Introducing the Safe Capsule for CdS Quantum Dots as Bio-Labeling Device

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Abstract

Bio-labeling applications of quantum dots (QDs) have grabbed scientists’ attention due to their numerous benefits to human life. The quality properties such as strong, narrow, tunable and size-dependent emission of broad absorption spectrum QDs, combined with water-solubility and non-toxicity, are needed for QDs to perform as excellent labeling tools in biological applications. Besides, predictable and reliable controls to achieve perfect and biologically safe QDs with excellent optical properties are expected. In this study, we managed to synthesize cadmium sulfide (CdS) QDs with a mean size of 8 nm by capping it with thioglycolic acid - which has hydrogen bonding - to make QDs soluble in aqueous solutions. Polyethylenimine has functioned to render the QDs to be water soluble and enhances photo-oxidation even in aqueous solution. It also acted as a ligand to modify CdS QDs to prevent surface trap and thus increase CdS QDs’ quantum yield. Furthermore, improved stability of the QDs in polymer sphere and non-toxicity is needed. Therefore, the ideal bio-labeling QDs should be dense and equal in size and shape to achieve constant photoluminescence peak. Hence, the CdS QDs have been capsulated with poly(2-vinylpyridine) (P2VP) homopolymers and apo-ferritin in order to compare which one is the best capsule for QDs with the desired properties for applications in bio-labeling.

Key Words: Quantum dots, Bio-labeling, Colloidal method, CdS, Capsule

I. INTRODUCTION

Nanoparticles 1-10 nm in diameter with diverse surface chemistry, tunable absorption and emission properties have shown great potential in disease diagnosis and therapy. Broad ranges of nanoscale inorganics such as water soluble quantum dots (QDs) have the potential to be applied in bio-detection, cellular imaging, photothermal therapy of tumors, optical barcoding, and drug carrier [1]. Ferrari 2005 has successfully prepared fluorescent magnetic nano particles (FMNPs) composed of silica-coated CdTe QDs and magnetic nanoparticles, and used as-prepared FMNPs to label biomolecules for tumor imaging and hyperthermia therapy [2-3].

Quantum dots (QDs) are fluorescent semiconductor nanocrystals with unique optical and electrical properties. Compared to organic dyes and fluorescent proteins, QDs possess near-unity quantum yields and much greater brightness than dyes (10-100 times). QDs also show broad absorption characteristics; a narrow, continuous and tunable emission spectra due to quantum size effects. Other properties include long fluorescence lifetime (5 to > 100 ns compared to 1-5 ns in organic dyes) and negligible photobleaching (100-1000 times less than fluorescent dyes) over minutes to hours [1].

Surface functionalization has further expanded the potential of QDs as probes for biomedical applications [1]. Therefore, hydrophobic conditions for synthesis of high-quality QDs are required as many biomolecules have limited solubility and stability in organic solvents. Ligand exchange and surface stabilizers can be used for surface modification of QDs to improve stability in aqueous conditions as well as biocompatibility. In this process, heterobifunctional ligands such as mercaptoacetic acid containing thiol functionalities are used, which can covalently bind to QDs and improve their hydrophilicity. However, the ligand exchange method may induce agglomeration and decreases fluorescence efficiency. Additionally, if QDs are to be used in biomedical sciences, their toxicity should be taken into considerations. Complete characterization of size, shape, charge, surface chemistry and material properties is important when correlating toxicity. QDs also may agglomerate in vitro or in vivo and may chemically degrade, making it difficult to measure toxicity level when the QDs are conjugated with other materials. Therefore, another approach to prevent these problems is by stabilizing the surface using amphiphilic polymers [4] and biomaterials such as protein and enzyme.

II. EXPERIMENTAL PROEDURE

A. Materials

Cadmium acetate, thioglycolic acid (TGA), sodium sulfide, sodium hydroxide (NaOH), P2VP homopolymer (\(M_w=2200\) \(M_w/M_n=1.09\)), apo-ferritin from horse spleen, polyethylenimine (PEI) were used in this experiment to produce CdS, CdS coated with PEI (CdS+PEI), CdS encapsulated with apo-ferritin coated with PEI (CdS-apo + PEI) and capsulated CdS.

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with P2VP homopolymer coated with PEI (CdS-p2vp +PEI).

B. Preparation of CdS Quantum Dots (QDs)

CdS quantum dots were synthesized via a colloidal method as mentioned in our previous report [5]. CdS-apoferritin complexes were prepared by referring to Kenji Iwahori’s [6] research with some adjustments made during synthesis. 1 M of ammonium acetate and 1 M of ammonia water was mixed with 100 mM of a cadmium acetate solution in 300 mL of distilled water. Then, 0.3 mg/ml of apo-ferritin solution is added to the resulting reactant. The reaction was allowed to stand for 10 minutes under nitrogen flow at room temperature to obtain ammonium complex of cadmium. Next, 0.02 mmol of sodium sulfide was added to the reaction solution. Then, the solution was allowed to stand for 24 hours at room temperature with vigorous stirring to yield CdS-apoferritin complexes in pH 9-10 before adding PEI at the end of the reaction. CdS capped with P2VP homopolymer was prepared using this same method by replacing apo-ferritin with 1 wt% P2VP homopolymer.

C. Sample Characterization

The size of CdS QDs and CdS-apoferritin complexes were measured using transmission electron microscopy (TEM). TEM micrographs of the colloidal dispersions were obtained using a JEM-2000FX instrument operated at 200 kV as the acceleration voltage. The optical properties of CdS QDs were measured using UV-vis Absorption spectrum and Photoluminescence spectrum. The UV-vis Absorption spectrum for the colloidal dispersions of CdS QDs as the corresponding ionic precursors was measured using a Hitachi U-3010 spectrometer to determine the adsorption peak of each sample. Photoluminescence spectrum was performed using a F-7000 spectrofluorometer (Hitachi, Japan) with a quartz cell (1cm × 1cm). The fluorescence spectra were recorded at $\lambda_{ex} = 335$ nm.

III. RESULTS AND DISCUSSIONS

Safe bio-labeling with unique properties is the desired bio-imaging, perfect for tumor imaging and treatment. In this study, the in vitro experiment method was introduced for preparing safe bio-labeling based on CdS QDs for medical treatments. It is well known that CdS has great optical properties but it is also considered toxic due to the cadmium metal (Cd). To overcome this problem, we have introduced two kinds of capsules; apo-ferritin and hydrophilic P2VP homopolymer, to encapsulate the CdS QDs, with the purpose of determining which the best capsule is for these QDs.

Fig. 1 (a) and Fig. 1 (b) show the absorption spectra and the photoluminescence spectra peaks for all studied samples, respectively. The apo-ferritin encapsulated CdS (CdS-apo + PEI) and P2VP capped CdS (CdS-p2vp + PEI) are smaller in size compared to the naked CdS and CdS capped with PEI only (CdS + PEI). On the other hand, the photoluminescence spectra in Fig. 1 (b) shows that the CdS-apo + PEI spectrum is the second highest peak after CdS + PEI. Based on these spectra, it is clear that CdS-apo + PEI has small size QDs with high peak photoluminescence. Therefore, we have concluded that the combination of apo-ferritin and PEI can produce CdS QDs that are water-soluble, less toxic plus small particles with a high emission peak by encapsulating the QDs inside the apo-ferritin cage. To determine the toxicity level of these QDs, further in vivo research and clinical test are extremely needed.

Fig. 1 (a) Absorption spectra and (b) photoluminescence spectra peaks of CdS, CdS coated with PEI (CdS+PEI), CdS encapsulated with apo-ferritin coated with PEI (CdS-apo + PEI) and capsulated CdS with P2VP homopolymer coated with PEI (CdS-p2vp +PEI).

The fundamental absorption, which corresponds to electron excitation from the valence band to conduction band, can be
used to determine the value of the optical band gap [7]. The connection between the band gap \( (E_g) \) and maximum wavelength \( (\lambda_{\text{max}}) \) can be written as

\[
E_g = \frac{hc}{\lambda_{\text{max}}}. \tag{1}
\]

where, \( E_g \) is the band gap of the material, \( h \) is Planks constant, \( c \) is speed of light and \( \lambda_{\text{max}} \) is the absorbance peak wavelength.

Band gap energy is illustrated as a graph of \((ahv)^2\) versus photon energy as shown in Fig. 2, where \( a \) is absorbance constant and \( hv \) is photon discrete energy, respectively. For the band gap energy in the absence of PEI, the photon energy is 2.90 V and this value increased to 2.99 eV in the presence of PEI through calculations using the complex equation available in our previous report [8]. Meanwhile, using the same calculations, the photon energy for encapsulated CdS with apo-ferritin in the presence of PEI is 4.8 eV which increases to 5.0 eV for capping with P2VP homopolymer.

As the size of CdS-apo + PEI and CdS-p2vp + PEI are being too small and emission under 365 nm wavelength was hardly obtained, hence these two samples were placed under UV-light with 254 nm wavelength as shown in Fig. 3 (c) and (d). Fig. 3 (a) shows the CdS without any capping agent has emitted orange emission under 365 nm wavelength of UV-light. Meanwhile, the colour of the emitted light has changed from bright yellow under 365 nm wavelength (Fig. 3 (b)), with the absence of capsules, to a yellow greenish emission for encapsulation with apo-ferritin (Fig. 3 (c)) and to a bluish yellow for encapsulation with P2VP homopolymer (Fig. 3 (d)) under UV light at 254 nm. The size of core CdS QDs with presence capsules has obtained blue shifted and this result is fitted to optical properties results, where encapsulation with P2VP homopolymer’s size is smaller than encapsulation with apo-ferritin. On the other hand, these two samples are smaller than naked CdS QDs and also CdS QDs with PEI.
with the size and shape of the ferritin protein. Additionally, by introducing apo-ferritin, the aggregation of dark circles around the CdS QDs (Fig. 4 (a)) was prevented if compared to QDs encapsulated with P2VP homopolymer (Fig. 4 (b)), even though the size of the QDs much smaller than those encapsulated with apo-ferritin.

Fig. 4  TEM images of (a) encapsulated CdS with apo-ferritin and (b) capsulated CdS with P2VP homopolymer.

Meanwhile Masanobu Naito et al. 2010 [9] prepared CdS inside ferritin without PEI coating and as our experiment has shown, by adding PEI, the CdS QDs obtained great PL peaks of smaller size at maximum wavelength of 455 nm compared to Masanobu Naito et al. 2010, where their CdS-ferritin gave a broad PL band with wavelength of 780 nm. In reference to Hanying Zhao et al. 2001 [10], they introduced CdS nanoparticles into PS-\(b\)-P2VP block copolymer micelles. In our study, however we used P2VP homopolymer to encapsulate CdS purposely to increase hydrophilicity of CdS QDs and also to control CdS QDs’ size. Their study also mentioned that a single micelle is composed of P2VP-Cd\(^{2+}\) complexes in the core and PS blocks in the shell. In the case of our study, the P2VP molecules were just attached to CdS particles’ surface purposely to control their size. Hence, the use of PS-\(b\)-P2VP is as a stabilizer. With that said however, we did not only use P2VP as a stabilizer but also for preventing CdS QDs from toxicity problems. Based on this study, we found that encapsulated CdS with apo-ferritin resulted better capsulation when compared to P2PV homopolymer as the capsule.

IV. SUMMARY

CdS QDs encapsulation process has resulted in CdS QDs with small particle size compared to CdS QDs without capsule. Therefore, the developed CdS QDs with high band gap energy has the possibility of being employed as bio-labeling due to the water solubility and non-toxicity properties due to use apo-ferritin as a capsule. Although nanoparticle-based molecular imaging applications are moving towards clinical applications, formulation challenges such as aggregation and storage in clinical settings remain a challenge such as mentioned by S. K. Nune et al. 2009 [1]. The possibility of using apo-ferritin as capsules for nanoparticles might just be the solution for the aggregation problem and CdS QDs can be used as a tracer without doubt about its toxicity problem that is sourced from cadmium metal.

REFERENCES