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Aqueous synthesis of high bright Ag_2Se–ZnSe quantum dots with tunable near-infrared emission

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ABSTRACT

Efficient aqueous synthetic methods for near-infrared quantum dots as bioimaging agents are urgently required. In this work, a simple and fast synthesis of highly luminescent, near-infrared Ag_2Se quantum dots (QDs) in aqueous media is reported. The method avoids high temperature, pressure and organic solvents to directly generate water-dispersible Ag_2Se QDs. The photoluminescence emission of Ag_2Se QDs ranges from 835 to 940 nm by different Ag:Se molar ratio. Using the ZnSe as a shell, the quantum yield reaches up to 42%. The Ag_2Se–ZnSe QDs with high quantum yield, near-infrared and low cytotoxic could be used as good cell labels, showing great potential applications in bio-imaging.

1. Introduction

Quantum dots (QDs) have been widely investigated for both fundamental research and technical applications over the last three decades because of their appealing optical properties [1–4]. These unique properties render them desirable for various technological applications in a wide range of fields, such as biolabeling, solar cells, and light-emitting devices [4–6]. For bioimaging, near-infrared (NIR) QDs are of great interest for the study, because the fluorescence they emit could penetrate deeply into the body and reduce the interference from blood and tissue autofluorescence and absorption [7–9]. Previous works on NIR QDs are mainly based on II–VI (CdTe) [10], III–V (InAs) [11] and IV–VI (PbS) [12] QDs with narrow band gaps. However, the intrinsic toxicity of Cd, As or Pb is an obstacle to practical bioapplications of these QDs, because of the risks these materials pose to human health and the environment [13,14]. In recent years, ternary I–III–VI NIR QDs ($CuInS_2$, $AgInS_2$, $AgInSe_2$) have been synthesized [15–18]. However, it is hard to control their chemical composition, which is an important factor in controlling the optical properties of ternary I–III–VI QDs.

Ag_2Se, which has low toxicity and narrow band gaps (~0.15 eV), is a potential alternative for NIR QDs. Several reports on Ag_2Se nanocrystals have been published [19–24]. Yarema et al. prepared Ag_2Se QDs with two fluorescent wavelengths of 1030 and 1250 nm in an organic phase by tuning the sizes of Ag_2Se with a relatively low quantum yield (QY) of 1.7% [19]. Wang et al. synthesized Ag_2Se QDs with emission at 1300 nm by a solvothermal method [21]. After surface modification, the Ag_2Se QDs possess good watersolubility, high colloidal stability and photostability, which are further successfully performed in imaging of organs and vascular structures. Tian et al. synthesized Ag_2Se QDs with tunable FL emission in 1080–1330 nm in an organic phase with about 9.58% QY [22]. After being transferred to the aqueous phase, the QY decreased to 3.33%. In order to avoid additional ligand exchange, synthesizing nanocrystals in aqueous phase is a direct strategy to obtain water-dispersible nanocrystals for bioapplications. However, few aqueous synthetic strategies for water-soluble NIR fluorescent Ag_2Se QDs have been reported to avoid a further phase transfer process for bioapplication. Pang et al. prepared the water-soluble Ag_2Se QDs with an ultrasmall size in aqueous media, while the QY of the

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samples is about ~3% [25]. The low QY limited its development in deeper tissue penetration and higher spatial and temporal resolution for bioimaging. Therefore, it is urgent to develop a new aqueous method to obtain high QY Ag_2Se nanocrystals for bioapplications.

In this work, we report a simple and fast method for the synthesis of highly luminescent Ag_2Se QDs directly in aqueous media. We examined the luminescence properties of the samples as a function of the initial Ag: Se molar ratio, reaction temperature and reaction time. Moreover, highly luminescent Ag_2Se–ZnSe QDs was prepared by a second injection of Zn^{2+} precursor directly into the same reactor with existing Ag_2Se cores without purification. The QY of the obtained Ag_2Se–ZnSe QDs reached 42%. These QDs exhibit excellent water solubility and colloidal stability as well as very low cytotoxicity, showing great potential applications for fluorescent probes.

2. Experimental section

2.1. Materials and methods

Selenium powder (99.95%), sodium borohydride (>96%), silver nitrate (99.9%) and zinc acetate (99.0%) were purchased from Sinopharm Chemical Reagent (Shanghai, China). 3-Mercaptopropionic acid (MPA, 98%) was purchased from Aladdin, Shanghai, China. All chemicals were of analytical reagent grade and used without further purification. Ultrapure water with a resistivity of 18.2 MΩ cm^{-1} was used in all syntheses.

2.2. Preparation of precursor solution

The silver precursor solution (Ag^+ solution) was prepared by dissolving silver nitrate (0.0340–0.1360 g) in water (20 mL). A QD stabilizing solution was prepared by dissolving MPA (0.1–0.6 g) in water (10 mL), adjusting the pH of the solution to 12 by adding aqueous NaOH (3.0 M) drop-wise with stirring. Zinc solutions (Zn^{2+} solution) were prepared by dissolving zinc acetate (0.0878 g) in water (20 mL). A literature method was used to synthesize NaHSe [26]. All precursor solutions were prepared immediately before use.

2.3. Synthesis of Ag_2Se and Ag_2Se–ZnSe QDs

In a typical reaction, 20 mL Ag^+ solution was loaded into 100 mL three-necked flask. Then the MPA stabilizing reagent solution (0.4 g) was added and degassed for 30 min by nitrogen flow. The mixture solution was incubated at 90 °C. Then a fresh 0.02 M NaHSe solution (20 mL) was injected into the three-necked flask at 90 °C. The reaction processed 10–60 min.

For preparing the Ag_2Se–ZnSe QDs, Ag_2Se QDs were synthesized following the procedure described above, except that the 0.03 M NaHSe (20 mL) was used, and the experiments were conducted at 90 °C for 45 min. After synthesizing the Ag_2Se QDs, the reaction mixture was cooled to 80 °C. Without further separation or purification, the Zn^{2+} solution was added dropwise by a syringe pump (KD Scientific KDS100, USA) at a flow rate of 0.11 mL min^{-1} for 3 h.

After the reaction was complete, the reaction mixture was cooled to room temperature. The obtained suspension was diluted with acetone, causing a precipitate to form. The flocculent was centrifuged at 8000 rpm for 6 min to separate the particles from the suspension. To remove excess stabilizing reagent, the particles were redispersed in acetone and centrifuged for 2 more times. The final solid QDs were redispersed and stored in 10 ml deionized water for characterization.

2.4. Characterization

An X-ray diffractometer (D/MAX-2550, Rigaku, Japan) with Cu-Ka radiation ($\lambda = 1.54$ Å) was used to perform the X-Ray diffraction (XRD) patterns. A JEM-2100F TEM (JEOL Tokyo, Japan), operating at 200 kV, was used to take the Transmission electron microscope (TEM) images. Lambda 35 (Perkin Elmer, America) spectrophotometer was used to measure the UV–Vis absorption spectra. The photoluminescence (PL) spectra were carried out by using a JASCO FP-6600 spectrofluorometer (Japan). The PL QYs of various samples were comparatively studied by taking rhodamine 6G (R6G) as a reference fluorescent dye with known QY (95%). This method has been discussed extensively elsewhere [15]. The solution concentration of samples or R6G was based on the solution absorbance. The QD samples and R6G were all diluted to yield an absorbance of 0.1 at the 422 nm, in order to minimize possible reabsorption effects. The PL QYs of the as-prepared QDs were calculated using the following equations: $QY = (F_{QD} \times A_{R6G} \times QY_{R6G})/(F_{R6G} \times A_{QD})$, where F is the integrated fluorescence emission, A is the absorbance at the excitation wavelength.

2.5. Cytotoxicity evaluation by MTT assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is investigated on the ability of viable cells to reduce the soluble and yellow MTT into the insoluble and blue formazon product. Human hepatoma cells SMMC-7721, breast carcinoma cells MCF-7 and lung cancer cells A549 were plated in 200 μL media per well in three 96 well plates respectively. Each left 12 wells empty for blank controls. Then the plates were incubated (37 °C, 5% CO_2) 24 h to allow the cells attaching to the wells. The QDs were sterilized in the super-clean worktable by ultraviolet irradiation (254 nm, 20 W) for 2 h, and then serial dilutions of the nanoparticles at concentrations of 25, 50, 100, 200, 500, 1000 and 1500 μg mL^{-1} were added to the culture wells to replace the original culture medium and the plates were incubated for another 24 h in 5% CO_2 at 37 °C. 12 duplicates were obtained for each concentration. A 2.5 mg*mL^{-1} stock solution (50 mL) of MTT was prepared in phosphate buffer (PBS) and 20 μL stock solution was added to each well. Then the plates were covered by aluminium foil for protection from the light and incubated at 37 °C and 5% CO_2 for 4 h. During this period, viable cells reduce the MTT to formazan pigment, which were dissolved in dimethyl sulfoxide (DMSO). After incubation, 100 μL DMSO was added to each well. Then the plates were placed on a shaking table for 40 min, to thoroughly mix the formazan into the solvent. The absorbance of the suspension was recorded using a microplate reader at 570 nm. The cell viability was calculated by normalizing with the results obtained with no QD-loading.

3. Results and discussion

A direct aqueous synthetic approach was used to prepare Ag_2Se and Ag_2Se–ZnSe QDs. In contrast to the conventional organic phase synthetic route, this method is simple, fast and allows for biocompatibility without further phase transfer process. Fig. 1 depicts the formation of Ag_2Se QDs and Ag_2Se–ZnSe QDs. When we prepared the Ag_2Se QDs, a cold (room temperature) solution of Se precursor was injected into a hot solvent (water, 90 °C) containing Ag precursors as well as the MPA as a capping ligand. After a few minutes, the colorless reaction solution turned light yellow, indicating the start of the nucleation process. With increasing reaction time, the solution becomes canary yellow, showing the particles grown. Precipitation of the product occurs when the reaction time is over 60 min because of aggregation. Ag_2Se–ZnSe QDs were