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Original Article

Functionalized $YVO_4:Eu^{3+}$ nanophosphors with desirable properties for biomedical applications

Tran Thu Huong [a, *], Ha Thi Phuong [a, b], Le Thi Vinh [a, c], Hoang Thi Khuyen [a], Tran Kim Anh [d], Le Quoc Minh [a, d]

[a] Institute of Materials Science, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay Distr., Hanoi, Viet Nam
[b] Department of Chemistry, Hanoi Medical University, Viet Nam
[c] Department of Chemistry, Hanoi University of Mining and Geology, Viet Nam
[d] Duy Tan University, K7/25 Quang Trung, Da Nang, Viet Nam

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ABSTRACT

Highly luminescent nanophosphors (NPs) containing rare earth (RE) ions were successfully prepared by careful control of nanosynthesis. The $YVO_4:Eu^{3+}$ NPs formed core/shell structures with sizes from 10 nm to 25 nm. The NPs were functionalized with biocompatible groups such as OH, NH_2 and SCN. A chemical coupling reaction connected the functionalized $YVO_4:Eu^{3+}$ NPs with Biotin via a direct reaction between the functional groups or an intermediate linker. Under UVIS excitation, $YVO_4:Eu^{3+}$ NPs exhibited strong red luminescence with narrow bands corresponding to the intra 4f transitions of $^5D_0-^7F_j$ ($j = 1, 2, 3, 4$) Eu^{3+}. The peaks were found at 594 nm ($^5D_0-^7F_1$), 619 nm ($^5D_0-^7F_2$), 652 nm ($^5D_0-^7F_3$) and 702 nm ($^5D_0-^7F_4$) with the strongest emission at 619 nm. The fluorescence intensity and stability of the functionalized $YVO_4:Eu^{3+}$ NPs have been increased. This is a promising result in sense of using the conjugates of $YVO_4:Eu^{3+}$ and a bioactive molecule, Biotin for the development of a fluorescent label tool in biomedical analysis.

1. Introduction

Detection analysis of biomolecules is crucial for many applications in biochemistry, molecular biology and medicine. Typical analytical methods such as fluorescent immunoassay (FIA) along with other, labeling and imaging techniques have thus been developed for decades. These detection techniques, however, are often limited by the optical (fluorescent) properties of the available probes. This has been one of the main motivations for the development of new probes, either for biomolecule labeling or detection of an intracellular signaling species.

Among the newly developed fluorecent probes, nanophosphors (NPs) containing rare earths have become of great interest in biochemistry, molecular biology and biomedicine applications because of their non-toxicity and strong luminescence properties [1–7]. There are several kinds of nanophosphors containing rare earth ions with high luminescent efficiency up to several tens of percent such as $YVO_4:Eu^{3+}$ nanoparticles [8–13], $LnPO_4 \cdot H_2O$: Eu, Tb nanomaterials [14–19] and $ZrO_2:Yb^{3+}$, Er^{3+} nanoparticles [20], which have been developed for molecular biology, agrobiological and medical applications.

In previous studies, there has been success in synthesizing nanorods of Tb^{3+}, Eu^{3+} ions [21,22] and nanoparticles of $YVO_4:Eu^{3+}$ [23,24]. The nanoscale and high-emission characteristics of these nanomaterials are more effective for ultrahigh sensitive fluorescent label for biomolecules, cell and tissue.

For the biological applications, surface functionalization of the nanomaterials is an important step. The objective is first to ensure good dispersion of the nanomaterials in biological media, that is, in water at neutral pH and at high ionic strength. Next, nanomaterials should contain specific organic or bioorganic groups which aim at targeting specific receptor sites, and/or ensuring innocuity in the case of in vivo experiments. In addition, the luminescence properties of functionalized nanomaterials should not be lost. Therefore, in this report, focus is paid on the surface functionalization of $YVO_4:Eu^{3+}$ NPs. Then, the compatibility of $YVO_4:Eu^{3+}$ nanomaterials with a biological system is investigated. The structure, morphology and

* Corresponding author.
E-mail addresses: tthuongims@gmail.com, huongtt@ims.vast.vn (T.T. Huong).
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luminescence properties of the functionalized $YVO_4:Eu^{3+}$ NPs have been studied by powder X-ray diffraction, field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM) and photoluminescence spectroscopy. The average size of the functionalized $YVO_4:Eu^{3+}$ nanophosphors are about 10–25 nm. The functionalized $YVO_4:Eu^{3+}$ NPs exhibit red luminescence with narrow bands corresponding to the intra 4f transitions of $^5D_0-^7F_j$ ($j = 1, 2, 3, 4$) Eu^{3+}.

To develop a new conjugate suitable for labeling we focused on some strong bioaffinity molecules and organisms such as biotin, protein IgG or bovine serum albumin (BSA). Based on the immune-reactions between antibody of the conjugate and antigen of virus/vaccine one can be detected by a fluorescence microscope and imaged by a digital camera. These results indicate that, the bioactive molecule linked nanoparicles $YVO_4:Eu^{3+}$ can be potentially applied in a variety of fields of application, especially in fluorescent labeling for biochemical and biomedical application.

2. Experimental

2.1. Synthesis of $YVO_4:Eu^{3+}$ nanophosphors

The $YVO_4:Eu^{3+}$ NPs were prepared by the controlling nano-synthesis method. In a typical synthesis, 0.55 g sodium orthovanadate Na_3VO_4 90% (Sigma–Aldrich) were completely dissolved in 50 ml H_2O. Subsequently, 0.91 g Yttrium (III) nitrate hexahydrate $Y(NO_3)_3 \cdot 6H_2O$ 99,8% (Sigma–Aldrich) and 0.13 g Europium (III) nitrate pentahydrate $Eu(NO_3)_3 \cdot 5H_2O$, 99,9% (Aldrich) were added to the solution in a 100 ml round-bottomed flask. This was followed by magnetic stirring for 60 min. Various pH values of the reaction solution were made in the range of 10–12 by using NaOH. After that, the reaction solution was transferred into an autoclave and heated at 200 °C for 1–24 h, and then cooled down slowly to room temperature. The resulting products were collected and centrifuged at 5900 rpm. The precipitate was washed several times in water and then dried in air at 60 °C for 6 h–24 h.

2.2. The primary silica shell as protecting layer

10 ml of Tetraethylorthosilicate (TEOS) (1/2) in absolute ethanol and 10 ml of as-synthesized $YVO_4:Eu^{3+}$ solution was mixed by magnetic stirring at room temperature for 24 h. The pH of this solution was adjusted to the range of 11–12 by adding NH_4OH. The resulting products were collected, centrifuged and cleaned several times with ethanol and distilled water. The final products were dried at 60 °C in 6h–24 h on air. The results experimented several times, showed good reproducibility.

2.3. The surface functionalization

Surface functionalization of materials with functional groups on their surfaces can be designed from substrates with standard bulk material properties. It is well-known that, the functionalization of the materials is a key step toward the aforementioned applications, since it determines the control of the coupling between the materials and the biological species of interest.

Functional silane compounds containing an organo-functional or organo-reactive arm can be used to conjugate biomolecules to inorganic substrates. The appropriate selection of the functional or reactive group for a particular application can allow the attachment of proteins, oligonucleotides, whole cells, organelles, or even tissue sections to substrates. The organosilanes used for these applications include functional or reactive groups such as hydroxyl, amino, aldehyde, epoxy, carboxylate, thiol, and even alkyl groups to bind molecules through hydrophobic interactions as discussed by [25].

3-Aminopropyltrimethoxysilane is among the most popular choices for creating a functional group on an inorganic surface or particle. This reagent contains a short organic 3-amino propyl group, which terminates in a primary amine. The 3-Aminopropyltrimethoxysilane reactive portion contains a trimethoxy group. Thus, the trimethoxy compound is more reactive and can be deposited on a substrate using 100 percent organic solvent without the presence of water to promote hydrolysis of the alkoxy groups prior to coupling. In this case, the organic solvent deposition processes described in the previous section can be used to covalently bond a layer of aminosilane to substrates. The advantage of this process is that a thinner, more controlled deposition of the silane can be made to create a monolayer of aminopropyl groups on the surface.

Isocyanate groups are extremely reactive toward nucleophiles and will hydrolyze rapidly in aqueous solution [25]. They are especially useful for covalent coupling to hydroxyl groups under non-aqueous conditions, which is appropriate for conjugation to many carbohydrate ligands. 3-(Triethoxysilyl) propylthiocyanate (TESCN) contains an isocyanate group at the end of a short propyl spacer, which is connected to the triethoxysilane group useful for attachment to inorganic substrates. Silanation can be accomplished in dry organic solvent to form reactive surfaces while preserving the activity of the isocyanates. An isocyanate reacts with amines to form isourea linkages and with hydroxyls to form carbamate (urethane) bonds.

Both reactions can take place in organic solvent to conjugate molecules to inorganic substrates. The solvent used for this reaction must be of high purity and should be dried using molecular sieves prior to adding the silane compound.

The functionalization of $YVO_4:Eu^{3+}$ nanophosphors with NH_2/SCN was performed by using 3-aminopropyltrimetoxysilane (APS) with -NH_2 group and 3-(Triethoxysilyl) propylthiocyanate (TESCN) with – SCN group, respectively. In these typical syntheses, 22.5 ml of absolute ethanol and 2 ml of APTMS (TESCN) were put in a 100 ml three-necked flask under magnetic stirring at room temperature for 30 min. The solution is heated up to 60 °C under reflux. Then, 5 ml of the $YVO_4:Eu^{3+}$ with silica shell nanomaterial solution at pH 7 is added drop wise. The reaction time is about 5 h. The solution is next gently stirred for 20 h. The resulting products were collected by three centrifugation/dispersion steps in a water/ethanol mixture (2:5, v/v). The final products were again washed with deionized water and then dried at 60 °C for 24 h in air.

2.4. Biotin binding with sol–gel functionalized nanophosphors

Coupling of the protein immunoglobulin to the -NH_2/-SCN groups functionalized nanomaterial, was achieved using the amine reactive linker glutaraldehyde by forming a thioure linker. The APS/TESCN treated $YVO_4:Eu^{3+}$ nanomaterials solution and glutaraldehyde were dispersed in vanadate buffered saline (PBS, 0.1 M, pH 5) with concentration of 5 gl^{-1}. The above solution is added to different concentrations of Biotin (Aldrich). These reaction mixtures were incubated at 30 °C for 4 h. The resulting products were collected, centrifuged at 5900 rpm, and washed several times by using ethanol/water and distilled water. The Biotin linked silica coated $YVO_4:Eu^{3+}$ SCN products were stored in closing box at 4 °C in a refrigerator.

3. Characterization methods

The morphology of the as-synthesized samples was observed by using field emission scanning electron microscopy (FE-SEM, Hitachi, S-4800) and transmission electron microscopy (TEM, JEM-1010). X-ray diffraction (XRD) measurements of the products