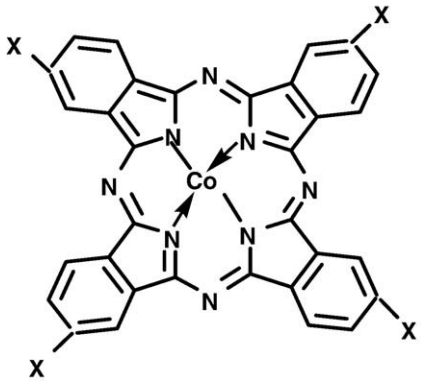

Fig. 1. Structure of cobalt tetraamino phthalocyanine (CoPTA), where X= NH2.