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CdTe Quantum Dots as Fluorescent Probes for Josamycin Determination

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A new method for the determination of josamycin has been developed based on quenching of the fluorescence of 3-mercaptopropionic acid-capped CdTe quantum dots (MPA-CdTe QDs) by josamycin in ethanol. Reaction time, interfering substances on the fluorescence quenching, and mechanism of the interaction of CdTe QDs with josamycin were investigated. Under optimum conditions, the relative fluorescence intensity was linearly proportional to the concentration of josamycin between 12.0 and 120.0 μg mL^{-1} with a correlation coefficient of 0.9956 and a detection limit of 2.5 μg mL^{-1}. The proposed method was successfully applied to commercial tablets, and the results were satisfactory, i.e. consistent with those of high-performance liquid chromatography (HPLC).

Key Words : Quantum dots, Josamycin, Fluorescence quenching, Determination

Introduction

Josamycin(JOS) is a 16-membered macrolide antibiotic that is particularly indicated for the treatment of infections of the skin, respiratory tract, ear, nose and throat. It is therefore used in human and veterinary practice. Like other macrolide antibiotics, JOS is a lipophilic molecule with a central lactone ring of 16 atoms to which several amino and sugar moieties are bound.[1,2] Up till now the detection and quantification of JOS in biological fluids, tissues, eggs and milk has mainly been performed by kinetic spectrophotometric,[3,4] Voltammetry method,[5] liquid chromatography,[6-9] liquid chromatography-mass spectrometry (LC-MS)[10,11] and capillary electrophoresis.[12] However, kinetic spectrophotometric methods is based on reaction for a fixed-time of 20 ± 2 min at 70 ºC. LC needs great amount of organic solvent, large instrument and skilled operator. Capillary electrophoresis is simple than HPLC, but its reproducibility is poor. Moreover, they are also time-consuming, rather complicated and expensive, and cannot be used for routine clinical analysis. So there still exists a need for improved a cheaper and simpler method for the measurement of JOS.

Fluorimetric techniques are becoming increasingly important for quantitative determination of pharmaceutical drugs[13,14] due to their high sensitivity, short run time, cheap instrumentation and selectivity, *etc.* Compared to organic fluorophores, QDs exhibit higher quantum yield, colour availability, good photostability, large surface-to-volume ratio and surface functionality. Thus, QDs have been widely employed in some applications, such as the determination of enrofloxacin,[15] ranitidine hydrochloride,[16] roxithromycin,[17] polycyclic aromatic compounds[18] and cytochrome c[19] in analytical chemistry. However, the use of MPA-CdTe QDs to the study of the interaction between QDs and JOS by fluorescent technique has not been reported so far. In this study we found that the fluorescence intensity of CdTe QDs quenched in the presence of JOS at ethanol. The quenched intensity of fluorescence was proportional to the concentration of JOS. Based on this phenomenon, a new method for the determination of JOS by the fluorescent technique was developed. The method has been applied to the determination of JOS in commercial tablets from different manufactures with satisfactory results.

Experimental

Apparatus. UV-vis absorption spectra were recorded with a UV-2102 PC UV/vis Spectrophotometer (UNICO (Shanghai) Instrument Co., Ltd., China). The spectra and intensity of fluorescence were measured with a RF-5301 spectrofluorimeter (Shimadzu, Japan). A quartz cuvette (1 × 1 cm cross section) was used. The slit-width for excitation and emission was set at 5 nm and 3 nm, respectively. All optical measurements were performed at room temperature under ambient conditions.

Reagents. JOS reference standard, Cadmium chloride, sodium borohydride ($NaBH_4$), tellurium powder, 3-mercaptopropionic acid (MPA), sodium hydroxide and absolute ethanol were purchased from Aladdin-Reagent Company (Shanghai, China). All chemical reagents were of analytical grade and used as received without further purification.

Synthesis of Quantum Dots. The preparation of MPA-CdTe QDs was *via* a modified method adopted from literature.[20] The CdTe QDs colloidal solution was prepared using the reaction between Cd^{2+} and NaHTe solution at pH 12.0 with MPA as the stabilizing reagent. The molar ratio of Cd^{2+}/Te^{2-}/MPA was 1:0.4:2.3. Briefly, 1.26×10^{-4} mol $CdCl_2 \cdot 2.5H_2O$ was dissolved in 90 mL water, and 2.88×10^{-4} mol of MPA was added under stirring, the pH of mixture was adjusted to 12 using 1 mol L^{-1} NaOH, the mixture was bubbled with N_2 atmosphere for 60 min. Under vigorous stirring, 5.04×10^{-5} mol freshly prepared oxygen-free NaHTe,

which was prepared by reaction of 80 mg Te powder and 80 mg $NaBH_4$ in 2 mL water at 0 °C for 8 h, was injected to the above solution. Afterward, the resulting solution mixture was heated to 100 °C and refluxed for 2 h.

Procedures. An appropriate volume of working solution of JOS were added to a 10 mL volumetric flask with 0.40 mL CdTe QDs, then diluted with ethanol to the mark and mixed thoroughly. The fluorescent spectra were obtained by scanning the emission from 350 to 600 nm on the spectrofluorimeter (with 5 nm and 3 nm slite width for excitation and emission, respectively), and the fluorescent intensity F for the reaction product and F_0 for the reagent blank were measured at the maximum emission wavelength, $\Delta F = F_0 - F$.

Sample Treatment. For the pharmaceutical analysis, 10 tablets were reduced to a homogeneous fine powder in a mortar. An amount of this powder corresponding to about 500.00 μg mL^{-1} solution was accurately weighed and dissolved in about 25 mL ethanol. After sonicated for 10 min, the solution was filtered, transferred into a 50 mL calibrated flask and completed to volume with the same solvent.

Results and Discussion

UV-vis and Fluorescence Spectroscopy of CdTe QDs. The absorption spectra and fluorescence spectra of the CdTe QDs were obtained and shown in Figure 1. The fact that the emission maximum lay close to its absorption onset indicated the emission rose from direct recombination between conduction and valence band charge carriers. It can be seen that the line width of the fluorescence spectra was narrow, which also revealed that the as-prepared CdTe QDs were nearly monodisperse and homogenous.

The Fluorescence Quenching of CdTe QDs by JOS. The emission spectra of CdTd QDs in the absence and presence of JOS were recorded in ethanol, the results of which are shown in Figure 2. The observed fluorescence band was centered at 561 nm (excitation 350 nm). When JOS was added to the CdTe QDs, a significant decrease of QDs fluorescence emission was observed. Considering this

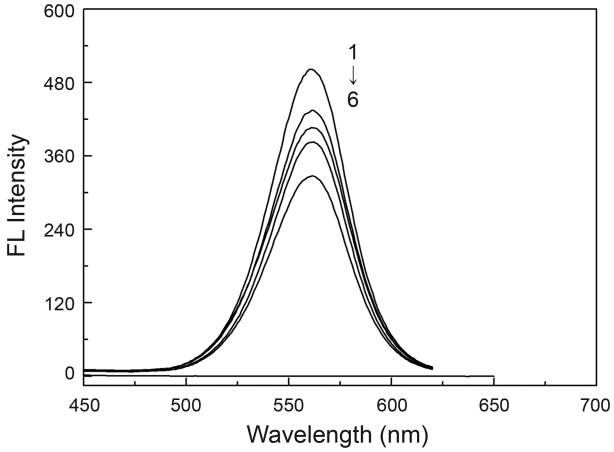

Figure 2. Fluorescence emission spectra of CdTe QDs in the absence and presence of different concentrations of JOS. Concentration of CdTe QDs from (1) to (5): 0.2 mmol L^{-1}, (6): 0 mmol L^{-1}; JOS from (1) to (6): 0, 24, 32, 40, 60, 40 μg mL^{-1}.

significant quenching of fluorescence intensity, the possibility of developing sensitive methods for JOS based on spectrofluorometry has been evaluated.

Mechanism of the Interaction of CdTe QDs with JOS. Quenching of FL emission from QDs may occur by several mechanisms: energy transferring, charge diverting, surface absorption, and surface bound complexation equilibrium attraction.[21,22]

To explore the mechanisms of reaction, the UV-vis absorption spectra of CdTe QDs were investigated in the absence and presence of the JOS (Figure 3). As showed in Figure 3, JOS had no absorption in the 400-700 nm wavelength range. This shows the quenching of FL emission of CdTe QDs was not attributed to the absorption of the emission wavelength by JOS. No obvious change was observed for the CdTe QDs absorption spectra before and after successive addition of JOS, which also means CdTe QDs do not aggregate or become smaller after addition of JOS. If FL emission quenching of CdTe QDs occurred because of a charge transfer process, the absorption of

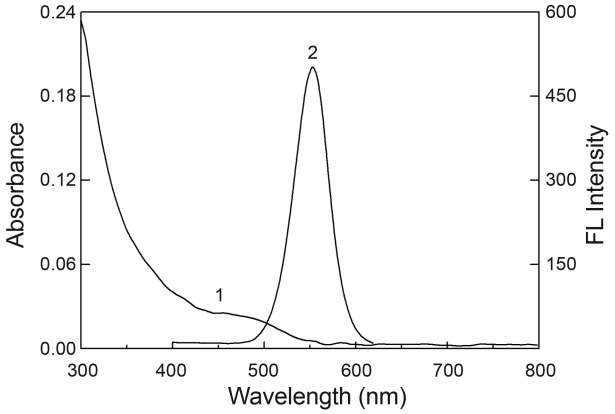

Figure 1. Absorption spectra (1) and fluorescence spectra (2, excited at 350 nm) of CdTe QDs.

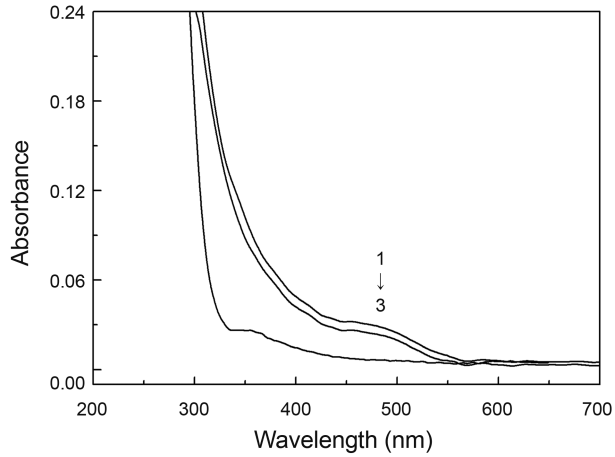

Figure 3. Absorption spectra of CdTe QDs in the presence of JOS (1), CdTe QDs (2), JOS (3).

sufficient light energy causes excitation of electrons from the valence band (VB) to the conduction band (CB) resulting in the formation of positive charge termed as a hole (h^+) in the VB and a free electron (e^-) in the CB (eqn. (1)).

$$\mathrm{CdTe} + h\nu \rightarrow \mathrm{CdTe}^* + e^-(\mathrm{CB}) + h^+(\mathrm{VB}) \quad (1)$$

Without the presence of electron acceptor, the electron and hole would recombine and emit the fluorescence. Based on the presence of the conjugated double bonds, hydroxyl groups of macrocyclic lactone ring are traps of the holes, which could block the recombination of electron and hole and quench the fluorescence of nanocrystals. Similar results have been reported by Yang *et al*., they have reported the photoluminescence quenching of nanoparticles through the direct oxidation of catechol earlier.[23]

Reaction and Stability. Under room temperature, the effect of time on the fluorescence intensity of the system was tested. Results show that the intensity of fluorescence intensity reaches quickly a maximum when all reagents are mixed together. No additional incubation time is needed. The intensity of ΔF is stable for at least 2.0 h at room temperature. Therefore, 5 min of incubation time was recommended.

Effect of Temperature on the FL Intensity of QDs. Temperature has great effect on the fluorescence intensity of CdTe QDs (Figure 4). The influence of the temperature on the fluorescence intensity shows a nearly linear (negative) relationship between temperature and fluorescence intensity of CdTe QDs. When the temperature is decreased, the fluorescence is enhanced. Similar results have been reported by Yu *et al*.[24]

Temperature coefficient (T_{coef}), in percentage, was calculated from the expression

$$T_{coef}(\%) = \left[\frac{2.303}{\Delta T}\log\frac{I_{F_1}}{I_{F_2}}\right] \times 100 \quad (2)$$

Where I_{F1} and I_{F2} are the fluorescence intensity at any two temperatures which define the temperature increase (ΔT). The calculation of T_{coef} affirms the non-radiant deactivation process is the internal conversion when $|T_{coef}| \leq 1\%$;[25] the value obtained for CdTe QDs with a temperature coefficient of 0.48%. It showed non-radiant deactivation process occurred for CdTe QDs. Thus, as for the CdTe QDs, the increased temperature results in the decreased quantum yield of fluorescence, which contributes to the declining FL intensity when it is excited with the same energy.

Calibration Curves and Sensitivity. Under the optimum conditions, the calibration curves for determination were built under the temperature of 25 °C (Figure 5). The linear range was 12.0-120.0 μg mL^{-1} for JOS. The linear regression equation was $\Delta F = 2.6996C + 1.267$, the correlation coefficient was 0.9956. It demonstrated that there was a good linear relationship between the concentration of JOS and ΔF. The limit of detection (LOD) was 2.5 μg mL^{-1}, which was given by the equation $\mathrm{LOD} = KS_0/S$, where K was a numerical factor chosen according to the confidence level desired, S_0 was the standard deviation (S.D.) of the blank measurements ($n = 11$, $K = 3$), and S was the slope of the calibration curve. For five successive determinations of a 40.0 μg mL^{-1} JOS solution, the relative standard deviation (RSD) for the FL Intensity was 1.65%. The comparison of CdTe quantum dots as fluorescent probes for JOS determination with other methods was listed in Table 1. It can be seen that the CdTe quantum dots offered a wide linear range for JOS detection and better reproducibility than some of previous reports. These results indicated that CdTe quantum dots is an appropriate fluorescent probes for the determination of JOS.

Tolerance of Foreign Substance. The tablets often contained the following excipients: sodium dodecyl sulphate, glucose, sucrose, starch, mannitol and o-phthalic acid.[26] The results showed that for 40.0 μg mL^{-1} JOS, at least 2.5-fold concentration of mannitol; 2-fold concentration of sodium dodecyl sulphate; 0.1-fold concentration of Glucose, sucrose, starch and o-phthalic acid; 0.01-fold concentration of EDTA and $CaSO_4$ do not interfere with fluorescence intensity of the standard solution (signal change below 5%). However, the

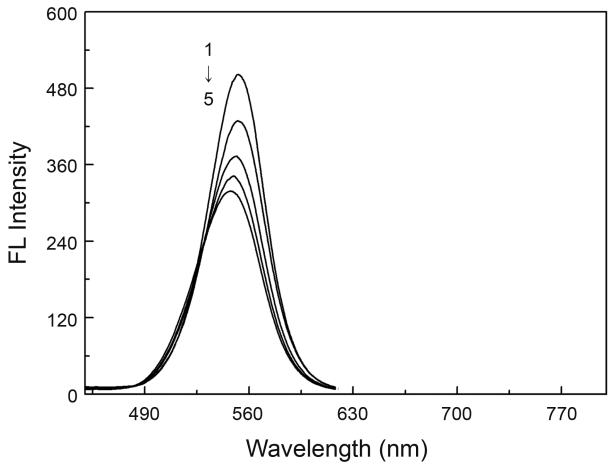

Figure 4. Effect of the temperature on fluorescence intensity of CdTe QDs, from 1 to 5: 20, 24, 28, 32, 36 °C.

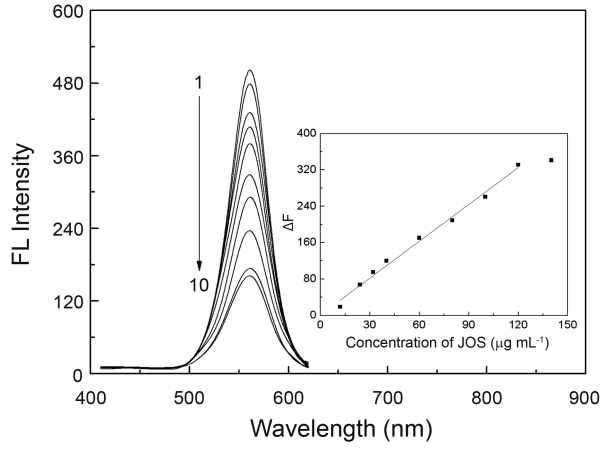

Figure 5. Fluorescence emission spectra of CdTe QDs in the absence and presence of different concentration of JOS: 0.0, 12.0, 24.0, 32.0, 40.0, 60.0, 80.0, 100.0, 120.0 and 140.0 μg mL^{-1} from 1 to 10.

Table 1. Comparison of the main characteristics for fluorimetric determination of JOS with other methods

Method	Linear range (μg mL^{-1})	Correlation coefficient	LOD (μg mL^{-1})	Reproducibility (RSD, %)	Ref.
kinetic spectrophotometric	2-10	0.9960	1.0	2.60[a]	[3]
kinetic spectrophotometric	5.0-30.0	0.9996	1.0	3.43[a]	[4]
Voltammetric	6-50	0.9987	1.6	1.08[a]	[5]
HPLC	0.05-0.5	0.998	0.013	5	[6]
LC	1.0-10.0	0.9987	0.76	0.45	[7]
HPLC	0.05-0.4		0.02	10.6	[8]
HPLC	0.1-3.0	0.9932	0.025	3.99	[9]
LC-MS	0.001-0.1	0.9954		2.8-13.5[a]	[10]
capillary electrophoresis	0.01-5.0	0.9993	0.0031	2.2	[12]
fluorimetric	12.0-120.0	0.9956	2.5	1.65	Our work

[a]SD.

Table 2. Analysis of pharmaceutical formulations by proposed procedures

Pharmaceutical formulation[a]	Labelled amount (mg)	Amount found (mg)		Recovery (%) (n = 5)
		Reference procedures[b]	Proposed procedures[c]	
Tablets T_1	200	198.6	199.5 ± 1.31	96.2-101.6
Tablets T_2	200	197.4	198.2 ± 1.73	96.4-101.9

[a]T1: Batch No. 090502, Expiry date: 04/2011, from Guilin Pharm; T2: Batch No. 100601, Expiry date: 05/2013, from Guilin Pharm. [b]HPLC method. [c]mean ± S.D.

solubility of these interfering substances in ethanol was very low, so that their concentration in the sample solution was low. After being dissolved in ethanol and then filtered, the sample was directly detected without other treatments.

Application. The present method was applied to determine JOS in commercial tablets. The results obtained by the proposed and reference (HPLC method[9] for the assay of JOS was adopted) procedures for pharmaceutical formulations are compared in Table 2 and are in good agreement. The recovery of the method was in the range of 96.2-101.9%.

Conclusion

The article described a simple, rapid and economical fluorescence method for the determination of JOS in commercial tablets. The primary advantage of this method is its simplicity and rapidity. Under the optimum conditions, the method has a linear range of 12.0-120.0 μg mL^{-1} with a 0.9956 correlation coefficient. The limit of detection was 2.5 μg mL^{-1}. The presented method has been applied successfully to the determination of JOS in pharmaceutical formulations.

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References

1. Bryskier, A. J.; Butzler, J. P.; Neu, H. C.; Tulkens, P. M. *Macrolides: Chemistry, Pharmacology and Clinical Uses*; Amette Blackwell: Paris, France, 1993.
2. Reeves, D. S., Wise, R., Andrews, J. M., White, L. O., Eds., *Clinical Antimicrobial Assays*; Oxford University Press: Oxford, UK, 1999.
3. Al-Majed, A. A.; Belal, F.; Ibrahim, K. E. E.; Khalil, N. Y. *J. AOAC Int*. **2003**, *86*, 484.
4. Al-Majed, A. A.; Belal, F.; Khalil, N. Y.; Ibrahim, K. E. E. *J. AOAC Int*. **2004**, *87*, 352.
5. Belal, F.; Al-Majed, A.; Ibrahim, K. E. E.; Khalil, N. Y. *J. Pharm. Biomed. Anal*. **2002**, *30*, 705.
6. Gomis, D. B.; Ferreras, A. I. A.; Álvarez, M. D. G.; García, E. A. *J. Food Sci*. **2004**, *69*, 415.
7. Daidone, F.; Heuvelmans, R.; Aerden, L.; Hoogmartens, J.; Adams, E. *J. Pharm. Biomed. Anal*. **2008**, *48*, 347.
8. Tad, M.; Biarez, O.; Nicolas, P.; Petitjean, O. *J. Chromatogr*. **1992**, *575*, 171.
9. Räder, K.; Wildfeuer, A.; Schwedass, A.; Laufen, H. *J. Chromatogr*. **1985**, *344*, 416.
10. Kikuchi, Y.; Teramura, T.; Sekino, J.; Nishimura, T.; Miura, H.; Watanabe, T.; Higuchi, S. *J. Chromatogr. B* **1998**, *720*, 81.
11. Berrada, H.; Borrull, F.; Font, G.; Marcé, R. M. *J. Chromatogr. A* **2008**, *1208*, 83.
12. Deng, B.; Kang, Y.; Li, X.; Xu, Q. *J. Chromatogr. B* **2007**, *859*, 125.
13. Amjadi, M.; Manzoori, J. L.; Orooji, M. *Bull. Korean Chem. Soc*. **2007**, *28*, 246.
14. Zhu, X.; Gong, A.; Yu, S. *Spectrochim. Acta A* **2008**, *69*, 478.
15. Chen, J.; Xu, F.; Jiang, H.; Hou, Y.; Rao, Q.; Guo, P.; Ding, S. *Food Chem*. **2009**, *113*, 1197.
16. Liu, M.; Xu, L.; Cheng, W.; Zeng, Y.; Yan, Z. *Spectrochim. Acta A* **2008**, *70*, 1198.
17. Peng, J.; Hu, X. *J. Luminesc*. **2011**, *131*, 952.
18. Carrillo-Carrión, C.; Simonet, B. M.; Valcárcel, M. *Anal. Chim. Acta* **2009**, *652*, 278.
19. Cao, M.; Cao, C.; Liu, M.; Wang, P.; Zhu, C. *Microchim. Acta* **2009**, *165*, 341.
20. Zhang, H.; Zhou, Z.; Yang, B.; Gao, M. *J. Phys. Chem. B* **2003**, *107*, 8.

21. Wu, H.; Liang, J.; Han, H. *Microchim. Acta* **2008**, *161*, 81.
22. Liang, J. G.; Zhang, S. S.; Ai, X. P.; Ji, X. H.; He, Z. K. *Spectrochim. Acta A* **2005**, *61*, 2974.
23. Ma, Y.; Yang, C.; Li, N.; Yang, X. *Talanta*. **2005**, *67*, 979.
24. Yu, D.; Wang, Z.; Liu, Y.; Jin, L.; Cheng, Y.; Zhou, J.; Cao, S. *Enzyme Microb. Tech*. **2007**, *41*, 127.
25. Seitz, W. R. *Treatise on Analytical Chemistry*; Wiley: New York, 1981.
26. Liang, J.; Huang, S.; Zeng, D.; He, Z.; Ji, X.; Ai, X.; Yang, H. *Talanta* **2006**, *69*, 126.