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Highly luminescent and long-term anti-photobleaching $Eu(TTA)_3(TOP)_3$ conjugated poly(St-*co*-DVB-*co*-NaSS-*co*-MAA) nanoparticles

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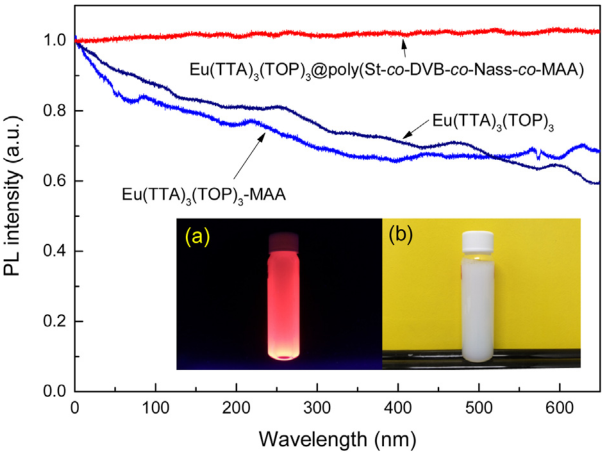
HIGHLIGHTS

- Highly luminescent and anti-photobleaching for a long period, Eu^{3+} complexes labelled polymer nanoparticles are synthesized.
- Highly monodispersed Poly(St-*co*-DVB-*co*-NaSS-*co*-MAA) nanoparticles with a diameter of 77 nm are synthesized.
- Coordination of carboxylic acid on $Eu(TTA)_3(TOP)_3$ complex shows a high anti-photobleaching effect for a long term.
- Eu doped latex nanoparticles show relatively low cytotoxicity in the concentration range of <100 µg/mL.

GRAPHICAL ABSTRACT



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ABSTRACT

Highly luminescent and stable against photobleaching for a long period, Eu^{3+} complexes labelled polymer nanoparticles with a quantum yield of 78% are presented. Europium complexes are prepared by chelating Eu^{3+} with 4,4,4-trifluoro-1-(2-Thienyl)-1,3-butanedione (TTA) and trioctylphosphine (TOP). Poly(St-*co*-DVB-*co*-NaSS-*co*-MAA) (St = styrene; DVB = divinylbenzene; NaSS; 4-styrenesulfonic acid sodium salt hydrate; MAA = methacrylic acid) with a diameter of 77 nm are modified with $Eu(TTA)_3(TOP)_3$. Scanning electron microscopy (SEM) results shows that $Eu(TTA)_3(TOP)_3$ modified Poly(St-*co*-DVB-*co*-NaSS-*co*-MAA) is monodispersed with a diameter of 83 nm. The intensity fractions of $^5D_0 \rightarrow {}^7F_2$–$^5D_0 \rightarrow {}^7F_1$ of EuNPs defines that Eu^{3+} could be existed as a more polarized structural states and comparatively stronger hypersensitive performance at $^5D_0 \rightarrow {}^7F_2$ transition. The results show that the role of MAA in this system is important to maintain the continuous polarization of Eu^{3+} ions against photobleaching because carboxylic group from MAA protects the dissociation of the symmetry around the Eu^{3+} ions due to the influence of pH or external light source. The cell viability of PC 12 cells shows that in the range of 10–100 µg/mL concentrations is more than 70%. Thus, EuNPs show relatively low cytotoxicity in the concentration range of <100 µg/mL and incubation times examined in this study.

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1. Introduction

Fluorescent particles are becoming increasingly applied for nanomedicine fields in cellular and molecular imaging and immunodiagnostic kits [1,2] because of their superior physiochemical properties such as strong photobleaching and high quantum yield. Red fluorescent organic nanoparticles with diameters from 60 to 100 nm were prepared with three cyano-substituted diarylethlene derivatives R-OMe (-H, $-CF_3$) with a surfactant Pluronic F127 for cell imaging application [3]. Mesoporous silica nanoparticles (MSNs) as a dual functional vesicles were reported by directly in cooperating aggregation-induced emission (AIE) materials of fluorogens (9,10-distyrylanthracene with alkoxyl end group) and a cationic surfactant cetyltrimethyl ammonium bromide as structure-oriented master and cytotoxic agent. It shows excellent properties both in cell imaging and cancer therapy [4,5]. Cy5 containing four primary amines are covalently encapsulated in fluorescent silica nanoparticles (FSNPs). They exhibit highly stable, good biocompatibility as well as low cytotoxicity and can be applicable in biological targeting and fluorescent cell imaging [6].

Especially, europium complexes show quite constant behaviors and biocompatible properties among Ln^{3+} based fluorescent complexes. Photoluminescence bioimaging with nanoparticles such as quantum/carbon dots and Au/Ag nanoparticles possesses prospective applications in biological studies [7] and clinical uses due to its highly delicate detection level [8]. Ln^{3+} based organometallic complexes can be used for photonics [9] and diagnostic agents due to their rather strong photoluminescence, quantum yield and good compatibility against aqueous phase. In addition, the final molecular structures of Ln^{3+} complexes can be modified with specific functional groups that could be used for covalently conjugation of defined biomolecules [10]. The photoluminescence of Ln^{3+} (Eu^{3+}, Sm^{3+}, Tb^{3+} and Dy^{3+}) can be drastically tuned by coordinating with specific organic molecules [11]. It is intensively investigated that Ln^{3+} complexes have unique optical emission spanning from UV to IR- near IR. The Ln^{3+} complexes have somewhat high photostability with a strong resistance against self-quenching effect. In this reason, europium based organometallic complexes show stable emission spectra with an exceptionally high fluorescence intensity under broad excitation wavelengths. The ultrasensitive properties of Eu^{3+} complexes could enable immunoassays with a detection level of sub pico gram per mL. Currently, Ln^{3+} complexes modified with carboxylic group form л conjugation shows strong energy absorption under UV irradiation. The carboxylic acid modified Ln^{3+} complexes have not only prominent emission spectra but also good photobleaching and physiochemical stability [12]. The polymer based Ln^{3+} complexes show good chemical stability, and can effectively form colloid without precipitation and prevent decomposition of ligand components [13].

The role of hydrophilic core materials [14] is particularly inevitable to induce the colloidal stability of the hydrophobic compounds, i.e. europium complexes, in water phase. In general, the core materials should be preferred as a monodispersed spherical particles with a narrow size distribution. Second, the refractive index (RI) of the core materials should be equivalent to dispersing solvents. Third, the zeta potentials related to surface charge of the core materials should be located in the range of ±30 mV to formulate the stable colloids in specific solvents. Based on those prerequisite, only few materials such as sulfonated latex nanoparticles, silica nanoparticles and polymethylmethacrylate (PMMA) etc. can be utilized as core materials. $Eu(TTA)_3Phen$ are directly incorporated in latex nanoparticles during the polymerization [15]. Yan et al. prepared europium complexes with vinyltrimethoxysilane and methacrylic acid to modify the surface of inorganic/organic nanoparticles [16]. However, the long term stability influenced by carboxylic acid is not investigated. $Eu(TTA)_3Phen$ is directly incorporated into several polymeric core materials [17].

In this work, it is first time report on highly luminescent and extremely long term anti-photobleaching fluorescent nanoparticles. The poly(St-*co*-DVB-*co*-NaSS-*co*-MAA) (St = styrene; DVB = divinylbenzene; NaSS; 4-styrenesulfonic acid sodium salt hydrate; MAA = methacrylic acid) nanoparticles with a diameter of 83 nm prepared by stepwise process of chelation of europium complexes. Europium complexes are prepared by chelating Eu^{3+} with 4,4,4-trifluoro-1-(2-Thienyl)-1,3-butanedione (TTA) and trioctylphosphine (TOP). The concentration of TOP is varied to change the coordination status in order to investigate the influence on PL intensity. The influence of carboxylic acid on long term photobleaching is also investigated. The present work thus differs from aforementioned reports both in long term stability study and influences of concentration of TOP in europium complexes, and in the breadth of the study.

2. Experiment details

2.1. Materials

Europium(III) chloride hexahydrate ($EuCl_3\cdot 6H_2O$) and 4-styrenesulfonic acid sodium salt hydrate ($H_2C{=}CHC_6H_4SO_3Na\cdot xH_2O$, NaSS) are purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Sodium bicarbonate (SBC) and ammonia solution (28.0%–30.0%) are purchased from Samchun chemicals (Kyunggido, South Korea).

4,4,4-trifluoro-1-(2-Thienyl)-1,3-butanedione (TTA), trioctylphosphine (TOP, 99.0%), divinylbenzene (DVB, 80%), potassium persulfate (PPS), methacrylic acid (MAA), tetrahydrofuran (THF, anhydrous, 99.9%, inhibitor-free) and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) are purchased from Sigma-Aldrich Chemical Co. (St. Louis, U.S.A.). Ethyl alcohol (EtOH, 99.9%) is purchased from Duksan Reagents. Polyvinylpyrrolidone (PVP) is supplied by Biosesang Inc. Styrene (St) is supplied by Junsei Chemicals (Chuo-Ku, Tokyo). The rat C6 glioblastoma cells are purchased from Korean Cell Line Bank (KCLB). All the chemicals are used without further purification. Triple distilled and deionized water is used throughout.

2.2. Characterization

The size and morphology of nanoparticles are characterized by field emission-scanning electron microscope (FE-SEM) with JEOL, JSM-6700F. Fourier transform infrared (FTIR) spectra are recorded at 20 °C using an Alpha FTIR Spectrometer equipped with Platinum ATR (single reflection diamond attenuated total reflectance) from Bruker Optics. The samples are dried in oven at 50 °C overnight. Spectra are measured at the wavenumber ranges of 500–4000 cm^{-1} with a resolution of 1 cm^{-1}.

Dynamic light scattering (DLS) measurements are carried out on the poly(St-*co*-DVB-*co*-NaSS-*co*-MAA) with a Malvern Nano ZS instrument, equipped with a He–Ne 633 nm laser. The zeta potentials of the poly(St-*co*-DVB-*co*-NaSS-*co*-MAA) nanoparticles were measured at 25 °C using a Photal Otsuka Electronics ELSZ-2000ZS (Hirakata, Japan) particle analyzer at wavelength of 633 nm. For electrophoretic mobility measurements the conditions are as follows: dielectric constant 78.3, current 0.55 mA, fluid refractive index of 1.33, cell field of 33.49 V/cm, viscosity of 0.89 cP and conductivity of 0.33 mS/cm.

Excitation and emission spectra are examined by Fluorescence Spectrophotometer with SHIMADZU, RF-5301PC equipped with a 150 W xenon lamp. Luminescence is measured at an angle of 90° with respect to excitation light beam. The resulting particles are