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Synthesis and characterization of fluorescent Chitosan-ZnSe/ZnS nanoparticles for potential drug carrier

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Abstract: The objective of the study is to describe a new approach of combining quantum dots into chitosan as an anti-cancer drug carrier. Novel Chitosan-ZnSe/ZnS (CS-ZnSe/ZnS) nanoparticles were synthesized by one-step ionic gelation technique, ZnSe/ZnS quantum dots (ZnSe/ZnS QDs) as cross-linking agent and fluorescent labeling. The approach not only avoids the use of emulsifiers and chemical cross-linking agents but also prevents the possibility of damage to drugs. The fluorescent CS-ZnSe/ZnS nanoparticles were about 100-500 nm in size and stable in physiological environment. Low cytotoxicity was ensured by investigated with mice lung carcinoma cells. The cell viability remained 99% when the concentration of CS-ZnSe/ZnS nanoparticles increased to 200 μg/mL. *In vitro* drug release experiment, 5-fluorouracil (5-Fu) loaded within CS-ZnSe/ZnS nanoparticles had more preferable sustained-release performance and longer equilibrium time compared with that of the

pure 5-Fu. The fluorescent CS-ZnSe/ZnS nanoparticles were expected to be used as biological fluorescent labeling and drug carrier.

Key Words: ZnSe/ZnS quantum dots; chitosan; fluorescent; nanoparticles

1. Introduction

In recent years, luminescent materials have attracted tremendous attention due to its ideal optical properties and a wide range of potential biomedical applications in bioassays and intracellular labeling, [1-2] ion detection, [3] tumor cell targeting,[4] and the essay of telomerase activity.[5] Compared with conventional organic fluorophores, quantum dots (QDs) have size-tunable spectral properties, symmetric fluorescence emission spectrum, excellent photostability and other advantages.[6] Recently, traditional cadmium chalcogenide (CdS, CdSe and CdTe) QDs have become the most common materials, but the inherent toxicity may hinder *in vivo* application and cadmium-containing products are eventually caused environmentally problematic.[7-9] Thus, it is natural to seek for substituting cadmium ions and producing low toxic labeling materials.[10, 11] In view of blue light emitting, free from toxic elements, excellent optical property, ZnSe QDs are potential candidates for the biological application.[12, 13] In order to improve the stability and surface passivation, ZnS represents a nearly-ideal candidate for the construction of core/shell structures around the ZnSe core. ZnSe/ZnS core/shell QDs that had uniform size, water-soluble, low toxic and emission of blue-green fluorescence have been synthesized in aqueous phase.[14-16]

Chitosan, (1,4)-2-amino-2-deoxy-β-D-glucan, a natural biopolymer generally obtained by alkaline deacetylation of chitin with one amino group and two hydroxyl groups, is hydrophilic, nontoxic, biocompatible and biodegradable.[17, 18] Due to unique cationic polysaccharide character, it has been investigated extensively as a carrier for a wide application. To take better advantage of QDs in the fields of multiplexed bioassay, intracellular study and imaging of tumor, several works about QDs/chitosan systems have been reported.[19, 20] It was known that ZnSe/ZnS quantum dots have low cytotoxicity and good biocompatibility. Therefore, chitosan-ZnSe/ZnS (CS-ZnSe/ZnS) nanoparticles will be expected to be a potential drug delivery material with fluorescent labeling. To the best our known, the synthesis of CS-ZnSe/ZnS nanoparticles is scarcely reported.

Recently, different techniques for incorporating QDs into polymer microsphere have been reported, such as cross-linking the composite particles with glutaraldehyde,[19] counterion complexation by EDTA,[21] via the reactions between amino groups of chitosan and carboxylic groups on the surfaces of QDs by EDC.[22] The emulsifying agents and chemical cross-linking agents were integrant for these procedures, which are toxic to organisms and damage to drugs.

Compared with above techniques, a facile one-step ionic gelation procedure was employed to embed ZnSe/ZnS QDs into chitosan between amino groups of chitosan and carboxylic groups of ZnSe/ZnS QDs (schematically shown in Fig. 1). ZnSe/ZnS QDs were carboxylic-functionalized and can be used as counterion and fluorescent labeling. The approach not only avoids the use of emulsifiers and chemical

cross-linking agents but also prevent the possibility of damage to drugs.[23-25]

In this research, the fluorescent CS-ZnSe/ZnS nanoparticles and 5-fluorouracil (5-Fu) loaded fluorescent nanoparticles (5-Fu-CS-ZnSe/ZnS) were obtained based on one step ionic gelation procedure. The obtained fluorescent CS-ZnSe/ZnS nanoparticles were characterized by various techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR), photoluminescence spectra (PL), X-ray diffraction (XRD) and thermogravimetric analysis (TG). The cell viability was estimated by mice lung carcinoma cells line Lewis. The hydrophilic drug 5-Fu was used as a model drug to investigate controlled release properties of the fluorescent nanoparticles. The obtained CS-ZnSe/ZnS nanoparticles may be used as biological fluorescent labeling and a carrier for guest material.

2. Experimental

2.1. Materials

Chitosan with 85.0-90.0% degree of deacetylation, acetic acid (HAc), sodium borohydride ($NaBH_4$), sodium hydroxide (NaOH), dimethyl-sulfoxide (DMSO) and dialysis bag (M_w = 14000) were obtained from Sinopharm Chemical Reagent Co., Ltd. Zinc acetate dihydrate ($Zn(OAc)_2{\cdot}2H_2O$) was purchased from Xingta chemical plant (Shanghai, China). 3-mercaptopropionic acid (3-MPA, 98%) and 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, >98%) were purchased from Aladdin. Sodium sulfide (Na_2S) was purchased from Tongya

chemical plant (Shanghai, China). Selenium powder was obtained from Shanghai Meixing Chemical Co., Ltd. 5-fluorouracil (5-Fu) was obtained from Shanghai Zhuorui Chemical Co., Ltd. Deionized water was used throughout. The mice lung carcinoma cell line Lewis was obtained from School of Medicine, Jiangsu University.

2.2 Synthesis of 3-MPA-capped ZnSe/ZnS core/shell QDs

3-MPA-capped ZnSe/ZnS core/shell QDs (ZnSe/ZnS QDs) were synthesized following a previously reported method with minor modifications.[26] The NaHSe solution was prepared through the reaction between selenium powder and $NaBH_4$ solution.[27]

2.2.1 Preparation of 3-MPA-capped ZnSe core QDs

In a typical procedure, 0.6 mmol $Zn(OAc)_2 \cdot 2H_2O$ and 100 μL 3-MPA were dissolved in 20 mL of deionized water, and the pH value of the solution was adjusted to 9.5 by dropwise adding 1.0 M NaOH solution under stirring. The Zn precursor solution was transferred into a 50 mL three-neck flask. The air in the system was replaced with N_2, and subsequently 1.0 mL of fresh NaHSe solution (0.1 M) was added through a syringe. The system was heated to 100 °C under nitrogen flow and maintained at this temperature for 1 hour (Fig.1, step1). Ice bath was used to terminate the reaction. By this approach one can obtain ZnSe QDs with the average size of 3-4 nm.

2.2.2 Preparation of 3-MPA-capped ZnSe/ZnS core/shell QDs

Typically, as-prepared ZnSe QDs solution 20.0 mL was added into a 250 mL three-neck flask. Then, 70 mL Zn precursor solution (1.3 mmol $Zn(OAc)_2 \cdot 2H_2O$ and

100 μL 3-MPA) was added. The pH value of the mixture solution was adjusted to 8.7 with the addition of 1.0 M NaOH solution. Subsequently, the reaction mixture solution was heated to 100 °C and kept at this temperature for 30 min with H_2S blowing. Vigorous stirring and nitrogen atmosphere were needed during the whole process. Ice bath was used to terminate the reaction. ZnSe/ZnS QDs were obtained (Fig.1, step2). Similarly, a series of ZnSe/ZnS QDs solutions with different content (e.g. 4 $mg \cdot mL^{-1}$, 8 $mg \cdot mL^{-1}$ and 12 $mg \cdot mL^{-1}$) were obtained.

2.3 Synthesis of CS-ZnSe/ZnS nanoparticles and 5-Fu loaded fluorescent nanoparticles

The CS-ZnSe/ZnS nanoparticles were prepared by ionic gelation technique, which is based on the electronic interaction between amino groups of chitosan and carboxylic groups of ZnSe/ZnS QDs. (Fig.1, step3). In a typical procedure, Chitosan was dissolved in 1.0 % (v/v) acetic acid solution and diluted into 1.5 wt‰. Then, 4 mL ZnSe/ZnS QDs solution was dropped slowly into 20 mL chitosan solution using a fine syringe under vigorous stirring at room temperature and the pH value of the solution was adjusted to 4.5-5.5 by dropwise adding 1.0 M NaOH solution. The resultant mixtures were continuously stirred for 6 h and centrifuged at 12,000 rpm for 30 min. The resulting fluorescent nanoparticles were washed with deionized water and then lyophilized. 5-Fu loaded fluorescent nanoparticles (denoted as 5-Fu-CS-ZnSe/ZnS) were prepared according to the same process, only the required amount of 5-Fu was firstly dissolved in chitosan solution.

2.4 Characterization of QDs and CS- ZnSe/ZnS nanoparticles

The crystalline phases of samples were analyzed by X-ray diffraction (XRD) using Bruker D8 diffractometer with Cu *K*α radiation (λ = 1.5418 Å) within the range of 2θ = 10-80°. The morphology and structure of the ZnSe/ZnS QDs were examined by Transmission Electron Microscopy (TEM, JEM-2100, JEOL). The Morphology of the CS-ZnSe/ZnS nanoparticles was examined by Field Emission Scanning Electron Microscope (SEM, JSM-7001F, JEOL). The chemical composition of the CS-ZnSe/ZnS nanoparticles was determined by X-ray energy dispersion spectrum (EDS). Ultraviolet-visible (UV-vis) absorption spectra were obtained using a UV-2401 PC Recording Spectrophotometer (Shimadzu, Japan) from 200 to 400 nm. Photoluminescence (PL) spectra were taken with Cary-Eclipse spectrofluorophotometer (Varian, America) with excitation wavelength of 370 nm. Fourier transform infrared spectra (FT-IR) of samples were recorded on a Nicolet Avatar-370 spectrometer. Thermogravimetric analysis (TG) was done on STA-449C Jupiter (NETZSCH Corporation, Germany). The experiment temperature ranged from 25 ^{o}C to 700 ^{o}C at a constant heating rate of 10 ^{o}C·min^{-1} in N_2 atmosphere.

2.5 Evaluation of drug loading capacity and encapsulation efficiency

5-Fu-loaded fluorescent nanoparticles suspensions were separated by a centrifuge at 10,000 rpm for 30 min. The concentration of 5-Fu in the supernatant was determined through the absorbance at a maximum absorption at 266 nm.[28, 29] The loading capacity (LC) and drug encapsulation efficiency (EE) of 5-Fu-CS-ZnSe/ZnS nanoparticles were calculated by the following formulas:[30]

$$LC\ (\%) = (W_t - W_f) / W_n \quad (1)$$

$$EE\ (\%) = (W_t - W_f) / W_t \quad (2)$$

Where W_t represents the total amount of 5-Fu, W_f is the amount of free 5-Fu in the supernatant and W_n is the weight of nanoparticles after freeze-drying. Each sample was assayed in triplicates.

2.6 Cytotoxicity of CS-ZnSe/ZnS nanoparticles

The cytotoxicity of the CS-ZnSe/ZnS nanoparticles was evaluated by MTT assay. Lewis cells were seeded in a 96-well microtiter plates to a total volume of 100 μL/well. Plates were maintained at 37 ^{o}C in a 5% CO_2 incubator for 24 h. CS-ZnSe/ZnS nanoparticles of different concentrations were loaded into each well, with three duplicates for each sample. No CS-ZnSe/ZnS nanoparticles were added to the control cells. After 24 h of incubation, the supernatant was removed, and the cells were washed with PBS three times. To evaluate cell viability, 100 μL MTT solution (5 mg/mL in PBS) was added to each well and incubated the mixture at 37 ^{o}C for 4 h. After incubation, the remaining MTT solution was removed, and 100 μL of DMSO was added to each well to dissolve the formazan crystals. The optical absorbance was measured at 570 nm on a microplate reader (My Goal instrument, SpectraMax 190). Cell viability was calculated using the following equation:

$$\text{Cell viability (\%)} = \frac{\text{Intensity (sample)}}{\text{Intensity (control)}} \times 100$$

The results are the average values of triplicates.

2.7 *In vitro* drug release study

The drug release response from the 5-Fu-CS-ZnSe/ZnS nanoparticles was studied at the physiological pH 7.4 and 37.0 ^{o}C. In each experiment, 25 mg 5-Fu-CS-ZnSe/ZnS nanoparticles and 5 mL PBS (Na_2HPO_4-KH_2PO_4 buffer solution) were sealed in a dialysis bag. The dialysis bag was placed in a test tube containing 200 mL PBS. The test tube was maintained at 37.0 ^{o}C in a water bath. At selected time intervals, 5 mL release medium was taken for ultraviolet absorption analysis at a wavelength of 266

nm. Fresh *in vitro* medium was then added after each sampling. The *in vitro* release analyses for each sample were carried out in triplicates and the average values were obtained. The percentage of cumulative 5-Fu release (%, w/w) was investigated as a function of release time.

3. Results and discussion

Fig. 1 schematically represents the fabrication process of ZnSe/ZnS core/shell QDs and the drug-loading CS-ZnSe/ZnS nanoparticles. ZnSe/ZnS QDs were modified with carboxylic groups on the surface after introducing 3-MPA. Therefore, chitosan can be cross-linked via electrostatic interaction between its amino groups and carboxylic groups on the surface of ZnSe/ZnS QDs. Fluorescent 5-Fu-CS-ZnSe/ZnS nanoparticles were synthesized by adding 5-Fu into chitosan solution.

3.1 XRD analysis

The XRD patterns of chitosan, ZnSe/ZnS QDs, CS-ZnSe/ZnS nanoparticles, pure 5-Fu and 5-Fu-CS-ZnSe/ZnS nanoparticles were shown in Fig. 2. From Fig. 2A, it can be observed two peaks at 2θ of 11° and 20°, indicating partially crystalline chitosan[31]. For the XRD pattern of ZnSe/ZnS QDs, it can be observed one intense peak at 2θ of 28.5°, corresponding to ZnSe cubic structure (111) crystal face (JCPDS No.80-0021).[32] The peaks of ZnS crystals cannot be observed clearly. It indicates that the as-prepared ZnS layer is too thin to become well crystals.[32]

For CS-ZnSe/ZnS nanoparticles, the basic crystallization peak of chitosan at 20° is disappeared. This is due to ionic cross-linking reaction between chitosan and ZnSe/ZnS QDs, thus making the chitosan molecular chain less stacked. The other peak of chitosan at 11° and the peak of ZnSe/ZnS QDs at (111) can be both observed

in CS-ZnSe/ZnS hybrid nanoparticles. The results showed the coexistence of ZnSe/ZnS QDs and chitosan in the composite nanoparticles.

Several intense diffraction peaks can be observed in Fig. 2B, indicating the pure 5-Fu in a crystalline powder state.[33] However, broad diffraction peaks were observed in the 5-Fu-CS-ZnSe/ZnS nanoparticles. The ionic interactions formed in 5-Fu-CS-ZnSe/ZnS nanoparticles restrained the movement of the molecular chain of both polymers and 5-Fu. Thus, amorphous states of 5-Fu was observed in the fluorescent nanoparticles.[34]

3.2 Particle morphology and size distribution

The morphology and size of ZnSe, ZnSe/ZnS QDs and CS-ZnSe/ZnS hybrid nanoparticles were investigated by TEM and SEM. It can be observed that the size of ZnSe QDs was about 3-4 nm (Fig. 3A) whereas ZnSe/ZnS QDs with a larger size around 5-6 nm (Fig. 3B), suggesting the coating of ZnS on the ZnSe QDs. From Fig. 3C (HRTEM of ZnSe/ZnS QDs), the lattice fringes have a spacing of 0.326 nm, corresponding to interplanar spacing of (111) of cubic ZnSe (JCPDF).[32] From Fig. 3D, it can be observed that the spherical outline in shape for CS-ZnSe/ZnS nanoparticles within size from 100-500 nm. To identify the elemental composition of the specimen, the EDS analysis was shown in Fig. 2E, the selected areas of the CS-ZnSe/ZnS nanoparticles were found with Zn, O, Se, S, C, Si and Na elements. The high peak of silicon was attributed to silicon substrate and the Na elements were introduced by NaOH in the synthesis of CS-ZnSe/ZnS nanoparticles. The result is accordance with the composition of the nanoparticles. It was found that the amount of ZnSe/ZnS QDs

solution (2 mL-12 mL, 4 mg·mL^{-1}) has little effect on the morphology and size of CS-ZnSe/ZnS hybrid nanoparticles. In addition, when the concentration of chitosan solution was more than 5 wt ‰, the agglomeration of nanoparticles were found.

3.3 FT-IR spectroscopy

In order to examine the interaction between ZnSe/ZnS QDs and chitosan, the FT-IR spectra of pure chitosan, ZnSe/ZnS QDs and CS-ZnSe/ZnS nanoparticles are given in Fig. 4. For chitosan, the peaks at 1657, 1596, 1383 cm^{-1} and 1082 cm^{-1} are corresponding to amide I, II bands, bending vibration of C-H and stretching vibrations of C-O-C, respectively.[35]

For ZnSe/ZnS QDs, the peaks at 3421 cm^{-1}, 1560 cm^{-1} and 1392 cm^{-1} are attributed to the O-H stretching vibration, C=O asymmetric stretching vibration of carboxylic acid and S-CH_2 wagging vibration of MPA molecular, respectively.[27] The absence of the S-H stretching bond between 2700 cm^{-1} and 2550 cm^{-1} proves the attachment of the MPA molecular *via* covalent bonds between thiols and surface Zn atoms of ZnSe/ZnS QDs.[36]

However, in the case of CS-ZnSe/ZnS nanoparticles, compared with that of pure chitosan, three peeks at 1657 cm^{-1}, 1596 cm^{-1} and 1082 cm^{-1} shifted to the lower wave number 1631 cm^{-1}, 1561 cm^{-1} and 1066 cm^{-1}, illustrating the electrostatic interaction between chitosan and ZnSe/ZnS QDs.[37] In addition, it can be observed the absorption of CS-ZnSe/ZnS nanoparticles at 1561 cm^{-1} is stronger than that at 1066 cm^{-1}. The result is attributed to the enhanced absorption of amide bands for CS-ZnSe/ZnS nanoparticles.[22] According to FT-IR analysis, it can be concluded that ZnSe/ZnS QDs

were linked to chitosan via electrostatic interaction between amino groups of chitosan and carboxylic groups of ZnSe/ZnS QDs.

3.4 Absorbance spectra and fluorescence spectra

UV-vis absorption spectra of original ZnSe and ZnSe/ZnS core/shell QDs were shown in Fig. 5A. ZnSe/ZnS core/shell QDs demonstrate a red shift in absorption compared with initial ZnSe core. This phenomenon is caused by extensive delocalization of the electron into the surrounding shell and has been previously observed in ZnSe/ZnS QDs[32] and CdSe/CdS QDs,[38] suggesting the growth of the ZnS shell around ZnSe core. From Fig. 5B, it can be observed that the PL emission peak positions of ZnSe and ZnSe/ZnS QDs were at 425, 485 and 525 nm, the peak at 425 nm was assigned to the excitonic emission,[39] whereas the latter two peaks were assigned to a defect emission.[40] After formation of ZnS shell, the intensity of the excitonic luminescence spectra increases significantly compared to initial ZnSe core. The reason for the increase of fluorescent intensity was the coating of ZnS shell, which passivates surface defects of the ZnSe.[26] To investigate the CS-ZnSe/ZnS nanoparticles fluorescent intensity, PL emission spectra for CS-ZnSe/ZnS hybrid nanoparticles and 5-Fu loaded CS-ZnSe/ZnS fluorescent nanoparticles were shown in Fig. 5C, the PL emission peak position at around 425, 485 and 525 nm were in accordance with the PL emission spectra of ZnSe/ZnS QDs and 5-Fu- CS-ZnSe/ZnS nanoparticles also have strong fluorescence intensity.

In order to investigate the PL character of the ZnSe/ZnS core/shell QDs and the CS-ZnSe/ZnS nanoparticles, the digital camera was used to obtain the PL images

under UV light, which was showed in Fig. 4D. Pure deionized water shows no light under UV-light (Fig. 5D a). The ZnSe/ZnS QDs in deionized water show the strongest blue-green fluorescence (Fig. 5D b), the fluorescent CS-ZnSe/ZnS hybrid nanoparticles in deionized water (Fig. 5D c), 5-Fu-CS-ZnSe/ZnS nanoparticles in deionized water (Fig. 5D d) and 5-Fu-CS-ZnSe/ZnS nanoparticles in 0.9% sodium chloride solution (Fig. 5D e) also show blue-green light, indicating the good optical stability of 5-Fu-CS-ZnSe/ZnS nanoparticles in physiological environment. Therefore, these fluorescent CS-ZnSe/ZnS nanoparticles were expected to be used as biological fluorescent labeling and a carrier for guest materials.

Combined with the XRD, TEM, FT-IR, UV-vis and PL analyses, it can be concluded that novel fluorescent CS-ZnSe/ZnS nanoparticles and 5-Fu-CS-ZnSe/ZnS nanoparticles were successfully synthesized through the strong electrostatic interactions between chitosan amino groups and carboxylic groups of ZnSe/ZnS QDs.

3.5 Thermal properties

The thermal stability of CS-ZnSe/ZnS nanoparticles is compared with the pure chitosan. Thermograms of pure chitosan and CS-ZnSe/ZnS nanoparticles, obtained under the atmosphere of nitrogen, are shown in Fig. 6. The obtained results indicated that incorporation of ZnSe/ZnS QDs significantly alter the thermal properties of chitosan matrix. The thermal decomposition of chitosan is shifted towards higher temperature for about 100 ^{o}C in the presence of ZnSe/ZnS QDs. It should be noticed that residual mass is larger compared to the content of inorganic phase after decomposition of CS-ZnSe/ZnS nanoparticles. The observed results are characteristic

for polymers filled with inorganic particles,[41] suggesting chitosan was partially carbonized. Pronounced improvement of thermal stability of chitosan filled with ZnSe/ZnS QDs is consequence of the formation of chemical bonds between chitosan and ZnSe/ZnS QDs. Similar result has been found in CdS QDs-chitosan.[37]

3.6 Cytotoxicity evaluation

Lewis cells were treated with different concentrations of CS-ZnSe/ZnS nanoparticles for 24 h to determine the effect of concentration of the nanoparticles. It was found that the cell viability still remained 99% while the CS-ZnSe/ZnS nanoparticles concentration increased to 200 μg/mL. In addition, when the concentration was less than 200 μg/mL, the cell viability was greater than that of blank group (Fig. 7). The result showed that CS-ZnSe/ZnS nanoparticles were conducive to the growth of cells instead of toxic effect on the cells under suitable concentration (<200 μg/mL).

Above phenomenon may be due to following aspects: (i) chitosan is a biocompatible material; (ii) Zn and Se element was a kind of energy sources which can provide help for the growth and division of cell.[32]

3.7 Drug release response

To research the drug loading capacity and the drug releasing behavior of the CS-ZnSe/ZnS nanoparticles, the hydrophilic anti-cancer drug 5-Fu was loaded as a model drug. The encapsulation efficiency was higher than 40% and the loading percentage was up to about 15%. The dependence of percentage cumulative 5-Fu release from bare 5-Fu and 5-Fu-CS-ZnSe/ZnS nanoparticles at a temperature of 37

^{o}C in the PBS (pH = 7.4) was shown in Fig. 8. It can be observed that the release of bare 5-Fu was finished in 2 h. In the case of 5-Fu-CS-ZnSe/ZnS nanoparticles, the samples had a quick release within 5 h, which was due to the release of 5-Fu absorbed on the surface of nanoparticles. Subsequently the drug release rate slowed down and finally reached equilibrium about 40% in 48 h, which was attributed to the entrapped 5-Fu in the nanoparticles. The sustained-release performance may be due to the interaction among 5-Fu, chitosan and ZnSe/ZnS QDs. In view of the atoms (F, O and N) with strong negativity in 5-Fu-CS-ZnSe/ZnS nanoparticles, strong hydrogen bonds formed in 5-Fu, chitosan and ZnSe/ZnS QDs. In addition, it can be observed that with the increasing of the content of ZnSe/ZnS QDs (4 mg·mL^{-1}, 8 mg·mL^{-1}, 12 mg·mL^{-1}), the drug release in 5-Fu-CS-ZnSe/ZnS nanoparticles showed more slowly. Thus the release process can be controlled by adjusting the content of ZnSe/ZnS QDs.

4. Conclusion

Novel fluorescent CS-ZnSe/ZnS nanoparticles were successfully prepared via ionic gelation technique in aqueous solution. Compared with the current synthetic methods for preparing polymer-QDs nanoparticles, our approach not only avoids the use of emulsifiers and chemical cross-linking agents but also prevent the possibility of damage to drugs. The obtained CS-ZnSe/ZnS nanoparticles with the size of 100-500 nm have good fluorescent and colloidal stability in physiological environment. TG analysis indicated the thermal stability of the CS-ZnSe/ZnS nanoparticles was much higher than pure chitosan and the enhancement can be attributed to strong electrostatic interactions between chitosan amino groups and carboxylic groups of ZnSe/ZnS QDs.

The drug release response can be controlled by adjusting the content of ZnSe/ZnS QDs. We anticipate the biocompatible fluorescent CS-ZnSe/ZnS nanoparticles may be used as biological fluorescent labeling and drug carrier.

Acknowledgments

The authors appreciate the financial support of this work from the Foundation of the National Natural Science Foundation of China (Nos. 21406094, 21201086), Foundation of Jiangsu University (04JDG048) and A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

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Figure captions:

Fig. 1 Schematic representation of the fabrication process of drug-loading fluorescent CS-ZnSe/ZnS nanoparticles.

Fig. 2(A) XRD patterns of chitosan, ZnSe/ZnS QDs, CS-ZnSe/ZnS nanoparticles and (B) XRD patterns of pure 5-Fu, chitosan, fluorescent CS-ZnSe/ZnS nanoparticles and 5-Fu-CS-ZnSe/ZnS nanoparticles.

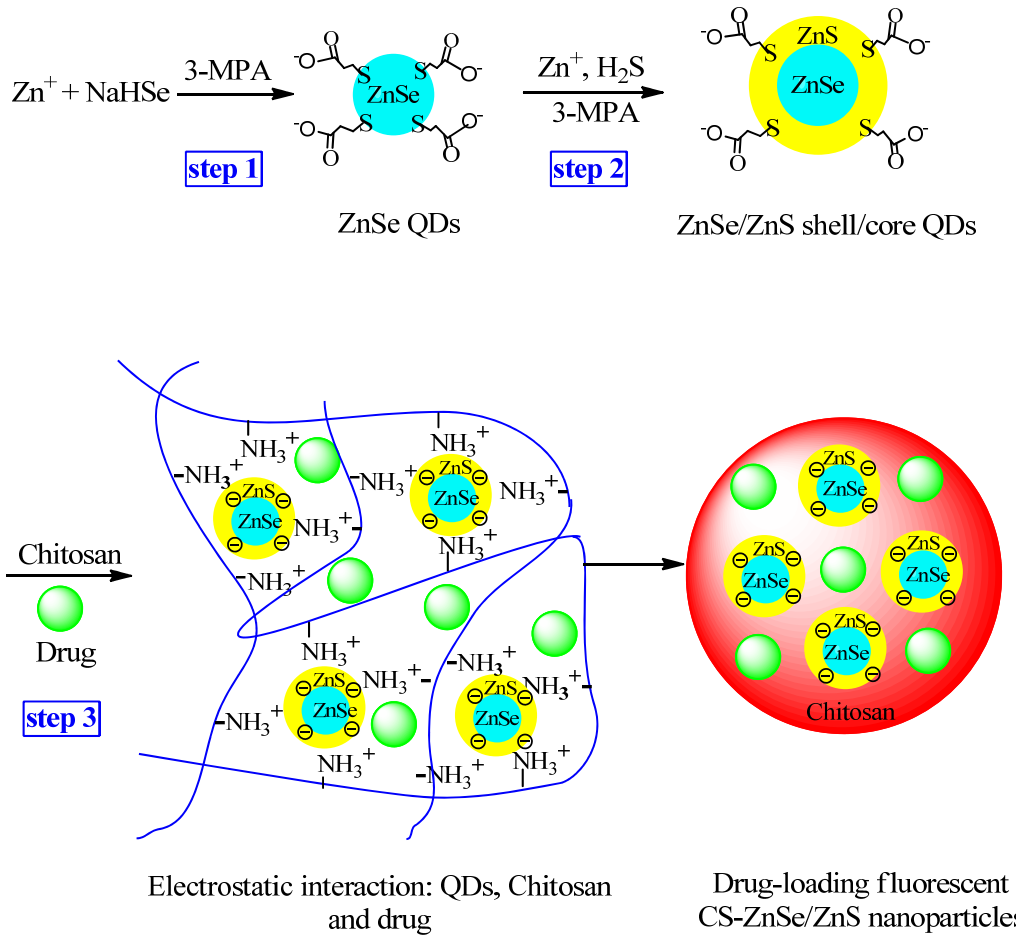
Fig. 3(A) TEM images of ZnSe QDs (B, C) TEM and HRTEM images of ZnSe/ZnS QDs, (D) SEM image of CS-ZnSe/ZnS nanoparticles and (E) EDX spectrum of CS-ZnSe/ZnS nanoparticles.

Fig. 4 FT-IR spectra of pure chitosan, ZnSe/ZnS QDs and CS-ZnSe/ZnS nanoparticles.

Fig. 5(A) UV-vis spectra of ZnSe QDs and ZnSe/ZnS QDs, (B) PL emission spectra of ZnSe QDs and ZnSe/ZnS QDs, (C) PL emission spectra of CS-ZnSe/ZnS nanoparticles and 5-Fu-CS-ZnSe/ZnS nanoparticles (ex = 370 nm) and (D) Photographs of ZnSe/ZnS QDs and CS-ZnSe/ZnS nanoparticles solution under UV irradiation: (a) pure deionized water, (b) ZnSe/ZnS QDs in deionized water, (c) CS-ZnSe/ZnS nanoparticles in deionized water, (d) 5-Fu-CS-ZnSe/ZnS nanoparticles in deionized water and (e) 5-Fu-CS-ZnSe/ZnS nanoparticles in 0.9% NaCl solution.

Fig. 6 TG curves of pure chitosan and CS-ZnSe/ZnS nanoparticles.

Fig.7 MTT assays of mice lung carcinoma cells.



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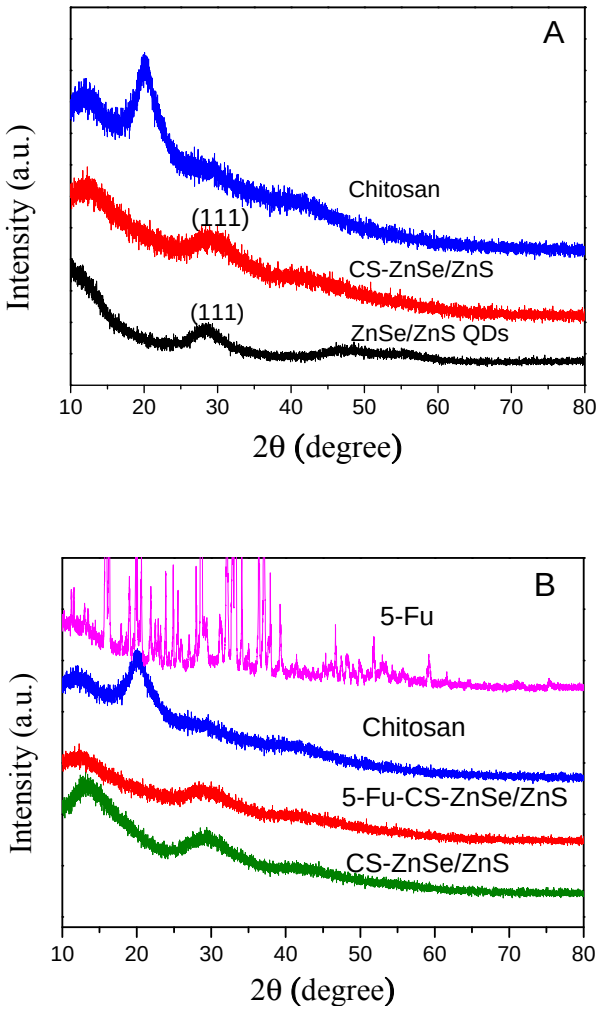

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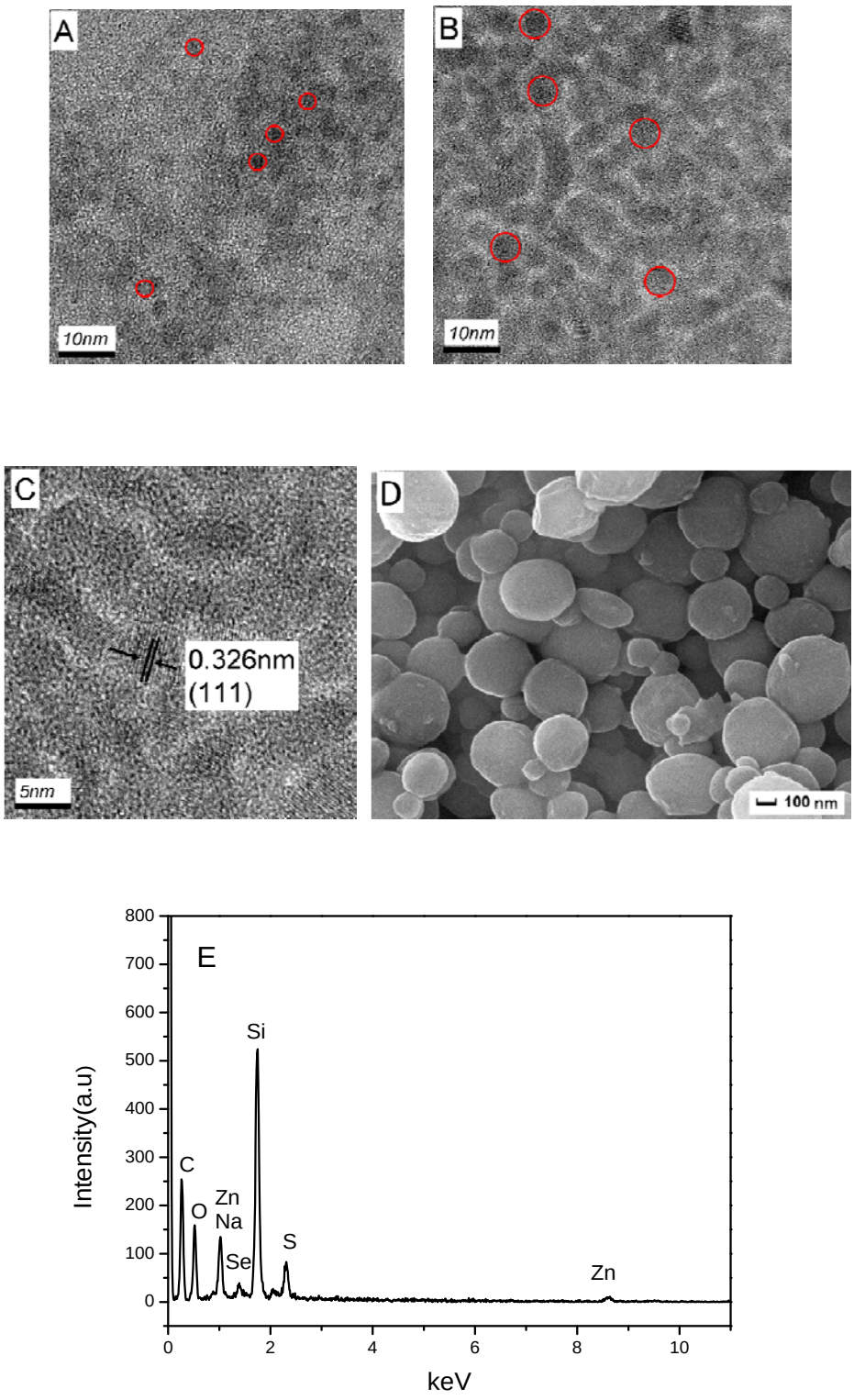

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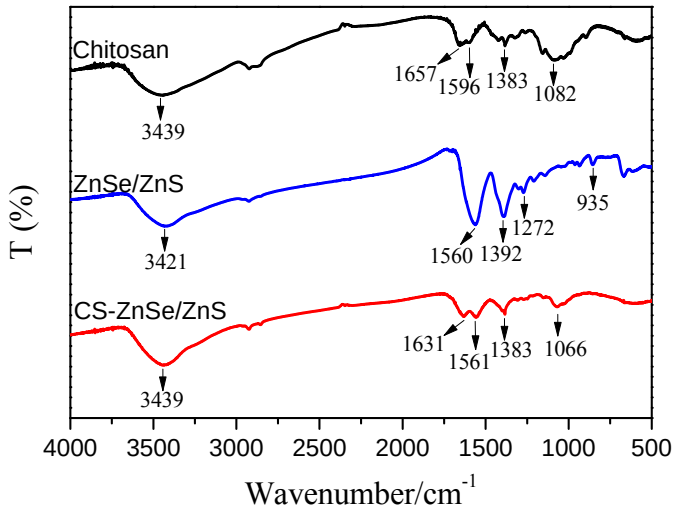

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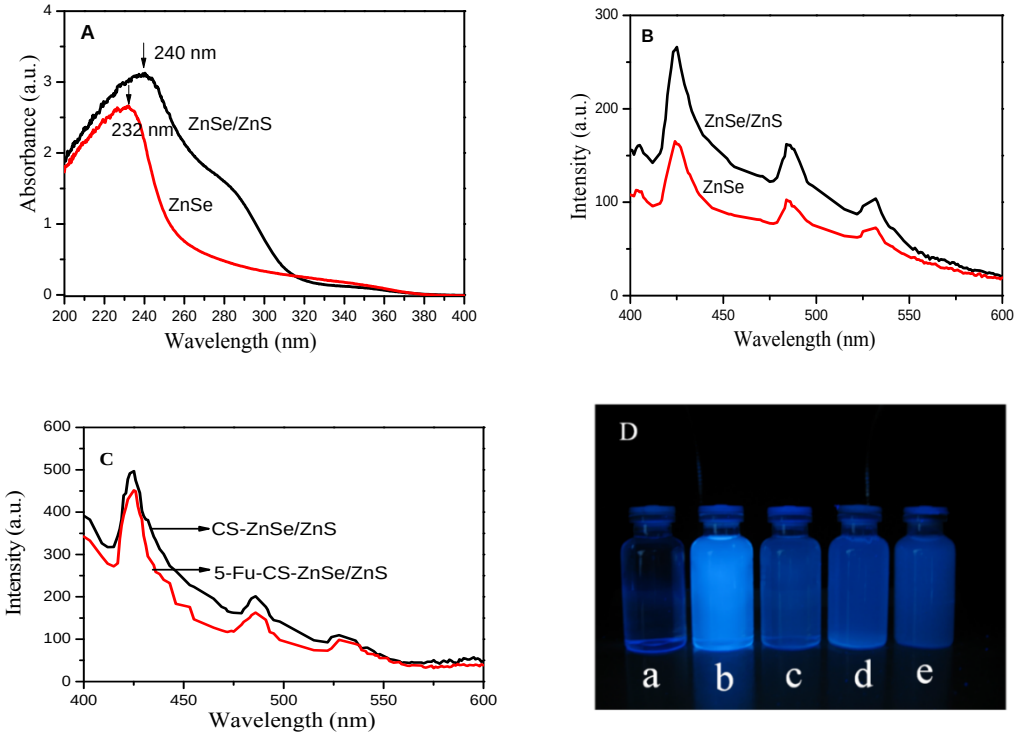

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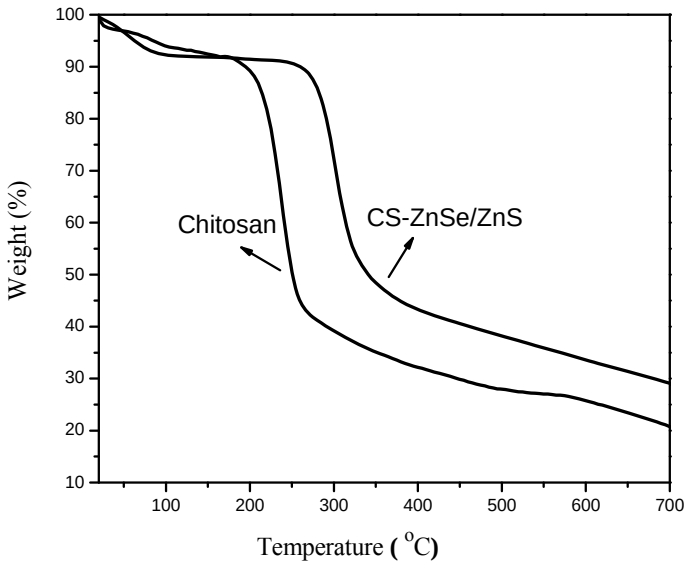

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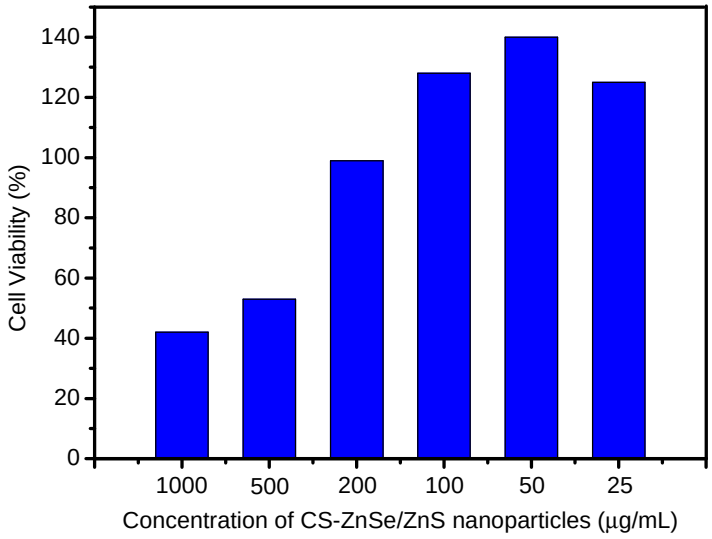

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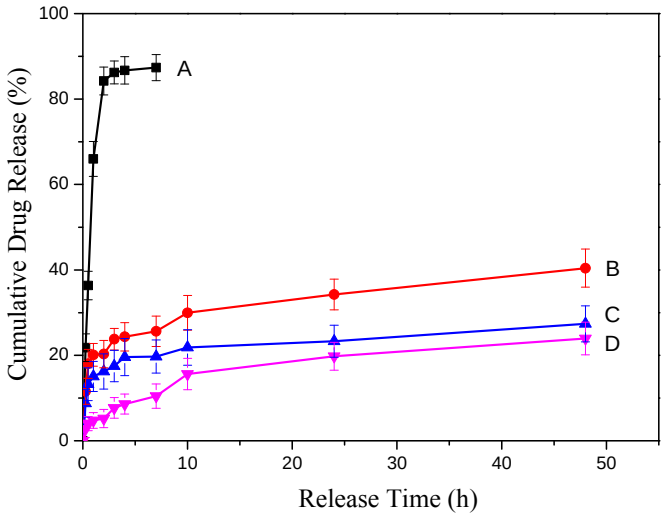

Fig. 8 Cumulative release curve of samples in pH 7.4 (PBS) at 37 ^{o}C: (A) bare 5-Fu, (B) 5-Fu-CS-ZnSe/ZnS nanoparticles obtained from 4 mg·mL^{-1} ZnSe/ZnS QDs, (C) 5-Fu-CS-ZnSe/ZnS nanoparticles obtained from 8 mg·mL^{-1} ZnSe/ZnS QDs, (D) 5-Fu-CS-ZnSe/ZnS nanoparticles obtained from 12 mg·mL^{-1} ZnSe/ZnS QDs.