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# Encapsulation of CdSe/ZnSe Quantum Dots by Liposome Complexes

SHAN, Gui-Ye[a,b](单桂晔) LI, Di[c](李迪) FENG, Li-Yun[b](冯力蕴)
KONG, Xiang-Gui[b](孔祥贵) LIU, Yi-Chun[b](刘益春)
BAI, Yu-Bai*[,a](白玉白) LI, Tie-Jin[a](李铁津) SUN, Jia-Zhong[a](孙家钟)

[a] *College of Chemistry, Jilin University, Changchun 130023, China*
[b] *Key Laboratory of Excited State Process, Changchun Institute of Optic Fine Mechanics and Physics, Chinese Academy of Sciences, Changchun 130033, China*
[c] *State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China*

A general method was developed to encapsulate CdSe/ZnSe quantum dots by phospholipids liposome complexes, which rendered the quantum dots water-soluble and biocompatible. The encapsulation process was confirmed by transmission electron microscopy and photoluminescence spectroscopy. The photoluminescence of CdSe/ZnSe quantum dots in the liposome complexes was quite stable; their fluorescence remained in the same order of magnitude even after 90 min of photobleaching. CdSe/ZnSe quantum dots in the liposome complexes were further applied as fluorescence marker in immune assay for the detection of rat monoclonal CD95 antibodies. Quantum dots encapsulated by this facile strategy could also be applied in DNA, enzyme and cell labeling, and contribute a lot to the development of *in vivo* imaging and probing of tissues.

**Keywords** quantum dot, liposome, immunoassay

## Introduction

Nano-scale semiconductor crystallites (quantum dots) have received great interest for their unique size-dependent chemical and physical properties. In particular, extensive research has been focused on II—VI quantum dots (QDs). Compared with conventional organic dye molecules, QDs offered advantages of their narrow and particle size tunable emission spectra, wide excited spectra and good photostability.[1-4] However, the main disadvantage of QDs lies in that they are capped by hydrophobic coating shell and water-unsoluble.[5,6] Effect on transferring the hydrophobic QDs into aqueous solution is the key focus in recent years. In one method, a more hydrophilic ligand was ligand-exchanged on the QDs surface.[7] In another method, QDs were coated on the surface with another outer hydrophilic shell, such as silica[8-11] or block-copolymer,[12,13] even though it was still hard to avoid the aggregation of QDs and the non-specific adsorption in aqueous media.

Dubertret[14] reported the encapsulation of QDs by phospholipid block-copolymer micelles and demonstrated both *in vivo* and *in vitro* imaging. Recently Wu[15], described that octylamine-modified amphiphilic polyacrylic polymer could be also employed to encapsulate the QDs to render them water-soluble. Petruska[16] employed an amphiphilic polymer network to complex QDs to enable the QDs to be incorporated into titania matrix. Larson[17] used these polymer materials to encapsulate QDs for multiphoton fluorescence *in vivo* imaging. However, the bio-compatiblity of the polymer can not be comparable with natural phospholipids.

Phospholipids are natural amphiphilic materials in cell membranes and vesicles. When suitably mixed with water, the oily parts of amphiphiles tend to associate while the more hydrophilic parts face inner and outer aqueous solution to form two interfaces of the membrane.[18-20] Therefore liposomes formed from amphiphilic lipids have spherically closed lamellar structures. In this article, we report the encapsulation of CdSe/ZnSe core/shell QDs by phospholipid liposome complexes. After encapsulation, the liposome complexes enabled the QDs water-soluble and biocompatible, while the photoluminescence remained. The liposome complexes were also applied as fluorescence marker in immune assay.

* E-mail: yubai@jlu.edu.cn; Fax: 0086-0431-8499192
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## Experimental

### Chemicals

Poly(ethylene glycol) (PEG, $M_w$=2000), cholesterol (Ch), propidium iodide (PI), rat monoclonal antibody CD95, anti-CD95 antibody, *L*-*α*-phsophatidyl-ethanolamine (PE, 98% from Bovine brain) and *L*-*α*-phosphatidylcholine (PC, 90% from egg) were purchased from Sigma and used as received. Trioctylphosphine (TOP, 90%), trioctylphosphine oxide (TOPO, tech. 90%), hexadecylamine (HDA, 90%), stearic acid (SA, 95%), zinc stearate (ZnSA, 90%), cadmium oxide (CdO, 99.9%) and Se powder (99.999%) were purchased from Aldrich and used without further purification. Ethyleneglycol-bis(*β*-amionoethylether)tetraacetic acid (EGTA), methanol (A.R.), hexane (A.R.) and acetone (A.R.) were purchased from Beijing Chemicals.

### Synthesis of CdSe/ZnSe core/shell QDs

CdSe nanocrystals with the average diameter of 4.5 nm were synthesized according to a modified Peng's method,[1-4] and the difference lay in that the added amount (mole) of Se was 10 times larger than that of Cd precursor, CdO. In brief, 0.1 mmol of CdO and 0.4 mmol of SA were added to a 50 mL three-neck flask and heated to 150 ℃ for 30 min to form $Cd(SA)_2$. The mixture was cooled to room temperature before the addition of 5 mmol of TOPO and 8 mmol of HDA. The resultant solution was heated to 150 ℃ again and the drying process was continued at this temperature for 20 min under vacuum. Then the solution was heated to 310 ℃ and stock solution of TOPSe prepared by dissolving 1 mmol of selenium in 2 mL TOP was quickly injected into the above mixture under vigorous stirring. The temperature was cooled to 280 ℃ for 2 min to grow the particle further.

The freshly prepared CdSe crude solution was cooled to 180 ℃. Then ZnSA was added into the Se rich mixture at this temperature. CdSe/ZnSe core/shell QDs were synthesized in a one-pot method. The thickness of the ZnSe shell was determined by the amount of ZnSA.[21] In a typical experiment, toluene solution of ZnSA (0.27 mmol) was injected into the CdSe solution by means of syringe pump at 180 ℃ and the corresponding thickness of the ZnSe shell was *ca*. 0.5 nm. The resulting mixture was refluxed under stirring for 1 h and cooled to room temperature without the addition of excess Se and TOP ligand, which was different from the protocol adopted by Reiss.[5] The as-prepared core/shell QDs were purified by precipitation with methanol and redissolved in hexane. The purification process was repeated four times and the resultant CdSe/ZnSe QDs were stored at ambient temperature for further experiment.

### Encapsulation of the CdSe/ZnSe core/shell QDs by the liposome complexes

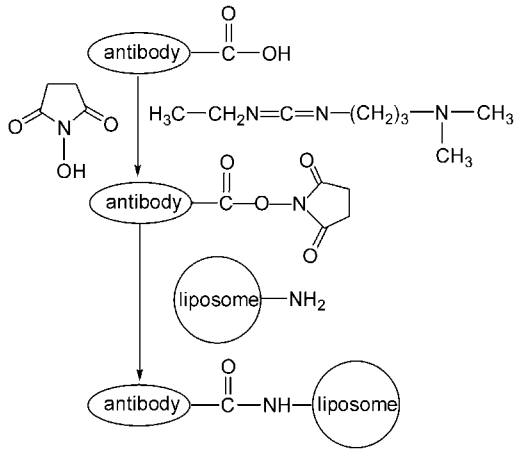
The fluorescence liposome complexes containing CdSe/ZnSe nanocrystals were prepared following two-step method. Firstly, PC, PE, PEG and cholesterol with the molar ratio of 8∶1∶4∶3 were dissolved in chloroform and heated to 35—45 ℃. Then 1 mL of chloroform solution of CdSe/ZnSe QDs (0.284 mg/mL) was added at this temperature. This was a point of departure from Dubertret's method,[14] in which temperature was heated to 80 ℃ and micelle structure was formed. After the materials were fully mixed, chloroform was completely removed under vacuum and the resultant thin film was dried in nitrogen. Secondly, 50 mL of phosphate buffer solution (pH=7.4) was added in the resulting mixture and sonicated to form the liposome (Scheme 1). The final solution was extruded through a polycarbonate filter (0.2 μm) to remove large part of liposomes.

### Bioconjugation of the liposome complexes with CD95 antibodies

The bioconjugation process of CD95 antibodies with the liposome complexes is shown as Scheme 1. Purified CD95 antibodies (10 μL, 0.287 mg/mL) in PBS buffer was mixed with dehydrating agent 1-ethyl-3-[3-dimethylaminopropyl]carbdiimide (EDC, 0.4 mg) and *N*-hydroxysulfosuccinimide (NHS, 0.6 mg), then 3 mL of liposome complexes (0.3 mg/mL) were added in the mixtures at ambient temperature and stirred for about 30 min. EDC reacts with the carboxyl group to form an amine-reactive intermediate, *O*-acylisourea. The intermediate is unstable in aqueous solution without stabilization by NHS.

Unreacted EDC was quenched by the addition of 1.4 μL of 2-mercaptoethanol and stirred for 15 min.

**Scheme 1** Anti-CD95 antibody bioconjugated with the liposome complexes in a two-step method




### Detection of the liposome complex labeled antibody by ELISA

The sensitivity of CD95 antibody was checked by detecting the fluorescence intensity. The 96-well microplates were previously coated with anti-CD95 antibodies and further incubated with 1% BSA in bicarbonate buffer for 3 h at room temperature. Then different amounts (0.1—30 ng/mL) of CD95 antibodies conju-

gated with the liposome complexes were incubated in the well for 2 h at room temperature. Subsequently, these plates were washed with PBS to remove excess CD95 antibody conjugated liposome complexes. The fluorescence intensity was measured with an automatic ELISA reader (Bio-Rad model 550) (excited wavelength＝450 nm). Dose-dependency curve was obtained by describing fluorescence intensity against CD95 antibody concentration.

## Results and discussion

The PL spectra of CdSe/ZnSe QDs with increasing amount of ZnSA precursor are shown in Figure 1a. In this experiment red shift in the PL spectra was observed, indicating the formation of the CdSe/ZnSe core/shell QDs, not $Cd_xZn_{1-x}Se$ alloy nanocrystals.[22] The PL of the CdSe/ZnSe was dependent on the thickness of the ZnSe shell, which was in turn determined by the amount of ZnSA precursor.[21] The largest PL intensity appeared in Figure 1a (E), indicating that the thickness of the ZnSe shell reached its maximum at that point. However further increasing amount of ZnSA precursor resulted in the decrease of PL intensity, which was interpreted as the formation of new interface strain. The interface stain would lead to new interface defects, which became electron and hole trapping centers and quench the PL of CdSe.[23-25] The curve in Figure 1b is the relationship between quantum yield of the core/shell QDs and the amount of ZnSA precursor. The quantum yield also reached its maximum, 93%, at this point, nearly four times larger than that of free CdSe.

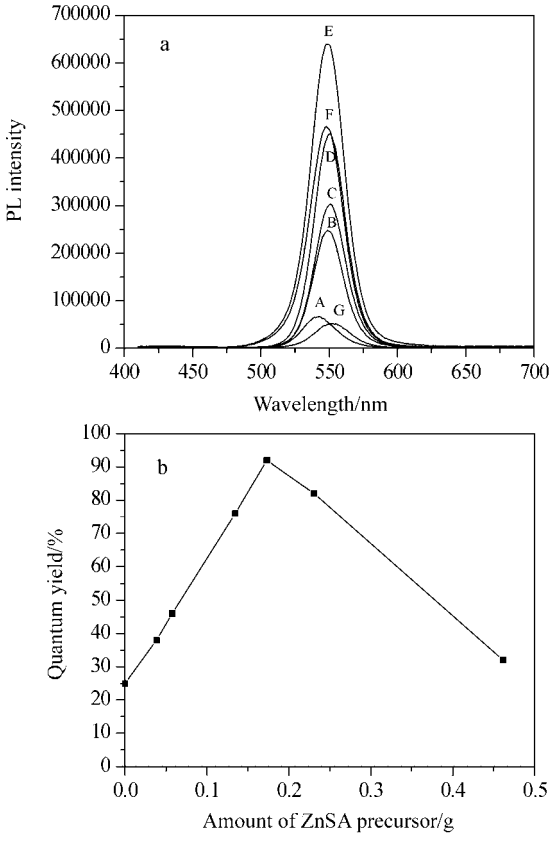



**Figure 1** (a) Photoluminescence spectral change of the CdSe/ZnSe QDs with increasing amount of ZnSA precursor, where A was photoluminescence spectrum of CdSe core [the amount of ZnSA precursor was 0.03845 g (B), 0.05768 g (C), 0.13458 g (D), 0.17303 g (E), 0.23070 g (F) and 0.46140 g (G), respectively and the excitation wavelength was 400 nm]; (b) relationship between the quantum yield and the amount of ZnSA precursor.

The TEM image of ZnSe/CdSe QDs is shown in Figure 2. The average diameter was (5±0.3) nm. The particles appeared spherical and no aggregation was observed.

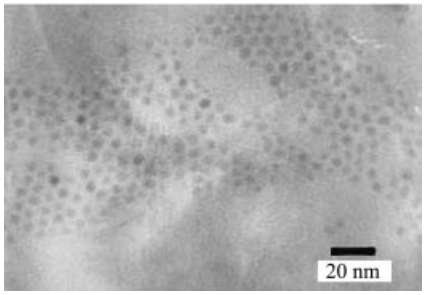



**Figure 2** TEM image of free CdSe/ZnSe QDs with diameter of (5±0.3) nm prepared by a one-pot method.

During the encapsulation process, the hydrophobic coating on the surface of nanocrystals interacted with hydrophobic regions of liposome via hydrophobic-hydrophobic interaction, while the hydrophilic region of liposome exposed to water phase. Therefore, the liposome not only enabled QDs to transfer into aqueous phase but also provided biocompatible condition for bioconjugation. Meanwhile, PEG is an important biomaterial with excellent properties of high hydrophilicity, biocompatibility and nontoxicity.[26-28] Until now, PEG has been widely utilized in modifying the surface of the biological macromolecules. The TEM image of the liposome complexes with the diameter range of the liposome from 60 to 100 nm is shown in Figure 3. The wide size distribution was the characteristic of solvent evaporation methods commonly used to prepare liposome. It could be clearly seen that the CdSe/ZnSe QDs existed as darker dots in the liposome complexes and a dim layer of PEG on the outer surface of the liposome. The layer of PEG on the surface of the liposome complexes could avoid the aggregation of liposome and prevent nonspecific absorption.

Compared with Dubertret's work,[14] the liposome complexes containing QDs were prepared at a relatively low temperature (35—45 ℃) in our experiment and the high temperature (80 ℃) needed for formation of micelle was avoided. Therefore activity of the phospholipids in the liposome complexes would be much better, which also means that the biocompatibility of the liposome complexes is better than that of micelle.

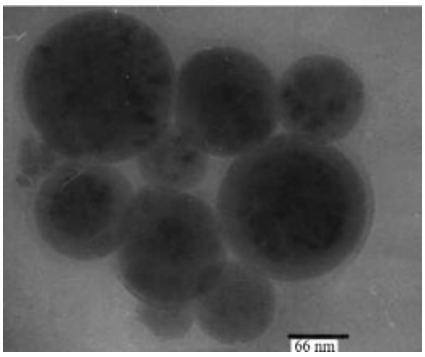



**Figure 3** TEM image of CdSe/ZnSe QDs encapsulated by the liposome complexes with diameter of the liposome complex ranging from 60—100 nm.

After encapsulation by the liposome complexes, QDs in the liposome complexes could be dispersed in aqueous media. It is reasonable to doubt whether their PL remains. The emission spectra of the free QDs (a) and QDs in the liposome complexes (b) at $\lambda_{ex}$ 400 nm are compared as shown in Figure 4. From the analogy of the emission band position (Figure 3a and 3b) it is indicated that PL of the CdSe/ZnSe QDs was well preserved. The decrease of PL intensity resulted from that the chloroform solvent was replaced by water.

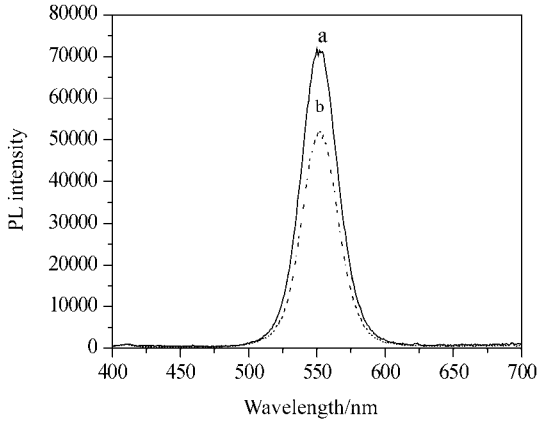



**Figure 4** Photoluminescence spectra of the CdSe/ZnSe QDs before (a, in chloroform solution) and after (b, in aqueous solution) encapsulation by liposome complexes at the excitation wavelength of 400 nm.

The photostability of QDs in the liposome complexes was further compared with that of one organic dye molecule PI, which is commonly used in cell and DNA labeling. QDs and PI were illuminated with UV lamp (12 W) at the wavelength of 365 nm. Figure 5 plots the time-dependent PL intensity against photobleaching of QDs in the liposome complexes. The decay in the PL intensity of QDs encapsulated by the liposome complexes was quite low and only lost 30% after 90 min of UV bleaching, while the photostability of organic dye PI (Figure 5, insert curve) was quite poor and the fluorescence faded within 2 min. The photostability of the QDs in the liposome complexes will enable them to be fine fluorescence marker in immune assay.

Further experiments were carried out to apply the liposome complexes as fluorescence marker in immune assay. CD 95 antibodies were bioconjugated with the liposome complexes via a classical reaction (Scheme 1). The detection of CD95 antibodies was conducted by a direct binding fluorescence-based immune assay. The dose-dependency curve is shown as Figure 6. The fluorescence signal for the CD95 antibodies was measured over a concentration range of 0.1—30 ng/mL. The PL intensity was increased linearly through the concentration range and the linear relation indicated that liposome complexes were bonded to microplate via specific interaction between antibody and second antibody.

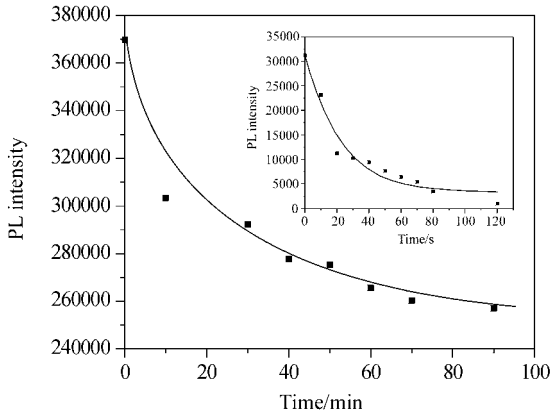



**Figure 5** Photostability of QDs in the liposome complexes [time dependent PL intensity of QDs in the liposome complexes plots against photo bleaching by illumination with a UV lamp (12 W) at the wavelength of 365 nm (insert curve shows the time-dependent PL intensity of organic dye PI under the same condition)].

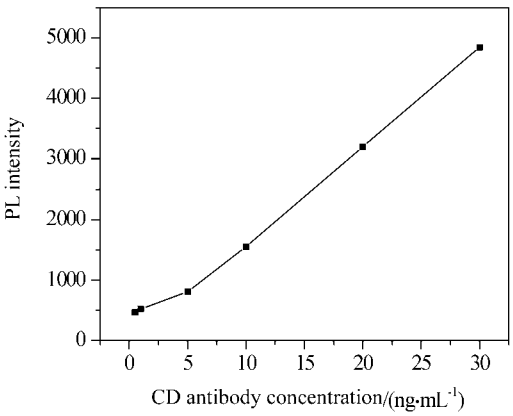



**Figure 6** Calibration curve describing the fluorescence intensity against CD95 antibody concentration.

## Conclusion

In conclusion, a novel method was developed to encapsulate QDs by phospholipids liposome complexes. QDs encapsulated by the liposome complexes were biocompatible and could be dispersed in aqueous media.

PL of QDs in liposome complexes was rather stable and our primary results indicated that they could be employed as fluorescence marker in immune detection assay. This facile liposome encapsulation strategy could also enable QDs be applied to DNA, enzyme and cell labeling, in particular, *in vivo* imaging.

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