Dye fluorescence quenching by newly synthesized silver nanoparticles

Ghasem Rezanejade Bardajee*, Zari Hooshyar, Mohaddeseh Khanjari

Department of Chemistry, Payame Noor University, PO Box 19395-3697, Tehran, Iran

ABSTRACT

New types of silver nanoparticles (Ag NPs) were prepared by the reduction of silver trifluoroacetate with diamine-sarcophagine (DiAmsar) in DMF solution. Nanoparticles growth, morphology, and chemical composition were determined by ultraviolet–visible spectroscopy, transmission electron microscopy, and Fourier transform infrared spectroscopy, respectively. The effect of prepared Ag NPs on the fluorescence quenching of the dyes rhodamine 6G and 4-hydroxycoumarin were investigated in different temperatures. The results of the quenching experiments were analyzed using Stern–Volmer equation. The Stern–Volmer constant values showed that the quenching efficiencies decreased as the temperature increases indicating that the quenching process was a dynamic-type quenching. Moreover, the energy transfer behavior between Ag NPs and dye molecules were interrogated using Förster/fluorescence resonance energy transfer and nanomaterial surface energy transfer models. The anti-bacterial activities of Ag NPs were assessed against the *Staphylococcus aureus* and *Escherichia coli* bacteria. Results showed that the entitled Ag NPs have excellent antibacterial properties.

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1. Introduction

Fluorescence quenching is one of the most commonly powerful techniques used in chemical, biological and medical sciences because of its useful information on the partition and mobility of small molecules in the micro phases of the organized systems and also on the properties (micropolarity and microviscosity) of the systems in the surroundings of the probe and the quencher [1–6]. It refers to any process which fluorescence intensity of the solute decreases by variety of molecular interactions such as excited state reactions, molecular rearrangements, energy transfer, ground-state complex formation (static quenching) or collisional interactions (dynamic quenching). In this regard, fluorescence quenching depends on the nature of fluorophore, quencher molecule, polarity of the solvent medium, and range of quencher concentration.

Nowadays, with the development of nanotechnology, fluorescence quenching of laser dyes (e.g. coumarins and rhodamines) by noble metal nanoparticles (NPs) have attracted great attention in biophotonics as well as in material science [7–10]. In the close proximity of metal NPs, it has been reported that the quenching of the fluorescence intensity of dye can be attributed to the reduction in ratio of the radiative to nonradiative decay rate and the quantum yield of the dye due to the dipole energy around the NPs. In general,

in interaction between a dye and a metal NP, metal NP as a universal energy acceptor can interact with dye as an energy donor, leading to energy transfer process in a way similar to that occurs during Förster/fluorescence resonance energy transfer (FRET) [11,12]. However, several theoretical and experimental studies have been explained the energy transfer between a dye molecule and a metal NP to be more appropriate by nanomaterial surface energy transfer (NSET) [13,14]. The main differences between FRET and NSET mechanisms are in the physical bases and the distance between the NP and the dye molecule. In comparison to the dipole–dipole interaction in FRET, the metal–dye interaction has been considered as the interaction between a dipole and a metal surface in NSET. The distance in FRET mechanism follows R^{-6} (<100 Å), whereas in NSET mechanism follows R^{-4} (~220 Å). Thus, these mechanisms can act as a spectroscopic ruler around the NPs. The efficiency of these mechanisms strongly depends on the several factors such as the spectral overlap between the spectrum of dye emission and the absorption spectrum of the NP, the desirable orientation of the dye and NP' dipoles, size and shape of the NPs. Therefore, the synthesis of NPs and investigation on their interaction with dyes are of great importance for both fundamental research and technical applications [8,10].

Among noble metal NPs, silver (Ag) NPs are extensively used to alter the emission behavior of a dye molecule because their plasmon resonance bands are located in the visible range of the spectrum and match the absorption and emission bands of dyes [15–17,11]. Moreover, their high compatibility and stability with biomoloecules, strong affinity for thiols, antibacterial activity toward germs without release of toxic biocides and exceptional
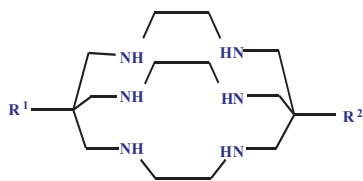
Fig. 1. Molecular structure of some sarcophagine derivatives.

physico-chemical properties offer excellent prospects for biological sensing and various medical applications [18,19]. The most popular approach for the preparation of Ag NPs has been chemical reduction using variety of organic and inorganic reducing agents, electrochemical techniques, physicochemical reduction, and radiolysis [20–26]. In these methods, to control the size of Ag NPs, stabilizers such as alkylthiols, alkylamines, fatty acids, carbon disulfide, and polymers have been generally required to prepare Ag NPs with appropriate sizes. However, most of these methods have difficulty to apply to large-scale synthesis, owing to its highly diluted and exothermic condition. Moreover, the derived Ag NPs with the above methods are hard to be dissolved in organic solvents, which limit their applications.

To overcome these problems, the choice of suitable stabilizer is a key step for synthesis of Ag NPs. It is thought that the cage-type ligands derived from 3,6,10,13,16,19-hexaazabicyclo[6.6.6]icosane (commonly called sarcophagines, Fig. 1) can be a good choice to this aim. Main advantage of these cage-like frameworks is the preservation of chromophore and redox characteristics of the metal in a range of chemical environments [27,28]. This cage was first synthesized by Sargeson and co-workers in the 1970 using the cobalt (III) ion as a template to hold the reacting organic fragments [29]. After that, considerable efforts have been made to encapsulate transition metal ions into sarcophagines. However, the obtained Co (III) complexes are extremely stable both kinetically and thermodynamically. Therefore, the metal ion may be effectively removed with either cyanide or concentrated HBr. After the isolation of the free ligand, other metal ions including Cu(II), Ni(II), Co(II), Fe(II), Mn(II), Mg(II), Zn(II), Cd(II), Hg(II), Cr(III), Ga(III), In(III), V(II), Ag(II), Re(I), Ru(II), Rh(II), Ir(III) and Pt(II) have been encapsulated within the sarcophagine cage [30–32]. The transition metal complexes of sarcophagines are expected to find various specific applications in molecular sensors, electron transfer agents, and radiopharmaceuticals [33,34].

In this work, for the first time, we use diamine-sarcophagine (DiAmsar) for the synthesis of Ag NPs at room temperature in organic medium. DiAmsar was chosen as a reducing agent as well as capping agent because of the presence of six nitrogen atom donors into its cage-like framework. The resulting Ag NPs were characterized by ultraviolet–visible (UV–vis) spectroscopy, transmission electron microscopy (TEM), and Fourier transform infrared spectroscopy (FT-IR). Furthermore, the effects of Ag NPs on the bacterial activity of *Staphylococcus aureus* and *Escherichia coli* were examined. Also, fluorescence intensity of rhodamine 6G

(Rh 6G) and 4-hydroxycoumarin (4HC) were investigated. The observed results and the probable explanations are detailed in this paper.

2. Experimental

2.1. 2.1. Materials

Double-distilled water was used when necessary. Silver trifluoroacetate, acetonitrile, *N,N*-dimethylformamide (DMF), tetrahydrofuran (THF), ethanol, tri (ethylenediamine) cobalt (III) chloride dehydrate, formaldehyde, sodium hydroxide, hydrochloric acid, methanol, stannous chloride dihydrate, hydrochloric acid, sodium hydroxide, cobalt (II) chloride, sodium cyanide, rhodamine 6G (Rh 6G), 4-hydroxycoumarin (4HC), and acetonitrile were obtained from Acros, Merck and Sigma–Aldrich Chemicals and used without further purification.

2.2. Instrumentation

TEM observation was performed with a Hitachi H-700 CTEM. FT-IR spectra were recorded on KBr pellets by a Jasco 4200 FT-IR spectrophotometer. UV–vis spectra of samples were collected by a Shimadzu UV–visible 1650 PC spectrophotometer from 200 to 800 nm with 1.00 cm path length quartz cuvettes. Fluorescence measurements were performed on a SCINCO's fluorescence spectrometer FluoroMate FS-2. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 400 spectrometer and a Bruker Avance III spectrometer, respectively with deuterium oxide as solvent. Chemical shifts reported are referenced against the deuterated solvent used.

2.3. Preparation of DiAmsar

Multi-step processes are needed for the synthesis of DiAmsar which are outlined as followed (shown in Scheme 1a–c) [27,29]:

(A) [Co-(NO$_2$)$_2$-sar]$^{3+}$,Cl$_3$: the complex of tri (ethylenediamine) cobalt (III) chloride dihydrate (1 eqiv., 2 g) was dissolved in a mixture of water (3 mL) and aqueous formaldehyde 37% (10.4 eqiv., 4.5 mL). Then, nitromethane (3.45 eqiv., 1.06 mL) was added to the solution. The resulting solution was cooled at 4 °C in an ice-water bath. Aqueous sodium hydroxide (4 M, 3.45 eqiv., 5 mL) was cooled to 4 °C and mixed with the resulting solution above. The combined solution was stirred magnetically for 90 min in ice-water bath while maintaining the temperature at 4 °C. The mixture rapidly turned deep brown from the initially orange color. After 90 min, the reaction temperature was allowed to come to room temperature. At the end, the reaction was quenched by the addition of concentrated hydrochloric acid (HCl 37%, 60 mmol, 5 mL). The orange precipitate was collected by filtration after cooling on ice for 90 min, and washed with methanol. Then, the orange powder was dried at room temperature. (^{1}H NMR (400 MHz, D$_2$O) δ (ppm): 2.94–2.96 (d, J = 8 Hz, 6H, −NH−CH$_2$−CH$_2$−NH−), 3.36–3.40 (d, J = 11 Hz, 6H, −NH−CH$_2$−C−NO$_2$), 3.58–3.60 (d, J = 8 Hz, 6H, −NH−CH$_2$−CH$_2$−NH−), 3.89–3.93 (d, J = 11 Hz, 6H, −NH−CH$_2$−C−NO$_2$). ^{13}C NMR (400 MHz, D$_2$O) δ (ppm): 51.28, 54.62, 87.53. HRMS (EI, *m/z*) calcd. for C$_{14}$H$_{31}$CoN$_8$O$_4$ 431.3565 [M−3 HCl]$^+$, Found 431.2689).

(B) [Co-(NH$_3$)$_2$-sar]$^{5+}$,Cl$_5$: The stannous chloride dihydrate (13 eqiv., 3 g) was introduced into a round bottom flask under nitrogen atmosphere. Then, concentrated hydrochloric acid (37%, 145 eqiv., 12 mL) was added followed by the addition of ethanol (6 mL). The resulting solution was stirred magnetically and heated to 70 °C under nitrogen until the