

A Highly Selective Turn-On Fluorescent Chemosensor for Zinc Ion in Aqueous Media

Zeng-chen Liu · Zheng-yin Yang · Yan-xia Li · Bao-dui Wang · Yong Li · Tian-rong Li · Yong-jie Ding

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Abstract In the paper, a novel rhodamine6G based fluorescent chemosensor bearing 3-carbaldehyde chromone was designed and synthesized. According to the fluorescence behavior toward several metal ions, it showed highly selectivity and sensitivity to Zn(II) over other commonly coexistent metal ions (Cu(II), Cd(II), Hg(II), Mg(II), K(I), Pb(II), Fe(III) and Cr(III)) in aqueous environment (pH=7.4). Meanwhile the binding constant between Zn(II) and chemosensor achieved $6.21 \times 10^{11} \text{ M}^{-1}$ in aqueous media. Moreover, according to the Job plot, 1:1 stoichiometry between Zn(II) and sensor was deduced in aqueous media (pH=7.4). The good selectivity and sensitivity in aqueous media effectively enhanced the application value of the fluorescent chemosensor for Zn(II).

Keywords Chemosensor · Zn(II) selectivity · 3-carbaldehyde chromone-rhodamine6G hydrazone · Turn on · Binding constant

Z.-c. Liu (✉) · Y.-x. Li · Y.-j. Ding
College of Chemistry, Zhoukou Normal University, Zhoukou
466001, People's Republic of China
e-mail: liuzch07@lzu.edu.cn

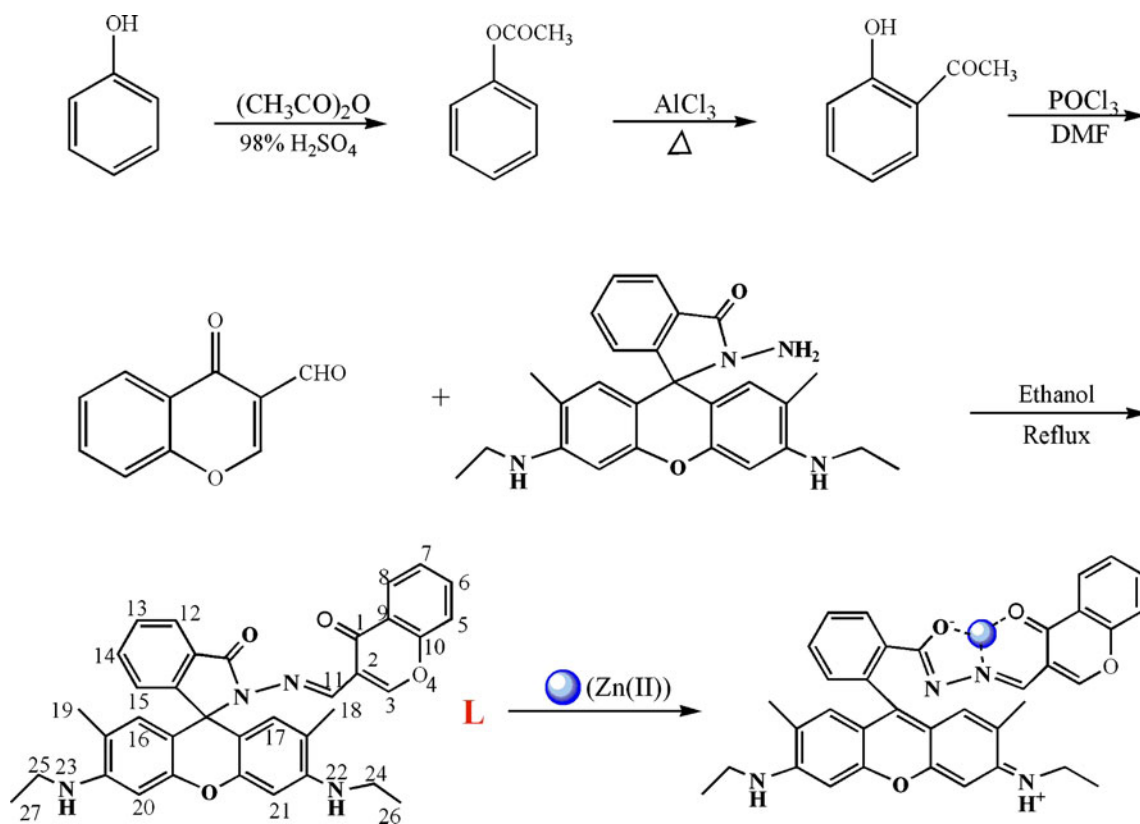
Z.-c. Liu · Y.-x. Li · Y.-j. Ding
The Key Laboratory of Rare Earth Functional Materials and
Applications, Zhoukou 466001, People's Republic of China

Y. Li
Faculty of Material Science and Chemistry Engineering, China
University of Geosciences, Wuhan 430074, People's Republic of
China

Z.-y. Yang · B.-d. Wang · T.-r. Li
College of Chemistry and Chemical Engineering and State Key
Laboratory of Applied Organic Chemistry, Lanzhou University,
Lanzhou 730000, People's Republic of China

Introduction

The highly selective and sensitive chemosensors toward metal ions in aqueous environment are of great importance in studying and tracking the chemical and physiological functions in the wide range of chemical and biological processes [1–5]. Because the analysis of relevant metal elements like copper, zinc, iron and cobalt et al. in living organism can provide some valuable information about our physical state. At present, owing to its particular structural property, the rhodamine framework is an available mode to construct OFF-ON fluorescent chemosensors, consequently, considerable efforts have been devoted to developing fluorescent chemosensors based on rhodamine derivatives toward corresponding metal ions due to their simplicity, high selectivity and sensitivity [6–10]. The Zinc is a biologically essential element and plays an important role in living organism. Zinc ions serves as key structural components of a large of proteins and also performs catalytic roles in enzymes, which is relevant to many cellular processes. Moreover, in the physiological processes such as brain, prostate and intestine locations, abnormal level of zinc ions even can cause some relevant diseases like Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and parkinson's disease [11–14]. Additionally, in environment, an excess of zinc is regarded as a pollutant, which may lead to the death of plants and are harmful to useful soil [15, 16]. But the incompletely understood of zinc ions remains active topics of research. Thus, there is a considerable need to design highly selective fluorescent chemosensors, which are capable of detecting the presence of zinc in biological and environmental system in aqueous media at a physiological pH value condition. In addition, it is also a challenging task for designing the chemosensor for Zn(II) via chelation enhanced fluorescence (CHEF) [17, 18]. To date, some selective fluorescent chemosensors for Zn(II)



Scheme 1 The synthesis and probable complexation mechanism of corresponding compounds

have been achieved. However, to the best of our knowledge, very few fluorescent sensors in aqueous media have been designed.

When aiming at the rational development of specific fluorescent chemosensors for targeting zinc ion (Zn(II)), the choice of binding motif is a critical factor, because in the complicated

environment containing, many metal ions such as Cu(II), Fe(III), Hg(II) and Cd(II) et al. can interfere with zinc binding [19, 20]. It is well known that the chelating group such as $-C=N-$ and $-C=O-$ exhibits a high affinity to transition metal cations, but less binding affinity toward alkali and alkaline earth metal cations [21–23]. Thus, it is significative to design

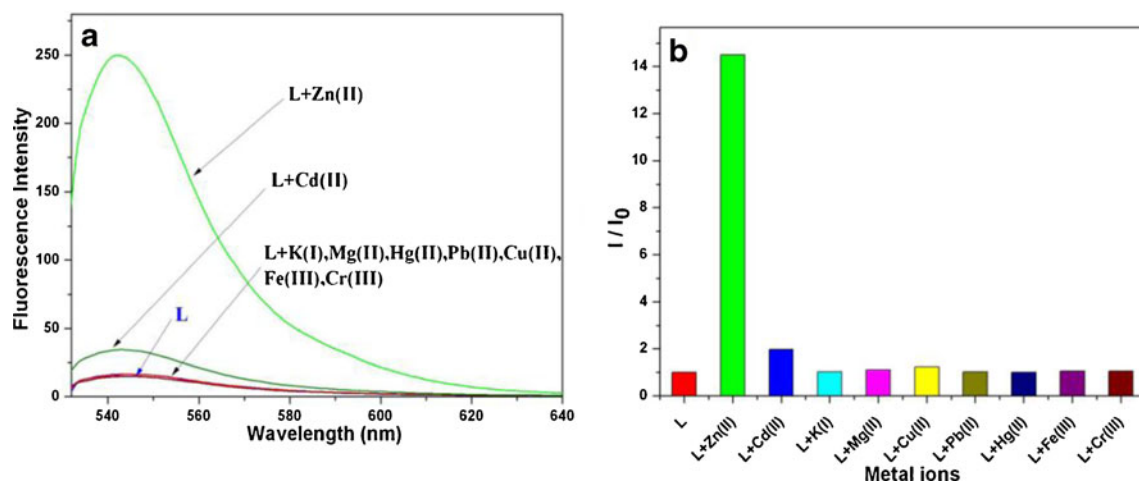
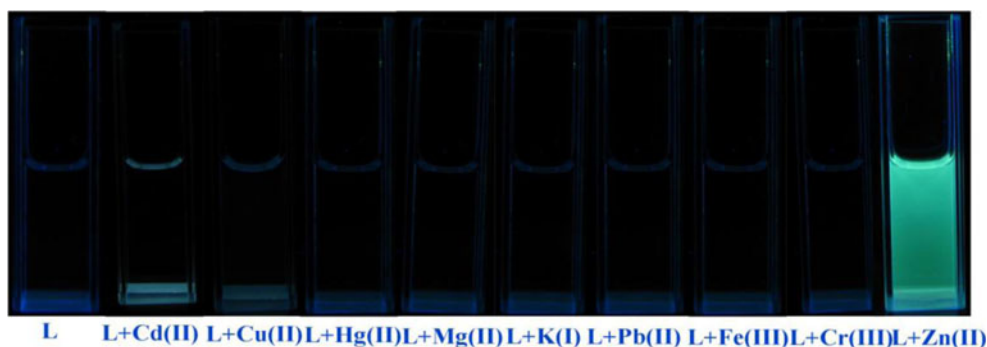


Fig. 1 **a** The changes in the fluorescence spectra (excitation at 525 nm, Slit: excitation/emission=3/3) of **L** (1.0×10^{-5} M) in the presence of different metal ions (4.0×10^{-5} M) in ethanol-HEPES buffer solution (9:1, pH=7.4). **b** the histogram of selectivity for various metal ions

Fig. 2 The fluorescence image of **L** (2.0×10^{-5} M) and the fluorescence images of **L** with addition of various metal ions (4.0×10^{-5} M)



relevant fluorescent chemosensors containing the functional group. In the present investigation, novel 3-carbaldehyde chromone-rhodamine6G hydrazone architecture is developed to increase the selectivity and sensitivity for detecting Zn(II). According to the spectra analysis, 3-carbaldehyde chromone-rhodamine6G hydrazone based fluorescent sensor exhibits highly selectivity toward Zn(II) over other metal ions in aqueous buffer media (pH=7.4).

Experimental Sections

Materials

All the chemicals containing solvents were of reagent grade and were used without further purification.

Synthesis of 3-Carbaldehyde Chromone-Rhodamine6G Hydrazone (L)

Rhodamine6G hydrazine and 3-carbaldehyde chromone was prepared by the reported methods [24–26]. Their synthesis routine was shown in Scheme 1.

An ethanol solution (20 mL) of rhodamine6G hydrazine (0.1 mol, 0.428 g) was added to another ethanol (50 mL) containing 3-carbaldehyde chromone (0.1 mol, 0.1174 g). Then the solution was reflux for 12 h and cooled to room temperature. The mixture was filtered and dried under vacuum. Recrystallization from MeOH/H₂O (V:V=1:1) gave 3-carbaldehyde chromone-rhodamine6G hydrazone (L), which was dried under vacuum. Yield, 75 %. m.p: 283–285 °C. ¹H-NMR (DMSO-*d*₆ 400 MHz): δ 8.55 (1H, s, -C³-H), δ 8.46 (1H, s, -C¹¹H=N-), δ 8.3–8.46 (1H, d, -C⁸-H), δ 8.00–8.03 (1H, m, -C¹²-H), δ 7.56–7.60 (1H, m, -C⁷-H), δ 7.44–7.49 (2H, m, -C^{13,14}-H), δ 7.37–7.39 (1H, d, -C⁵-H), δ 7.31–7.34 (1H, m, -C⁶-H), δ 7.04–7.06 (1H, m, -C¹⁵-H), δ 6.44 (2H, s, -C^{20,21}-H), δ 6.31 (2H, s, -C^{16,17}-H), δ 3.71–3.72 (2H, m, -C^{22,23}-H), δ 3.19–3.20 (4H, d, -C^{24,25}-H), δ 1.869 (6H, s, -C^{18,19}-H), δ 1.28–1.31 (6H, m, -C^{26,27}-H).

All spectroscopic measurements were performed in ethanol-HEPES (9:1) buffer solution (pH=7.4).

Stock solutions (1.0×10^{-3} M) of metal ions (metal chloride) were prepared in two-distilled water. The stock solution of 1.0×10^{-3} M 3-carbaldehyde chromone -rhodamine6G hydrazone (L) was prepared in ethanol. In titration experiments, each time 2 mL aqueous solution containing 20 μL

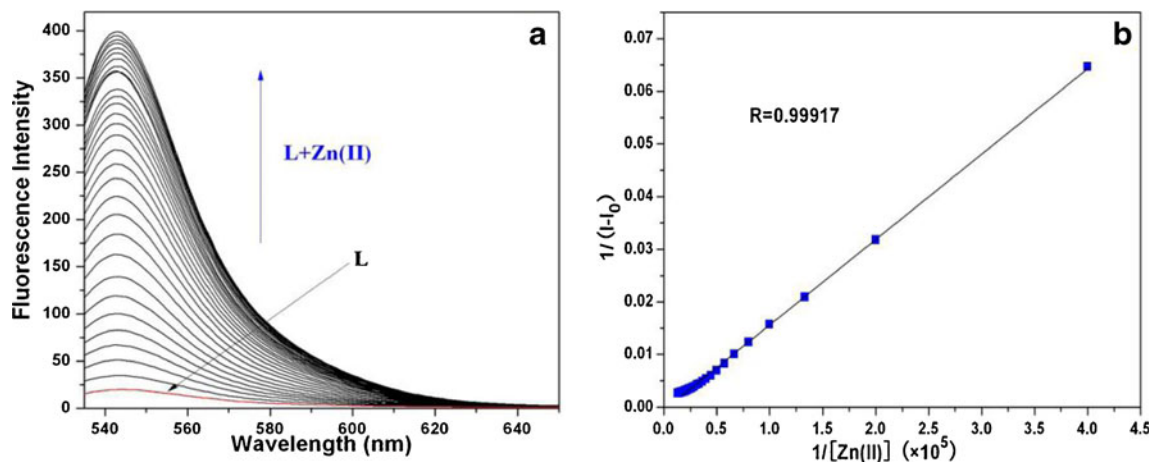


Fig. 3 **a** Fluorescence spectrum of **L** (1.0×10^{-5} M) upon addition of **Zn(II)** (0–8 equiv) in ethanol-HEPES solution (pH=7.4). Excitation at 525 nm. **b** Fitting of Fluorescence titration curve of **L** in ethanol-

HEPES solution (pH=7.4). The linear equation is $Y=0.0161X-3.3975E-4$, the linear range of concentration of Zn(II) is from 1.0×10^{-7} M to 4.0×10^{-5} M

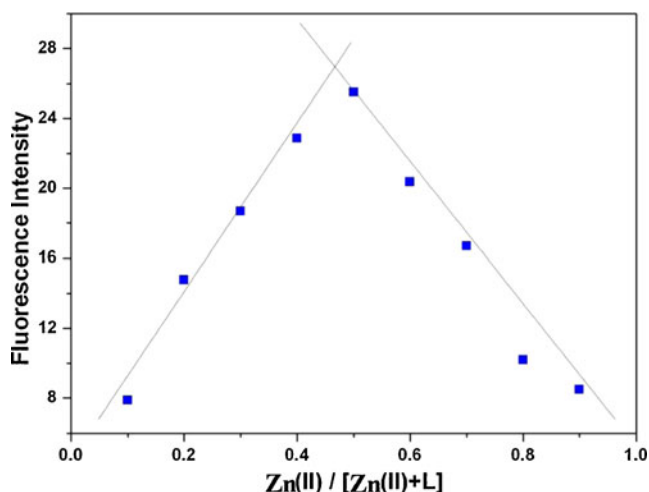


Fig. 4 Job's plot according to the method for continuous variations, indicating the 1:1 stoichiometry for L-Zn(II) (the total concentration of L and Zn(II) is 1.0×10^{-5} M). ($\lambda_{\text{ex}}=525$ nm, Slit: excitation/emission=3/3)

solution of L (1.0×10^{-3} M) was filled in a quartz optical cell of 1 cm optical path length. Then equal amount of Zn(II) stock solution (5 μ L) was added to the compound solution with micro-pipette. Spectral data was recorded at 2 min after the addition. In selectivity experiment, the test samples were prepared by placing appropriate amounts of metal ion stock solution into 2 mL aqueous solution of L (1.0×10^{-5} M). For fluorescence measurements, excitation wavelength is at 525 nm.

The binding constant between L and Zn(II) was calculated by the linear Benesi-Hildebrand expression [27, 28]

$$\frac{1}{I-I_0} = \frac{1}{[L]} + \frac{1}{K_s} \cdot \frac{1}{[L][M]}$$

Where I is the change of fluorescence intensity in the presence of Zn at 545 nm, K_s is the stability constant, and [L] and [M] are the concentration of L and Zn(II), respectively.

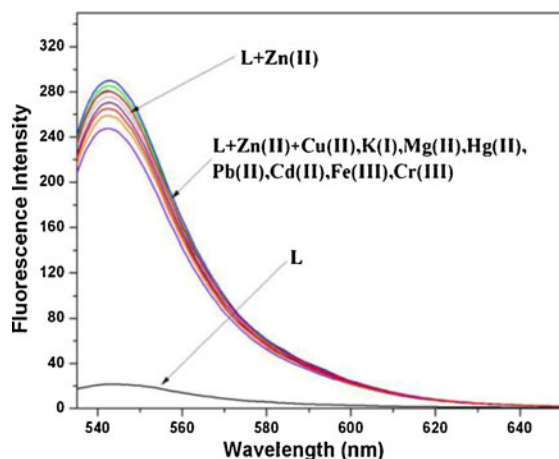


Fig. 5 The selectivity of L for Zn(II) in the presence of other metal ions (Cu(II), Cd(II), Hg(II), Mg(II), K(I), Pb(II), Fe(III), Cr(III)) in ethanol-HEPES media (pH=7.4). Excitation at 525 nm. The response is normalized

I_0 is the fluorescence intensity of L in the absence of Zn(II). On the basis of the plot of $1/(I-I_0)$ versus $1/[Zn(II)]$, the stability constant can be obtained.

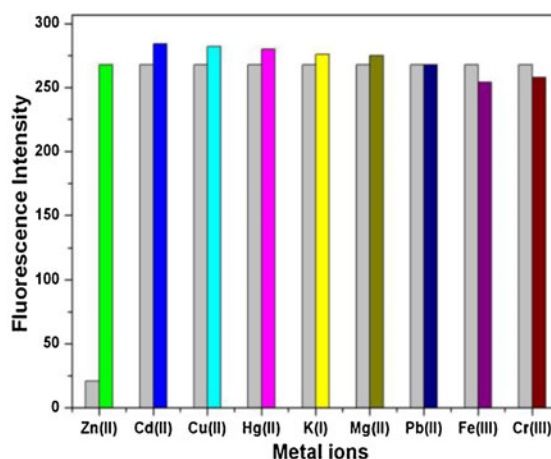
Physical Measurement

$^1\text{H-NMR}$ spectra were recorded on a Varian VR400-MHz spectrometer with TMS as an internal standard. The melting points of the compound were determined on a Beijing XT4-100X microscopic melting point apparatus. The UV-vis spectra were recorded on a Perkin-Elmer Lambda-35 UV-vis spectrophotometer. Fluorescence spectra were obtained on a Shimadzu RF-5301 spectrophotometer at room temperature.

Results and Discussions

Spectra Investigation

To investigate the fluorescence selectivity toward special metal ion, the fluorescence selectivity experiments of various metal (Zn(II), Cu(II), Cd(II), Hg(II), Mg(II), K(I), Pb(II), Fe(III), Cr(III)) were conducted in ethanol-HEPES (9:1) buffer solution at pH=7.4. As Shown in Fig. 1, in the absence of metal ions, the 3-carbaldehyde chromone-rhodamine6G hydrazone exhibited a rather weak fluorescence signal in the range from 520 nm to 700 nm in ethanol-HEPES medium (pH=7.4). However, with addition of four equivalent various metal ions, only the addition of Zn(II) lead to approximately 15-fold fluorescence enhancement in ethanol-HEPES media (pH=7.4). Except for weak fluorescence increase for Cd(II), other metal ions did not cause obvious fluorescence changes of L. It's deduced that the coordination between Zn(II) and L aroused the opening of rhodamine6G ring and resulted in the formation of Zn(II)-L with enhanced fluorescence intensity.



with respect to fluorescence intensity of the free L (1.0×10^{-5} M) with addition of Zn(II) (4.0×10^{-5} M). Then other metal ions were added (4.0×10^{-5} M)

The fluorescence behavior preliminarily indicated 3-carbaldehyde chromone-rhodamine6G hydrazone could act as an fluorescence probe for Zn(II) under physiological pH conditions. In addition, according to the histogram, the highly selectivity of L for Zn(II) over other metal ions were indicated obviously. To illustrate the selectivity of L toward Zn(II) in aqueous media, the fluorescence images under UV light were also made (Fig. 2). Upon addition of two equivalent metal ion, only the solution of L with Zn(II) showed remarkable green fluorescence under UV light, which indicated 3-carbaldehyde chromone-rhodamine6G hydrazone could act as a highly selective turn-on fluorescent chemosensor for Zn(II) in aqueous media (pH=7.4).

Fluorescence titration experiment (Fig. 3a) of L with Zn(II) was performed in aqueous buffer media (pH=7.4) at room temperature. Upon addition of Zn(II), the fluorescence signal at 545 nm significantly enhanced. It was explicit that the binding between 3-carbaldehyde chromone-rhodamine6G hydrazone and Zn(II) induced the open ring of spirolactam of chemosensor, which was responsible for the fluorescence intensity change. It was also clear that the OFF-ON process of chemosensor, which was switched on by coordinative Zn(II) as the excitation at 525 nm, resulted in the emission of rhodamine6G fluorophore with a maximum at 545 nm. As shown in Fig. 3b, the association constant between L and Zn(II) was estimated to be $6.21 \times 10^{11} \text{ M}^{-1}$ in aqueous media (pH=7.4) by fitting the data to the Benesi-Hildebrand expression with a good linear relationship. And it exhibited wide linear range of concentration of Zn(II), which is from $1.0 \times 10^{-7} \text{ M}$ to $4.0 \times 10^{-5} \text{ M}$. Additionally, by the Job plot shown in Fig. 4, the binding mode between L and Zn(II) was investigated systemically. We could deduce that there was a 1:1 stoichiometry between them. Moreover, in accordance with the 1:1 stoichiometry, the turn-on fluorescent chemosensor

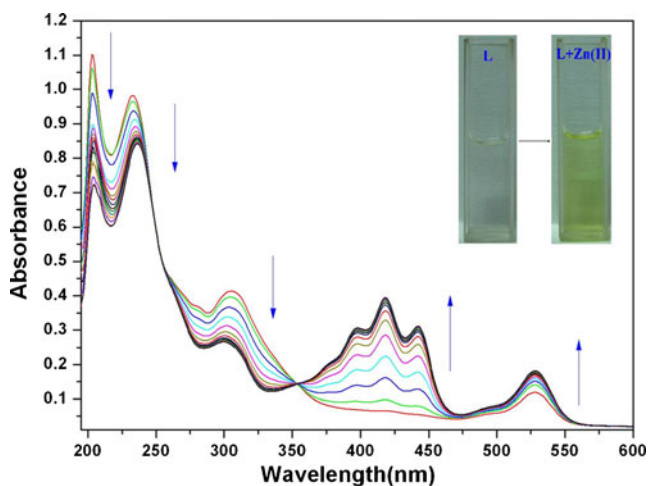


Fig. 6 The absorption spectra of L ($1.0 \times 10^{-5} \text{ M}$) in ethanol-HEPES media in the presence of different amounts of Zn(II) (0–4 equiv). Inset: the color change of chemosensor solution with addition of Zn(II)

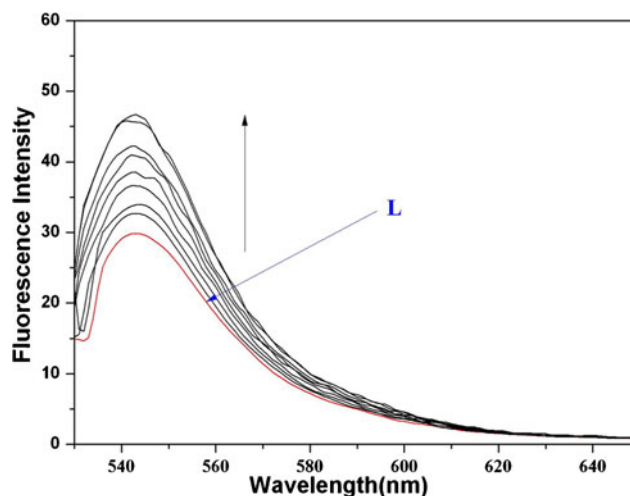


Fig. 7 Fluorescence spectra (excitation at 525 nm, Slit: excitation/emission=5/5) of L ($1 \mu\text{M}$) with addition of Zn(II) ($0.1 \mu\text{M}$) in ethanol-HEPES media (pH=7.4). Red line represents L

was most likely to chelate with zinc ion via its carbonyl O, imino N and 3-carbaldehyde chromone O atoms.

Furthermore, to validate the high selectivity of L for Zn(II), the fluorescence competitive experiments of Zn(II) with other cations were also investigated. Four equivalent Zn(II) was added to the aqueous solution of L ($1.0 \times 10^{-5} \text{ M}$), then equivalent amount of other metal ions (Cu(II), Cd(II), Hg(II), Mg(II), K(I), Pb(II), Fe(III) and Cr(III)) were also inserted into the solution. Their fluorescence intensities were recorded, respectively. The histogram of fluorescence changes were listed in Fig. 5. As shown in Fig. 5, no significant variation in the fluorescence emission of L-Zn(II) was observed by comparison with the fluorescence property with addition of other metal ions. All the experimental results indicated the high selectivity of L toward Zn(II) over other co-existent metal ions in aqueous media (pH=7.4).

As shown in Fig. 6, the absorption spectrum of L ($1.0 \times 10^{-5} \text{ M}$) in aqueous media (pH=7.4) exhibited a very weak

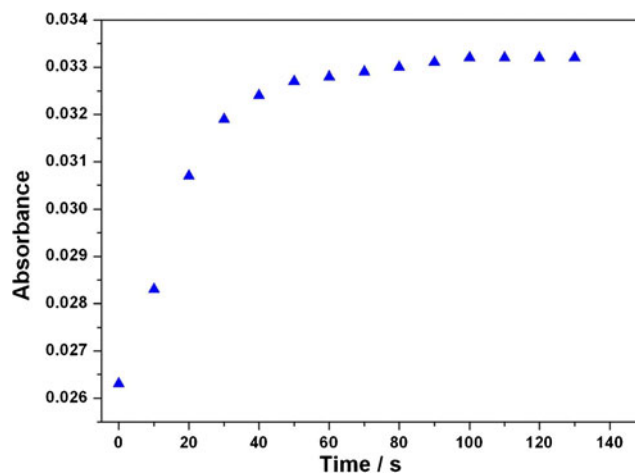


Fig. 8 Time course of the response of L ($1 \times 10^{-5} \text{ M}$) to 1 equiv Zn(II) in ethanol-HEPES solution (pH=7.4)

peak at the range 500–560 nm, which was ascribed to the closed spirocyclic lactam of chemosensor molecule L. With addition of Zn(II), the absorption spectrum changed significantly with the solution turning from colorless to yellow, clearly indicating the formation of ring-opened amide form of L with Zn(II) binding (Fig. 6 inset). Additionally, several new bands appeared obviously at the range 400–450 nm upon addition of Zn(II), which also implied that the coordination between L and Zn(II) lead to the fluorescence emission of chromone fluorophore. To evaluate the sensitivity of chemosensor L for Zn(II) in aqueous media, the detection limit of L in recognizing Zn(II) was also tested using fluorescence spectra (Fig. 7). The fluorescence titration profile of L (1.0×10^{-6} M) with Zn(II) demonstrated the detection of Zn(II) in aqueous media (pH=7.4) was at the part 1.0×10^{-7} M. Under the conditions, the fluorescence intensities of chemosensor L solution was still proportional to the amount of Zn(II). Simultaneously, the time course of response of L to equal Zn(II) in aqueous media (pH=7.4) was also investigated (Fig. 8). The experiment demonstrated that the interaction between L and Zn(II) was completed in less than 90 s. Therefore, the fluorescent chemosensor could be applied in real-time tracking of Zn(II) in organism.

Conclusions

In summary, we have developed a novel rhodamine6G based turn-on fluorescent chemosensor for Zn(II). It exhibits high selectivity and sensitivity toward Zn(II) over other metal ions in aqueous media (pH=7.4). The closed spirocyclic lactam ring of 3-carbaldehyde chromone-rhodamine6G hydrazone is opened with addition of Zn(II) in aqueous media, then intense green fluorescence appears under the combined action of rhodamine6G and chromone fluorophores. Moreover, according to the investigation, 1:1 stoichiometry complex between L and Zn(II) is formed. The excellent selectivity of chemosensor 3-carbaldehyde chromone-rhodamine6G hydrazone for Zn(II) in aqueous media (pH=7.4) indicates its potential application value in the biological monitoring and tracking of zinc ions.

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