Preparation of a Novel Fluorescence Probe of Terbium Composite Nanoparticles and its Application in the Determination of Ascorbic Acid

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Abstract. Novel Tb/acetilacetone (acac)/poly (acrylic acid) (PAA) composite nanoparticles were successfully synthesized using the ultrasonic method. The nanoparticles are water soluble, stable and have extremely narrow emission bands and high internal quantum efficiencies. They were used as fluorescence probes in the determination of ascorbic acid (Vc) based on the fluorescence quenching of nanoparticles in the presence of ascorbic acid. The method is based on the complexation between ascorbic acid and nanoparticles in the absence of the oxidant. The influence of different buffers and concentration of the nanoparticles on the relative fluorescence intensity was tested, and Tris-HCl buffer solution proved to be the best. Under the optimum conditions, a liner calibration graph was obtained over the Vc concentration range of 0.05–10 μg mL\(^{-1}\). The corresponding detection limit is 0.008 μg mL\(^{-1}\) and the relative standard deviation is 1.4% for 0.5 μg mL\(^{-1}\) (n = 7). The method proved to be simple, rapid, and specific, and the recovery and relative standard deviation are very satisfactory. A mechanism explaining the process is also presented.

Key words: Composite nanoparticles; terbium; ascorbic acid; fluorescence.

Due to the importance of ascorbic acid (Vc) in human beings, the determination of Vc has gained increased significance in several areas of analytical chemistry such as in pharmaceutical, clinical and food applications. Numerous analytical procedures have been reported including spectrometry [1], chemiluminescence [2], HPLC [3], enzymatic methods [4], flow-injection spectrophotometry [5, 6] and amperometry [7]. Recently, fluorimetric determinations of Vc have been developed based on the condensation reactions of Vc with \(\alpha\)-phenylenediamine (OPDA) [8, 20], 2,3-diaminonaphthalene (DAN) [18] and 2-cyanoacetamide [8]. Each method has its advantages and disadvantages.

The narrow line luminescence of terbium ions makes them important components in energy-efficient optical devices [9]. Lanthanides in aqueous solution are known to be either non-fluorescent or weakly fluorescent due to their low molar absorptivities and poor quantum yields [10]. The problem due to low molar absorptivity is overcome by employing the technique of ligand-sensitized fluorescence. In this technique, the weakly fluorescent lanthanide ion is complexed with a ligand of higher molar absorptivity. Then the ligand is excited in its absorption band. Some of the excited energy from the ligand is transferred to the lanthanide ion by intramolecular energy transfer. Various ligands have been used to sensitize and enhance lanthanide fluorescence [11–13].
Since the first report on an EL device with Tb/acac as the emissive center, more and more work has been done with rare earth β-diketonate complexes [14–16]. The complex of Tb3+ with a β-diketone emits characteristic fluorescence with long lifetimes due to intramolecular energy transfer from the excited β-diketone to Tb3+. These ligands have several attractive features. For example, their steric and electronic properties can be readily tuned by an appropriate choice of starting materials used in their synthesis, and they can form π systems due to electron delocalization within the ligand. However, the fluorescence of lanthanide complexes in water is unstable, which limits its application.

In the last decades more and more attention has been paid to rare earth (RE) polymer complexes because they have both the rare earth ions’ and the polymers’ advantages of good luminescence and simple synthesis, respectively. In a polymer matrix, rare earth ions, especially Eu3+ and Tb3+, exhibit excellent fluorescence via the energy transfer from the organic ligand. They display a long emission lifetime in the millisecond time scale and high quantum yields even at room temperature.

In this paper, we used acetylacetone which has high absorptivity, and acrylic acid which has high polymerization as ligands. Under ultrasonic irradiation, we successfully synthesized Tb/acac/PAA composite nanoparticles. The synthesis of these nanoparticles led to a particularly simple, inexpensive and sensitive method. These nanoparticles are highly resistant to photobleaching and emit narrow and steady fluorescence, and were employed as a fluorescence probe for the determination of ascorbic acid (Vc). The method permitted a limit of detection of 0.008 μg mL−1 for Vc. The potential mechanism has also been studied.

**Experimental**

**Apparatus**

A VCX 500 (Sonic USA) ultrasonic processor was used in the ultrasonic synthesis of nanoparticles. Transmission electron microscopy (TEM) images of the nanoparticles were obtained using a Hitachi H-600 (Tokyo) transmission electron microscope. Colloidal solutions of the nanoparticles in water were dropped onto 50 Å thick carbon-coated copper grids with the excess solution being immediately whisked away. Fluorescence spectra and intensities were obtained using a Hitachi F-4500 (Tokyo) spectrofluorometer, which operates with a high-pressure xenon lamp and a quartz cell. The pH measurements were made with a model pHS-3C pH meter (Dazhong Analytical Instruments Factory, Shanghai, China).

**Reagents**

All chemicals were of analytical reagent grade. All aqueous solutions were prepared with doubly distilled water. Stock standard solution of ascorbic acid (Vc) (Biochemistry Institute, Beijing, China) was prepared by directly dissolving Vc in water at a final concentration of 100 μg mL−1 and storing in a brown vessel. Standard TbCl3 solution of 0.01 mol L−1 were prepared by dissolving terbium oxide (Tb2O3, 99.9%, Chemical Company, Shanghai, China) in 6 M hydrochloric acid, evaporating to near dryness and diluting to the mark. Acetylacetone (acac) was distilled just before use and prepared in ethyl alcohol. Acrylic acid (AA) and potassium persulfate (KSP) were acquired from Acros (http://www.alfa.com). The buffer solution was prepared by adjusting 0.1 mol L−1 catylytrimethyl ammonium bromide.

**Procedure**

The Tb/acac complex was prepared as follows [17]. The synthesis of the composite nanoparticles was carried out in a 1 L three-necked round-bottomed flask. 620 mL water, 13.0 mL of 0.01 mol L−1 TbCl3 and 10.0 mL polyethylene glycol were added into the flask, respectively. Under vigorous stirring, 6.5 mL of 0.06 mol L−1 acac was added to the mixture dropwise.

The optical character of the prepared nanoparticles is not very stable. Therefore, under vigorous stirring, 100 μL AA and 0.0004 g KSP were added into the flask dropwise, and the pH was adjusted to about 7.50 with 0.1 mol L−1 NaOH. The composite nanoparticles were prepared under ultrasonic irradiation within 30 min. The reaction system was flushed with nitrogen throughout.

The nanoparticles are water soluble and stable at room temperature for at least one month. The nanoparticles were used to detect Vc via changes in the fluorescence intensity of the system. The following procedure was adopted. To a 10 mL volumetric flask, 2.0 mL of buffer, a certain volume of nanoparticles, and an appropriate volume of Vc were added, the mixture was diluted to the volume with water and mixed before the fluorescence intensity was measured. The excitation wavelength was set at 317 nm and the emission wavelength at 549 nm.

**Results and Discussion**

**TEM Images of Nanoparticles**

TEM images of Tb/acac nanoparticles and Tb/acac/PAA composite nanoparticles are shown in Fig. 1. The average diameter of the Tb/acac nanoparticles is about 18 nm, and the average diameter of the composite nanoparticles is about 25 nm. In addition, the TEM images showed that the nanoparticles are homogeneously distributed and not aggregated.

**Reaction and Spectral Characteristics**

The reaction between nanoparticles and Vc at room temperature occurs rapidly within 20 min. As shown in Fig. 2, the system has characteristic peaks at 496 nm and 549 nm, and the fluorescence intensity is quenched by Vc. From Fig. 2 it can be seen that PAA