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A sensitive phosphorescent method based on MPA-capped Mn-doped ZnS quantum dots for detection of Diprophyllin

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Short title: Detection of Diprophyllin Based on Mn-doped ZnS QDs

Category: Sensors

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Abstract: A new strategy for diprophyllin (DPP) detection was developed by utilizing room-temperature phosphorescence (RTP) of the MPA-capped Mn-doped ZnS QDs. Upon the addition of diprophyllin, the phosphorescence intensity could be regularly quenched with the increase of diprophyllin and a linear relationship could be observed in the range of 1.97~197 nM with the limit detection (LOD) of 0.893 nM. The phosphorescent quenching mechanism was preliminarily ascribed to electron transfer from photo-excited Mn-doped ZnS QDs to DPP that served as electron acceptor. Induced from Mn-doped ZnS QDs to diprophyllin, the elections were able to result in significant phosphorescence quenching of Mn-doped ZnS QDs. This room-temperature phosphorescence system for diprophyllin detection is label-free, convenient, sensitive and selective, which can be used to detect diprophyllin in real samples.

Keywords: Quantum dots (QDs); Room-temperature phosphorescence (RTP); Detection; Diprophyllin; Electron transfer
1. Introduction

Diprophyllin (DPP) is a kind of methyl xanthine derivative with important pharmacy functions, which is clinically used in treating bronchial asthma, panting bronchitis, blocked emphysema, and cardiogenic asthma. Due to instable metabolism and narrow treatment index, it is of crucial importance to closely monitor DPP levels for the sake of health. So far, many efforts have been devoted to the detection and quantification of DPP.

The conventional methods for DPP detection mainly include titrations\(^1\),\(^2\), spectrophotometry\(^3\),\(^5\), polarography\(^6\), high-performance liquid chromatography (HPLC)\(^7\),\(^10\), HPLC & chemometric assisted spectrophotometric methods\(^11\) and quantum dot fluorescent method\(^12\). However, there are some defects existing in these methods, such as, lacking of specificity, long processing, high cost and potential interference from other factors, which have greatly limited its actual application. Therefore, a convenient, sensitive and rapid method for recognizing and detecting DPP is still highly demanded at present.

Recently, the room-temperature phosphorescence (RTP) QDs detection has attracted much attention, which is widely studied for developing sensors with great success\(^13\),\(^19\), especially Mn-doped ZnS QDs\(^18\),\(^20\),\(^21\). RTP QDs detection shows satisfactory reliability, due to long lifetime of phosphorescence, which allows an appropriate delay time and thus avoids the interferences from autofluorescence and scattering light\(^13\),\(^15\),\(^17\),\(^22\). The selectivity is also enhanced, because phosphorescence is a less usual phenomenon than fluorescence\(^14\),\(^23\). Although the use of phosphorescent
QDs for optical detecting is at the infant stage, it is proved to be very promising\textsuperscript{15,16,24-27}.

In this paper, a sensitive, simple, and selective method for the detection of DPP based on RTP of Mn-doped ZnS QDs is proposed, which has been successfully applied in detection of DPP in real samples.

2. Experimental

2.1 Materials and apparatus

MPA (J&K Scientific, Beijing, China), Zn(Ac)\textsubscript{2}·2H\textsubscript{2}O, Mn(Ac)\textsubscript{2}·4H\textsubscript{2}O, and Na\textsubscript{2}S·9H\textsubscript{2}O (Tianjing Kermel Chemical Reagent Co., China) were used in preparation of Mn-doped ZnS QDs. Ultrapure water (18.2 MΩ cm) was obtained using a WaterPro water purification system (Labconco Corporation, Kansas City, MO). DPP was purchased from Sigma Corporation of America.

The morphology and microstructure of QDs were characterized by a JSM-7500F transmission electron microscope (TEM, Japan). Phosphorescence was measured on a Cary Eclipse fluorescence spectrophotometer (Varian American Pty Ltd., America) in the phosphorescence mode, equipped with a plotter unit and a quartz cell (1 cm × 1 cm). The slits for excitation and emission were 10 nm and 20 nm wide, respectively. Ultraviolet/visible (UV / Vis) absorption spectra were recorded using a Shimadzu UV-29100 UV / Vis spectrophotometer, and pH was measured with a pH meter (Jinpeng Analytical Instruments Co. Ltd, China).

2.2 Experimental procedure

2.2.1 Synthesis of the MPA-capped Mn-doped ZnS QDs
The synthesis of MPA-capped Mn-doped ZnS QDs was performed according to the reference\textsuperscript{15, 28, 29} with some modification. Briefly, prepare a three-necked flask, followed by addition of 170 µL of 0.04 M MPA, 5 mL of 0.1 M Zn(Ac)\textsubscript{2}, and 2 mL of 0.01 M Mn(Ac)\textsubscript{2}. Second, adjusted the pH value of mixture to be 11 with 1 M NaOH and stirred under argon bubbling at room temperature for 30 min. Then 5 mL of 0.1 M Na\textsubscript{2}S was quickly injected into the solution under isolation from the air. After stirring for 20 min, the solution was aged at 50 °C in open air for 2 h to form MPA-capped Mn-doped ZnS QDs. Finally, QDs were purified by precipitation with the same volume of ethanol, centrifugation, washing with ethanol, and vacuum-drying at room temperature. The obtained QD powder was highly water-soluble.

2.2.2. Analytical procedures

For the detection of quenching effect of DPP on RTP of Mn-doped ZnS QDs, Mn-doped ZnS QDs and different amounts of DPP were incubated in phosphate-buffered saline solution (PBS buffer, pH 7.4, 20 mM) at room temperature for 20 min. Mn-doped ZnS QDs were dissolved in water to obtain a solution of 2.0 mg mL\textsuperscript{-1} and then the QDs solution (100 µL) was added to each of the above DPP solutions. The solution was mixed thoroughly to measure the phosphorescence. Experiments were repeated three times. No further pretreatment was employed in sample preparation.

2.2.3 Samples pretreatment

The DPP injections were diluted as the standard solution in further experiment, and urine was collected from healthy volunteers, which were subjected to a 100-fold
3. Results and discussion

MPA-capped Mn-doped ZnS QDs were synthesized in the experiment part, so does the preparation of diprophyllin samples. In this section, we characterized QDs, optimized experimental conditions, studied the interference from coexisting substances, explored reaction mechanism and eventually applied to the analysis of real samples. Therefore, a DPP detection method was establish with strong feasibility on the basis of a fact that the RTP intensity of Mn-doped ZnS QDs was quenched regularly after the addition of DPP. The specific operation process of the system was carried out according to the following sections.

3.1 Characterization of the MPA-capped Mn-doped ZnS QDs

The morphology features and diameter of MPA-capped Mn-doped ZnS QDs are characterized by TEM and XRD (Fig. S1). Clearly, these nanoparticles are uniform and mono-dispersed in shape. The average diameter of the Mn-doped ZnS QDs is about 3.5 nm (Fig. S1a). Meanwhile, the crystal structure of Mn-doped ZnS QDs is characterized by X-Ray diffraction (XRD), and a zinc-blend structure is typically exhibited by the prepared Mn-doped ZnS QDs as shown in the distinguishable (111), (220) and (311) planes in XRD patterns (Fig. S1b). In addition, a maximum excitation peak and a narrow emission band in the phosphorescence spectra of Mn-doped ZnS QDs are shown at 295 nm and 590 nm respectively, which could be attributed to the energy transfer from the band gap of ZnS to Mn$^{2+}$ and the subsequent transition from the triplet state ($^4T_1$) to the ground state ($^6A_1$) of Mn$^{2+}$ (Fig. S1c)$^{26,30-32}$. All these
results reveal that MPA-capped Mn-doped ZnS QDs were prepared successfully.

3.2 Phosphorescence detection of DPP with Mn-doped ZnS QDs

Fig. 1A shows how the RTP intensity of Mn-doped ZnS QDs changed with concentration of DPP. Apparently, with increase of DPP concentration, the RTP intensity of Mn-doped ZnS QDs was quenched at 590 nm. When the concentration of DPP reaches 197 nM, the RTP intensity of Mn-doped ZnS QDs is basically stabilized. As shown in Fig. 1B, the RTP intensity of 40 mg L\(^{-1}\) Mn-doped ZnS QDs is quenched by 197 nM DPP as much as 4.5 times. This phenomenon indicates that DPP concentration has great effect on the RTP intensity of Mn-doped ZnS QDs. In this case, the possibility of developing a sensitive method for DPP detection is established.

![Graph A](image-1)  
![Graph B](image-2)

**Fig.1.** (A) DPP concentration-dependent RTP emission of the MPA-capped Mn-doped ZnS QDs; (B) Show the change of the RTP intensity with the increase of the concentration of DPP

3.3 Optimization of the system

The effects of variable factors on RTP intensity of DPP / Mn-doped ZnS QDs system were analyzed, and pH was found to be one of most important variables that affect the system. As pH values can not only affect the RTP intensity of Mn-doped
ZnS QDs, but also affect the interaction between Mn-doped ZnS QDs and DPP, it is important to select an appropriate pH value for DPP assay. Observed from Fig. S2a, it can be found that the RTP intensity is decreasing gradually with pH value ranging from 5.7 to 9.0, but the decrease from 7.4 to 8.5 is almost invariant. The pH value of biological fluids is in the scope of 7.35~7.45, pH 7.4 is selected as a mild condition, in this study.

Then, the effect of time and salt concentration on RTP intensity of DPP/Mn-doped ZnS QDs system is studied. As shown in Fig. S2b, the RTP intensity of Mn-doped ZnS QDs is basically stable within 40 min, so 20 min is adopted in following experiments to ensure the adequately reaction of Mn-doped ZnS QDs with DPP. As shown in Fig. S2c, the salt concentration poses no significant effect on the RTP intensity of DPP/Mn-doped ZnS QDs system.

3.4 Calibration curves and sensitivity

As shown in Fig. 2A, under the optimal conditions, a linear calibration plot of quenched phosphorescence intensity (P / P₀) versus DPP concentration (C, in nM) is drawn in the range of 1.97~197 nM with a correlation coefficient of 0.9985 and a linear regression equation of P₀/ P = 0.0031C + 0.9546 (where C is the concentration of DPP in nM). The relative standard deviation (RSD) of 11 continuous parallel detections is 2.36 %, and the detection limit for DPP is 0.893 nM.

In contrast with the reported methods \(^1,4,7,10,12\) for detection DPP, as shown in Table 1, the RTP method we established displays a relatively lower detection limit in the light of spectrophotometry, HPLC, and fluorescence method, but higher than that of HPLC.
& chemometric assisted spectrophotometric methods, not only its linear range is narrow but with complicated operation process. Compared with fluorescence method, RTP method suffers from less background interference by biological fluids as well, such as, no background interference is found under the phosphorescence detection, although the interference on fluorescence background of urine samples is extremely serious, as shown in Fig.2B. Therefore, the universal process used for biological sample treatment can be omitted in this project, because the sample can be tested after simple dilution, which simplifies the procedure of analysis and can be used to detect DPP content in complex biological fluid.

**Fig.2.** (A) Plots of $P / P_0$ as a function of DPP concentration show linear range. (B) The RTP (curves 1) and fluorescence spectra of urine (curves 2) Buffer, 20 mM PBS (PH 7.4); MPA-capped Mn-doped ZnS QDs, 40 mg L$^{-1}$.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Detection range(nM)</th>
<th>LOD(nM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrophotometric</td>
<td>$3.93 \times 10^2$ ~ $3.93 \times 10^3$</td>
<td>5.31</td>
<td>3-5</td>
</tr>
<tr>
<td>Polarography</td>
<td>$5 \times 10^1$ ~ $1.6 \times 10^5$</td>
<td>$7 \times 10^2$</td>
<td>6</td>
</tr>
<tr>
<td>HPLC</td>
<td>3.93~78.66</td>
<td>3.93</td>
<td>7-10</td>
</tr>
<tr>
<td>HPLC and C-S</td>
<td>39.33~98.33</td>
<td>0.001</td>
<td>11</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>$1.67 \times 10^7$ ~ $1.33 \times 10^8$</td>
<td>$2.24 \times 10^5$</td>
<td>12</td>
</tr>
<tr>
<td>Phosphorescence</td>
<td>1.97~197</td>
<td>0.893</td>
<td>This work</td>
</tr>
</tbody>
</table>

**Table 1** Comparison of the proposed method with different analytical techniques reported for
3.5 Selectivity of the method

Selectivity is a very important parameter to evaluate the performance of the phosphorescence system, especially for system with potential applications in biomedical sample, therefore, a highly selective response to the target over other potentially competing species is necessary. In this case, the selectivity experiments for our system are evaluated to detect various coexisting substances. Table S1 shows the interference effect of metal ions, inorganic anions and biological molecules on the detection of DPP, a relative error of ± 5.0 % is considered to be tolerable. As shown in Table S1, the tolerable concentration ratios of coexisting substances to DPP is over 100 fold for K⁺, Cl⁻, Br⁻, NO₃⁻, 50 fold for Na⁺, SO₄²⁻ and Glucose, 30 fold for Ca²⁺, Mg²⁺ and L-Cys and 17 fold for L-Gly. From the results, we can find there’s a little interference from commonly existing substances. Thus, the sensitive method displays high selectivity for the detection of DPP.

3.6 Possible Mechanism

The phosphorescence intensity of a compound can be decreased by a variety of molecular interactions, such as excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation and collision quenching⁸. Collision or dynamic quenching refers to a process that the phosphor group and quencher come into contact with each other during the lifetime of excited-state. Whereas the static quenching is a process that the phosphorescence intensity is weakened resulting from the formation of the complex⁹ between phosphor and a quencher at the ground state.
In dynamic quenching, the quenching agent and phosphorescent material interact in the excited state, which results in the decrease of phosphorescence intensity, so the mechanism can be analyzed quantitatively by Stern-Volmer’s equation (Eq. 1). In static quenching, the quenching agent and phosphorescent material are coordinated in the ground state to form a complex, which results in the decrease of phosphorescence intensity, this can be described by Stern-Volmer’s equation (Eq. 2).

$$\frac{P_0}{P} = 1 + K_{SV} C_q$$  \hspace{1cm} (1)

$$\frac{1}{(P_0 - P)} = \frac{1}{P_0} + \frac{K_{LB}}{(P_0 C_q)}$$  \hspace{1cm} (2)

where $P_0$ and $P$ are the RTP intensity of the Mn-doped ZnS QDs with absence and presence of a quencher, $C_q$ is the concentration of the quencher (DPP), $K_{SV}$ and $K_{LB}$ are the dynamic and static quenching constants respectively \(^3, 5\).

As shown in Fig. 3A, the linear relationship between $(P_0 - P)^{-1}$ and $C_q$ does not follow the Lineweaver-Burk equation, but the relationship between $\frac{P_0}{P}$ and $C_q$ followed Stern-Volmer’s equation, which indicates the occurrence of dynamic quenching. These results imply that, the interaction between DPP and Mn-doped ZnS QDs in the excited state, may cause the decrease of RTP intensity.

The characteristic structure of substance is obtained by using UV-vis absorption spectroscopy. In order to further distinguish the type of RTP quenching of Mn-doped ZnS QDs by DPP, UV-vis absorption spectra of DPP-QDs system is investigated as well (Fig. 3B). Assuming that RTP quenching by DPP is a static quenching, the quenching progress is the formation of ground state complexes between QDs and quenchers (DPP) probably. Moreover, the ground state complexes can cause the
change of UV-vis absorption spectra of quencher or phosphorescence substance.\textsuperscript{33} After comparing UV-vis absorption spectra of the quencher alone with quencher added with QDs (curve b and c), there are basically unchanged. Hence, the RTP quenching type of Mn-doped ZnS QDs by DPP is not belonging to static quenching congruously, but pertains to dynamic quenching which is resulting from collision encounters between the excited state of Mn-doped ZnS QDs and quenchers.

In order to make certain reason of RTP quenching of Mn-doped ZnS QDs by DPP, the absorption spectrum of DPP and emission spectrum of Mn-doped ZnS QDs are studied. As there is no overlap between the emission from Mn-doped ZnS QDs and absorption of DPP (Fig. 3C), the possibility of resonance energy transfer upon laser irradiation is excluded. Thus, electron transfer from the excited Mn-doped ZnS QDs to quenchers becomes the most probable reason for RTP quenching of Mn-doped ZnS QDs in our study.

As shown in Fig. 3D, DPP, a kind of methyl xanthine derivative condensed by theophylline and monochloropropanediol, is a positively charged compound due to a plurality of nitrogen atoms. On the contrary, MPA-capped Mn-doped ZnS QDs in aqueous dispersion are negatively charged because of carboxyl ionization in MPA. Hence, electrostatic interaction is likely to be occurred between DPP and
Fig.3. (A) The relationship between $P_0 / P$, $(P_0 - P)^{-1}$ and the concentration of DPP; (B) UV-vis absorption spectrum of a) the MPA-capped Mn-doped ZnS QDs, b) DPP, c) DPP (with Mn-doped ZnS QDs as reference); d) date of a plus b; (C) UV-vis absorption spectrum of DPP (curve 1), and emission spectrum of MPA-capped Mn-doped ZnS QDs (curve 2); (D) Chemical structure of DPP.

MPA-capped Mn-doped ZnS QDs. Further considering the phosphorescence emission principle of Mn-doped ZnS QDs resulted from electron transition, it is well known that, after excitation of QDs, the electron ($e^-$) of Mn-doped ZnS QDs will transit from valence band (VB) to conduction band (CB) that has higher energy than VB, and leaves a hole ($h^+$) consequently, this process is known as electron-hole pair generation.
While non-radiative electron-hole recombination results in luminescence of QDs via the release of energy in the form of photons\textsuperscript{34-36}. Thus, the DPP’ quenching effect on Mn-doped ZnS QDs RTP is mostly resulted from the effective electron transfer occurred between QDs and DPP.

DPP, as the possible electron donation\textsuperscript{37-39}, can not only induce the electrons transfer from DPP to the VB hole of excited state of Mn-doped ZnS QDs, but also hamper the recombination of electron–hole pair and, decrease the probability of recombination of electron-hole pair, which will lead to the RTP quenching of Mn-doped ZnS QDs\textsuperscript{40}. Please find the illustration of quenching process from Scheme 1.

Scheme 1 Mechanism of the phosphorescence quenching process of Mn-doped ZnS QDs by DPP

3.7 Real sample analysis

In order to evaluate the applicability of the proposed method to real samples, the RTP system with practical feasibility is applied to detect DPP in DPP injections and 1 % urine sample (healthy human) in 20 mM PBS buffer (pH 7.4). The results are shown in Table 2. Recoveries of DPP injection samples are mainly from 92 to 103 % and the recovery of urine samples is larger than 91 %, which demonstrates a good precision of this method. All of these results indicate the reliability and practicality of
this method.

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>DPP (nM)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>2.0</td>
<td>97±3</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>105±4</td>
</tr>
<tr>
<td></td>
<td>16.0</td>
<td>91±2</td>
</tr>
<tr>
<td>DPP injection</td>
<td>2.0</td>
<td>103±3</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>95±5</td>
</tr>
<tr>
<td></td>
<td>16.0</td>
<td>92±4</td>
</tr>
</tbody>
</table>

Table 2 Recovery for the detection of DPP in samples (Mean ± s; n = 3)

4. Conclusions

As the RTP intensity of MPA-capped Mn-doped ZnS QDs could be selectively quenched by DPP due to electron transfer reaction, a very sensitive and selective RTP method for DPP detection is developed, which provides a wide working range of 1.97~197 nM with low detection limit of 0.893 nM, and can be used to detect DPP in body fluid. Additionally, this method requires no complicated separation process to remove the interference from other substances that may coexist with DPP, so the analytical application is simplified. Finally, the strategy used in this method may offer a new approach to develop low-cost system for other biological and environmental applications as what we expected.

Acknowledgement

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*Advanced Science Letters*, 2011, **4**, 3490-3495.
Graphical abstract

Mechanism of the phosphorescence quenching process of Mn-doped ZnS QDs by DPP