

SYNTHESIS OF CORE/SHELL QUANTUM DOTS FOR DIAGNOSTICS

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Abstract: In this paper, synthesis of colloidal core and core/shell quantum dots (QDs) was described. First, CdTe QDs capped with glutathione, thioglycolic or mercaptopropionic acid were prepared in aqueous phase, and used for synthesis of colloidal core/shell CdTe/ZnS QDs. Core/shell QDs were used for conjugation with bovine serum albumin (BSA) or immunoglobulin G (IgG) via different crosslinkers (CDI, EDC/NHS, EDC). QDs as well as QDs-protein/antibody conjugates were characterized via UV-Vis spectroscopy and capillary electrophoresis (CE). Based on UV-Vis spectroscopy results it was found that, with increasing concentration of BSA, fluorescence intensity of QDs decreased. CE confirmed formation of QDs-BSA and QDs-IgG conjugates.

Keywords: Quantum dots, conjugation, glutathione, mercaptopropionic acid, thioglycolic acid, bovine serum albumin, immunoglobulin G.

1. INTRODUCTION

In the past decade there have been many studies on semiconductor quantum dots (QDs) that have shown great promise for labelling DNA, tissue, proteins, specific cells or cell structures due to their unique optical properties. These nanoparticles have many advantages over the conventionally used organic dyes, such as broad absorption spectra, narrow emission spectra, controllable surface characteristics, high photostability, high photoluminescence quantum yields (QYs) and high resistance to photobleaching [1-3].

Up to present day, many approaches have been developed to prepare fluorescent QDs, such as organic and aqueous route. QDs synthesized in aqueous way exhibit good reproducibility, low toxicity, excellent biological compatibility, stability and are less expensive [2]. However, QDs obtained through aqueous route have lower QY (<30%) [4], which is associated with surface defects. These defects can be minimized or entirely eliminated by using capping agents such as thioglycolic acid (TGA), mercaptopropionic acid (MPA), mercaptosuccinic acid (MSA), glutathione (GSH), L-arginine, L-cysteine [4]. GSH is intracellular non-protein present in all mammalian tissues participating in numerous cellular functions and is very prospective capping agent due to its key function in detoxification of heavy metals (cadmium) in organism. Especially reduced GSH plays an important role in detoxification of peroxides and radicals [4, 5].

When conjugated with biomolecular affinity ligands, such as proteins and antibodies, QDs can be used to target specific molecules such as DNA, proteins or cells. Conjugation of QDs and other biomolecules can be achieved by covalent coupling. One of the most frequently used methods is the cross-linking through an EDC or EDC/NHS reaction. These methods were used for detection of various proteins and antibodies such as anti-human BMP-7 antibody, insulin, goat anti-mouse IgG and many others. Another method for covalent conjugation is via carbonyldiimidazole (CDI) which is a highly reactive compound, the first which has been shown to be an excellent agent forming an amide bond in peptide synthesis. CDI has been used to conjugate QDs with different proteins/antibodies such as anti-ovalbumin, annexin and anti-PCNA antibody [6].

In this paper, different water soluble CdTe QDs capped with GSH, TGA or MPA and water soluble core/shell CdTe/ZnS QDs were successfully prepared via aqueous route and used for detection of BSA or IgG. Conjugation of QDs with protein/antibody was achieved via covalent coupling using different crosslinkers (EDC, EDC/NHS and CDI). QDs and QDs-protein/antibody conjugates were characterized via UV-Vis spectroscopy and CE in order to determine the quenching effect of BSA or IgG on fluorescence intensity of QDs.

2. MATERIALS AND METHODS

2.1. REAGENTS AND APPARATUS

Sodium borohydride (NaBH_4 , 99%), BSA (96%), CDI (97%), Sodium citrate dihydrate (99%), *N*-Ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide-hydrochloride (EDC, 99%), cadmium chloride (CdCl_2 , 99%), IgG (95%), hydroxysuccinimide (NHS, 98%), sodium tellurite (Na_2TeO_3 , 99%) were purchased from Sigma Aldrich. Sodium hydroxide (NaOH, 98%) and zinc chloride (ZnCl_2 , 99%) were purchased from Penta. MPA (98%), reduced GSH (98%) and TGA (97%) were purchased from Merck. Isopropyl alcohol (99.7%) was purchased from Lach-Ner.

Fluorescence measurements of the QDs and QDs-protein/antibody conjugates were performed on Infinite M200 Pro, Tecan in the range 400-750 nm. Capillary electrophoresis of QDs and QDs-protein/antibody conjugates was carried out using 7100 Agilent Technologies.

2.2. SYNTHESIS OF CdTe QDS CAPPED WITH GSH, MPA OR TGA

To obtain CdTe-GSH QDs, CdCl_2 solution (0.04 mol/l, 4ml) was diluted to 46 ml in one-necked flask and then GSH (300 mg), sodium citrate dihydrate (100 mg), Na_2TeO_3 (0.01 mol/l, 4 ml), NaBH_4 (50 mg) were added under vigorous stirring. Solution was then refluxed at 95°C for 3 h [2].

To obtain CdTe-MPA QDs, CdCl_2 solution (91.6 mg) was diluted to 50 ml in one-necked flask and sodium citrate dihydrate (200 mg) was added followed by addition of MPA (52 μl). The pH of the solution was adjusted to 10.5 using NaOH (1 mol/l), followed by addition of Na_2TeO_3 (22.15 mg) and NaBH_4 (50 mg) under vigorous stirring. Solution was then refluxed at 95°C for 4 h [5].

To obtain CdTe-TGA QDs, CdCl_2 solution (183 mg) was diluted to 48 ml in one-necked flask and TGA (104 μl) was added followed by adjustment of pH to 10.5 using NaOH (1 mol/l). Then sodium citrate dihydrate (50 mg), Na_2TeO_3 (0.01 mol/l, 2 ml) and NaBH_4 (20 mg) were added under vigorous stirring. Solution was then refluxed at 95°C for 4 h [5].

2.3. SYNTHESIS OF CdTe/ZnS QDS

To obtain CdTe/ZnS QDs, CdTe (40 mg) capped with GSH, MPA or TGA were diluted to 50 ml in one-necked flask followed by addition of ZnCl_2 (6.8 mg) and GSH (61.3 mg) were added under vigorous stirring. The pH of the solution was adjusted to 8 using NaOH (1 mol/l) and solution was then refluxed at 95°C for 3 h [3].

All obtained QDs, core and core/shell, were precipitated by isopropyl alcohol with centrifugation at 6000 rpm for 30 min and resultant precipitates were dried at 80°C for 2 h under vacuum.

2.4. PREPARATION OF QDS-BSA OR QDS-IGG CONJUGATES VIA CDI

Briefly, to the solution of core/shell CdTe/ZnS QDs (1 mg/ml), CDI (10 μl , 10 mmol/l) containing PBS buffer (100 mmol/l, pH=7.4) was added and the solution was then incubated at room temperature for 30 min in order to activate carboxyl groups. Then, IgG (0.1 mg/ml) was added to the solution and incubated at room temperature for 2 h [5].

2.5. PREPARATION OF QDS-BSA CONJUGATES VIA EDC/NHS

Briefly, to the solution of core/shell CdTe/ZnS QDs (200 μ l, 0.1 mg/ml) EDC (200 μ l, 50 mmol/l) and NHS (200 μ l, 5 mmol/l) were added and solution was then incubated at 32°C for 30 min. Then, BSA (200 μ l; 0, 0.0005, 0.002, 0.005, 0.05 mg/ml) was added to the solution and incubated at 32°C for 2 h while shaking [5].

2.6. PREPARATION OF QDS-BSA CONJUGATES VIA EDC

Briefly, to the solution of core/shell CdTe/ZnS QDs (250 μ l, 0.1 mg/ml) BSA (250 μ l; 0, 0.05, 0.5, 1 a 1.5 mg/ml) and EDC (57 μ l, 10 mg/ml) were added. The solution was then incubated at room temperature for 2 h [5].

3. RESULTS

3.1. CHARACTERIZATION OF CORE/SHELL QDS

Core/shell CdTe/ZnS QDs were prepared through aqueous route and characterized via UV-Vis spectroscopy. Figure 1 illustrates emission spectra of different core/shell CdTe/ZnS QDs. From the results it is observable that the highest intensity of fluorescence is in the case of CdTe-MPA/ZnS QDs (95277 CPS at 626 nm) while the lowest intensity of fluorescence is in the case of CdTe-GSH/ZnS QDs (10525 CPS at 512 nm). Another parameter that was observed is full width at half maximum (FWHM) which indicates the uniformity of size distribution of QDs. FWHM is 86 nm, 42 nm and 86 nm in the case of CdTe-MPA/ZnS QDs, CdTe-TGA/ZnS QDs and CdTe-GSH/ZnS QDs. This indicated that the best uniformity of size distribution of QDs is in the case of CdTe-TGA/ZnS QDs.

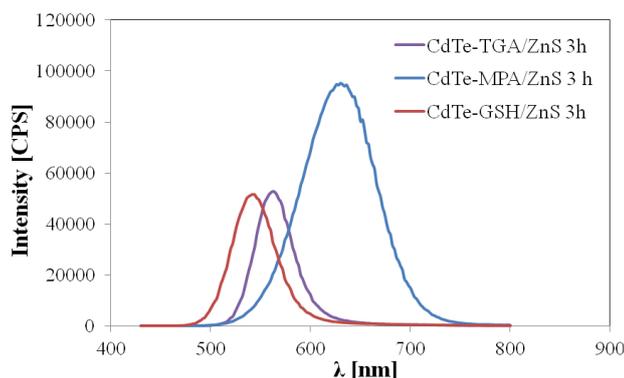


Figure 1: Fluorescence spectra of different CdTe/ZnS QDs

3.2. THE EFFECT OF BSA ON FLUORESCENCE INTENSITY OF CORE/SHELL QDS

Core/shell QDs and BSA were conjugated via covalent coupling using carboxyl groups of cross-linkers (EDC and EDC/NHS). The interaction between QDs and BSA was studied by UV-Vis spectroscopy. The fluorescence quenching is usually described by the linear Stern-Volmer equation:

$$I_0/I = 1 + K_{sv}[C] \quad (1)$$

where I_0 and I are the steady-state fluorescence intensities in the absence and presence of BSA, respectively. K_{sv} is the Stern-Volmer quenching constant, and $[Q]$ is the concentration of BSA.

From Figure 2 it is obvious that in case where EDC alone was used as crosslinker, the highest quenching effect was noticeable in the case of CdTe-MPA/ZnS QDs. In case of CdTe-GSH/ZnS QDs the quenching effect was the least obvious. In the case where conjugation is achieved via EDC/NHS reaction, the highest quenching effect was noticeable in the case of CdTe-MPA/ZnS QDs while in case of CdTe-GSH/ZnS QDs the quenching effect was the least obvious.

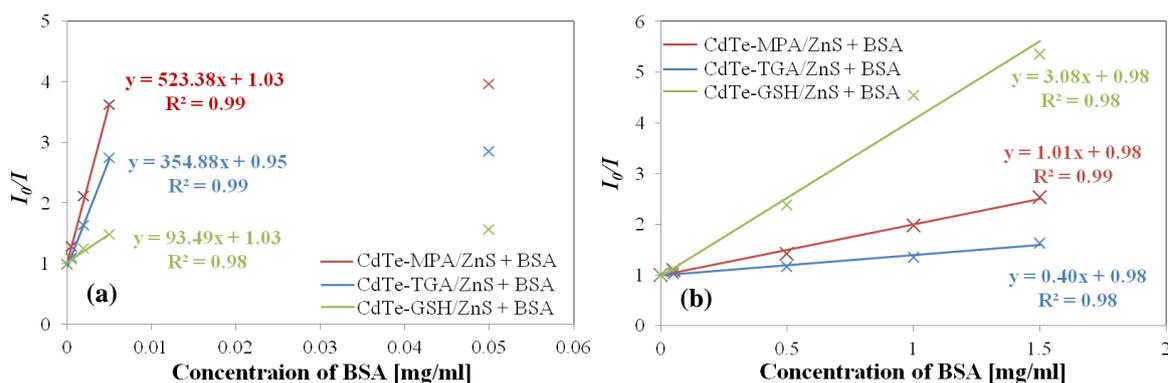


Figure 2: Stern-Volmer plots for quenching of QDs fluorescence by different concentrations of BSA. Conjugation via EDC/NHS (a), via EDC (b).

3.3. THE EFFECT OF IGG ON FLUORESCENCE INTENSITY OF CORE/SHELL QDS

Core/shell QDs and IgG were conjugated via covalent coupling using carboxyl groups of CDI. The interaction between QDs and IgG was studied by UV-Vis spectroscopy. Results illustrated the quenching effect of IgG on fluorescence intensity of QDs (Figure 3). The quenching effect can indicate that QDs-IgG conjugates were formed. In the case of CdTe-MPA/ZnS QDs, quenching effect is most noticeable while in the case of CdTe-GSH/ZnS was the least observable.

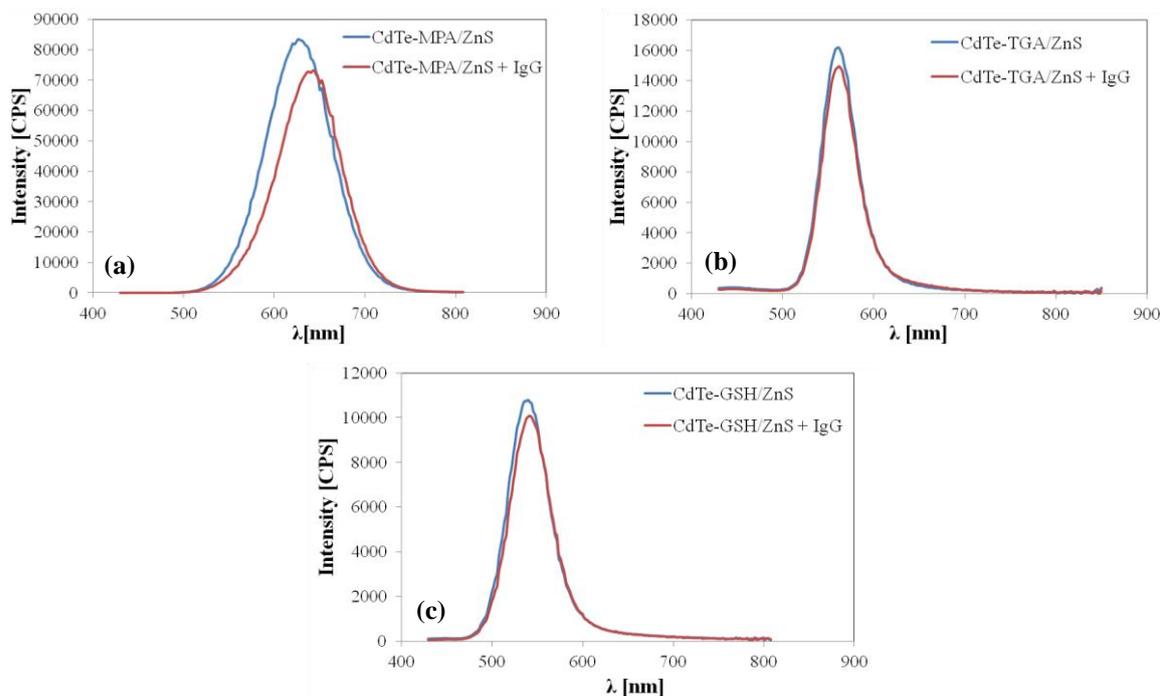


Figure 3: Fluorescence spectra of different core/shell QDs and core/shell QDs-IgG conjugates. CdTe-MPA/ZnS-IgG (a), CdTe-TGA/ZnS-IgG (b), CdTe-GSH/ZnS-IgG (c).

3.4. CHARACTERIZATION OF QD CONJUGATES BY CE

Figure 4 shows the electropherograms of the CdTe-MPA/ZnS QDs, QDs-BSA and QDs-IgG conjugates. The conjugates used for CE analysis were prepared using CDI as crosslinker. In the separation electrolyte (20 mmol/l sodium borate, pH=9.2), both QDs-BSA and QDs-IgG are well separated with free QDs by CE within 6 min 13 s. In comparison to free QDs, QDs-BSA showed a wider peak, which can be attributed to the adsorption of protein (BSA) to the capillary inner wall. The surfaces of CdTe QDs, used for synthesis of CdTe-ZnS, were in this case coated with MPA, and

carried negative charges. Since the migration direction of QDs conjugates was different from that of electroosmotic flow, the QDs-BSA and QDs-IgG conjugates were first eluted within 4 min 54 s in the case of QDs-BSA conjugate, and within 3 min 50 s, in the case of QDs-IgG conjugate.

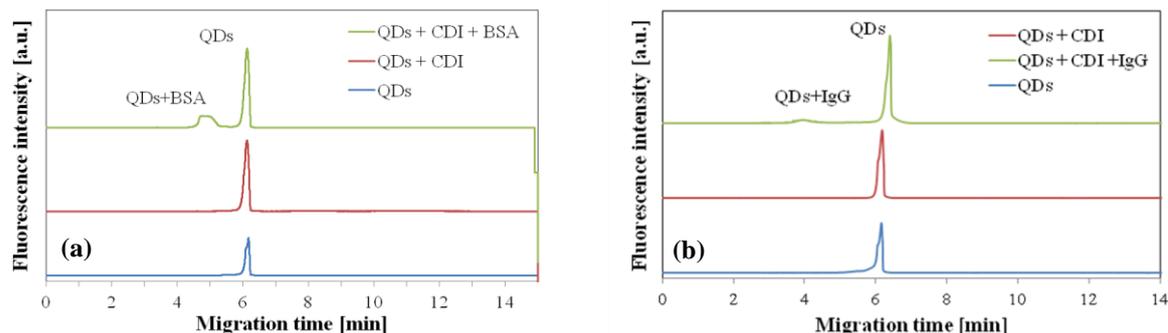


Figure 4: Electropherograms of QDs-BSA (a) and QDs-IgG (b) conjugates.

4. CONCLUSIONS

In this study, a method for synthesis of colloidal core and core/shell QDs and three methods for preparation of core/shell QDs-BSA/IgG conjugates were described. QDs and protein/antibody conjugates were analyzed via UV-Vis spectroscopy and CE. The results of UV-Vis spectroscopy illustrated quenching effect of different concentrations of BSA or IgG on fluorescence intensity of QDs while results of CE confirmed that QDs-BSA or QDs-IgG conjugates were in fact formed.

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