CdTe/CdSe quantum dots as a fluorescent probe for kanamycin determination in the pharmaceutical formulation and human serum

YONGQIANG MAO[a,b], NA LI[b], YE HAN[a], ZHIJIANG ZHOU[a,*]
[a]*School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China*
[b]*College of Science, Liaoning Technical University, Fuxin 123000, China*

A new method for the determination of kanamycin (KANA) was developed based on enhancing the fluorescence intensity of thioglycollic acid (TGA) modified CdTe/CdSe quantum dots (QDs) by KANA. Under optimal conditions, the fluorescence intensity was proportional to the concentration of KANA between 1.0 to 10.0 μg mL^{-1} with a correlation coefficient of 0.9981 and a detection limit of 25 ng mL^{-1}. Moreover, the proposed method was successfully applied to the determination of KANA in the pharmaceutical formulation and human serum, and the results were satisfactory. In addition, the reaction mechanism was also discussed in detail.

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1. Introduction

Kanamycin (KANA) is a broad spectrum aminoglycoside antibiotic produced by fermentation of *Streptomyces kanamyceticus*. As an important bactericidal agent of treating Gram-negative and Gram-positive bacteria infections, KANA is widely used in human and veterinary practice [1]. However, the use of KANA can lead to potential side-effects of ototoxicity and nephrotoxicity [2]. Therefore, it is necessary to develop an accurate and quick determination technique for KANA in pharmaceutical formulations and human serum. Various analytical methods, such as spectrophotometry, liquid chromatography (LC), gas chromatography-mass spectrometry (GC-MS), liquid chromatography–tandem mass spectrometry (LC–MS/MS), capillary zone electrophoresis (CZE), electrochemistry and enzyme-linked immunosorbent assay (ELISA) have been reported [3-9]. However, these methods required expensive instruments, time consuming and skilled personnel, and cannot be used for routine clinical analysis.

Recently, quantum dots (QDs) have gained increased attention because of their unique properties, including broad excitation, narrow emission, size-tunable optical property and photostability [10]. On the basis of these properties, QDs have been extensively used to the study in material science, analytical chemistry, biology and medicine. Thus, QDs have been widely employed for the quantitative determination of many pharmaceutical molecules [11, 12].

In this paper, we found the fluorescence intensity of CdTe/CdSe QDs was significantly enhanced in the presence of KANA. Furthermore, the fluorescence intensity of QDs was linearly proportional to the concentration of KANA. The present method has been used to determine trace amounts of KANA in the pharmaceutical formulation and human serum with the satisfactory results. Based on this phenomenon, a novel method for the determination of KANA by the fluorescent technique was developed.

2. Experimental

The CdTe/CdSe QDs were prepared according to previous method with some modification [13]. Briefly, freshly made NaHTe solution was injected into an oxygen-free mixture of $CdCl_2$ and TGA at pH 9.5. The crude solution was refluxed at 100 ^oC for 3 h to promote the growth of CdTe QDs. The degassed mixture of $CdCl_2$, TGA and Na_2SeSO_3 were then injected into the CdTe QDs solution and refluxed for 30 min. Thus, the first layer of CdSe shell was formed. Repeating the above procedure four times, CdTe/CdSe QDs was finally synthesized.

In a 5.0 mL calibrated test tube, 0.5 mL of the CdTe/CdSe QDs solution, 0.5 mL of Tris-HCl buffer solution and 0.4 mL of KANA solution with standard concentration or extracts from samples were sequentially added. The mixture was diluted to volume with double deionized water, shaken and waited for 10 min for equilibration. The fluorescence intensity was measured with the excitation wavelengths of 350 nm and the emission wavelengths of 600 nm. The excitation and emission slit widths were 2.0 nm.

The fluorescence spectra were obtained by a F-4500 Fluorescence Spectrophotometer. The absorption spectra was acquired on a Hitachi U-3010 Spectrophotometer. Transmission electron microscopy (TEM) images of the QDs were performed on a JEOL-2010 transmission electron microscope using an acceleration voltage of 200 kV. The pH values were measured with a PHS-3C digital meter. A Sigma 3-30K refrigerated centrifuge was used to centrifugate samples of human serum.

3. Results and discussion

Fig. 1 shows the UV-vis absorption spectrum of CdTe/CdSe QDs with a shoulder centered at 550 nm. In addition, the maximum emission appeared at 600 nm. It can be seen that the full width at half maximum of the fluorescence spectra is narrow, which revealed that the us-prepared CdTe/CdSe QDs were nearly monodisperse and homogenous. As shown in Fig. 2, the average size of CdTe/CdSe particles was 4.5 nm.

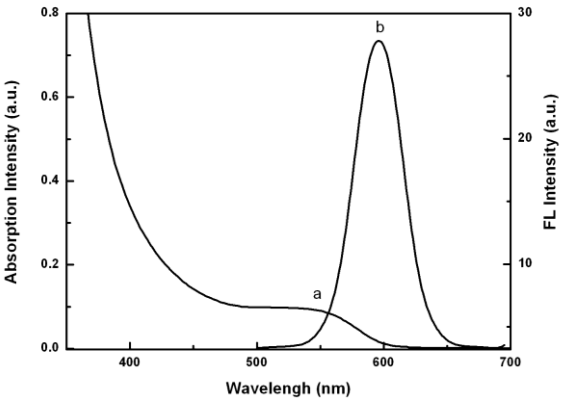

Fig. 1. UV-vis absorption (a) and fluorescence spectra (b) of CdTe/CdTe QDs.

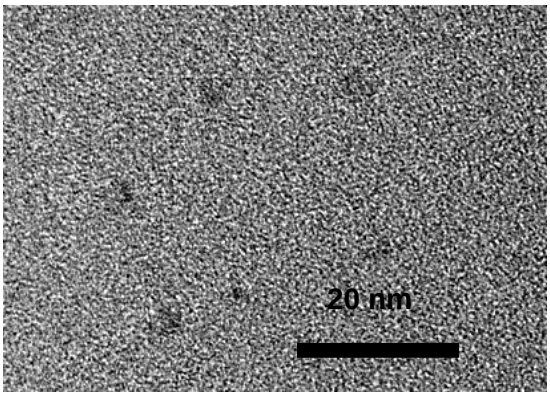

Fig. 2. The TEM images of CdTe/CdSe QDs. The scale bars was 20 nm.

Different pH buffer solutions have great effect on the fluorescence intensity of CdTe/CdSe QDs (Fig. 3). The fluorescence intensity kept increasing as the pH values ranged from 5.0 to 7.5, and then decreased dramatically when the pH values increased to 9.0. According to that, pH 7.5 was recommended for subsequence experiments.

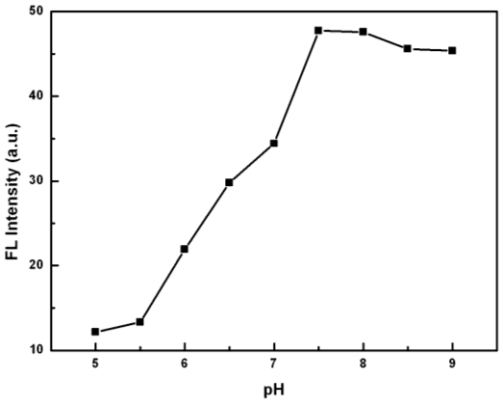

Fig. 3. Effect of pH on the fluorescence intensity of CdTe/CdSe QDs.

It was well known that the concentration of QDs affected the sensitivity and the linear range of the calibration graphs [14]. When the concentration of CdTe/CdSe QDs was low, the fluorescence intensity changed significantly and the sensitivity was relatively high, which might result in the narrow linear range. However, with increasing the concentration of CdTe/CdSe QDs, the linear range became wider whereas the sensitivity decreased. Take into account these factors, 15 μmol L^{-1} of CdTe/CdSe QDs solution was employed for further research. As shown in Fig. 4, the reaction between CdTe/CdSe QDs and KANA reached the equilibrium within 10 min, and the fluorescence signals remained stable at least for 20 min. Hereby, the fluorescence intensity were recorded after reaction for 10 min.

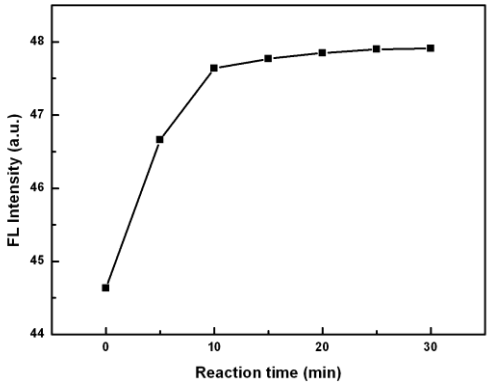

Fig. 4. Effect of reaction time on the fluorescence intensity of CdTe/CdSe QDs.

In order to further evaluate the possibility of practical application, the interference from some metal ions and excipients used together with KANA in medicine was

investigated. The results showed most of them could be allowed at relatively high concentrations. Whereas, some heavy metal ions, such as Hg^{2+}, Ag^{+}, Pb^{2+} and Cu^{2+} could be tolerated at relatively low concentration levels. In fact, their contents in pharmaceutical excipients are strictly limited, so that they could hardly interfere with the determination of KANA.

Under optimal conditions, the calibration graphs for the determination of KANA were shown in Fig. 5. The fluorescence enhancement intensity was proportional to the concentration of KANA ranging from 1.0 to 10.0 μg mL^{-1} with the correlation coefficient of 0.9981. The linear regression equation was $\Delta F=F-F_0=0.069+2.095C$ (μg mL^{-1}), and the limit of detection (LOD, S/N=3) was 25 ng mL^{-1}. The relative standard deviation for seven replicate measurements of a 5.0 μg mL^{-1} KANA solution was 2.0 %.

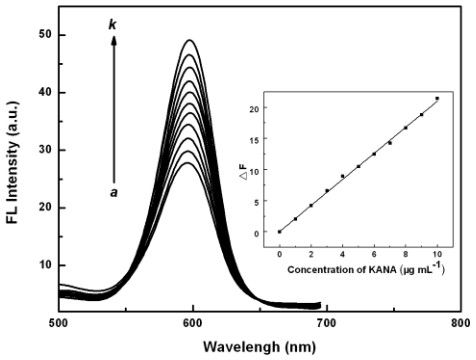

Fig. 5. Fluorescence emission spectra of CdTe/CdSe QDs in the absence and presence of different concentration of KANA (0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 μg mL^{-1} from a to k). Inset: Plot of fluorescence enhancement [ΔF] of CdTe/CdSe QDs at emission peak versus [KANA].

Table 1. Determination of KANA injections and human serum by the proposed method and the HPLC method.

Sample	This method		HPLC	
	Average value (μg mL^{-1})	RSD (%) (n=6)	Average value (μg mL^{-1})	RSD (%) (n=6)
KANA injections	8.44	2.3	8.36	1.6
Human serum	3.12	2.6	3.06	1.8

The human serum samples were added acetonitrile and centrifugated to remove proteins. KANA in the pharmaceutical formulation and human serum were determined by the proposed method and the conventional HPLC method respectively (Table 1). The results obtained by the present method were in good agreement with those obtained by HPLC method, which suggested that the proposed method had great potential in practical applications.

The fluorescence intensity of CdTe/CdSe QDs was significantly enhanced in the presence of KANA, which indicated that a coordination compound between KANA and QDs was possibly formed. Since the surface of TGA-capped CdTe/CdSe particles contain carboxyl group which appear as negative charge, while the amino group of KANA display positive charge [15]. It is easy to see that KANA molecule bind to the surface of CdTe/CdSe QDs through electrostatic attraction. Moreover, with increasing amount of KANA, the compound shows positive charge properties and it is prone to binding to another negative charged QDs, which would finally induce QDs aggregation. Therefore, it was reasonable that the fluorescence enhancement of CdTe/CdSe QDs was due to the formation of QDs aggregation.

4. Conclusions

In summary, a simple, rapid and economic fluorescence method for the determination of KANA was described. Under the optimum conditions, the method has a linear range of 1.0-10.0 μg mL^{-1} with the correlation coefficient of 0.9981. The limit of detection (LOD, S/N=3) was 25 ng mL^{-1}. The proposed method has been applied to the determination of KANA in the pharmaceutical formulation and human serum, and the satisfactory results were obtained. The possible mechanism of reaction might be due to the formation of QDs aggregation.

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*Corresponding author: zzjtju@126.com