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Evaluation of interparticle interaction between colloidal Ag nanoparticles coated with trisodium citrate and safranine by using FRET: Spectroscopic and mechanistic approach

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ABSTRACT

Current study employs fluorescence spectroscopy, UV–Vis absorbance spectroscopy, dynamic light scattering (DLS) and cyclic voltammetry (CV) to investigate the interaction of safranine dye with spherical shaped silver nanoparticles (AgNPs) coated with trisodium citrate. In fluorescence spectroscopic study we used the AgNPs and safranine dye as component molecules for the construction of FRET, whereas AgNPs serve as donor fluorophore and safranine as acceptor. The fluorescence quenching of AgNPs followed by sensitization of safranine occurs almost simultaneously by addition of safranine dye with different concentrations, indicating fluorescence energy transfer observed between them. Interaction between safranine and AgNPs is also confirmed by using UV–Vis absorption spectroscopy. Addition of safranine results in the significant decrease in the absorbance of AgNPs at 423 nm and simultaneous increase in the absorbance of safranine at 518, 276 and 248 nm which is indication of rapid binding of safranine molecules with AgNPs. However CV measurements reveals that the safranine molecule does not alter the redox properties of the AgNPs but the safranine molecule lose their redox properties upon getting bonded with AgNPs. This clearly confirms that the safranine molecules get attached on the surface of AgNPs which was also supported by the DLS as well as zeta potential measurement.

1. Introduction

Fluorescence is one of the most leading technology commonly used in life sciences because of its versatility and wide spread potential applications in the areas of luminescence tagging, imaging, medical, diagnostics, multiplexing and most recently in biosensors. The resonance energy transfer (RET) is a widely prevalent photophysical process, through which an electrically excited donor molecule transfers its excitation energy to an acceptor molecule such that the excited state life time of the donor decreases. If the donor is a fluorescent molecule, RET is referred to as FRET although the process is non-radiative where the acceptor may or may not be fluorescent. It is commonly used in biological research to measure the molecular distances or donor to acceptor proximity. It occurs through the dipole–dipole interaction between the excited donor (D) and an acceptor (A) molecule. Energy conservation requires that the energy gap between the ground state and excited states of participating donor and acceptor molecules are nearly the same. This in turn implies that the fluorescence emission spectrum of donor must overlap with absorption spectrum of acceptor and they should be within a minimal spatial range for donor to transfer its excitation energy to acceptor [1,2].

The nanoparticles such as Ag, Au have prominent absorption spectrum in the visible region and are employed as either the acceptor or as both donor and the acceptor [3,4]. Among the various metal nanoparticles, AgNPs have been widely investigated because they exhibit unusual optical, electronic and chemical properties, depending on their size and shape with respect to technological applications [5]. The metal enhanced fluorescence (MEF) have attracted significant interest due to the widespread use of molecular fluorescence based measurements and devices in chemistry, molecular biology, material science, photonics and medicine [6,7]. However, the strength of enhancement or quenching might be influenced by many factors such as size and shape of metal NPs and several other factors which are of interest to be explored. The interactions of fluorophores with metal nanoparticles results in fluorescence enhancement, increased photostability, decreased lifetimes because of increased rates of system radiative decay and increased transfer distances for FRET [8]. Resonance energy

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transfer (RET) systems consisting of organic dye molecules and noble metal nanoparticles have recently gained considerable interest in biophotonics [9] as well as in material science [10]. Several theoretical and experimental studies have been published on energy transfer from a dye to metal surface [11–13]. As a result of the recent findings, its seems to be crucial to investigate the interaction of dye with the medium at the molecular level which is reflected in its visible and fluorescence spectra. Most of previous researchers were focused on the absorption features of silver nanoparticles. In this paper, we have synthesized the stable colloidal solution consisting of monodisperse AgNPs in aqueous solution at room temperature (RT) with very high quality and these nanoparticles were characterized for size, optical properties. The quenching of AgNPs was followed by enhancement of safranine dye with isoemissive point at 540 nm to prove the Stern–Volmer relation. Also dynamic light scattering (DLS), zeta potential and CV measurements can be successfully used to study the spectroscopic interaction between safranine (Fig. 1) and AgNPs for the first time.

2. Experimental section

2.1. Chemicals

Safranine and high quality silver nitrate was procured from Hi Media and S.D. Fine Chem. Limited, Mumbai, respectively. The doubly distilled water was used for preparing the solutions. All spectroscopic measurements were performed at room temperature.

2.2. Instrumentation

The spectroscopic analysis were carried out using the stable dispersion. The UV–Vis absorption spectra of AgNPs were recorded at RT using UV–VIS–NIR spectrophotometer (Shimadzu Model-UV 3600). All fluorescence measurements were recorded with FP-750 spectrofluorimetric (Jasco, Model-F.P.-750, Japan) equipped with 1 cm quartz cell. The X-ray powder diffraction (XRD) pattern was recorded using Material Analysis and Characterization (MAC) advanced powder X-ray diffractometer with Cu Kα radiation ($\lambda = 1.54056$ Å) in 2θ ranging from 20–90°. The particle size distribution and zeta potential of AgNPs in aqueous suspension was measured by dynamic light scattering with a Zetasizer Nano ZS (Malvern Instruments Ltd., UK). Cyclic voltammeter (CV) measurements were performed with model Princeton Applied Research Potentiostat 263 AEG & G.

2.3. Synthesis of silver nanoparticles (AgNPs)

Silver nitrate was used as precursor of silver to prepare AgNPs in colloidal solution. The silver colloid was prepared by using reported literature chemical reduction method by Lee and Meisel [14]. In a typical synthesis procedure, an aqueous solution containing 50 ml of 1×10^{-3} M Silver nitrate ($AgNO_3$) which was heated at 85 °C for 12–15 min. To this warm solution 3 ml of 1% trisodium citrate ($Na_3C_6H_5O_7$) was added drop by drop and the solution was mixed vigorously for half an hour. Solution was heated until color's change (pale yellow) is evident. The solution of silver nitrate turns pale yellow indicating the formation of AgNPs. Trisodium citrate acts as both reducing as well as stabilizing agent (Scheme 1). AgNPs synthesized by any physical means require expensive equipments, high temperature, vacuum condition however chemical methods are most convenient and reproducible. All solutions of reacting materials were prepared in doubly distilled water.

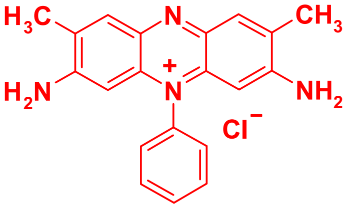

Fig. 1. Structure of safranine.

2.4. Fluorimetric titration

A 3 ml solution containing appropriate concentration of Ag NPs (5×10^{-5} M) in a quartz cell, was titrated by successive addition of (0–1.8 ml) 5×10^{-5} M stock solution of safranine. The fluorescence emission spectra were measured in the range of 450–650 nm at $\lambda_{ex} = 260$ nm at R.T. with slit width maintained at 10 nm.

3. Characterization of colloidal AgNPs

It is known that the nanoparticles shape, size and morphology govern its properties and thus its characterization is important for understanding and co-relating the results. Thus, synthesized colloidal AgNPs were characterized by following methods.

3.1. UV–Vis absorption spectra

UV–Vis absorption spectra can be used to confirm the formation of silver colloid nanoparticles which is prepared by chemical reduction method. In the present study citrate stabilized AgNPs show intense absorption peak also known as surface plasmons resonance, at $\lambda_{max} = 423$ nm confirming the formation of AgNPs (Fig. 2).

3.2. XRD characterization of AgNPs

The colloidal AgNPs drop coated on glass substrate were used for XRD analysis. The XRD spectrum (Fig. 3) showed that the peaks corresponding to hkl planes [1 1 1], [2 0 0], [2 2 0], [3 1 1], and [2 2 2] are characteristic of fcc AgNPs which is in agreement with the JCPDS card 03-0921. The average particle size estimated from Scherrer's equation was found to be 22 nm.

3.3. Dynamic light scattering measurements

Dynamic light scattering (DLS) experiments was also carried out to measure particle size of AgNPs in the suspension. Fig. 4 shows the typical size and size distribution of synthesized colloidal AgNPs measured by DLS. The average size of AgNPs is determined by DLS found to be 21 nm and size distribution was obtained within the range of 15–30 nm which is in good agreement with size calculated from Scherrer's equation. In order to confirm the effectiveness of the citrate capping, zeta (ζ) potential measurement was performed for AgNPs in order to characterize the surface charge of AgNPs as well as stability. The obtained ζ potential of the AgNPs was found to be −31.5 mV. For small enough nanoparticles, a high ζ potential will confer stability i.e. the solution or dispersion will resist aggregation. It means that nanoparticles remain stable in the colloidal suspension with trisodium citrate performing dual role as reducing as well as capping agent.