Ultrasmall Quantum Dots: A Tool for in Vitro and in Vivo Fluorescence Imaging

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Abstract. Fluorescence bioimaging is an increasingly popular approach in biomedical research and diagnosis, where semiconductor nanocrystals or quantum dots (QDs) have proved to be excellent fluorescent labels. The use of ultrasmall QDs in nanoprobes extends the possibilities of bioimaging owing to an enhanced capacity for penetrating through cell membranes. However, the QDs synthesis is accompanied by the rapid growth of nanocrystals in colloidal medium what prevents obtaining sufficiently small QDs prepared by conventional approaches. Here, a one-pot injection technique of QD synthesis in an organic medium, with the reaction terminated at an early crystal growth stage and excess precursors eliminated by gel permeation chromatography, is proposed. This technique yields defect-free cadmium selenide QD cores about 1.5 nm in size emitting at the wavelengths less than 500 nm. Coating of these QDs with epitaxial shells of different compositions ensures a photoluminescence quantum yield approaching 100%. The resultant ultrasmall QDs are promising components of nanoprobes to be used for imaging intracellular and intranuclear events down to the molecular level.

1. Introduction

Quantum dots (QDs) are semiconductor nanocrystals (NCs) several nanometers in size capable of photoluminescence (PL) in a wide spectral range. Their energy structure and characteristics of light absorption and fluorescence strongly depend on the QD size because of the quantum confinement effect [1,2]. Advanced methods of one-pot injection synthesis with one component injected into a hot solution of the other component [3] allow obtaining QDs with controllable sizes and, hence, PL parameters by varying the reaction time, temperature, and/or types of precursors. Core/shell QDs consisting of a fluorescing core (usually, CdSe) and a thin epitaxial inorganic shell of a semiconductor with a higher band gap (usually, CdS or ZnS) emitting in the range from 500 to 650 nm, depending on the core size, are currently the most common type of QDs. The shell enhances charge carrier localization in the core, protects the core from the environment, and suppresses blinking [4,5].

QDs are increasingly widely used in various fields [6,7], including biomedical research and medicine [8,9]. A typical biomedical application of QDs is their use as fluorescent labels, which are advantageous over the routinely used organic dyes due to the unique optical properties and high stability. In this field, the QD shell provides and additional advantage in that it facilitates the

attachment of hydrophilic ligands to the QD surface to make the QDs water-soluble. These QDs can serve as components of complex suspension microarrays or nanoprobes, whose PL may be excited in either the one-photon [10] or the two-photon [11,12] modes.

In recent years, the possibility of imaging intracellular events down to the molecular level by means of QDs has raised special interest [13]. In this case, of particular importance are small sizes of QDs and the absence of PL blinking [14]. In addition, ultrasmall CdSe cores fluorescing in the blue spectral region are promising components of next-generation light-emitting diodes. Therefore, the development of ultrasmall QDs with a shell effectively enhancing fluorescence is an urgent task.

The main difficulty with obtaining QD cores smaller than 2 nm [15] is that the NC growth at early stages is so rapid that larger crystals are formed almost instantaneously. To date, methods for obtaining the so-called magic-size clusters with a high thermodynamic stability and very narrow optical transitions [16] in the UV and blue spectral regions have been developed . However, this type of NCs is unsuitable for biomedical applications because attempts at coating them with inorganic shells effectively enhancing the PL quantum yield (QY) and protecting the core have thus far failed. Apparently, the reason is that these clusters are structurally different from common QDs [16], which makes the standard coating techniques ineffective.

In this study, we suggest a new procedures for synthesizing CdSe cores with diameters as small as 1.5 nm and coating them with one or several layers of an inorganic shell to obtain CdSe/ZnS and CdSe/ZnS/CdS/ZnS core/shell QDs with highly uniform sizes and a PL QY as high as 90% fluorescing in the region between 480 and 540 nm.

2. Results

Our procedure, described in detail elsewhere [17], is based on the high-temperature injection synthesis method. In this study, the growth of QD cores was monitored by recording their absorption and emission spectra. The PL QY was measured relative to Coumarin 102 and fluorescein dyes.

Our first objective was to obtain CdSe NCs of strictly controllable size, which was fulfilled by precise control of the reaction time and removal of all unreacted precursors.

First, we measured the kinetics of NC growth by monitoring of the position of the first excitonic transition in the light absorption spectrum, and, by using the relationship between the latter and the NC diameter [18] we plotted an approximated calibration curve. The measurement showed that the reaction had to be terminated within about 45 s to obtain QD cores smaller than 1.5 nm in diameter. However, the commonly used method of terminating the synthesis by cooling the reaction flask with an air flow takes more than 1 min to cool the mixture to a temperature below 100°C, during which time the crystal growth gradually slows down. This leads to size heterogeneity of the resultant NCs.

Therefore, we put the reaction mixture into liquid nitrogen after 45 s of synthesis to stop the reaction instantaneously. The frozen mixture was collected into excess isopropyl alcohol, and when it warmed to room temperature, the mixture was centrifuged to settle the cores. Then, the cores were purified by dispersing them in hexane and coagulating with acetone. The purification was repeated two times. The absorption spectrum of the resultant CdSe cores had a peak at 401 nm, which, according to Ref. 18, corresponds to a core diameter of 1.5 nm. Their PL spectrum had a peak at 442 nm and a full width at half maximum (FWHM) of 30 nm.

The next problem consisted in that the rapid termination of synthesis left considerable amounts of unreacted precursors in the mixture. Cadmium precursors, in the forms of cadmium alkyl phosphonates and carboxylates, cannot be totally removed by the dispersion/coagulation procedure, because they are adsorbed on the NC surface [19], the process being further facilitated by the small size and, hence, large relative surface area of the nanocrystals. Moreover, these remaining precursors are involved in reaction during the subsequent formation of the shell, either causing additional growth of the core or being included in the shell and disturbing its designed stoichiometry.

This problem was addressed by additional purification of the CdSe cores by gel permeation chromatography (GPC) [19] using the SX-1 cross-linked polystyrene beads (BIO-RAD) as the

stationary carrier phase. In this method, QD cores rapidly migrate along the stationary phase, while low-molecular-weight precursors are removed as a result of retention in the pores of the carrier [19].

In order to estimate how this additional purification improves the quality of the resultant QDs, we compared the optical characteristics of the core-shell QDs grown with or without GPC-purification of the cores. The results are shown in Table 1 and Figure 1.
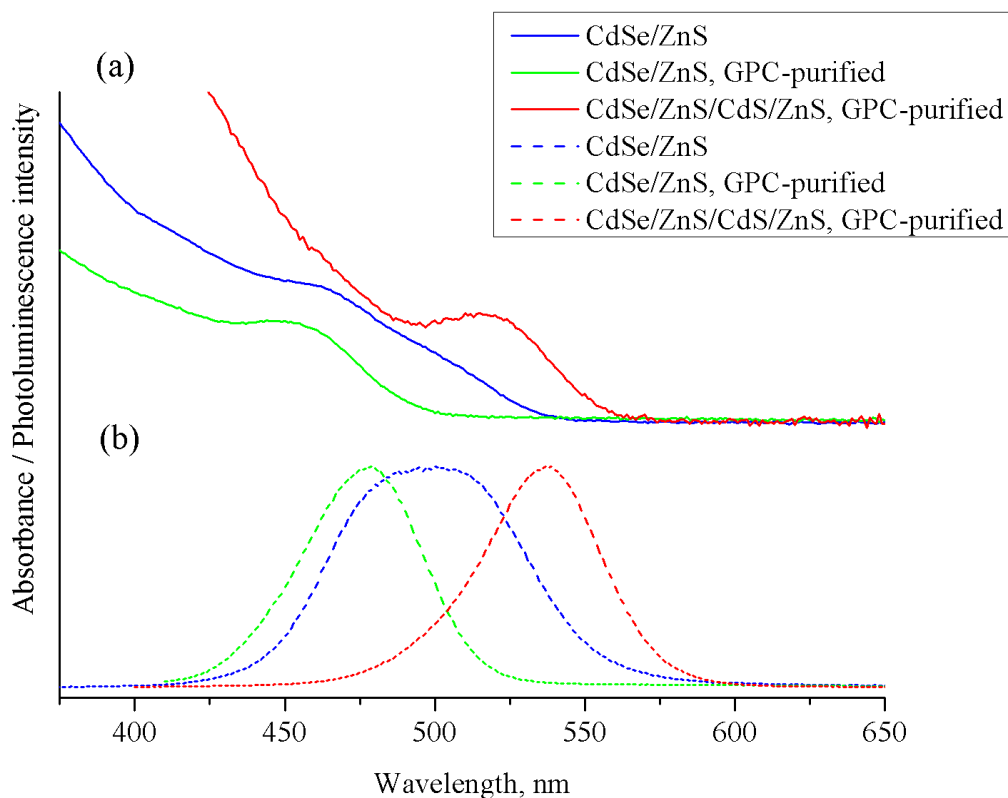
Figure 1. Absorption spectra (a) and fluorescence spectra (b) of quantum dots with different shell structures and procedures for nanoparticles isolation and purification.

The CdSe cores purified by the dispersion/coagulation method alone (Sample 1 in Table 1) or dispersion/coagulation followed by GPC (Sample 2) were coated with three monolayers of ZnS. We used the method of successive ion layer adsorption and reaction (SILAR) [20,21], because it allows layer-by-layer coating with a predetermined number of layers. Some GPC-purified cores were coated with a multicomponent (ZnS/CdS/ZnS) shell three monolayers in thickness (Sample 3). This type shell was earlier shown [17] to ensure an enhanced charge localization, thereby substantially increasing the PL QY. The final size of the CdSe/ZnS and CdSe/ZnS/CdS/ZnS QDs was less than 4 nm.

The incomplete removal of cadmium precursors from the solution of CdSe cores led to the formation of an inner CdS layer or a layer of a more complex and varying composition ($Cd_xZn_{1-x}S$) in the shells of QDs in Sample 1. As can be seen from Table 1 and Figure 1, this has "blurred" the excitonic transition due to the formation of several types of QDs, which is expressed in a wide FWHM of both absorbance and PL peaks. Sample 1 is also characterized by a 56-nm red shift of the PL peak. CdSe/ZnS QDs in Sample 2 are considerably more homogeneous, as evidenced by their more than 1.5-fold narrower PL band. In addition, the red shift of the PL peak is also smaller (37 nm). Thus, the additional GPC purification of CdSe cores has substantially improved the optical parameters of the resultant QDs.

As expected, CdSe/ZnS/CdS/ZnS QDs in Sample 3, which also are based on GPC-purified cores, were as homogeneous as Sample 2, as judged by the FWHM of their PL spectrum (Table 1). The multicomponent shell has ensured an improved charge carrier localization in the core and their better protection from the environment, which has resulted in a higher PL QY (90%). The large red shift of the PL band of these QDs (79 nm) is explained by the complex energy band structure of their multicomponent shell [3,17].

Table 1. Optical characteristic of quantum dots with different shell structures and procedures for nanoparticles isolation and purification.

No.	Sample	Absorbance peak, nm / PL peak, nm / FWHM, nm	PL QY, %
1	CdSe/ZnS	- / 498 / 73	63 (relative to Coumarin 102)
2	CdSe/ZnS, GPC-purified	449 /479 / 46	68 (relative to Coumarin 102)
3	CdSe/ZnS/CdS/ZnS, GPC-purified	517 / 537 / 46	90 (relative to fluorescein)

Thus, the experimental results confirmed the efficiency of our new procedure for the synthesis of ultrasmall CdSe cores with rapid termination of the reaction and additional GPC purification of the cores. We plan to use the CdSe/ZnS and CdSe/ZnS/CdS/ZnS QDs designed in this study as fluorescent labels in next-generation nanoprobes for *in vivo* and *in vitro* bioimaging [22].

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