

A Ratiometric Fluorescent Sensor for Zn^{2+} Based on *N,N'*-Di(quinolin-8-yl)oxalamide

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Abstract A new ratiometric fluorescent sensor (DQO) based on *N,N'*-Di(quinolin-8-yl) oxalamide has been designed and synthesized for selective detection of Zn^{2+} . The fluorescence ratio ($I_{536\text{ nm}}/I_{450\text{ nm}}$) of DQO was enhanced 10-fold when Zn^{2+} was present in a buffer aqueous solution at pH 8.66. The sensor showed linear response toward Zn^{2+} in the concentration range 0–15 μM , and the detection limit was calculated to be 2.4 μM . A Job's plot implied the formation of a DQO/ Zn^{2+} complex with 1:1 stoichiometry, and the apparent association constant of DQO/ Zn^{2+} complex was computed to be $1.5 \times 10^4\text{ M}^{-1}$.

Keywords Oxalamidoquinoline · Sensor · Zinc ion · Ratiometric fluorescence

Introduction

Zinc is an essential trace element for all organisms [1–3]. Thus, a variety of methods to detect Zn^{2+} have been developed, such as photometric determination [4], atomic

absorption spectrometry [5] and laser ablation-inductively coupled plasma-mass spectrometry [6]. However, to some extent, these methods are hampered by the sophisticated pre-treatment procedures and/or expensive instrumentation, and they are inappropriate for in-situ non-destructive monitoring [7]. Considering the high selectivity, sensitivity, and relatively simple handling, fluorescence detection has been widely implemented in biology and clinical medicine, especially for the detection of zinc ion [8–10].

Although fluorescent chemical sensor has many advantages, some limitations still exist in these single emission intensity-based sensors, such as influences of sensor concentration and excitation intensity, and errors from detection environment [11]. Fortunately, ratiometric fluorescent sensors can overcome the limitation of intensity-based fluorescent sensors because they usually obtain two emission peaks on the same excitation wavelength and the ratio of the two fluorescence intensities can eliminate the interference from sensor concentration, excitation light source, and background fluorescence [12].

Zinc and cadmium are in the same group of the periodic table and have similar properties. Therefore, similar fluorescence changes including the change of intensity and the shift of wavelengths are usually observed when they are coordinated with fluorescent sensors [13, 14]. Accordingly, it is critical to develop the new fluorescent sensors to detect Zn^{2+} without interference of Cd^{2+} [9].

In general, most fluorescence sensors have two components, a fluorophore and a binding site. The reported fluorophores include quinoline [15], rhodamine [16], naphthalimide [17], BODIPY [18] and so on. Among these sensors, amidoquinoline-based sensors [19–22] have obtained widely attention because of good water solubility, easy modification and well-biocompatibility. Thus, many researchers have made great effort to study 8-carboxamidoquinoline-

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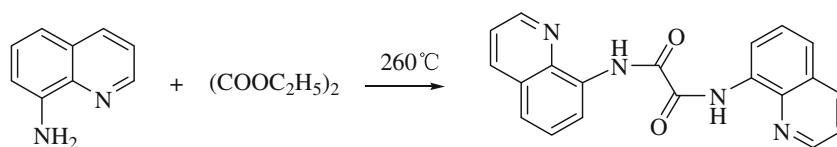
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Scheme 1 Synthesis of DQO



based sensors [23–29]. But it is worth mentioning that the above-mentioned sensors based on quinoline were acetamidoquinoline derivatives. Recently, we found an asymmetrical oxalamidoquinoline derivative could serve as a fluorescence turn-on sensor for Zn^{2+} in solution and living cells [30].

Herein, a new fluorescent sensor that was a symmetrical oxalamidoquinoline derivative, namely *N,N'*-Di(quinolin-8-yl)oxalamide (DQO), was synthesized by one-step reaction of 8-aminoquinoline and diethyl oxalate. The results showed DQO was a fluorescence ratiometric sensor for Zn^{2+} and could prevent the interference of Cd^{2+} .

Experimental

Reagents and Apparatus

All solvents were obtained from commercial suppliers (analytical grade) and used as received without further purification. Water was purified by Milli-Q purification system. The metal ion solutions (0.050 M) were obtained from NaCl, KCl, $\text{Mg}(\text{ClO}_4)_2$, $\text{Ca}(\text{NO}_3)_2$, $\text{Cr}(\text{NO}_3)_2$, FeSO_4 , CoSO_4 , NiSO_4 , $\text{Cu}(\text{NO}_3)_2$, $\text{Zn}(\text{NO}_3)_2$, AgNO_3 , CdSO_4 , HgCl_2 , $\text{Pb}(\text{NO}_3)_2$, and AlCl_3 . All of the detections of metal ions were operated at pH 8.66 maintained with tris- HClO_4 buffer (10 mM) in methanol/water (9:1, v/v) mixed solvent. All fluorescence data were recorded with the excitation of 330 nm, and the slit widths of excitation and emission were 2.5 nm.

NMR spectra were recorded on a Bruker Avance-600 spectrometer and referenced to internal TMS. ESI-MS spectrum was measured with Agilent 6310 ESI-Ion Trap Mass spectrometer. FT-IR spectrum was measured with Nicolet Avatar-370. Melting point was got on X-6 μ melting point apparatus. Fluorescence spectra and UV-vis absorption spectra were recorded on a Hitachi F-7000 and Puxi TU-1901 spectrophotometers, respectively. All pH measurements were made using a Sartorius basic pH-meter PB-10.

Synthesis of DQO

8-Aminoquinoline 501 mg (3.46 mmol) and diethyl oxalate 257 mg (1.76 mmol) were mixed in a 5 mL round-bottomed flask. Then the mixture was stirred for 1.7 h at 260 °C to obtain a black solid which was washed with diethyl ether, and filtered. The filter-cake was further purified by column chromatography on silica gel with chloroform as eluent to obtain *N,N'*-di(quinolin-8-yl)oxalamide (DQO), white needle-like crystals. Yield: 207 mg (37.8%), Mp: 311.7–313.6 °C. ^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ ppm 11.60 (s, 1H), 9.04 (d, $J = 3.0$ Hz, 1H), 8.76 (d, $J = 7.8$ Hz, 1H), 8.49 (d, $J = 7.8$ Hz, 1H), 7.83 (d, $J = 7.8$ Hz, 1H), 7.72 (t, $J = 6.3$ Hz, 1H). ^{13}C NMR (150 MHz, CDCl_3): δ ppm 158.0, 149.0, 139.1, 136.1, 133.2, 128.0, 127.1, 123.1, 122.0, 117.1. FT-IR (KBr) ν cm^{-1} : 3311, 1613, 1518 and 496–862. ESI-MS m/z [$\text{M} + \text{H}^+$] Calcd. 343.1117, Found 343.1192. The synthesis of DQO was shown in the Scheme 1.

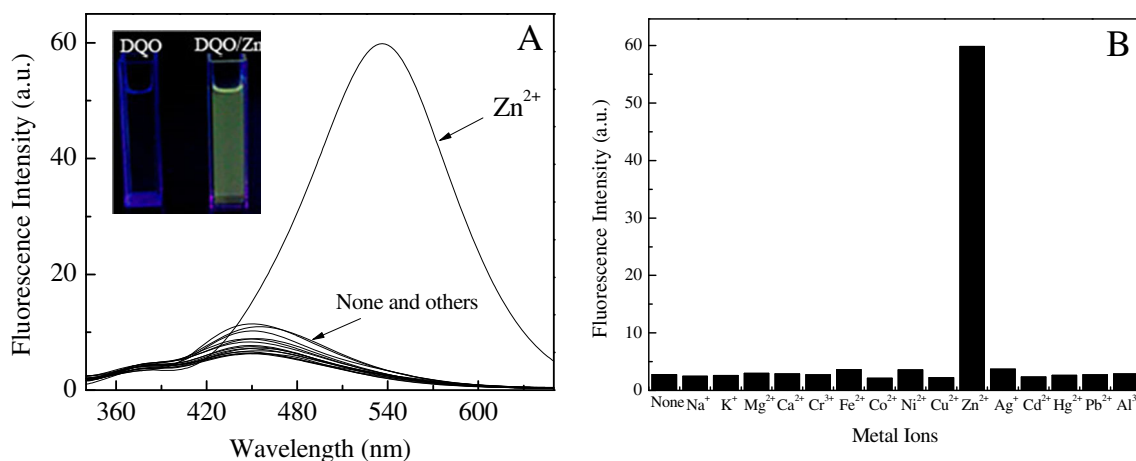


Fig. 1 Fluorescence spectra (a) and fluorescence intensities (b) at 536 nm of DQO (10 μM) with different metal ions (50 μM). Inset: Visible emission observed from DQO and DQO/ Zn^{2+} . (methanol/water = 9:1, v/v, 10 mM Tris- HClO_4 , pH = 8.66, λ_{ex} = 330 nm)

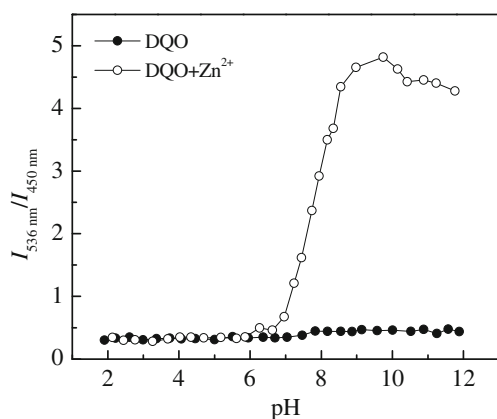


Fig. 2 The pH titration profiles of DQO and DQO/Zn²⁺ complex in solution (methanol/water =9:1, v/v)

Results and Discussion

Cation-Sensing Properties of DQO

Fluorescence and absorbance spectra of DQO were got upon treatment with different common metal ions (5.0 equiv.) in methanol-water Tris-HClO₄ buffer solutions. As shown in Fig. 1, DQO shows weak emission centered at 450 nm with a low quantum yield, 0.0015. In presence of different common cations, namely Na⁺, K⁺, Mg²⁺, Ca²⁺, Cr³⁺, Fe²⁺, Ni²⁺, Co²⁺, Cu²⁺, Ag⁺, Cd²⁺, Hg²⁺, Pb²⁺, and Al³⁺, no obvious changes in emission properties were observed. However, a significant fluorescence change was found that a new fluorescence peak at 536 nm appeared dramatically, when Zn²⁺ was added.

As illustrated in the inset of Fig. 1a, a fluorescent color change from blue to yellow-green could be seen by the naked eye as Zn²⁺ was added and the solution was irradiated with a 365 nm UV lamp. The absorbance spectra of DQO were

shown in the Fig. S1 in present of different common cations. After adding different metal ions in the system, the absorption spectra of the compound will be changed. When Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺ added, respectively, a new absorption peak was emergence at 329 nm, 372 nm, 387 nm, 360 nm. After added Fe²⁺, the absorption at 330 nm was significantly enhanced.

The fluorescence intensity of DQO at 536 nm was almost enhanced 22-fold in present of Zn²⁺ in Fig. 1b, while its fluorescence intensities at 536 nm were scarcely changed by other metal ions. More importantly DQO has no response to Cd²⁺, which is akin to Zn²⁺ and has closed-shell d¹⁰ configuration [31, 32].

Effect of pH on the Sensor Performance

The effect of pH on the fluorescence of DQO in the absence and presence of Zn²⁺ was studied to eliminate the disturbance by the variation of pH values during detection. As shown in Fig. 2, when pH value ranged from 2 to 6.8, the values of $I_{536\text{nm}}/I_{450\text{nm}}$ were almost overlapping, which means that DQO cannot response to Zn²⁺ in this pH value range. Then it increased rapidly with the increasing of pH value when Zn²⁺ is present and the maximum appears at about pH 9. This is due to the deprotonated of nitrogen atom in amide group and the formation of the complex DQO/Zn²⁺. So that, 8.66 was chosen as test pH value.

Zn²⁺-Titration and Spectral Responses

The titration experiments were carried out by adding the increasing amounts of Zn²⁺ to a solution of DQO. The emission spectrum of free DQO displays a broad band with a maximum at 450 nm in an aqueous Tris-HClO₄ buffer solution (Fig. 3a). When Zn²⁺ was added to the solution of

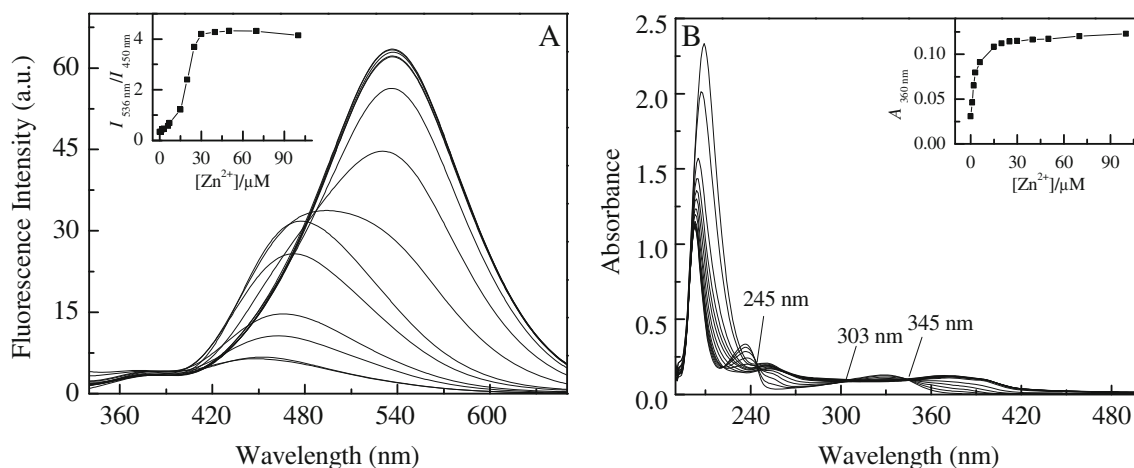


Fig. 3 Fluorescence (a) and absorbance (b) responses of DQO (10 μM) upon addition of Zn²⁺ in Tris-HClO₄ buffered aqueous solution (methanol/water =9:1, v/v; pH = 8.66; λ_{ex} = 330 nm.). Insets: the

fluorescence rate ($I_{536\text{ nm}}/I_{450\text{ nm}}$) (a) and absorbance intensity (b) of DQO as a function of Zn²⁺ concentration

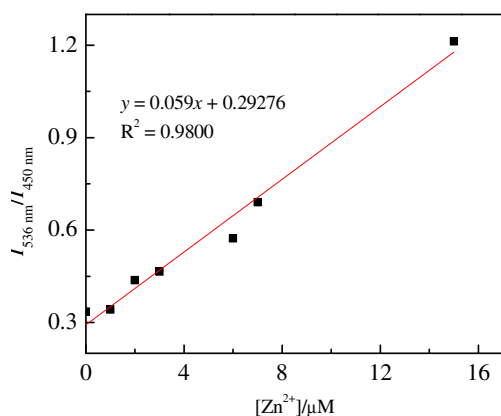


Fig. 4 Curve of fluorescence rate ($I_{536\text{ nm}}/I_{450\text{ nm}}$) of DQO (10 μM) versus the concentrations of Zn^{2+} (10 mM Tris-HClO₄, methanol/water =9:1, v/v, pH = 8.66, λ_{ex} = 330 nm)

DQO, an 86 nm red-shifted band was observed (from 450 nm to 536 nm). The inset in Fig. 3a exhibited the dependence of the intensity ratios of emission at 536 nm and 450 nm ($I_{536\text{ nm}}/I_{450\text{ nm}}$) on Zn^{2+} , and it got stabilized after the amount of Zn^{2+} ions reached 3 equiv. with a defined emission point. A satisfactory linear relationship between $I_{536\text{ nm}}/I_{450\text{ nm}}$ and Zn^{2+} concentration was observed and the linear equation was found to be $y = 0.29276 + 0.059x$ (linearly dependent coefficient: $R^2 = 0.9800$), as shown in Fig. 4. The quantitative detection concentration range is 0–15 μM . The limit of detection (LOD) was evaluated to be 2.4×10^{-6} M with $3\sigma/\text{slope}$ [33, 34]. As shown in Fig. 3B, UV–vis spectra of DQO exhibited an absorption band at 235 nm as well as 330 nm. Upon addition of Zn^{2+} , the absorbance both at 235 nm and 330 nm decreased obviously, whereas new absorption peaks appeared at 250 nm and 360 nm with three isosbestic points at 245 nm, 303 nm and 345 nm, respectively. This phenomenon can be construed as the complex and monomer both in the presence of the ground state [35].

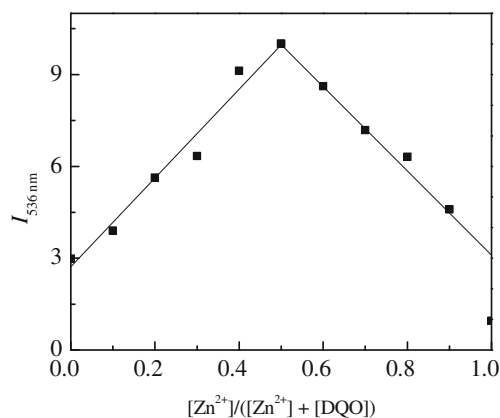


Fig. 5 A Job's plot for DQO (10 mM Tris-HClO₄, methanol/water =9:1, v/v, pH = 8.66. The total $[\text{DQO}] + [\text{Zn}^{2+}] = 10\ \mu\text{M}$)

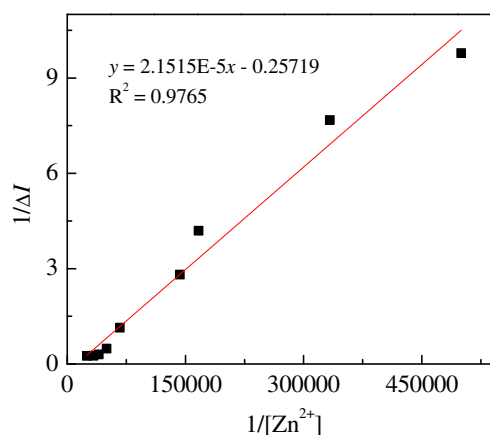


Fig. 6 Benesi–Hildebrand plot from fluorescence titration data of DQO (10 mM Tris-HClO₄, methanol/water =9:1, v/v, pH = 8.66, λ_{ex} = 330 nm)

Binding Model and Bonding Strength

To determine the stoichiometry of DQO and Zn^{2+} in the complex, Job's method was employed by using the emission changes at 536 nm as a function of molar fraction of Zn^{2+} . From Fig. 5, the maximum emission was observed when the molar fraction of Zn^{2+} reached 0.5. This result indicated that a 1:1 complex formed between DQO and Zn^{2+} . The association constant was calculated to be $1.5 \times 10^4\ \text{M}^{-1}$ by a Benesi–Hildebrand plot (Fig. 6) [36, 37].

Metal Ion Competition

To utilize DQO as a selective sensor for Zn^{2+} , the effect of competing metal ions has been examined by recording the fluorescence spectra of DQO (10 μM) with Zn^{2+} (5 equiv.)

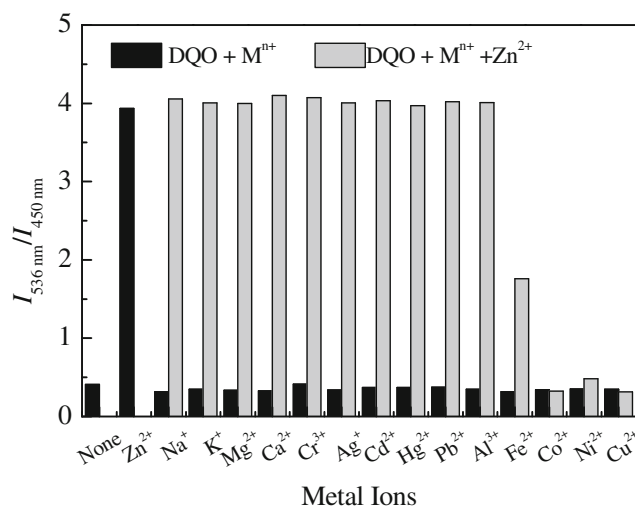


Fig. 7 Metal ion competition of 10 μM DQO in the presence of Zn^{2+} (5 equiv.) and additional metal ions (5 equiv.) at pH 8.66 (10 mM Tris-HClO₄, methanol/water =9:1, v/v, λ_{ex} = 330 nm)

Table 1 Determination of zinc ion concentrations in real samples ($n = 3$)

Samples	Contents (μM)	Added (μM)	Found ^a (μM)	Recovery (%)	RSD(%)
Lake water	0	5	5.7	114	0.75
		10	9.4	94	1.3
Tap water	0	5	5.1	102	0.66
		10	9.1	91	2.2

^a Average value of three determinations

in the presence of a competing metal ion (5 equiv.). As shown in Fig. 7, the presence of Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cr^{3+} , Ag^+ , Cd^{2+} , Hg^{2+} , Pb^{2+} , and Al^{3+} has little influence on the fluorescence response of DQO to Zn^{2+} . However, some paramagnetic metal ions, such as Co^{2+} , Ni^{2+} , and Cu^{2+} , quenched the fluorescence, which is probably due to the displacement of Zn^{2+} by Co^{2+} , Ni^{2+} , and Cu^{2+} [38].

Practical Applications

In order to verify the practical applicability of the ratiometric sensors, the determination of Zn^{2+} in tap water and lake water were evaluated using the standard addition method. Lake water was taken from the Labor Lake, and the further processing was use of acticarbon. The recovery of Zn^{2+} in lake water from 114 to 94% and the tap water from 102 to 91% were obtained, indicating the appreciable practicality of the presented sensors in the micromolar range (Table 1).

Conclusions

In summary, a new ratiometric fluorescent sensor for Zn^{2+} based on symmetrical derivative of oxalamide has been designed and synthesized in one-step. The sensor exhibits turn-on responses toward Zn^{2+} and an 86 nm red-shift of fluorescence emission upon binding Zn^{2+} in buffer solution are observed. Additionally, DQO can quantitatively detect for Zn^{2+} with a linear range 0–15 μM , and the detection limit was calculated to be 2.4 μM . Moreover, we hope that this oxalamidoquinoline derivative would provide ideas to the development of fluorescent sensor towards Zn^{2+} .

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