Synthesis and Photoluminescence Properties of Water-Soluble ZnS Quantum Dots for Biomarkers

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Abstract. In this work, we investigated the effects of the surfactant L-cysteine (L-cys) and folic acid (FA) on photoluminescence (PL) properties, water-solubility and biotoxicity of ZnS quantum dots (QDs). It was found that capping L-cys improved the water-solubility of ZnS QDs. The emission intensity of ZnS QDs was found to be also enhanced after capping with L-cys. The maximum emission intensity of ZnS capped with L-cys QDs was about 4.5 times stronger than the uncapped ZnS QDs. FA decreased the PL intensity of L-cys/ZnS QDs and increased water-solubility the of L-cys/ZnS QDs. The L-cys/ZnS and FA@L-cys/ZnS quantum dots were proved to be nontoxic by the MTT assay.

1. Introduction

ZnS quantum dots (QDs) as a bio-probe due to its low-toxic and excellent PL properties [1–5]. The vital factor for ZnS QDs to have more practical applications in biomedicine, is that they must own better water solubility and higher luminous intensity. One of methods for improving the photoluminescence and water-soluble of ZnS QDs is coat them with surfactant agents, and many important achievements have been obtained [6-8]. K. Deka et al. [6] reported that 2-mercaptoethanol capped Mn-doped ZnS nanocrystals with different doping contents (0.5%, 1%, 1.5% and 2%) have the potential for development of phosphorescence sensor for detection of extremely low concentration of ions, particularly Ni^{2+} and Mn^{2+}. Kuppayee et al. [7] used a co-precipitation method to synthesize Cu^{2+}-doped ZnS nanoparticles with methyl methacrylate (PMMA) and cetyltriethylammonium bromide (CTAB) surfactants, and they found a 2-fold enhancement in the luminescence after Cu^{2+} doping. A.A. Bol et al. [8] found that the quantum efficiencies of nanocrystallines ZnS:Mn^{2+} capped with polyvinyl butyral (PVB), polyethylene glycol (PEG), MA (methacrylic acid), or sodium polyphosphate (PP) was higher than that of nanocrystallines without a passivating polymer.

Although, L-cys is a commonly used capping agent for the synthesis of various un-doped and doped nanocrystals, most research groups have paid attention to either the water-soluble and photoluminescence property behavior of QDs [9, 10]. N. Ertas et al. [9] used L-cys capped Mn doped ZnS quantum dots/Idarubicin (IDA) nanohybrids as novel room temperature phosphorescence (RTP) sensor to detect double stranded deoxyribonucleic acid (ds-DNA)/drug interaction. D. Diaz-Diestra et al. [10] used L-cys to cap ZnS: Mn QDs, and found that QDs display a prominent orange emission band peaking at 598 nm. R. K. Sajwan et al. [11] studied the Quenching response of cyst capped CdSe QDs. It was found that due to formation of complex of Zn^{2+} ion onto QDs surface which would restrict ligand rotation that enhanced the conformational rigidity of the surface substituent, resulting in...
suppress the quenching of Cyst capped CdSe QDs. This serves as an object of this present work to study the effects of L-cys on both of the luminescence and water-solubility of ZnS QDs.

Tumor microenvironment is of great significance for tumor markers and treatment. The tumor microenvironment can be regarded as a high expression of folate receptor [12]. Due to folic acid (FA) can improve drug targeting tumor cells and water solubility [13]. FA is usually used as a synthetic of tumor marker. In this experiment, FA was used to modify the water-soluble L-cys/ZnS quantum dots, and it was made with the function of labeling tumor.

In our previous work [14], ZnS QDs was prepared by low temperature solid-state reaction method. In this work, we intended to investigate the effects of L-cys on PL properties, water-soluble behavior of ZnS QDs prepared by a solid-state reaction method. Due to the tumor marker effect of folic acid (FA). FA was modified on the surface of L-cys/ZnS. It was aim to synthetize the quantum dots with innocuity and labeling function.

2. Experimental

2.1. The synthesis of L-cys/ZnS QDs
The as-synthesized ZnS quantum dots used in the present study were synthesized by a solid-state reaction method reported earlier [15] and labeled with sample A. For ZnS QDs capped with L-cys, L-cys (A. R.) was mixed with ZnS QDs according to the mass ratio of L-cys to ZnS (0.4:1, 0.8:1, 1.2:1, 1.26:1, 1.4:1, 1.6:1) and labeled with L1, L2, L3, L4, L5 and L6, respectively. The exact amounts of these starting materials used in the synthesis were detailed in Table 1. After ZnS QDs was washed three times with n-hexane and acetone, respectively, 5 ml n-hexane and L-cys were added with grinding until n-hexane completely evaporated. And then the products were moved to the water solution. The L-cys/ZnS QDs was obtained.

2.2. The synthesis of FA@L-cys/ZnS QDs
The corresponding 4 groups of FA were dissolved in 30ml dimethylsulfoxide (DMSO). Add the carbodiimide (EDC) and stir until the FA was dissolved. Then 500 mg sample L5 was added and stirring 24h at room temperature. The supernatant was removed by centrifugation and the solid was washed with water for three times. In the end, ultra pure water was added to the ultrasonic reaction for 10min. The solution was centrifuged and dried at 60 °C for collection. The samples of FA@L-cys/ZnS QDs were detailed in Table 2 and labeled with F1, F2, F3, F4.

The crystal structure of the samples was measured by an X-ray diffraction (XRD) (Japan, XRD6100, CuKα) at room temperature. The measured scanning speed and procedure were 2°/min, 0.02° in the range of (2θ = 25-80 °). The transmission electron microscope (TEM, TECNAI G² 20 S-TWIN) was used to analysis the surface morphology of the samples. Fourier transform infrared (FTIR) spectra were obtained by a Nicolet 5700 spectrometer. The emission spectra were measured by a photoluminescence spectroscopy with a He-Cd laser (Zolix, OmniPL-LF325) with a spectral slit width of 1 nm. Scanning speed and measurement steps were 60 nm/min and 1 nm, respectively.

For MTT assay, the sample L5 and F1 was set as 2 concentrations groups (10 and 20 μg•ml⁻¹), respectively. A control group was set up to calculate the cell viability. Each group was established three holes and cultured with fibroepithelial L929 for 48 hours. The morphology of the cells were observed by the inverted microscope nikon (Nikon ECLIPSE Ti). After Dimethyl Sulfoxoydum (DMSO) was added, the optical density (OD) of each hole was measured by a microplate reader (BioTek). Finally, the cell survival rate was calculated by the OD value of experimental group dividing the OD value of blank group.

Table 1. Amounts of starting materials used in the synthesis of L-cys/ZnS QDs.

|        | A  | L1  | L2  | L3  | L4  | L5  | L6  |
|--------|----|-----|-----|-----|-----|-----|-----|
| ZnS (g) | 0.5| 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| L-cys (g) | 0  | 0.2 | 0.4 | 0.6 | 0.631| 0.7 | 0.8 |
Table 2. Amounts of starting materials used in the synthesis of FA@L-cys/ZnS QDs.

|     | F1 | F2 | F3 | F4 |
|-----|----|----|----|----|
| L5 (mg) | 500 | 500 | 500 | 500 |
| FA (mg) | 1   | 5   | 10  | 50  |

Figure 1. XRD patterns of L-cys/ZnS QDs (a) and FA@L-cys/ZnS QDs (b).

3. Result and discussion

Figure 1 presents the XRD patterns of L-cys/ZnS QDs and FA@L-cys/ZnS QDs. The results indicated that all of samples were of cubic zinc blende ZnS crystal structure. Which were consistent with the XRD card number 05-0566, the corresponding crystallographic plane of each peak was marked by the short line in the picture. Diffraction peaks from high angles have submerged in the background in the XRD patterns because of large line broadening, which may be on account of the nanosized of particles [16]. The broadening of the XRD peaks indicates the nanocrystalline nature of the samples. In addition, there were no diffraction peaks from manganese impurities were detected. The average crystallite size of all samples can be calculated by Scherrer’s equation [17]:

\[
d = \frac{k\lambda}{\beta\cos\theta}
\]

Where \( k = 0.89 \), \( \lambda \) is the wavelength of the X-ray and equals 1.54056 Å, \( \beta \) is the half-peak width of the diffraction peak and \( \theta \) is the Bragg angle. According to the equation (1), The average crystallite size of seven samples was obtained to be about 4.6, 4.4, 5.1, 4.4, 4.3, 4.2 and 4.3 nm for the samples A (ZnS), L1, L2, L3, L4, L5 and L6, respectively. In Figure 1b, the samples diameter of samples was calculated to 3.5, 3.4, 3.5 and 3.9 nm for F1, F2, F3 and F4, respectively. The diameter of ZnS QDs increased slightly with L-cys capped, but it was decreased by the FA modification. This was due to ZnS QDs was capped with L-cys sucessfully. The particle size of FA@L-cys/ZnS quantum dots decreased for the preparation process.

Figure 2 gives the TEM and high-resolution (HR) TEM images of sample A (Figure 2a), L5 (Figure 2b) and F1 (Figure 2c). There were still some reunion phenomena in the picture. As seen from HRTEM of Figure 2a, the as-synthesized QDs average diameter was about 4.0 nm and the samples were nearly spherical shape. Compared to the as-synthesized samples, it can be seen that the sizes of sample L5 and sample F1 were same to the average diameter of 4-5 nm, which is consistent with the values calculated by the Scherrer’s formula. The inset pictures of Figure 2b and Figure 2c showed that ZnS was modified with L-cys and FA dispersed well, indicating that these two substances could reduce the agglomeration of quantum dots.
FTIR spectra of L-cys, sample A and L5 were recorded in the range 4000-400 cm\(^{-1}\) and are shown in Figure 3. The absorption peak at about 669, 1008, 1334, 1400, 1570, 2078, 2554, 2972 and 3184 cm\(^{-1}\) are assigned to C-S, C-NH\(_2\), C-O, C=O, N-H, S-H, N-H and O-H, respectively [18]. It can be seen that, compared to L-cys sample, the absorption peak at 2554 cm\(^{-1}\) in the capped QDs (sample L5), which originates from the stretching vibration of -SH in L-cys, was disappeared, but the peak of the stretching vibration of C=O was still observed at about 1600 cm\(^{-1}\). This indicated that ZnS QDs were capped with L-cys [19]. Also, from Figure 3, the result indicated that as compared with the sample A, the absorption peak of sample L5 shifted to lower wavenumber, which is due to the manifestation of size confinement. The results showed that Sample L5 has a larger grain size, which is consistent with XRD and TEM analysis.

Figure 4a shows the emission spectra of ZnS samples without and with L-cys under 325 nm laser excitation. The emission spectra for all samples were found to show only one emission band at 350-600 nm due to the radiative recombination of the defect state in ZnS nanocrystals [15]. It was clear that the emission intensity of ZnS QDs was enhanced after capping with L-cys. The PL of the samples increased with the increase of the L-cys concentration and reached a maximum when the mass ratio of L-cys to ZnS = 1.4 : 1, then decreased after that. ZnS QDs surface has a large number of dangling bonds that can cause defects and adsorption, while the surface dangling bond state located inside the band gap usually inhibits the PL intensity. However, the number of dangling bonds in the surface of
QDs is reduced by capping L-cys [20]. Accordingly, when the L-cys compensated for the surface hanging bond of ZnS and the coincidence luminescence of ZnS was enhanced, so the luminescence of ZnS was enhanced. When the amount of cysteine was equal to the dangling bonds of the quantum dots, the intensity of luminescence reached the maximum. Beyond this value, the intensity of luminescence would decrease. That is why the luminescence of the samples followed the order L5 > L6. It also can be seen in Figure 4a that the emission intensity of the sample L5 was estimated to be 4.5 times greater than that of the ZnS QDs without L-cys. From the inset in Figure 4a, it can be observed that L-cys can improve the water-solubility of ZnS QDs. This indicated that water-soluble ZnS QDs can be obtained by this method. Figure 4b presents the Emission spectra of FA@L-cys/ZnS QDs under 325 nm excitation. The diagram showed that the intensity of luminescence follows the sequence: L5 > F1 > F3 > F2 > F4. This indicated that the modification of FA weakened the luminescence intensity of sample L5. The existence of FA groups obscured the luminescence of L5 quantum dots. Even so, FA@L-cys/ZnS QDs still had high luminous intensity and had the effect of tumor markers after the grafting of FA. Both the sample L5 and sample F1 expressed excellent water-solubility in the inset image of Figure 4b.

![Figure 4](image_url)

**Figure 4.** Emission spectra of ZnS and L-cys/ZnS (a), L5 and FA@L-cys/ZnS QDs (b).

![Figure 5](image_url)

**Figure 5.** The MTT assay of sample L5 (a:10 ug/ml, b:20 ug/ml) and sample F1 (c:10 ug/ml, d:20 ug/ml).
Figure 5 displays the MTT assay of L-cys/ZnS QDs and FA@L-cys/ZnS QDs. The pictures were obtained by a Nikon inverted microscope. Different concentrations of L5 and F1 were incubated with fibroepithelial L929 for 48 hours. By comparing the experimental group with the blank group, the enzyme labelled instrument was used for detection. The survival rate of cells in each group were a:102.45%, b:98.33%, c:97.11%, d:96.03%. With the increasing of concentration, the black spots were also increasing in the picture. The cell viability of all samples were approached 100% and almost unchanged. It indicated that L-cys/ZnS and FA@L-cys/ZnS QDs were nontoxic.

4. Conclusion
In summary, the resulted revealed that ZnS, L-cys/ZnS and FA@L-cys/ZnS QDs had a cubic blende crystal structure. The average crystallite sizes of all samples were in the range from 3.5 to 5.1 nm. The surface topography of ZnS QDs had no obvious change with the L-cys and FA modification. L-cys had increased the PL of ZnS. The result indicated that when the mass ratio of L-cys to ZnS was 1.4:1. The emission of ZnS QDs (the sample L5) was the most, and was about 4.5 times greater than that of ZnS QDs without L-cys. It was observed that capping with L-cys and FA helped to improve the water-solubility of ZnS QDs. FA decreased the PL and endowed it with labeling function of L-cys/ZnS QDs. MTT experiments showed that ZnS quantum dots modified with L-cys and FA were nontoxic, which was of great significance to biomarkers and detection.

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