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ORIGINAL PAPER

Synthesis of Functionalized ZnSe Nanoparticles and Their Applications in the Determination of Bovine Serum Albumin

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Abstract Luminescent quantum dots (QDs)-semiconductor nanocrystals were promising alternative to organic dyes for fluorescence-based applications. In this paper, we developed procedures to use mercaptoacetic acid (MAA) to modify ZnSe nanoparticles and made the nanoparticles to be soluble for the quantitative and selective determination of bovine serum albumin (BSA). Maximum fluorescence intensity was produced at pH 7.0, with excitation and emission wavelengths at 242 and 348 nm, respectively. Under optimal conditions, the straight line equation: $^{\triangle}F$= 0.38+0.34 C (μg/ml) was found between the relative fluorescence intensity and the concentration of BSA in the range of 9.6–124.8 μg/ml, and the limit of detection was 2 μg/ml.

Keywords Quantum dots · Spectrofluorimetric detection · ZnSe nanoparticles · Bovine serum albumin

Introduction

Semiconductor nanocrystals, known as "quantum dots" (QDs), have attracted increasing interest for bioimaging, biolabelling, biodetection and clinical diagnosis [1–4] especially in the biodetection, such as tracing and diagnosis of cell or tumor [5], the image research of tissues and the animated cells [6–9]. Due to quantum confinement effects [10–12], QDs have high luminescence efficiency, continuous excitation spectrum, stability against photobleaching, and controllable and narrow emission bands compared with the traditional organic fluorescent dyes [13], so they are ideal fluorescent dyes to replace organic fluorescent dyes. Since the report on the aqueous synthesis of mercaptoethanol—and thioglycerol—capped nanocrystals was appeared in 1996 [14], solubility of QDs has been resolved, and it has been widely used in bioanalytics.

Serum albumin is the most abundant protein in the circulatory system, and it has many physiological and pharmacological functions. It is able to carry drugs as well as endogenous and exogenous substances. And numerous experiments with the aim of characterizing the binding capacity and sites of albumins have been carried out [15, 16]. The most important property of serum albumin is that it serves as a depot protein and as a transport protein for a variety of compound. Bovine serum albumin (BSA) has been one of the most extensively studied of this group of proteins.

There have been many methods reported to determine bovine serum albumin, such as resonance light scattering techniques [17–19], chemiluminescence [20], Bradford assay method [21], circular dichroism, fluorescence spectroscopy [22], Fourier transform infrared spectroscopy [23], ultraviolet absorption spectroscopy, differential scanning calorimetry [24] and etc. Because proteins bear tryptophan, tyrosine and phenylalanine residue, which have intrinsic fluorescence, it is seen that fluorescence spectroscopy could serve as an effective and cheaper method for such studies [25, 26]. At the same time, the fluorescence spectroscopy method is simple, fast, high sensitive and enough selective. So it is an effective method to detect the proteins.

In this paper, ZnSe nanoparticles have been synthesized with hydrothermal synthesis and functionalized with mercaptoacetic acid(MAA). Under the optimum conditions in our study, the fluorescence of functionalized ZnSe nano-

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particles could be enhanced through binding with bovine serum albumin, and the extent of the fluorescence intensity enhancement were proportional to the concentrations of BSA. The proposed method led to a particularly sensitive and quantitative assay, the concentration of BSA was in the range of 9.6–124.8 μg/ml, and the limit of detection was 2 μg/ml.

Experimental

Preparation of nano-ZnSe-MAA

In a typical procedure, the mixture of 0.7120 g Zn powder (99.95%) and 0.2886 g Se powder (99.95%) was put into a teflon-lined autoclave with 30 ml capacity. And then 20 ml 3 mol/L NaOH solution was added into the autoclave. The autoclave was sealed, maintained at 180 °C for 24 h, and then cooled to room temperature naturally. At last, the yellow product of ZnSe solid powder was collected by filtration, washed with distilled water and ethanol, and then dried at 80 °C in vacuum.

Under vigorous stirring, the ZnSe nanoparticles reacted with the mercaptoacetic acid(MAA) in the circumstances of buffer solution of phosphate (PBS, pH=7) for 3 h. And then the colloidal solution was sealed lightproofly for one night. Then the functionalized nanoparticles solution was taken into a 500 ml brown jar and was stored in buffer solution at room temperature. The obtained solution was ready for the following experiment.

Fluorescence emission spectra of BSA

For determining the concentration of BSA, the resulting solutions were used to monitor the change of emission fluorescence intensity by means of spectrofluorometer. The excitation wavelength and emission wavelength were selected at 242 nm and 348 nm, respectively. The slit widths of both excitation and emission were 3.0 nm. The scanning bound was from 265 to 480 nm.

Results and discussion

Morphology and structure

The morphologies and structures of products were investigated by transmission electron microscopy (TEM) and powder X-ray diffractometry (XRD). Figure 1a shows that monodisperse ZnSe nanoparticles with defined shape and diameter about 25 nm could be obtained via hydrothermal process. The corresponding electron diffraction pattern (Fig. 1b) and the XRD pattern (Fig. 2) revealed that the ZnSe nanoparticles were crystals of cubic sphalerite structure with lattice constant of a= 5.65 Å, which was consistent with the JCPDS card number 80-0021.

Fluorescence spectra of unfunctionalized and functionalized ZnSe nanoparticles

The fluorescence spectra of ZnSe nanoparticles and functionalized ZnSe nanoparticles are shown in Fig. 3. It can be seen that the fluorescence intensity is dramatically enhanced at 263 nm for the functionalized ZnSe nanoparticles, and also the fluorescence emission peak of functionalized nanoparticles is blue shifted compared with unfunctionalized. Also we can see that the fluorescence spectra of functionalized ZnSe nanoparticles with a peak at 263 nm of an intrinsic nanocrystal fluorescence is narrower and more symmetric than the fluorescence spectra of unfunctionalized ZnSe nanoparticles with a peak at

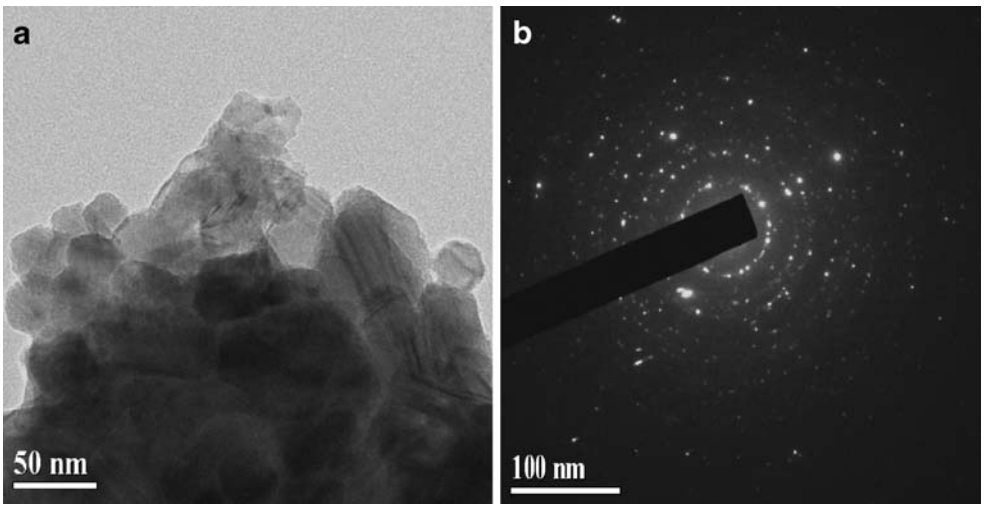

Fig. 1 **a** TEM images of as-prepared ZnSe nanoparticles **b** SAED pattern of ZnSe nanoparticles

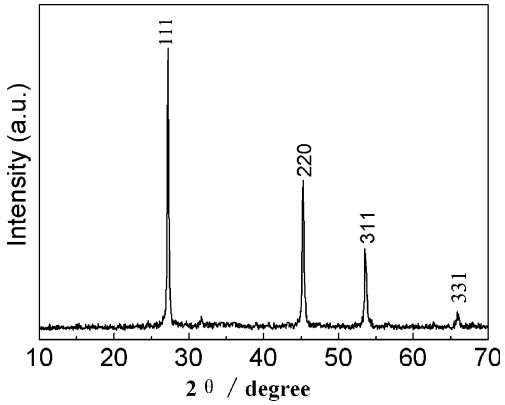

Fig. 2 XRD pattern of as-prepared ZnSe nanoparticles

306 nm from an extrinsic fluorescence band, strongly quenched in functionalized nanocrystals.

Optimization of the determination

The fluorescence emission spectra of many matters are sensitive to their surrounding environment. In order to develop a sensitive and rapid spectrophotometric method for the determination of BSA, the test conditions were improved by studying the effect of various factors such as pH value, buffer solution, incubation time, temperature and so on. The effect of pH value of the solution on the fluorescence intensity was studied, and the results were shown in Fig. 4. The optimal pH was 7.0. If the pH is too low, although the fluorescence intensity is higher, it is not suitable for the pH of animal body, contrary, if the pH is too high, the fluorescence intensity becomes lower. So we developed this action in the phosphate buffer solution of pH=7.0. And the optimal temperature in our experiment was 25 °C.

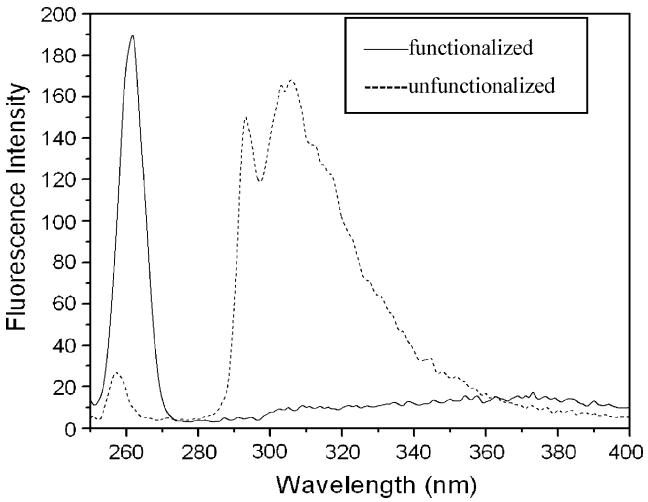

Fig. 3 Fluorescence spectra of ZnSe nanoparticles and functionalized ZnSe nanoparticles

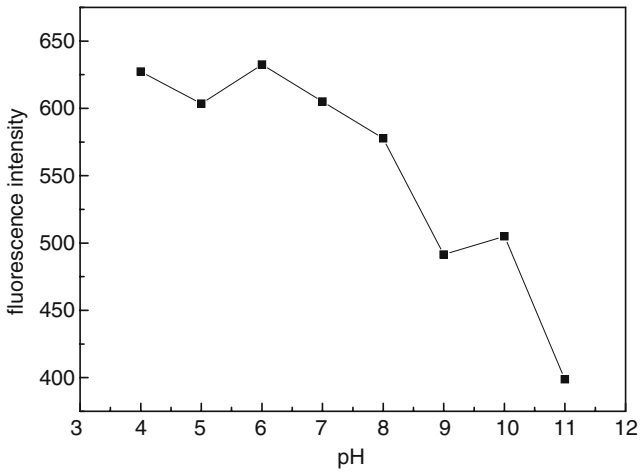

Fig. 4 Effect of pH on the fluorescence intensity of ZnSe-MAA-BSA system

Spectra characteristics and reaction

The reaction between functionalized ZnSe and BSA at room temperature occurred rapidly within 40 min. The fluorescence intensity was stable at least 4 h. Figure 5 is the fluorescence spectra of composite nano-ZnSe-MAA-BSA binding systems. It can be seen that the fluorescence signal of functionalized ZnSe nanoparticles at wavelength region of 275–450 nm is weak without presence of BSA in solution. However, the fluorescence signal of it has been enhanced by presence of BSA and has a characteristic peak at 348 nm. Results suggest that there are some interaction between functionalized ZnSe and BSA. As shown in Fig. 6,

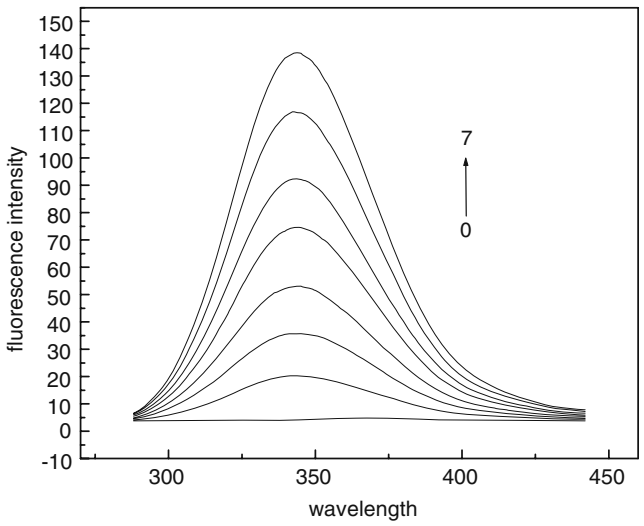

Fig. 5 Fluorescence emission spectra of nano-ZnSe-MAA complex (in the presence of different concentrations of BSA) obtained in phosphate buffer solution (PBS) of pH=7.0 with excitation wavelength at 242 nm. BSA (concentration of 600 μg/ml) added by volume (ml) was (from bottom to top): 0.0, 0.8, 1.6, 2.4, 3.2, 4.4, 5.6, 6.8

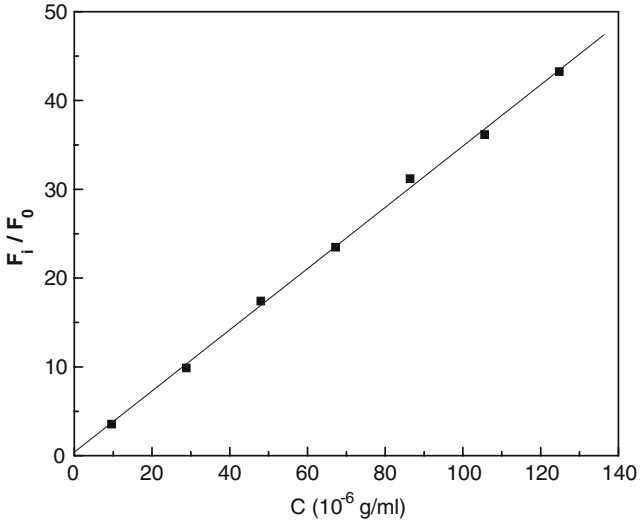

Fig. 6 The straight line equation $^{\triangle}F=0.38+0.34$ C (black line) was found between the relative fluorescence intensity and the concentration **C** of BSA in the range of 9.6–124.8 μg/ml. Points are experimental values

There are good linear relationships between relative fluorescence intensity and the concentrations (C) of BSA, the straight line equation is: $^{\triangle}F=0.38+0.34$ C (μg/ml), with the range of concentration of BSA in 9.6–124.8 μg/ml and correlation coefficient: $R=0.9990$.

Interference of co-existing foreign substances

Under the conditions of the recommended assay, a number of co-existing substances including metal ions, amino acids, salts, nucleic acids, glucose and citric acid are performed. Each foreign substance with various concentrations are mixed prior to the detection. Table 1 shows that a lot of the above substances do not interfere in the determination. However, such ions as Hg(II) and Pb(II) can be allowed only at very low concentration. Even though, diluting the samples with water can minimize the interference of coexisting substances in real samples. So it is possible to use the method for determination of proteins in the animal body fluid samples.

Table 1 Test for the interference of co-existing substances

Co-existing substance	Coexisting conc. (μgmL^{-1})	Relative error (%)
Glysine	25.0	−3.1
Arginine	25.0	−2.0
Citric acid	25.0	+3.1
Isoleucine	25.0	−2.8
Co^{2+}	10.0	−0.2
Cu^{2+}	2.0	−1.9
Fe^{2+}	5.0	−1.7
Lysine	25.0	−1.6
Glucose	25.0	−3.9
Ba^{2+}	20	−2.3
Pb^{2+}	10	−4.3
Mg^{2+}	20	−4.8
Hg^{2+}	16	−3.9

Analytical applications

To investigate the possibility of practical application, a systematic study of sequence of ZnSe-MAA-BSA binding systems in the determination of BSA was carried out. Table 2 gives the results of analysis for three samples that were based on the tolerance to foreign co-existing substances presented in Table 1. The average recovery (R) is defined by the equation $R=[(C_T-C_A)/C]\times 100\%$ [27], where C is the amount of BSA from synthetic samples, C_A is the added amount of standard solution of BSA, C_T is the amount of BSA after adding of standard solution of BSA and obtained from the linear equation $^{\triangle}F=0.38+0.34C$. From Table 2, it can be seen that the values found for the three samples are identical with the expected values. Moreover, the relative standard deviation (R.S.D.) is lower than 4%, showing a good precision on this method and the average recovery is between 99 and 105%, which meets the requirement of microanalysis. Therefore, the developed fluorescence method is applicable to the determination of proteins.

Conclusions

In this paper, ZnSe nanoparticles have been successfully synthesized with hydrothermal synthesis and modified with mercaptoacetic acid. Then the surface of the nanoparticles was covered with abundant carboxylic groups. The functionalized nanoparticles were water-soluble and biocompatible, so they could be used as fluorescence probes in the determination of BSA. The fluorescence intensity of the functionalized nanoparticles was greatly enhanced with the presence of trace BSA. Based on this, a novel fluorescence method has been developed for the determination of BSA. The use of

Table 2 Results of the analysis of synthetic samples ($n=3$, $P=95\%$)

Number	Amount (10^{-6}g/ml)	Addition of BSA (10^{-6}g/ml)	Average recovery%	RSD%
1	48	12	104	1.2
2	60	12	103	3.5
3	84	12	100	1.7

composite nanoparticles as a fluorescence probes leads to a particularly inexpensive, simple, and sensitive assay. Future studies will be developed for the applications.

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