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Hydrothermal synthesis of highly luminescent blue-emitting ZnSe(S) quantum dots exhibiting low toxicity

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ABSTRACT

Highly luminescent quantum dots (QDs) that emit in the visible spectrum are of interest to a number of imaging technologies, not least that of biological samples. One issue that hinders the application of luminescent markers in biology is the potential toxicity of the fluorophore. Here we show that hydrothermally synthesized ZnSe(S) QDs have low cytotoxicity to both human colorectal carcinoma cells (HCT-116) and human skin fibroblast cells (WS1). The QDs exhibited a high degree of crystallinity, with a strong blue photoluminescence at up to 29% quantum yield relative to 4′,6-diamidino-2-phenylindole (DAPI) without post-synthetic UV-irradiation. Confocal microscopy images obtained of HCT-116 cells after incubation with the QDs highlighted the stability of the particles in cell media. Cytotoxicity studies showed that both HCT-116 and WS1 cells retain 100% viability after treatment with the QDs at concentrations up to 0.5 g/L, which makes them of potential use in biological imaging applications.

1. Introduction

Semiconductor nanoparticles have unique optical properties such as narrow emission and wide absorption spectra, long decay lifetimes and large absorption cross-sections, promoting their usage in biological and medical imaging applications [1–4]. However, there are considerable restrictions to the effective use of QDs in bio-applications, such as water dispersivity, toxicity and the stability of the QDs in biological environments [5–7]. As toxicity is a serious concern in biological applications, the synthesis of QDs free of heavy metals such as Cd, has attracted much attention over the past decade, with less toxic nanoparticles including ZnSe(S) of particular interest [8–13].

Up until now, ZnSe nanoparticles of various morphologies have been synthesized using both organometallic [15,16,32] and aqueous synthetic pathways [14,17–19,22–31]. By avoiding toxic solvents, aqueous syntheses are more attractive for biological applications than competing organic routes. Moreover, the products of aqueous methods are often water dispersible, which is also an important requirement for bioapplications [20,21]. There have been several reports in the literature using a core experimental method based around the initial formation of an inorganic zinc complex, which is then further reacted to form ZnSe QDs upon addition of selenide under appropriate thermal conditions [14,17–19,22–31]. Most reports have focused on the preparation of ZnSe(S) QDs in which sulfur atoms from thiol groups in the capping agents alloy into the ZnSe lattice at high temperatures [17,18,22–25]. Such particles have been reported to show a higher quantum yield (QY) than native ZnSe QDs [23]. However, a number of reports of alloyed ZnSe(S) QDs prepared under reflux have resulted in relatively poor QYs [18,22–24], which has been overcome in some cases with a post-preparative UV irradiation of the ZnSe(S) QDs [23,24]. The chemical stability of the QDs decreased after post-preparative treatments, making this approach less attractive. An more direct route to alloyed ZnSe(S) QDs prepared them under 24 h of reflux at high pH, yielding QDs with a high QY and chemical stability, without the need for post-synthetic UV irradiation [22]. Metal-doped ZnSe QDs, including Mn, Cd and Cu-doped ZnSe QDs, have also been synthesized [28–30,41]. In order to reduce the reaction time, ZnSe(S) QDs have also been synthesized using microwave assisted methods, but the products had low QYs [17,25]. Hydrothermal reactions have previously been reported, including the synthesis of ZnSe(S)/ZnS nanoparticles with *N*-acetyl-L-cysteine as the capping agent [31] and ZnSe QDs in a single-step reaction with cetyltrimethyl ammonium bromide [32]. However, the hydrothermal synthesis of ZnSe(S) QDs in the presence of 3-Mercaptopropionic acid (MPA) has not been reported to date. Herein, we show that water dispersible ZnSe(S) QDs having high luminescence were synthesized using a hydrothermal method in a short reaction time; the QY is comparable to that obtained by other routes, but without the need for post-synthetic UV irradiation. The cytotoxicity of these

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particles with respect to two cell lines, human colorectal carcinoma cells (HCT-116) and human skin fibroblast cells (WS1) was determined. Images of HCT-116 cells after incubation with the QDs were recorded and the stability of QDs in cell media was investigated using confocal microscopy.

2. Experimental method

2.1. Materials

3-Mercaptopropionic acid (MPA) was obtained from Fluka. $ZnCl_2$, $NaBH_4$, Se (powder), NaOH, bisbenzimide (Hoechst 33342), 17-allyamino-17-demethoxy-geldanamycine (Hsp90 inhibitor 17-AAG) and 4′,6-diamidino-2-phenylindole (DAPI) were all obtained from Sigma-Aldrich. Human colorectal carcinoma cells (HCT-116) and human skin fibroblast cells (WS1) were obtained from ATCC (Manassas, Virginia, USA). Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), Penicillin, Streptomycin, L-glutamine and nonessential amino acids were received from Invitrogen. All reagents were used as supplied, without additional purification. Ultra-pure water was used in all syntheses.

2.2. Synthesis of QDs

Initially, a fresh sample of aqueous NaHSe (0.5 mmol in 20 mL) was synthesized according to literature methods [33,34]. Then, an aqueous Zn-MPA precursor solution was prepared by dissolving 1.09 g (8 mmol) anhydrous $ZnCl_2$ in 100 mL ultra-pure water and adding 2.5 mL MPA (~29 mmol) to the solution. The pH of the Zn-MPA solution was adjusted to 7.3 using 1 M NaOH. Then, 20 mL of fresh NaHSe (0.5 mmol of Se in 20 mL) was added to the Zn-MPA precursor solution, under a nitrogen atmosphere and a colourless solution was obtained. The Zn:Se:MPA molar ratio in the solution was 16:1:58. This was transferred to a Teflon-lined autoclave and placed in a conventional oven under hydrothermal treatment at T = 150 °C for 165 min to form ZnSe(S) water soluble QDs as a transparent colourless solution. The experimental parameters were based upon a range of experiments found to be optimal for CdSe(S) QDs [35] and were used here without further optimization.

2.3. Characterization of QDs

UV–visible absorption spectra were measured with a Varian Cary UV spectrometer. The photoluminescence spectrum of ZnSe(S) QDs was recorded using a J/B SPEX 270 M spectrometer equipped with a power max PMT detector and excitation laser at 350 nm. X-ray powder diffraction spectra (PXRD) were taken on an X'pert PRO Multi-purpose X-ray diffraction System (MPD system). X-ray photoelectron spectroscopy (XPS) was performed using an Escalab 250Xi spectrometer. Transmission electron micrographs (TEM) were recorded by a Philips CM200 instrument. ImageJ software was used to analyse the images.

2.4. Cytotoxicity assays

A total of five different solutions of QDs were prepared by diluting the aqueous solutions of the ZnSe(S) QDs with ultra-pure water to achieve different concentrations of 25, 50, 100, 250, 500 (μg/mL). Cell cultures of human HCT-116 and WS-1 cell lines were obtained according to the standard protocol [36], as described in the supplementary data S_1. The cells were then separately seeded in two 96-well plates (approximately 3000 cells/well) and allowed to adhere to the dish for 24 h in a humidified incubator. As a control, both the cell media alone and cell media in the presence of Hsp90 inhibitor 17-AAG (100 nM = 0.06 μg/mL) were also seeded. Finally, 10 μL of each aqueous solution of QDs (concentrations 25–500 μg/mL) was added to the plates. The plates were incubated in the presence of QDs for 72 h at 37 °C with 5% CO_2. The thus-prepared samples were used to determine proliferation of the cells using a Cell Counting Kit-8 assay [37].

2.5. Confocal microscopy studies

Live HCT 116 cells were incubated with ZnSe(S) QDs in solution for 24 h at a QD concentration of 100 μg/mL. Images of live cells in the presence of the ZnSe(S) QDs were recorded under a Zias LSM 780 confocal microscope. The cells were stained by adding a Hoechst solution (5 μg/mL) 10 min before recording the images. The emission spectra of the QDs in the cell medium were investigated, initially in the cell media solution, then in both cell media and aqueous solution, as detailed in Supplementary data S_2 & S_3.

3. Results and discussions

3.1. Synthesis of QDs

The hydrothermal synthesis of QDs was found to lead to the formation of highly crystalline ZnSe(S) QDs. PXRD data confirmed that the obtained ZnSe(S) QDs, were cubic in structure, with the particle size calculated using the Scherrer equation of 2.6 ± 0.1 nm, Fig. 1. The diffraction peaks of the QDs were found to be consistent with standard patterns of the cubic phases of ZnSe [38] and ZnS [39], and are characteristic of alloyed ZnSe-ZnS, Table S_1, Supplementary data S_4.

Lattice matching of ZnS and ZnSe facilitates efficient alloying of S into the ZnSe particles [17,40]. This was confirmed using XPS, which revealed the presence of zinc and selenium as the main elements in the QDs, with characteristic peaks of Zn_{2p} at 1021.5 eV and Se_{3d} at 54.5 eV. However, sulfur, originating from the mercaptan group in MPA, was detected at 163.5 eV in addition to three distinct carbons at 285, 286.5 and 288.5 eV, that correspond to the binding energies of C—S, C═O and C—H, indicate that ZnSe(S) QDs contain MPA in their structure (Fig. 2, a–d).

FT-IR spectroscopy confirmed that MPA is bound to the ZnSe(S) QDs. As shown in Fig. 3, MPA has absorbance bands at 1708, 1251 and 2574 cm^{-1}, corresponding to the C═O, C—O and S—H stretching bands respectively, along with the sharp absorption at 1427 cm^{-1} assigned to the OH bending. These peaks all disappeared in the infrared spectrum of ZnSe(S)–MPA capped QDs, confirming the formation of new covalent bonds between sulfur in the structure of MPA and of ZnSe(S) QDs. Meanwhile, two sharp and strong peaks at 1560 and 1406 cm^{-1}, corresponding to the symmetric and asymmetric stretch of COO^-, appeared in the FT-IR spectrum of ZnSe-MPA capped QDs, indicating that the carboxylic acid functional group of MPA had ionized

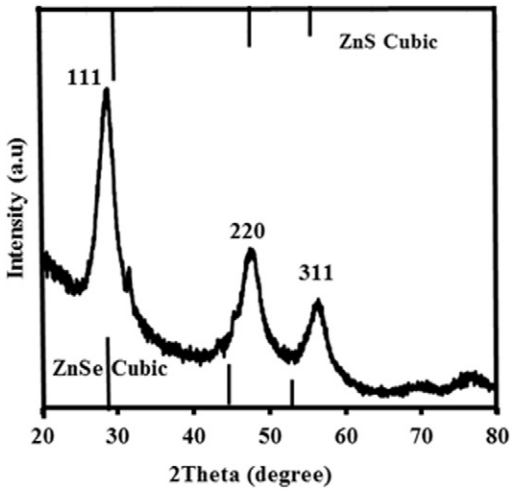

Fig. 1. PXRD pattern of ZnSe(S) QDs.