Water Synthesis of Glutathione- capped Cadmium Selenide Quantum Dots and Stabilization by PVP Coating at Room Temperature

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ABSTRACT

We report the water-based synthesis and effective stabilization of glutathione-capped cadmium selenide quantum dots (GSH-CdSe QDs) with PVP, and study their optical properties. X-ray diffraction and transmission electron microscopy were applied to elucidate the structure and morphology of the materials. The absorption spectra indicate that bare GSH-CdSe QDs exhibit a maximum absorption peak at ~340 nm, which was red shifted (~100 nm) upon coating the QDs with PVP. It was also observed that PVP-coated GSH-CdSe QDs show a strong red-orange emission band peaking at ~ 450 nm. No thiol group was observed on the QD surfaces, which is a clear indicator of the successful GSH-capping. FTIR analyses also reveal that the GSH signal completely disappears by coating the QDs with PVP, showing its characteristic bonds. Furthermore, our findings evidence that the polymeric coating provides the QDs with a high degree of stability suitable for biomedical applications, especially in cell bio-imaging. Stability studies were made to the QDs indicating a shift in the absorbance peak of the GSH QDs, while the QDs coated with PVP did not showed any shift. Proving that polymeric material can give stability to QDs.

Keywords: CdSe QDs, PVP, Glutathione, Fluorescence

1 INTRODUCTION

Several materials have been developed, such as organic dyes for bio-imaging applications. However, these dyes present many disavantages in multicolor experiments due mainly to problems related to their relatively low signal intensity, short lifetime, narrow excitation ranges, and broad emission spectra [1,2]. In addition, they are easily photobleached under normal imaging conditions [1,2]. New alternatives to develop new, improved, and more sensitive materials have been thus conducted for decades. As a result, interesting reports focused on these kinds of materials have recently appeared in the literature.

Among these alternatives, the processing of semiconducting nanocrystals have allured increased attention due to that their size, shape, structure and composition can be experimentally tailored. Quantum dots (QDs) offer many improvements in the optical quality for biological imaging and other exciting applications. QDs are materials within the 2-10 nm size range, and their optical,electrical and magnetic properties are size-dependent [3,4,5]. The most commonly used QDs are composed of semiconductors of periodic group II-IV (CdSe, CdTe, CdS, ZnSe, ZnS, PbSe, PbS, PbTe, SnTe) [4,5,6], which are widely used in solar cells, telecommunications, photodetectors, and photvoltaic and biomedical application [4,5,6]. It has been reported that QDs are brighter and more stable than traditional fluorophore, such as organic dyes [1,2,3,4], and they are photostable.

To date, the most used route of synthesis is the organic method. This method is performed in organic phase at high temperatures, and employs solvents (such as trioctylphosphine oxide (TOPO) or trioctylphosphine) with high boiling points, [1,2] [4,6]. Materials developed by this method is suitable for solar cell or photovoltaic applications, but not for biomedical applications given that it still presents certain constrains. For instance, the nanocrystals are capped with hydrophobic ligands[1], and they must be water soluble. Moreover, further intermediate steps must include: ligand exchange, silica encapsulation (usually) or polymer coating [1,2,4,7]. It has been also reported that QDs coated with polymers and/or silica have greater stability [6,7,8] and less toxicity. Moreover, the fact that the inorganic semiconductor materials are toxic to living systems [9] limits the use of QDs in biological systems. However, numerous experiments have reported that modified QDs exhibit less degree of toxicity [1,2,5]. The limited toxicity has been as discribed to surface modifications [2-5]. Some examples include, surface coating with polymers [7,9], silica encapsulation [7,8], and thiolate ligands [2-3,6-7].The understanding of the effect in capped nanocrystals is currently a hot topic in this research field.

An alternative to overcome these constrains without using a multistep synthesis is to synthetize the QDs in a direct aqueous medium [6]. Herein, we present a

comprehensive study on the synthesis of CdSe QDs in aqueous medium using glutathione (GSH) (a linear tripeptide) as capping ligand, and we study the effect of the polyvinyl pyrrolidone (PVP) coating on the stabilization properties of the QDs.

2 METHODOLOGY

2.1 Reagents and Solutions

Ultra-high pure water (18.2 MΩ) was obtained from an Aries Filter Works Reverse Osmosis System to ensure a trace metal free environment. Cadmium chloride ($CdCl_2$) (306576-100g Sigma-Aldrich St Louis, MO.), Selenium powder (229865-20g Sigma-Aldrich St Louis, MO.), Sodium Borohydride ($NaBH_4$) (452882-100g Sigma-Aldrich St Louis, MO.), L-Glutathione reduced (GSH, ≥ 98.0%) 0.5 M, Sodium Hydroxide (306576-100g Sigma-Aldrich St Louis, MO.) was used in the synthesis of Glutathione-Cadmium Selenide Quantum dots (GSH- CdSe QDs). Hydrochloric acid (HCl) and Nitric Acid (HNO_3) were used to prepare aqua regia (caution: aqua regia is highly corrosive and extremely dangerous).
Selenium powder (4 g) was dissolved in trace metal HNO_3 to prepare Se(IV) solution. After dissolution the solvent was left to evaporate the excess nitric acid. The solvent was then mixed and diluted to 100 mL with 10 % v/v HCl to reduce Se(VI) to Se(IV). The final concentration on Se(IV) stock was 0.5 M. Sodium borohydride solution was freshly prepared by dissolving 1.0g $NaBH_4$ in 0.1 % NaOH.

2.2 Synthesis of GSH- CdSe QDs

Glassware was roughly cleaned with Aqua Regia (HCl-HNO_3, 3:1 by volume), and rinsed in water prior to use. The pH in this synthesis protocol was adjusted to9.5. The GSH-CdSe synthesis was made via wet chemistry on an oxygen-free environment using the protocol reported for PbSe QDs with minor modifications [6]. The ratio between the GSH:Cd and Cd:Se was 11 and 0.20 respectively. On flask #1, a 1.0M solution of $CdCl_2$ was added to 250 mL of ultra-high pure water. The capping ligand, GSH 0.5 M, was then added to the solution. The pH value was then monitored by adding a solution of 5.0M NaOH. On flask #2, a solution of Se (IV) in HCl 10% was added. The system was then purged with Nitrogen for 30 minutes. A solution of $NaBH_4$ 10% in NaOH 0.1% (freshly prepared) was next dropwise to the flask #2. When all the Selenium was reduced (15-20 minutes) the second flask was disconnected, and the first flask was refluxed overnight.

2.3 Purification

The synthesis was purified and concentrated ten times (10X). QDs solutions were added to a HPLC acetone in several ratios. A systematic series of experiments was carried out to determine the that precipitates the QDs. The optimal ratio found is 1:2. The GHS- CdSe QDs were then centrifuged at 4500 rpm for 30 min. An IEC FL40R Centrifuge (Thermo Electron Corporation) was used to purify the QDs. The supernatant was discarded and the QDs were redispersed in 10 mL of ultra-high pure water.

2.4 PVP Coating Method

For the coating process, an aliquot of 4.0 mL of the QDs was taken and exposed to different concentrations of polymeric material, PVP (M.W 40,000) (3.75×10^{-3} M and 6.25×10^{-3} M). The aliquots were left at room temperature from 4-7 days (covered with aluminum foil) to ensure the coating of QDs.

2.5 Stabilization of QDs

Stability tests were performed with the bare QDs and covered with PVP. The aliquots were left for 1 month exposed to light (room temperature), and further analized by UV-Vis.

2.6 Characterization of QDs

DR 5000 UV-Vis Spectrophotometer and Varian Cary Eclipse Fluorescence Spectrophotometer were used to characterize the QDs. UV- Vis spectra were recorded in the range of 200 nm through 1000 nm. To observe the absorption peak of the GHS- CdSe, the QDs were diluted in 2000 uL of ultra-high pure water. X- Ray diffraction (XRD) and FTIR measurements were made with powders of the QDs. A Rigaku Miniflex X-Ray Powder Difractometer was used in 2θ configuration in the range of 20–80˚ with a scanning speed of 0.1˚ min^{-1} per scan. IR spectrum was obtained using an IRAffinity-1 Fourier Transformed Infrared Spectrophotometer (Shimadzu) in the range of 400 cm^{-1} to 4000 cm^{-1}.The morphology and size distribution of GSH-CdSe QDs were analyzed using a Carl Zeiss Leo 922 transmission electron microscope (TEM). TEM samples were prepared by spotting a drop of a stable suspension of GSH-CdSe and PVP coated QDs onto a lacey carbon Cu-supported grid (01895-F Ted Pella, Inc.). Bright-field TEM images at different magnifications were acquired under a continuous accelerating voltage of 200 kV. DLS experiments were performed in a Nanotrac 150 from Microtrac.

3 RESULTS AND DISCUSSION

3.1 Characterization of GSH- CdSe QDs and PVP- coated QDs

Synthesis of CdSe QDs using GSH as ligand was first made using a wet chemistry method, and replacing the oxygen environment with nitrogen, with a $[Cd^{2+}]$/[Se] and [GSH]/ $[Cd^{2+}]$ ratio of 0.2 and 11, respectively. The UV- Vis and fluorescence spectra of GSH- CdSe QDS are depicted in Figure 1. The maximum absorption peak was found at ~340

nm, whereas the red-orange emission band at ~450 nm. Note that the fluorescence intensity in the GSH-QDs is as strong as that reported for standard TGA-capped CdSe QDs, suggesting that they can be also used in the same way for biomedical applications.

The XRD pattern of GSH- CdSe QDS is depicted in Figure 2. The diffraction peaks ((111), (220) and (311)) are consistent with the diffraction planes of the cubic phase of CdSe[9]. The absence of additional diffraction peaks evidences that the products are of high crystalline quality and purity.

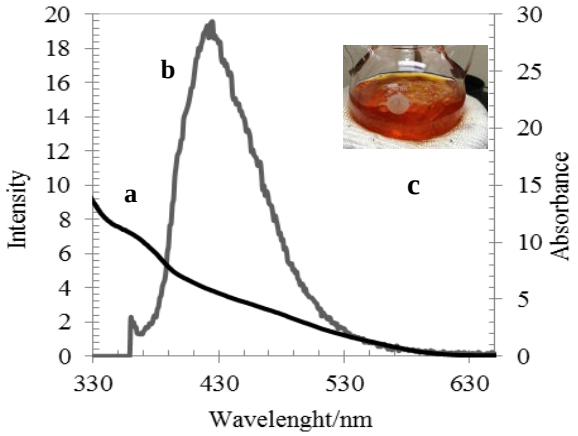

Figure 1: (a) UV-Vis and (b) fluorescence spectra of GSH-CdSe QDs. Inset shows an optical image of the so-synthesized QDs under suspension.

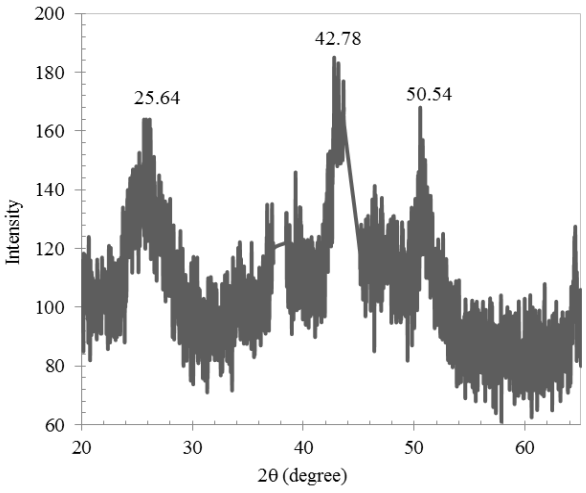

Figure 2: XRD diffraction pattern of GSH-CdSe QDs.

As described earlier, GSH was used as a capping agent. To ensure a bonding of the ligand, the pH was adjusted to 9.5. GSH has S-H bonds that show vibrations in the spectrum of the pure ligand located at 2513 cm^{-1} (not shown). However, this vibration vanishes on the GSH-CdSe capped QDs. Carboxylate moieties are confirmed by observing the bands within 1400–1470 cm^{-1} and 1616–1663 cm^{-1}, which can be assigned to symmetric ns(COO^-) and asymmetric nas(COO^-), respectively. [6]

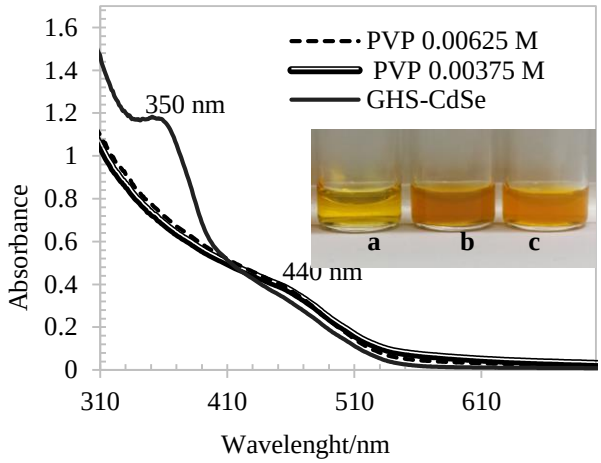

Figure 3: UV- Vis spectra of GSH-CdSe and PVP-coated QDs (at two different concentrations). Inset: optical images of (a) bare GSH-CdSe, (b) PVP-coated CdSe QDs at $3.75E^{-3}$ M, and (c) at $6.25E^{-3}$ M.

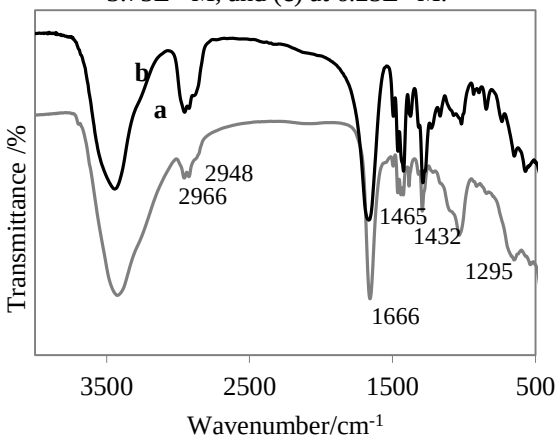

Figure 4: FTIR spectra of (a) PVP and (b) PVP- coated CdSe QDs after drying process.

As mentioned above, GSH-CdSe QDs were coated with a polymer (PVP) to increase the stability over the time, and decrease the degree of cytotoxicity. In Figure 3, it is shown that there exist a pronounced shift (~100 nm)of the absorption peak (PVP-coated QDs (440 nm) and GSH- CdSe QDs (330 nm)) after treatment with PVP. In addition, the fluorescence intensity at ~540 nm diminished sustantially when excited at the original absorbance (330 nm), indicating the successful coating of the QDs by the polymer.

FTIR results indicate that when PVP coating takes place, the GSH peaks disappeared and decreased broader nitrogen bonds (1491- 1414 cm-1) are observed instead, which are charasteristic of PVP (as shown in Figure 4). All the absorption peaks matches well with those reported in the literature [10,11]. Other characteristic peaks are found at the 1700-1600 cm^{-1} range corresponding to the C=O vibration. These results confirms that GSH-CdSe QDs were perfectly coated by PVP. These findings were further supported by

dynamic light scattering (DLS) and TEM analyses (see Figure 5)

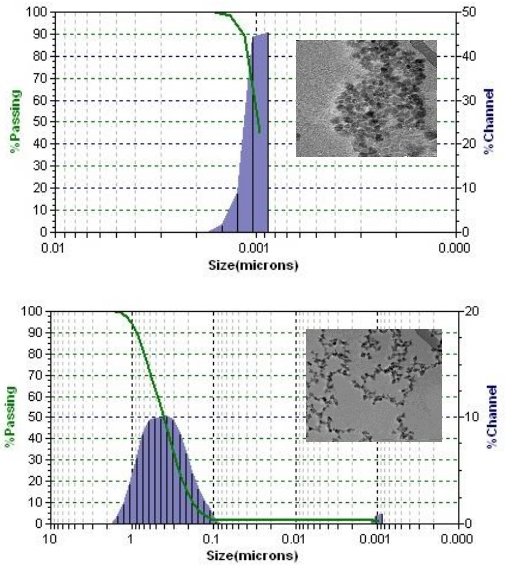

Figure 5: DLS profiles corresponding to bare GSH-CdSe (top) and PVP-coated CdSe QDs (bottom). Insets: TEM images.

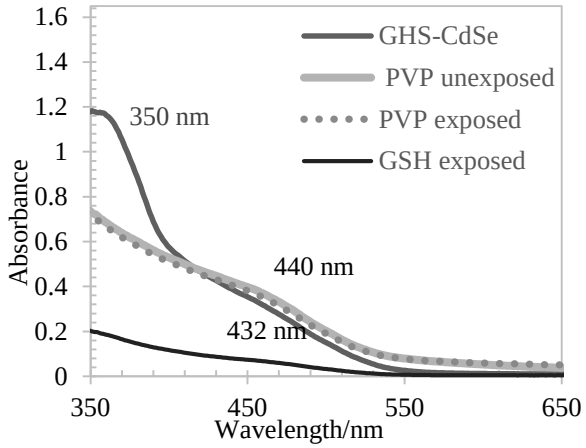

Figure 6: UV- Vis spectra comparison in stability process for GSH-CdSe and PVP- coated QDs in absence and when exposed to light.

The stability tests results showed a shift in the original absorbance peak in GSH-CdSe QDs. The original peak appeared at 350 nm, and after exposition to light at room temperature this absobance peak was redshifted to 432 nm. However, the effect of light on PVP- coated QDs was significant different. After 1 month being exposed to light the absorbance peak did not suffer any shift, it remained at 440 nm (see Figure 6). Our preliminary results thus indicate that the polymeric coating enable the QDs are more stable during the growth stage, which would improve the monitoring of their size. Nonetheless, further investigation is needed to find out the underlying mechanism.

4 CONCLUSIONS

In summary, we have successfully synthesized, in a aqueous solution, GSH- CdSe QDs. These QDs exhibited a greater fluorescence intensity compared to other standard QDs. In addition, the successful coating of the QDs with polymeric material revealed the possibility of stabilizing the growth rate, enabling an more effective path to monitor their size. This work represents a step ahead for the fabrication of biological markers. Future works with these nanomaterials include the cytotoxicity tests on eukariotic cells to observe not only their degree of cytotoxicity, but also quatum yield and quenching effect inside the cells for bioimaging purposes.

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